Molecular Therapy
Review

Novel Genetic and Epigenetic Biomarkers of Prognostic and Predictive Significance in Stage II/III Colorectal Cancer

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The therapeutic strategies of stage II/III colorectal cancer (CRC) patients after curative surgery remain controversial. In the clinical decision-making process, oncologists need to answer questions such as whether adjuvant chemotherapy is necessary or which therapeutic regimen should be given to each patient. At present, whether adjuvant chemotherapy should be applied is primarily based on histopathological features and clinical risk factors. However, only a fraction of patients can benefit from it. More rigorous stratifying biomarkers are urgently needed to help further distinguishing these populations of patients. Recent progress in next-generation sequencing and high-throughput technologies has greatly promoted biomarker discovery as well as our understanding of the underlying mechanisms in CRC. Novel genetic and epigenetic biomarkers that are associated with prognosis or therapeutic responses have emerged. In this review, we discuss the strategies of biomarker discovery and summarize the status and assess the utility of previously published biomarkers in CRC.

Colorectal cancer (CRC) was estimated to be the fourth most frequently diagnosed cancer and the second most common cause of cancer deaths worldwide in 2018.[1] CRC has been recognized as a disease affecting mostly developed countries. However, in recent years, the incidence of CRC has kept increasing in developing countries, such as China, possibly due to changes in lifestyle and nutritional habits.[2] Early-stage CRC is mostly asymptomatic, and many patients are diagnosed at advanced stages.[3]

The tumor-node-metastasis (TNM) stage remains the most potent prognostic factor in the clinical decision-making process. Based on current guidelines, most stage II CRC patients are treated surgically without adjuvant chemotherapy. However, almost 15% of these patients undergo tumor recurrence and death caused by disease progression.[4,5] Whether a stage II CRC patient should receive adjuvant chemotherapy after surgery largely depends on the recurrence risk assessed by a group of clinical factors.[6,7] Still, a considerable proportion of stage II patients who are evaluated as low-risk patients later suffer from recurrence and progression. Stage III patients routinely receive adjuvant chemotherapy after surgery.[8] However, evidence showed these patients did not respond equally.[9–12] Therefore, the need for biomarkers to more precisely identify stage II/III CRC patients that are suitable for adjuvant chemotherapy is highlighted.

The biomarkers that doctors use to predict CRC patients' prognosis and therapeutic response at present, such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9),[13,14] have relatively low sensitivity and specificity.[15,16] In this case, more meticulous biomarkers are needed. Thanks to the development of genomic high-throughput screening technologies, including next-generation sequencing and microarray analysis, a large number of molecular biomarkers and signatures with potential clinical prognostic and predictive values have been discovered via comprehensive association and bioinformatics analyses. Notably, several genomic-based biomarkers, such as the mismatch repair (MMR) or microsatellite instability (MSI) status, which plays a role as a predictive marker for adjuvant chemotherapy in patients with stage II CRC, have already entered into clinical practice and been validated.[17–20]

In this review, we summarize novel genomic and transcriptomic biomarkers as well as signatures reported recently with prognostic and predictive potentials in stage II/III CRC. We broadly examined current studies, focusing mainly on transcriptomic markers and signatures, which include newly discovered molecules such as microRNAs (miRNAs), long noncoding RNA (lncRNAs), and circular RNAs (circRNAs). We also assessed the clinical effectiveness of those already revealed and discussed their potential applications. The purpose of this review is to highlight some of the new candidate prognostic and predictive molecular markers for stage II/III CRC and assess their clinical utility for possible future applications.

Biomarker Discovering Strategies

Currently, two major strategies are adopted by researchers to discover new molecular biomarkers: mechanism-based strategies and

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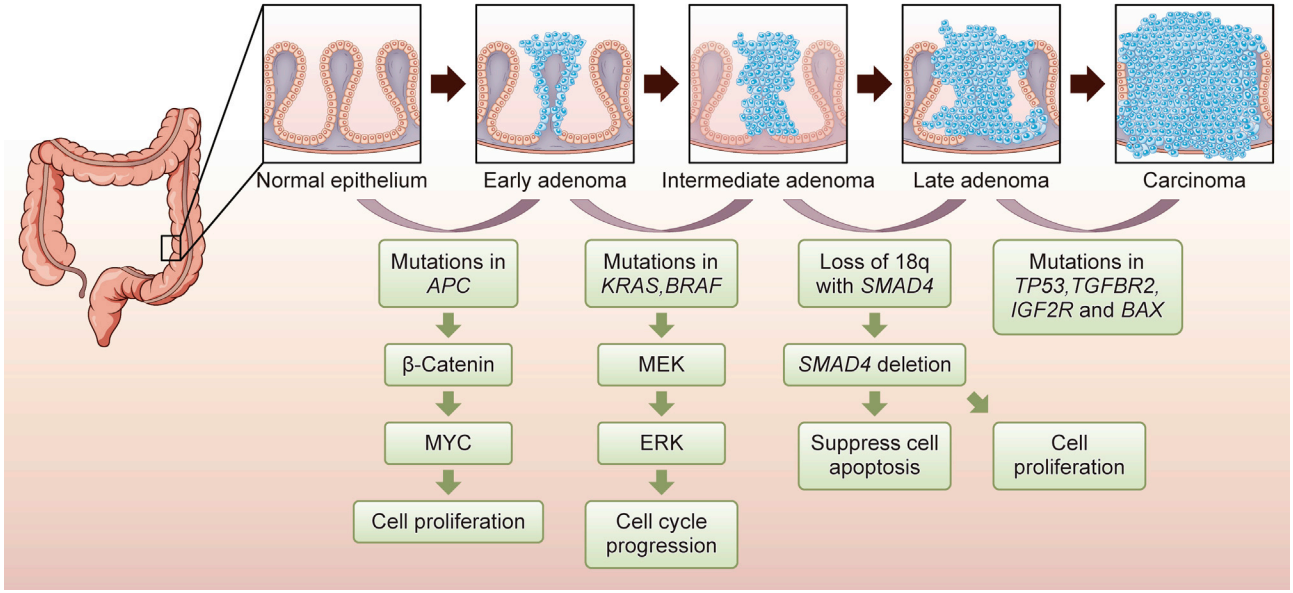

Figure 1. Sequential Progression Model in Colorectal Cancer

This is an oversimplified model describing the tumor progression sequence of CRC from adenoma to carcinoma. The development of CRC is a stepwise accumulation of genetic and epigenetic events, which align with clinical pathological changes.[24,27] The loss of *APC*, gatekeeping gene of CRC tumorigenesis, leads to small adenoma formation. Next, mutations in *KRAS* promote larger adenomas and gradually early carcinomas. The loss of SMAD family member 4 (*SMAD4*) and mutations in *TP53* as well as *PIK3CA* are acquired later in carcinoma. These serial alterations in genes that regulate cellular differentiation, proliferation, and apoptosis pathways give rise to CRC carcinogenesis.[28]

unbiased high-throughput screening. In the first scenario, a biomarker study often begins with candidate genes that play essential roles in tumorigenesis and cancer development with already defined molecular mechanisms that have been fully investigated both *in vitro* and *in vivo*. These genes are usually hallmark genes in biological processes such as the cell cycle, apoptosis, and drug metabolism.[21–23] In the case of CRC, Vogelstein et al.[24] proposed a model describing the sequential progression from adenoma to carcinoma process (Figure 1). Biomarkers developed by mechanism-based strategies include Kirsten rat sarcoma viral oncogene (*KRAS*), adenomatous polyposis coli (*APC*), and tumor protein p53 (*TP53*) that can be traced back to Vogelstein's model.[25,26] Investigators first group patients by clinical parameters (e.g., responders versus non-responders to fluorouracil-based chemotherapy), then they compare the expression levels of the candidate genes between groups and evaluate the power of these genes in distinguishing patients' grouping status. However, only discrete genes with well-known roles, but not new molecules, can be investigated and developed as biomarkers using this hypothesis-driven, mechanism-based strategy. Furthermore, this strategy might lead to unilateral conclusions considering the heterogeneity of CRC.

While target-based approaches can search only a small part of the genome, unbiased high-throughput profiling has opened new avenues for marker discovery by screening the whole genome. For example, whole-transcriptome expression can be profiled comprehensively by high-throughput technologies such as transcriptomic assays and RNA sequencing. Moreover, by the use of modified library construction or capture probes, as well as other bioinformatics analysis strategies, the technology can help develop novel RNA biomarkers such as miRNAs,[29] lncRNAs,[30] and circRNAs.[31] Specifically, CRC can be better characterized by transcriptomic subtypes that encompass not only information within the tumor but also stromal and immune components.[32] Genomic and bioinformatics technologies have yielded many potential biomarkers and related signatures, which may prove useful with prognostic and predictive values. Data acquired from high-throughput technologies such as microarrays are usually enormous and disordered. Researchers use hierarchical clustering analysis to examine these data.[33] Hierarchical clustering analysis is considered an unsupervised clustering method that is suitable for an exploratory analysis to determine how microarray groups cluster together according to similar features without taking experimental variables into account. Patients are sorted according to similarities in their gene expression profiles to predict different clinical outcomes.

Genomic Biomarkers

Genomic instability and oncogene mutations, tumor suppressor gene mutations, and mismatch repair genes can serve as biomarkers. Here, we summarize a few clinically approved genomic biomarkers in stage II/III CRC.

Chromosomal Instability (CIN) and MSI

CIN and MSI are two types of genomic instabilities. CIN refers to the increased rate of chromosomal gain and loss that leads to numerical

Table 1. Characteristics of Different RNA Molecules as CRC Biomarkers

Molecule	mRNA	miRNA	lncRNA	circRNA
Abundance	high	Low	low	low
Stability	+	++	+	+++
Detection method	q PCR sequencing	microarray (array-CGH), sequencing, qPCR	microarray (array-CGH), sequencing, qPCR	qPCR
Supporting evidence	weak	strong	intermediate	weak

CGH, comparative genomic hybridization.

or structural chromosome aberrations. MSI is defined as tumors having a defective DNA mismatch repair system due to the inactivation of either one of the following genes as the dominant genomic feature: mutL homolog 1 (*MLH1*), *MLH3*, mutS homolog 2 (*MSH2*), *MSH3*, *MSH6*, or PMS1 homolog 2 (*PMS2*).[26,34,35] CIN is more commonly presented in CRC than MSI.[36] The prognostic value of both CIN and MSI has been validated in a large meta-analysis.[37,38] Patients with CIN disease have a worse prognosis than those without CIN. In addition, MSI-high (MSI-H) patients have a better prognosis than microsatellite stable (MSS) patients in stage II CRCs[39] and maintain their survival advantages in the presence of 5-fluorouracil (5-FU) treatment;[40] however, MSI has no prognostic value in stage III CRCs.[41]

CpG Island Methylation Phenotype (CIMP)

Aberrant DNA methylation is the most broadly studied epigenetic alteration in cancer. The CIMP was first described in CRC in 1999 by Toyota et al.[42] It was characterized as a cluster of exceptionally hypermethylated CpG dinucleotides. Weisenberger et al.[43] later defined CIMP in CRC based on the methylation status of five genes: calcium channel voltage dependent alpha 1G (*CACNA1G*), insulin-like growth factor 2 (*IGF2*), neurogenin 1 (*NEUROG1*), RUNX family transcription factor 3 (*RUNX3*), and suppressor of cytokine signaling (*SOCS*). Multiple, extensive clinical studies have demonstrated that specific methylated DNA signatures could be developed as prognostic and predictive biomarkers in CRC. Several independent studies reported that CIMP-positive cancers were correlated with an unfavorable prognosis, including a cohort of more than 600 MSS CRC patients by Lee et al.[44] and a cohort of 206 stage III CRC patients by van Rijnsoever et al.[45] This conclusion was validated later in two other cohorts of stage II/III patients.[46,47] However, the opinions differ: some studies have suggested that the prognosis of CIMP-positive CRC patients depends on the MSI status of the tumor,[48,49] while other studies have suggested that the poor prognosis of CIMP-positive CRCs was from coexisting B-Raf proto-oncogene (*BRAF*) *V600E* mutations.[44,50–52] These contrary findings may be due to the heterogeneity of patient cohorts or the different CIMP criteria used in different studies. However, the CIMP status is still promising and can be further investigated as a prognostic factor.

KRAS *and* BRAF

The *KRAS* proto-oncogene encodes a small GTPase that affects cell proliferation and differentiation.[53] *KRAS* mutations in codons 12, 13, and 61 impair the GTPase activity of the encoded protein, which leads to the consistent activation of *RAS/RAF* signaling.[54] *KRAS* mutations occur at the early stage of CRC, accounting for one-third of all cases, and a large majority of the mutations are located in codon 12 (approximately 80%), followed by codon 13.[55] Many studies have evaluated *KRAS* as a prognostic biomarker based on its association with CRC outcomes.[50,55–58] The RASCAL study, a large meta-analysis, found that the glycine to valine substitution in codon 12 of *KRAS* was associated with a poor prognosis in stage III CRC patients.[56] However, several other large studies found no association.[50,57,58] In stage II CRC, no prognostic significance was found of the KRAS mutation type.[59] *BRAF* is the downstream target of *KRAS*. Mutations in *KRAS* and *BRAF* seem to be mutually exclusive.[60,61] *BRAF* mutation was considered a prognosticator for poor survival in stage II/III CRC patients receiving adjuvant chemotherapy.[62] The most famous *BRAF V600E* mutation was also reported to confer a worse prognosis in stage II/III CRC patients.[63]

Transcriptomic Biomarkers

More than 90% of the human genome is actively transcribed, but only less than 2% encodes protein-coding genes that produce translational messenger RNA (mRNA) transcripts, while most of these transcripts are noncoding RNAs (ncRNAs).[64] As protein-coding mRNAs have a short half-life and their expression changes enormously according to the physiological status, they are not an ideal prognosticator. Only a few studies have applied mRNA levels as biomarkers. For example, Takahashi et al.[65] demonstrated that high never in mitosis A (NIMA)-related kinase 2 (NEK2) mRNA levels were associated with a poor prognosis in 180 CRC patients.

ncRNAs have more advantages than translational mRNAs and thus can be developed into biomarkers based on their various types and remarkable stability. The characteristics of different types of RNA molecules and their potential as biomarkers are summarized in Table 1. In addition to the well-established types, such as transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), ncRNAs also comprise many recently identified novel transcripts, including miRNAs, circRNAs, small nucleolar RNAs (snoRNAs), PIWI interaction RNAs (piRNAs), and lncRNAs. Many studies have suggested that ncRNAs play important roles in various biological processes, such as the cell cycle, proliferation, migration, and apoptosis.[66] Several investigators have observed altered ncRNA expression patterns in human diseases, including CRC. ncRNA alterations can act as biomarkers to predict patient outcomes. Their potential is enormous. Compared to genomic markers, transcriptomic biomarkers are mostly quantitative markers rather than markers of a discrete gene status. Thus, the disease status can be better characterized by a continuous change than by a positive-negative index. This discovery is attributed to the rapid development of unbiased high-throughput screening methods. Quantitative real-time PCR is generally considered the "gold standard" method used to measure target RNA expression. It facilitates the possible clinical

application of transcriptomic biomarkers. Here, we summarize the studies that identified ncRNA biomarkers with clinical prognostic and predictive values using various patient cohorts with different clinical characteristics.

miRNAs

miRNAs mediate mRNA degradation and the inhibition of mRNA translation.[67–69] Croce and colleagues[70] first described the role of miRNAs in cancer in 2002. They found that the expression of miR-15 and miR-16 was decreased in patients with chronic lymphocytic leukemia. Since then, hundreds and thousands of miRNAs have been reported to be dysregulated in various human malignant diseases, including CRC, with a regulatory role in the expression of important oncogenes and tumor suppressor genes.[71] miRNAs are involved in multiple pathways that are important in CRC development, including the TP53, Wnt/β-catenin, RAS/mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathways.[72–75] They also play important roles in regulating drug resistance.[76–78] However, most of these results are based on *in vitro* studies and remain to be assessed in clinical sample sets.

miRNA biomarker studies in human cancer have emerged in large numbers during the past decade. The underlying reasons for this increasing interest are based on some of their unique characteristics. First, miRNAs are tolerant to RNase-mediated degradation because of their short length and hairpin-loop structure,[79] enabling extraction from a number of clinical specimens, including formalin-fixed, paraffin embedded (FFPE) tissues and various kinds of body fluids, including blood, saliva, urine, and feces. Second, miRNA stability is remarkable under a variety of laboratory conditions. Cell-free miRNAs are often contained in high-density lipoprotein particles, apoptotic bodies, microvesicles, and exosomes[80–83] and are thus protected from degradation. In addition, their binding to argonaute-2 (Ago-2) also increases their stability.[80] Third, cancer cells can secrete miRNAs into the blood and digestive tract.[84,85] Therefore, the stability of miRNAs and their extensive presence in the body (e.g., blood, feces, cancer cells, cells near the cancer) have made miRNAs promising candidates for prognostic and predictive biomarkers; thus, miRNAs have been widely investigated.

Schetter et al.[86] evaluated the expression levels of 389 miRNAs in 84 CRC patients and matched normal tissues by a microarray-based approach and validated the results by quantitative PCR. They found that high miR-21 expression was associated with poor survival in CRC patients. They also discovered that miR-21 overexpression was associated with a poor response to 5-FU-based adjuvant chemotherapy. Several other studies confirmed their conclusions independently. miR-21 has been proposed as both a prognostic and predictive marker for CRC.[87] Furthermore, Hur et al.[88] and Toiyama et al.[89] found that miR-200c was elevated in CRC, and they conducted a three-phase study using 446 colorectal specimens from both the serum and primary tumor from stage II and III CRC patients. They found that high serum miR-200c expression levels indicated lymph node metastasis and a poor prognosis. The let-7 family has also been broadly studied in the tumorigenesis of CRC. Members of this family were identified as tumor-suppressive miRNAs and downregulated in CRC tumor tissues.[90,91] Nakajima et al.[92] proposed that let-7g and miR-181b could act as indicators for the response to 5-FU-based adjuvant chemotherapy in CRC patients. In addition, many dysregulated miRNAs have been proposed as prognostic and predictive CRC markers by different researchers, as shown in Table S1.

lncRNAs

A lncRNA is another type of single-stranded ncRNA transcript that consists of more than 200 nt.[93] Only a few lncRNAs, such as H19 and Xist, were characterized in the pregenomic era. However, since the early 2000s, the elucidation of the human genome and transcriptome has led to an increasing number of studies on lncRNAs. Thousands of lncRNAs have been identified in the human genome, and this number continues to grow.[94,95] lncRNAs are the second most commonly studied ncRNA, following miRNAs. Many studies have suggested that lncRNAs are involved in multiple processes of cancer biology.[96] These studies have shown that lncRNAs function through a variety of regulatory mechanisms, including translational activation or inhibition, mRNA degradation, acting as RNA decoys or miRNA sponges, recruiting chromatin modifiers as scaffolds, and in the regulation of protein activity or stability.[97]

A growing body of literature indicates that the dysregulated expression of lncRNAs may be functional in human malignancies, including CRC, with clinical implications. For instance, HOX antisense intergenic RNA (*HOTAIR*) is reported to be an oncogenic lncRNA. *HOTAIR* cooperates with polycomb repressive complex 2 (PRC2) to reprogram chromatin organization and promote metastasis in breast cancer.[98] Kogo et al.[99] first demonstrated in CRC that high *HOTAIR* expression was associated with distant metastasis and a poor prognosis. Their finding was later validated by two other groups.[100,101] Li et al.[102] also discovered that a high expression level of *HOTAIR* was associated with a poor response to 5-FU-based treatment and predicted poor overall survival (OS) and recurrence-free survival (RFS) in CRC patients who received 5-FU-based chemotherapy. *HOTAIR* can be established as a both prognostic and predictive marker in CRC.

Another lncRNA, colorectal neoplasia differentially expressed (*CRNDE*), is overexpressed in a variety of cancers, including CRC.[103] Several independent studies conducted by different groups of researchers reached the same conclusion: high *CRNDE* expression is associated with a poor prognosis in CRC patients.[104,105] Metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) was originally identified as a metastasis predictor in non-small cell lung cancer patients.[106] Later, Zheng et al.[107] found *MALAT1* to be prognostic in CRC patients. In addition, several other lncRNAs were found to be prognostic in CRC, as shown in Table S2. lncRNAs have been detected in extracellular vesicles,[108,109] and some of these studies successfully detected and measured certain lncRNA levels in the serum or plasma,[30,110] indicating the potential of lncRNAs to serve as minimally invasive biomarkers in CRC.

circRNAs

circRNAs, with lengths of hundreds to thousands of nucleotides, are a group of naturally occurring endogenous ncRNAs.[111] circRNAs were first discovered in 1976 in viroids of RNA viruses as single-stranded covalently closed circRNA molecules.[112] Since then, circRNAs have been broadly found in different species, including humans.[113] The development of next-generation sequencing techniques and bioinformatics has allowed researchers to further investigate the functions and mechanisms of circRNAs.

Currently, circRNAs are much less studied than miRNAs and lncRNAs, with their functions remaining mostly unknown. A few circRNAs have been reported to be functional in CRC and may act as potential prognostic biomarkers. For example, the circRNA ciRS-7, one of the most studied circRNAs, is a miR-7 sponge that results in reduced miR-7 activity and increased levels of miR-7 targets.[114,115] miR-7 is considered a tumor suppressor in CRC, and it negatively controls the expression of several oncogenes.[115] Impairing miR-7 activity would have an important impact on the cell phenotype. Weng et al.[116] reported that ciRS-7 was overexpressed in CRC cancerous tissue and that high ciRS-7 expression indicated a poor patient prognosis. They performed a multivariate survival analysis in both training and validation cohorts and found that ciRS-7 expression was an independent risk factor for OS. A similar case was reported by Zeng et al.,[117] which indicated that circHIPK3 also acts as a miR-7 sponge, and that a high expression level of circHIPK3 is an independent prognostic factor of poor OS in CRC patients. The most reported mechanism of circRNAs is acting as miRNA sponges. They may also encode proteins with biological functions.[118] It has been reported that circRNAs can be transferred to exosomes and secreted into the circulatory system as well as other body fluids, indicating the possibility of circRNAs acting as noninvasive biomarkers.[119] However, investigations on circRNAs are mostly *in vitro* studies and lack validation in clinical specimens and patient cohorts, and the potential for prognostic biomarker discovery in circRNAs has yet to be exploited.

Transcriptomic Signatures

In addition to individual markers, gene panels comprised of ncRNAs have been developed to identify high-risk CRC patients. Specific expression patterns of several genes may yield higher power in distinguishing patients with different prognosis than a single gene. Signatures have higher sensitivity and specificity than do single gene biomarkers. CRC is not a one-gene show, as hundreds if not thousands of genes have been effective during the genesis and progression of the disease. It is too complicated to be simply represented by the status of one single gene. Using signatures as biomarkers, we generate scores that could more accurately represent the disease status, which allows clinicians to stratify patients more meticulously into groups, instead of just two groups depending on the positive/negative status of one gene.

Zhang et al.[29] examined the expression of 1,849 miRNAs in 40 paired stage II colon tumors and adjacent normal mucosa tissues using miRNA microarrays and found 35 differentially expressed miRNAs. They subsequently built a miRNA-based classifier comprising six miRNAs (miR-20a-5p, miR-21-5p, miR-103a-3p, miR-106a-5p, miR-143-5p, and miR-215) in a testing cohort of 138 patients and an independent validation cohort of 460 patients. The six-miRNA signature was able to identify stage II CRC patients with a high risk of recurrence. We recently developed a four-circRNA-based risk score (cirScore) (i.e., hsa_circ_0122319, hsa_circ_0087391, hsa_circ_0079480, and hsa_circ_0008039) to predict postoperative recurrence in stage II/III colon cancer patients using a cohort of 667 patients. Patients with high cirScores had shorter DFS and OS than did patients with low cirScores in the training cohort of 249 patients. The prognostic capacity of the classifier was validated in internal and external cohorts of 122 and 180 patients, respectively.[31]

Several other studies also developed different classifiers for patient stratification, including miRNA signatures, lncRNA signatures, and signatures comprised of both miRNAs and lncRNAs. However, there were more or less inherent differences in the patient characteristics of the cohorts; details are shown in Table S3.

CONCLUSIONS

Currently, the TNM stage and histopathological and clinical risk factors remain the most commonly applied risk factors in the clinical decision-making process. However, the recurrent risk assessment system of patients with stage II CRC needs further improvement to optimize the treatment strategy. Also, all stage III patients are unreasonably given adjuvant chemotherapy after curative surgery indistinguishably. With more accurate biomarkers, clinicians could better identify stage II patients who are more likely to go through recurrence and also spare those stage III patients who would not benefit from chemotherapy from the toxicities. Novel types of biomarkers are arising. Molecular-based biomarkers, such as genetic markers, epigenetic markers, and their signatures, enable physicians to more precisely stratify patients for personalized treatment. In addition, new methods of biomarker detection are also developing rapidly, such as liquid biopsy, which is minimally invasive and characterized by the isolating cancer-derived components from peripheral blood or other body fluids.[120] However, there are still issues remaining to be addressed before broad clinical application, such as the low amounts of circulating tumor cells or circulating tumor DNA or other molecules derived from the tumor.[120] However, it is reasonable to think that the liquid biopsy could play an important role in predicting relapses and monitoring metastases and treatment responses in CRC in the foreseeable future. Additionally, liquid biopsy could provide real-time genetic information of the tumor, which can help physicians better tailor the treatment to each patient.[121] Yet, with the discovery of novel biomarkers and ongoing development of detection methods, the application of biomarker-guided treatment for patients with CRC remains limited. CRC therapies are becoming increasingly target specific. In the era of personalized treatment, biomarkers will inevitably develop in tandem to play greater roles in predicting the prognosis and treatment response of CRC patients.

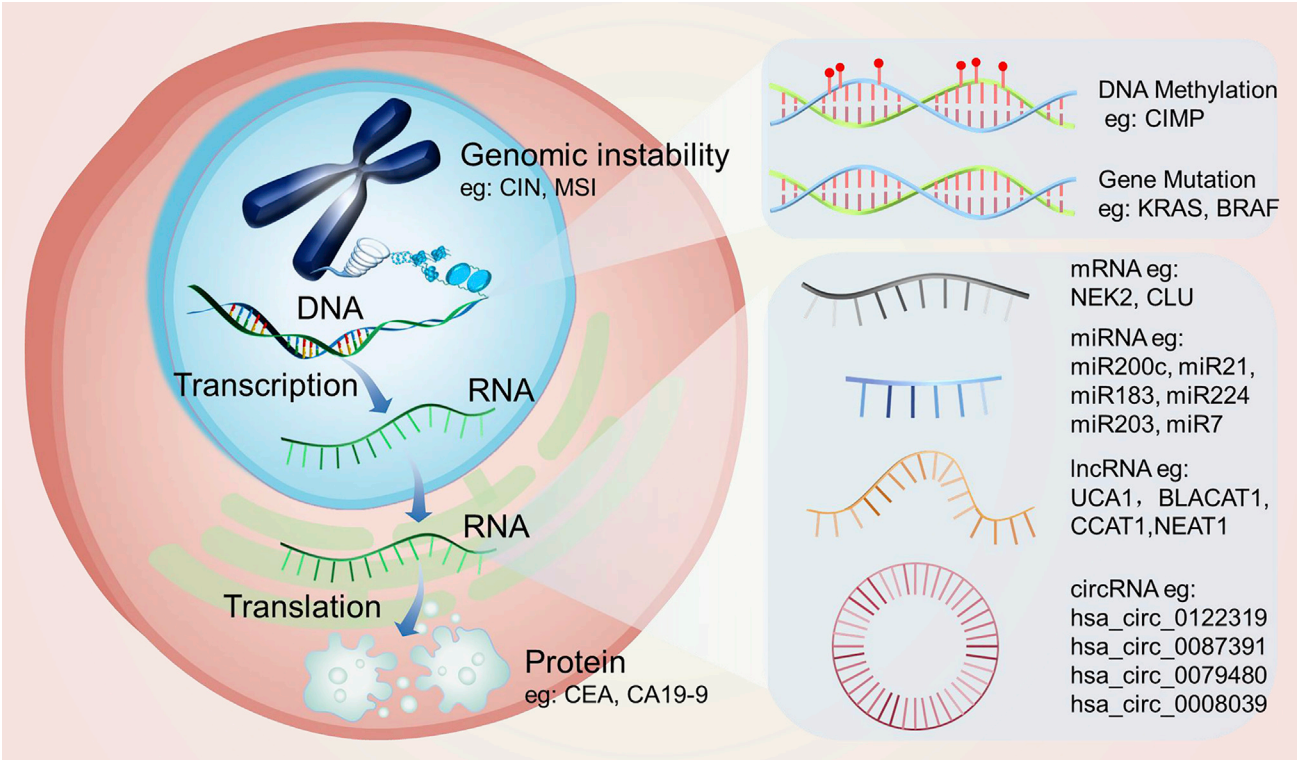

Figure 2. Different Categories of Prognostic and Predictive CRC Biomarkers in Bimolecular Level

Different categories in the bimolecular level of CRC biomarkers with potential prognostic and predictive values, which include genomic and transcriptomic aspects mainly, are summarized. Also, representative candidate genes that are currently under research subordinate to each category are enumerated.

Advances in high-throughput technology and bioinformatics have opened a gate for understanding the genetic and epigenetic alterations in CRC. In the last two decades, epigenetic alterations have become a burgeoning research hotspot for biomarker discovery. Epigenetic biomarkers, including methylated DNAs, miRNAs, lncRNAs, and circRNAs, for CRC prognosis and treatment response have emerged in large numbers (Figure 2). However, few markers have been validated and integrated into clinical practice. Most established biomarkers have a low clinical prevalence. Currently, the only marker with sufficient evidence to justify its use in routine clinical applications is *KRAS* mutation and the selection for anti-epidermal growth factor receptor (EGFR) therapy.[122] Different sample types, small cohorts, algorithms with poor efficiency, as well as high heterogeneity of the individuals pose challenges for biomarker discovery and development. In this setting, large-scale comprehensive molecular profiling from translational studies combined with artificial intelligence may offer an intriguing opportunity to develop improved molecular biomarkers and prediction algorithms and efficacious therapeutic targets. The transfer of these new biomarkers and targets from bench to bedside also necessitates larger-scale and multicenter trials to confirm their advantages. The discovery of potential biomarkers is opening the way to a more individualized practice of CRC, although the practical problems are many and difficult. The next frontier for novel molecular biomarkers is perhaps to answer the question of whether they might act as predictive and prognostic biomarkers for immunotherapy in CRC.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.ymthe.2020.12.017.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental Information

Novel Genetic and Epigenetic Biomarkers of Prognostic and Predictive Significance in Stage II/III Colorectal Cancer

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