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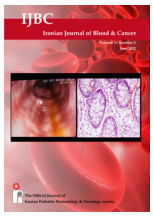

Review article

Role of Molecular Biomarkers in Colorectal Cancer

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Abstract

Colorectal cancer (CRC) is amongst the most widespread cancers and is the most common cause of cancer-associated mortality universally. Since previous decades, it has been clear that CRC develops owing to the buildup of a series of genetic and epigenetic changes in the normal colonic epithelium. Regardless of the current development in surgery and therapies, the overall survival of end-stage CRC patients is extremely low. Different biomarkers are valuable means found in tissue, blood, or stool samples usually, they are efficiently used for CRC monitoring because they occur in the initial stage of disease and provide better identification, diagnosis, prognostication, and prediction of cancer. An accurate biomarker-based treatment and prediction approach will help patients to get rid of unsuccessful treatments, involving clinical trial-based methods. It will also definitely inhibit under and over-dosages of treatment as well as it will decrease pointless harmful side effects. This review focuses on epidemiology, risk factors, molecular pathways, as well as Different DNA biomarkers and RNA biomarkers related to CRC. In this review, we will highlight the existing knowledge and advances in DNA-based biomarkers (RAS (Rat sarcoma), BRAF [B-Raf (RAF: rapidly accelerated fibrosarcoma)], cell-free DNA, DNA methylation-based biomarkers, and Microsatellite Instability) and RNA-based biomarkers (MicroRNAs, non-coding RNAs, circular RNAs, and Piwi-interacting RNA) for CRC. Herein, we emphasize gathering the latest facts related to biomarkers for obtaining their maximum possible advantages by promoting clinical practice. New studies show the frequent usage of biomarkers, which will help in optimizing the best possible treatment approaches ultimately.

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Abbreviations: CRC, Colorectal cancer; RAS, Rat sarcoma; BRAF, B-rapidly accelerated fibrosarcoma; FOBT, fecal detected occult blood; ESM1, Endothelial Cell Specific Molecule 1; CTHRC1, Collagen Triple Helix Repeat Containing 1; AZGP1, Alpha-2-Glycoprotein 1, MMP7, Matrix Metallopeptidase 7; PTGS2, Prostaglandin-Endoperoxide Synthase 2; TP53, Tumor protein p53; MYBL2, MYB,Proto-Oncogene Like 2; APC, adenomatous polyposis coli; MUTYH, mutY DNA glycosylase; MMR, mismatch repair; MLH1, MutL homolog 1; MSH2, MutS homolog 2; MSH6, MutS Homolog 6; PMS2PMS1, Homolog 2; BMI, body mass index; PIK3CA, phosphatidylinositol-4,5-bisphosphate3-kinase catalytic subunit alpha; SMAD4, SMAD family member 4; CIN, chromosomal instability; CIMP, CpG island methylator phenotype; MSI, Microsatellite Instability pathway; HER2, human epidermal growth factor receptor 2; PI3K, phosphatidylinositol-3-kinase; EGFR, epidermal growth factor receptor; RASGAPs, RAS-GTPase-activating proteins; RASAL1, RAS Protein Activator Like 1; KRAS, Kirsten rat sarcoma; NRAS, neuroblastoma RAS viral oncogene homolog; CLM, colorectal liver metastases; p16, cyclin-dependent kinase inhibitor 2A; mCRC, metastatic colorectal cancer; ERK, extracellular signal-regulated kinase; p38MAPK, p38 mitogen-activated protein kinase; cfDNA, Cell free DNA; CTCs, circulating tumor cells; ALU, sequences Arthrobacter luteus sequences; CEA, carcinoembryonic antigen; SEPT9, Septin 9; SHOX2, short stature homeobox 2; TCN1, Transcobalamin; TGFBI, transforming growth factor beta-induced; TAC1, tachykinin-1; SST, somatostatin; RUNX3, runt-related transcription factor 3; CpG, Cytosine preceding guanine; SCTR, Secretin Receptor; OS, overall survival; MSI, Microsatellite Instability; SRSs, simple sequence repeats; STRs, short tandem repeats; DFS, disease-free survival; MSI-H, MSI-high; HNPCC, hereditary nonpolyposis colorectal cancer; MiRNAs, Micro RNAs; NM, normal mucosa; TCGA, Cancer Genome Atlas; NcRNAs, Non-Coding RNAs; lncRNA, Long non-coding RNAs ; circRNAs, circular RNAs; PiRNAs, Piwi-interacting RNA.

1. Introduction

Colorectal cancer (CRC) is widespread cancer, and it has numbered third among the most common cancers in the US. It is almost equally common among both men and women. In 2018 according to studies, 2,595,326 men and 2,194,309 women were diagnosed with CRC [1]. Approximately, there are 41% of the proximal colon, 22% of the distal colon, and 28% of rectum cancer among all colorectal cancers which may vary regarding age and gender [2]. There can be different types of colorectal cancer i.e., squamous cell carcinoma, adenosquamous cell carcinoma, spindle cell carcinoma, undifferentiated carcinoma and the most common is adenocarcinoma which is 90% more than other types. There is the formation of adenoma or polyp from granular cells, and it produces mucous lining the large intestine. Of all types of adenomas, only 10% proceed to the cancerous tumor. As the polyp gets larger, the risk of arising adenocarcinoma becomes greater as well [3]. Almost 35-40% of cases arise due to inherited mutations and 60-65% have no inherited genetic mutations or no CRC family history [4].

CRC is also a multifactorial disease (involving many factors or genes) which has caused difficulties for both clinicians and researchers to cope with it. The main risk factors for CRC include hormonal disturbance, inflammation, microbial gut disorders, malfunction of immune responses, metabolic disorders, and genetic and epigenetic mutations [5]. The dysregulation of molecular signaling pathways is a unique feature of CRC. A list of reviews has pointed out that different signaling pathways are disordered and have the potential as therapeutic targets in CRC. While several molecular pathways (cell apoptosis, unfolded protein response, abnormal telomerase activity, and self-renewal) may also be altered to weaken the host defense and its survival rate [6]. However, the most common risk factors include smoking, high consumption of alcohol, poor dietary habits, reduce physical activity, and obesity. These factors can be modified to prevent the risk of disease as more than half of cases and deaths are a result of them [7]. CRC has numbered second worldwide among the deadliest cancers. Developed countries are at high risk for rectum and colon cancer due to the westernization of lifestyle habits. While the overall incidence rate is highly variable among different countries [8]. The incidence rate has increased by 90% from 1980 to 2016 and is continuously increasing due to the aging population [9]. Primary prevention is not possible. So, the five-year survival rate can be improved through secondary prevention which is early and advanced screening methods being administered in developed countries while the prognosis is still poor with only a 12.5% rate in the USA [10].

New advances in technology and a better understanding of the disease have led to the development of new screening methods and different treatments. The mortality rate has been reduced to 16% by the UK screening program which applied colonoscopy and fecal detected occult blood (FOBT). Flexible sigmoidoscopy used as a prime screening method has reduced mortality rate by 31% and CRC incidence by 23%. While these tests have less sensitivity for advanced adenomas [11]. So, there is also a need for biomarkers for early detection of disease prognosis, progression, and recurrence. Differentially expressed genes Endothelial Cell Specific Molecule 1 (ESM1), Collagen Triple Helix Repeat Containing 1 (CTHRC1), and Alpha-2-Glycoprotein 1, Zinc-Binding (AZGP1) in cancerous tissues are capable of discharging their secretions into saliva, urine, and blood so they can be predicted as candidate biological markers for colorectal cancer [12]. Tumor markers concentration can also help in the early diagnosis of cancer. Research has shown some of them to be important during disease treatment and long-term follow-up. The mRNA expression of the following genes i.e. Matrix Metallopeptidase 7 (MMP7), Prostaglandin-Endoperoxide Synthase 2 (PTGS2), tumor protein p53 (TP53), and MYB Proto-Oncogene Like 2 (MYBL2) in colonocytes showed that these biomarkers can identify CRC patients with a sensitivity rate of 58% and a specificity rate of 88% [13].

In this review article, we will explore different kinds of biomarkers that can be either DNA or RNA. We will summarize the biomarkers being utilized in the early prognosis of colorectal cancer as well as the future perspectives of biomarkers in the identification of cancerous disease and its treatment. The prevention and treatment through targeted therapies will also be focused on, along with the prevailing risk factors of colorectal cancer.

2. Epidemiology

Colorectal cancer (CRC) is a gastrointestinal malignancy and a significant human disease worldwide [1, 14]. Throughout the world, it is the third mostly spotted cancer and the second major reason for cancer-associated deaths [14, 15]. A rising trend is found in cancer incidence with increasing population and changing dietary routines towards western lifestyle [16]. Furthermore, the occurrence of CRC enhanced between 1991-2016 and is credited to lifestyle, environmental fluctuations, and aging populations [17, 18]. If colorectal cancer treatment and preventive measures will not be applied, then there is a substantial risk of having new CRC incidences above 2.2 million and 1.1 million CRC-related mortalities, by 2030 occurred [19]. So, CRC has turned out to be a potential danger to human well-being.

The prevalence of CRC varies in different countries. Many components are considered to have a role in the alterability of occurrence [20]. According to a statistical data measurement by the American Cancer Society, about 145,600 newly identified CRC patients and 51,020 CRC-associated deaths occurred in the US in the year 2019 [21]. While according to Globocan 2020, 521,490, 47,892, and 6421 CRC new cases and 245,263, 20,470, and 3022 new deaths occurred in China, UK, and Sweden respectively [22-24]. Developed nations are at the maximum threat of colon and rectal cancer. Countries having the uppermost occurrence of Colon cancer are Southern Europe, Australia/New Zealand, and Northern Europe. While for Rectal cancer, these countries are Eastern Europe, Australia/New Zealand, and Eastern Asia. Both cancers are maximally found in North America. The country which has an extremely high prevalence of CRC per 100,00 population is Hungary (70.6) for men and Norway (29.3) for women. While, entire areas of Africa and Southern Asia, have the smallest occurrence rates of colorectal cancer in both genders [14].

The survival in CRC is stage-dependent as 92% survival chances for stage I, equated to 10% in stage IV, yet, there is an enhancement in the continued existence for the 60–69 year age range group ascribed to screening [25]. Consequently, CRC persists as a widespread challenge regarding the control of cancer highlighting the requirement for timely diagnosis [11].

3. Risk Factors for Colorectal Cancer

3.1. Genetic risk factors

The risk of CRC is highly polygenic according to genetic analysis. Hereditary factors are estimated to account for about 35% of the overall risk of CRC [26]. Variations in the genes i.e., adenomatous polyposis coli (APC), mutY DNA glycosylase (MUTYH), and mismatch repair (MMR) cause 5% of all CRCs, which also includes less frequently altered syndrome-causing genes. These mutations account for almost 40% of all genetic modifications causing colorectal cancer.

Familial adenomatous polyposis is associated with variations in the adenomatous polyposis coli (APC) gene. This Adenomatous polyp is then converted into carcinoma over time. Bi-allelic somatic mutations in the APC gene are very common and found in around 80% of all CRC patients. As a result, APC becomes the primary gatekeeper gene in CRC progression.

Lynch syndrome is the most common inheritable CRC syndrome referring to almost 3% of all CRCs. Genetic variations in the MMR genes MutL homolog 1 (MLH1), MutS homolog 2 (MSH2), MutS Homolog 6 (MSH6), and PMS1 Homolog 2 (PMS2) have also shown an association with this syndrome as well [27].

3.2. Modifiable risk factors

Colorectal cancer outcomes may be influenced by gender, genetic susceptibility, behavior patterns, and ethnicity. Age, body mass index (BMI), serum cholesterol, and alcohol intake are all included as modifiable risk factors in numerous models among various screening populations. The risk of finding advanced neoplasia at the age of 50 has significantly increased from around 1% to > 3% according to screening colonoscopy analysis in Asia [1].

Obesity, lifestyle factors, unhealthy diets, alcohol consumption, and smoking are only a few of the characteristics linked to westernization that are predicted to drive up CRC prevalence in economically transitioning countries [4]. Colorectal cancer is becoming more common in the majority of low and middle-income countries. This could be due to aging populations, industrialization, and a rise in the prevalence of “western” lifestyles and poor dietary habits. The universal drift of colorectal cancer, which is typically attributed to a variety of adaptable risk

factors, has not been demonstrated elsewhere, but it has significance because of the implications for policies and preventative measures [1].

One study shows there is a difference in CRC risk factors among males and females [28]. Consuming alcohol, cigarettes, and diets lacking calcium, milk, and fiber are all significant potential causes of colorectal cancer in men. But in contrast, dietary risk factors, alcohol use or smoking, were found to have no considerable effect in females as well. The results of this analysis show that certain dietary risk factors have a higher global impact than smoking or alcohol usage. More particularly, a low-calcium diet has been identified as a primary contributing factor to colorectal cancer. Globally, no other study has proven the substantial burden linked to this dietary risk factor. So, to reduce the risk of disease, the necessity of improving diet through public health measures should be emphasized [5].

4. Molecular Pathways

Colorectal cancer is a heterogeneous condition that occurs gradually over time as a result of genetic changes [29]. Colorectal cancer is caused by at least three main molecular pathways including the chromosomal instability pathway (CIN), which comprises 85% of CRC cases, the CpG island methylator phenotype pathway (CIMP) which is the other major pathway to sporadic CRC4 and another molecular pathway is Microsatellite Instability pathway (MSI) which is ascribed to DNA MMR gene mutations [30]. CIN tumors are caused by the activation of oncogenic genes like Kirsten rat sarcoma (KRAS) and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), as well as the inactivation of tumor suppressor genes like APC, SMAD family member 4 (SMAD4), and TP53, which is followed by changes in tumor characteristics as the adenoma progresses into a carcinoma [31]. The CIN phenotype could be caused by abnormalities in the mechanisms that ensure correct chromosomal segregation. Checkpoint signaling defects cause chromosome mis-segregation and aneuploidy, with an incorrect number of chromosomes transferred to daughter cells [32]. Short DNA motifs of 1–6 bases, called microsatellites, are found in both coding and non-coding areas of the genome. A microsatellite's repeating structure makes it particularly susceptible to replication defects, which are generally corrected by the MMR system [33]. MSI is seen in around 15% of all colorectal carcinomas [29]. A distinctive aspect of dMMR/MSI-positive CRCs includes a preference for the proximal colon, poor differentiation, and an abundance of tumor-infiltrating lymphocytes [34]. CpG Island Methylator Phenotype (CIMP) is caused in the promoter region by the methylation of CG di-nucleotides of several genes at the same time, resulting in the silencing of transcription of the nearby genes [35, 36]. CIMP is a unique epigenetic tumor phenotype comprising 20% of colorectal cancers [37] In tumor suppressor genes like cyclin-dependent kinase inhibitor 2A (p16), hypermethylation of CpG islands leads to their transcriptional inactivation, silencing genes, function deterioration, and promotes tumor formation and progression. It also involves epigenome-wide abnormalities [38, 39].

CpG islands are linked to tumor suppressor genes' transcriptional inactivation in neoplasia. Multiple tumor suppressor genes such as asp16 and fhMLH1 are simultaneously inactivated by CIMP, which results in a mismatch repair defect [39]. Defects in KRAS and TP53 were also found to be related to CIMP [37]. There are also significant clinical, pathological, and genetic characteristics associated with CIMP tumors such as older age, tumor site on the right side, BRAF mutation, female gender, lack of differentiation; TP53 mutation rates, and mucus histology [38].

5. DNA Biomarkers for CRC

The molecular basis in colorectal cancer is like a network, accompanying changes at the genetic level. The genetic modifications are noticed in genes of the RAS proteins (KRAS (Kirsten rat sarcoma) and NRAS (neuroblastoma RAS viral oncogene homolog)), the RAF (rapidly accelerated fibrosarcoma) kinases (ARAF, BRAF, and CRAF), phosphatidylinositol-3-kinase (PI3K), epidermal growth factor receptor (EGFR), and human epidermal growth factor receptor 2 (HER2), etc. [40]. Herein, some DNA and RNA based CRC biomarkers as depicted in **Figure 1**, are discussed in detail in the text.

5.1. RAS

Mutations in KRAS genes are prognostic biomarkers for CRC [41]. Some mutations including KRAS, NRAS, and BRAF are found in above 50% of CRC

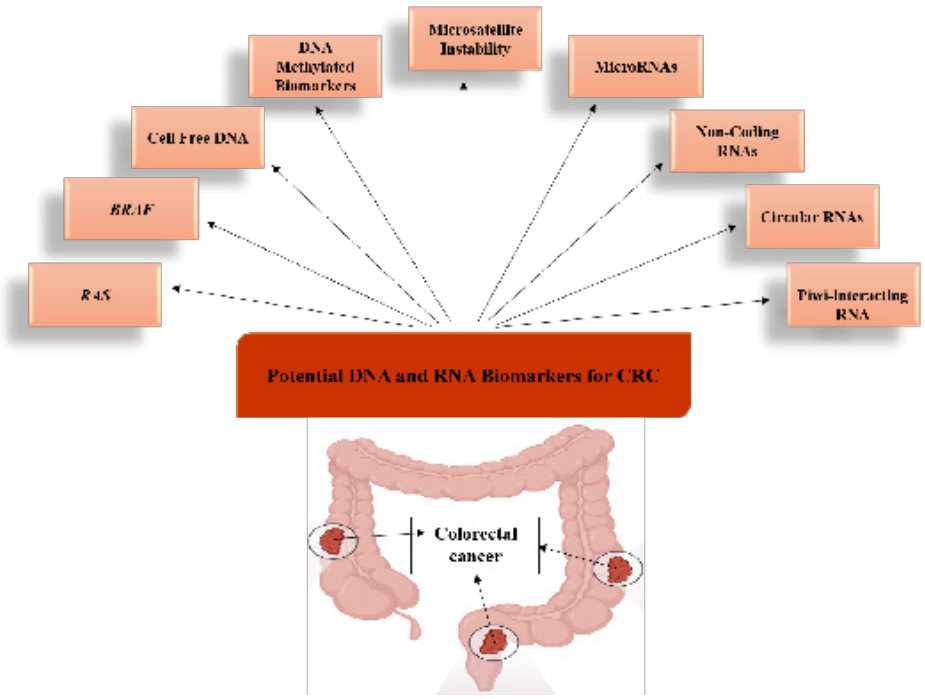

Figure 1: Potential DNA and RNA Biomarkers for CRC: Five major prognostic DNA-based Biomarkers known to affect the molecular basis in colorectal cancer are shown in this figure including RAS, BRAF, Cell free DNA, DNA Methylation based biomarkers, and Microsatellite instability. Four major RNA-based Biomarkers also known to affect the molecular basis in colorectal cancer are depicted in this figure including MicroRNAs, Non-coding RNAs, Circular RNAs, and Piwi-interacting RNA [41-48].

incidents [49]. KRAS is a GTPase protein, synthesized by KRAS proto-oncogene, involved in many pathways incorporating the epidermal growth factor receptor (EGFR) signaling pathway. According to many trials, only wild-type RAS tumors have a medical advantage for anti-EGFR antibody therapy, although KRAS modifications may also act as a negative predictive agent for EGFR inhibitors [50-53]. There are diverse clinicopathological characteristics related to mutation type in CRC. Specifically, KRAS exon 2 (codon 13), exon 3, and 4 variations possess well-defined medical, pathological, and molecular features, therefore, they should be wisely taken into account while evaluating the prognostic significance of RAS status [54].

Even though mutations in RAS and KRAS lead to changes in signaling and CRC progression but only mutations are not responsible for CRC. The function of RAS is altered by RAS-GTPase-activating proteins (RASGAPs) [55-58], and expression of only RAS Protein Activator Like 1 (RASAL1) was reduced in Colorectal cancer cells among 12 RASGAPs tested. RASAL1 expression is reduced in predominantly CRC cells having wild-type KRAS gene (mostly in progressive lesions as compared in small adenomas, signifying that RASAL1 acts in the advancement of benign colonic neoplasms) and rarely in mutant KRAS gene. The RAS signaling is very important for keeping in view the progression of colorectal neoplasms as well as RAS pathway is recommended as an appropriate curative target [57, 58]. Nevertheless, RasGAP has also been found to be a critical effector of mutant KRAS in colorectal cancer [59]. The V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) is often abnormally regulated in CRC. It causes the modification of numerous downstream effectors, that incorporate: Raf/Mek/Erk, PI3K/Akt, RalGDS/p38MAPK, and Rac/Rho, thus affecting carcinogenesis, progression of tumor cells, and opposition to treatment. There is a treatment approach by using drugs that target these pathways for metastatic CRC [60]. The drug Cetuximab has been permitted to use for CRC patients not having KRAS mutation, for patients having codon 12/13-KRAS mutations, and for EGFR expressing metastatic CRC [60, 61]. In the patients experiencing colorectal liver metastases (CLM) surgery, RAS mutations possess a larger negative effect on survival

of early-age onset CRC patients as compared to patients with patients having late-age onset CRC. Thus, it ought to be well-thought-out as a prognostic factor in multidisciplinary therapy design [62]. KRAS-mutated CRC was linked to statistically considerably poorer survival following diagnosis as compared with KRAS-wild-type CRC [63].

5.2. BRAF

BRAF mutations are prognostic biomarkers for CRC, for advanced and recurrent CRC [41, 64, 65]. BRAF-activating mutations found in advanced CRC patients are nearly 8% and in localized stage 2 and 3 CRC patients are 14% [66, 67]. Meta-analysis report by Li and Li showed, [68] the BRAF mutation was considerably linked with proximal colon tumor location, poor dif-ferentiation, tumor dimensions, and female gender [69, 70]. Patients having metastatic CRC (mCRC) undergo chemotherapy although it is widely identified that CRC entails different molecular and clinical populations. One of the sub-group is based on BRAF mutants [64]. Some researchers state that BRAF is imminent to KRAS. Even though above 40 somatic mutations have been observed in BRAF kinase domain, a particularly widespread change in different cancers is the exchange of GTG-GAG at the 1799th location of the 15th exon, which causes V600E amino acid alteration, ensuing key activation of the EGFR signaling pathway [71-74]. Whereas, some studies described that tumors due to BRAF mutations have changed clinical and histopathological characteristics equated to tumors due to KRAS mutations [75-77]. Mutation in BRAF is correlated with an exceptionally poor relapse-free survival in patients having hepatic resection. Information regarding mutations helps in optimizing medical judgment for whether an mCRC patient should be a possible candidate for liver surgery [78].

The CRC Patients who go through lung metastasectomy contain mutant KRAS and mutant BRAF as prognostic biomarkers [79]. It was known previously that in CRC, BRAF mutations affect tumor advancement [80]. In mismatch repair-deficient tumors, BRAF mutations are linked to a useful prognosis, while in the case of MMR-competent tumors, they were found to be disadvantageous [43, 70]. The distinctive expression of downstream MAPK pathway constituents, that get stimulated in BRAF mutants, can be credited to these changes. MMR-competent stage 3 cancers with BRAF mutations had a better prognosis when both extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinase (p38MAPK) are strongly phosphorylated in the nucleus. Moreover, one protein from both of these is possibly employed as a predictive biomarker medically in BRAF mutant competent MMR colorectal cancer patients, suggesting the agonist usage as a potential treatment option leading towards precision medicine [43].

5.3. Cell free DNA

Cell free DNA (cfDNA) and circulating tumor cells (CTCs) are easily approachable biomarkers for mCRC patients. They both are complementary for evaluating disease advancement [81]. Circulating cfDNA is nucleic acid in the blood flow coming from normal or tumor cells due to apoptosis or necrosis [82]. The length of cfDNA depends upon the synthesis process. Its length is almost 180bp in case of apoptosis (for healthy people) while lengthy DNA fragments are formed by necrosis in tumor cells [82]. So, the integrity number of circulating cfDNA is considered a better biomarker in CRC. As compared with healthy people, 5 times more concentration of cfDNA is found in serum, and 25-50 times more concentration is found in plasma for CRC [83-85]. When an elevated level of cfDNA is found it points outs drastically shorter overall survival and more risk for recurrence of CRC [84].

Some researchers have studied that cfDNA levels reduced after primary resection, nonetheless, cfDNA concentration significantly elevated upon CRC recurrence [85, 86]. Another study observed that post-surgical cfDNA level detection can help in monitoring relapse beforehand [87]. Hao, Shi [88] evaluated the clinical significance of circulating cfDNA in serum for observing diagnostic and progression aspects. The concentration of DNA in serum and integrity index are possibly effective biomarkers for CRC diagnosis at the beginning and for examining the advances in CRC. Arthrobacter luteus (ALU) sequences ALU115 and ALU247/115 index in serum are expressively elevated in CRC patients (primary stage) contrasted with healthy people. If ALU115, ALU247/115, and carcinoembryonic antigen (CEA) are collectively monitored, then CRC is proficiently identified [88]. Septin 9 (SEPT9) and short stature homeobox 2 (SHOX2) methylation are potent biomarkers in circulating cfDNA in CRC and give better directions for personalized medicine. The finding of these

biomarkers at the beginning leads to better treatment of patients. Post-therapeutically raised circulating cfDNA methylation levels give the idea of the existence of disease and distant metastases [89].

5.4. DNA methylation-based biomarkers

DNA methylation is the most widely investigated dysregulated epigenetic mechanism found in colorectal cancer cases. Hypermethylation of genes detected through blood specimens can be employed as a prognostic biomarker for CRC. However, more researches are consequently required to execute a genome-wide search to construct a group of sensitive and specific DNA methylation markers for monitoring CRC at its initial stages [44]. DNA methylation is accompanied by genomic instability and tumor beginning. Research showed two Hub genes Transcobalamin (TCN1) and transforming growth factor beta-induced (TGFBI) whose abnormal expressions occurred due to DNA methylation. Moreover, the amount of DNA methylation can detect CRC [90]. Methylated septin 9 (SEPT9) is an extensively analyzed and solitary FDA-accepted methylated biomarker for CRC. Other than SEPT9, more potential methylated biomarkers are there, comprising tachykinin-1 (TAC1), somatostatin (SST), and runt-related transcription factor 3 (RUNX3) in a variety of sample categories for CRC recognition [91]. Cytosine preceding guanine (CpG) islands are DNA regions with high GC content and are frequently found in promoter sites possessing CpG dinucleotides. In CRC, DNA is methylated unusually. CpG island methylator phenotype (CIMP) is found in almost 10-20% of CRC cases containing tremendously increased proportions of abnormally methylated CpG loci [39]. This abnormally high methylation in CpG islands leads to the silencing of genetic ac¬tivity ensuing in dysregulation of gene expression [92]. CIMP is generally described as hypermethylation of a minimum of three loci in a chosen group of five genes (hMLH1, p16, MINT1, MINT3, and MINT31) which are related to CpG islands [93]. Yet, most of the researchers stated that CIMP+/CIMP-high CRC patients exhibited a lesser prognosis than CIMP–/CIMP-high CRC patients [94].

Li, Zhang [95] found that abnormally high methylation of the SCTR (Secretin Receptor) gene at CpG islands in cfDNA is a potential diagnostic biomarker for CRC. But SCTR methylation usage as a non-invasive biomarker requires evaluation and validation from future research [95]. Another study investigated the prognostic efficiency of CIMP among individuals having CRC from levels I-IV. The overall survival (OS) between CIMP-high CRC and CIMP-low/negative CRC cases were compared. This study reported that the occurrence of CIMP autonomously determines poor OS in stage IV patients [96].

5.5. Microsatellite Instability (MSI)

Microsatellites (MS) also called simple sequence repeats (SRSs) or short tandem repeats (STRs) are repeating DNA sequences having 1–6 base pairs which may be present in coding and noncoding regions [45, 97, 98]. The mismatch repair (MMR) system deals with and corrects the DNA mistakes that appear in the course of rep¬lication [99]. Microsatellite instability (MSI) rises from deactiva¬tion of the MMR genes via sporadic MLH1 promoter hy¬permethylation (80% of MSI colorectal cancer cases) or hereditary muta¬tions in MMR genes for example MutL homolog 1 (MLH1), MutS homolog 2 (MSH2), MutS Homolog 6 (MSH6), PMS1 Homolog 2 (PMS2) (20% of MSI colorectal cancer cases) [45, 97, 98, 100]. MSI is a significant biomarker in CRC possessing critical analytical, predictive, and prognostic effects [101]. Initially, MSI was thought to be associated with hereditary defects in MMR genes appearing in affected people having Lynch syndrome (an autosomal-dominant hereditary disease) where > 90% of CRC cases display this phenotype [45, 102]. MSI condition is regularly evaluated in affected people with colorectal because it adds to Lynch syndrome initial screening, tumor prediction, and choosing patients for immunotherapy [103]. Afterward, it was found that besides germline cases, some sporadic CRC cases were also found i.e., nearly 12-15% although in them MSI establishes owing to methylation-induced inhibition of the MLH1 promoter [104, 105]. Patients having MSI-based CRCs usually retain improved prognoses [106]. Presently, standard reference approaches suggested for MSI/dMMR analysis comprise immunohistochemistry and pentaplex PCR-based analyses, though, innovative molecular-based procedures are evolving [103]. Takehara, Nagasaka [107] analyzed the potential of a group of four quasi-monomorphic mononucleotide-repeat markers (BAT26, NR21, NR27, and CAT25) for identifying dMMR CRCs which is a straightforward

and quick diagnosing method with increased throughput competency, sensitivity and specificity [107].

Five frequently used microsatellite markers are there, incorporating 2 mononucleotide repeats (BAT26 and BAT25) and 3 dinucle¬otide repeats (D2S123, D5S346, and D17S250). When some biomarker displays more than 30%–40% fluctuation, it is recognized as MSI-high [108]. MSI-high tumors show improved prediction as compared with MSI-low tumors, probably owing to some immune responses as they are inadequately and less differen¬tiated, comprise mucin and have subepithelial lymphoid ag¬gregates as well as intraepithelial lymphocytes [108, 109]. Guastadisegni, Colafranceschi [110] went through 13 different researches for assessing the predictive importance of MSI in CRC-having individuals and they stated that MSI among these patients displayed elongated overall survival and disease-free survival (DFS) as compared with microsatellite stable CRC patients [110]. Moreover, MSI-high (MSI-H) stage is linked to an improved prediction at the initial stage of CRC and minimum or no advantage from accessary treatment with 5-fluorouracil in stage II CRC. In recent times, MSI has appeared as a prognosticator of sensitivity to immunotherapy-based remedies [101]. Haghighi, Javadi [111] observed 5 mononucleotide biomarkers for developing a straightforward investigative approach to recognize patients having hereditary nonpolyposis colorectal cancer (HNPCC) and came out with the point that BAT25 and NR-21 biomarkers can offer more analytical advantage owing to their greater instability as compared to the other biomarkers [111]. Hveem, Merok [112] evaluated that for stage II CRC patients, instability of the genome is a valuable biomarker for recurrence and death after surgery for therapeutic goals [112]. Jorissen, Christie [113] found that wild-type APC is a biomarker for inadequate prognosis in Microsatellite stable proximal colon cancer [113]. **Figure 2**, given below summarizes the importance of above discussed potential DNA biomarkers. Furthermore, **Table 1** given below summarizes an overview of the roles of potent DNA Biomarkers for CRC.

6. RNA Biomarkers for CRC

Biomarkers are biological substances, features, or images that offer information about an organism's biological status. Their correct application necessitates an awareness of their sensitivity and specificity, as well as how and in what circumstances they should be used, and how to properly evaluate them [120]. Biomarkers are any objective testing and evaluation characteristics that serve as indications for normal biological processes, case processes, or pharmaceutical responses [12]. Biomarkers that aid in disease identification and prognosis prediction are highly sought to improve longevity and aid in deciding the best treatment for colorectal cancer patients [46]. In CRC, a variety of biomarkers have been found both in tumors, blood, and feces, with the host's genetic profile providing additional and essential supporting information [120].

6.1. MicroRNAs

MicroRNAs are single-stranded, non-coding RNA, about 18–25 nucleotide long, and are involved in post-transcriptional gene expression regulation [46]. MicroRNAs are relatively stable molecules with a hairpin-loop structure that limit protein translation by binding to target mRNAs that are critical in gene regulation networks [121, 122]. MiRNA genes make up 2% of the human genome and control the activity of protein-coding genes. The biology of miRNAs is now well understood and has undergone extensive review. There is compelling evidence that detecting non-coding RNAs, specifically microRNAs, in stool or blood offers a novel and effective therapeutic strategy for CRC screening [46].

The discovery of microRNAs triggered extensive research into their role in cancer and a variety of important pathways including development, epithelial-mesenchymal conversion, metastasis, and therapeutic resistance [123]. MiRNA expression deregulation has been reported in various types of cancers and could represent a new class of cancer biomarkers [12]. The RNA sequences can silence targeted genes and impede tumor invasion and progression via epithelial-mesenchymal transition to metastatic locations. The expression of miR-31 has been observed in CRC with BRAF mutations, which could be employed as a diagnostic biomarker [122]. MicroRNAs play a role in numerous physical and biochemical processes, as well as cancer genesis and progression [123]. Circulating miRNAs are stable and effective biomarkers for a variety of cancers, including solid tumors [121, 124]. Overexpression and silencing of specific miRNAs have

been associated with the emergence and development of colorectal cancer. The expression profile of miRNAs in tissue and blood has the potential to be used in the identification, screening, and surveillance of colorectal cancer [125].

Some miRNAs express differentially in the molecular progression of CRC from normal cells to malignant cancerous cells [47]. Microarray profiling discovered elevated and downregulated miRNAs that differed between CRC tissues and comparable non-malignant colorectal tissues. The researchers found miR-18a and miR-29a, which had dramatically different expression levels in CRC individuals and normal donors. For example, serum miR-29a levels were likewise shown to be considerably greater in CRC patients [121]. MiR-223 and miR-451 levels were highly expressed in CRC patients' feces. Furthermore, after tumor removal, miR-135b expression was reduced, and there was no association between miR-135b levels and colorectal lesion localization. The expression of MiR-20a in CRC tumors was considerably higher than in normal tissues nearby [46]. In CRC, 160 miRNAs were identified as to be deregulated. In a study, miR-20a and miR-31 were shown to be highly elevated, while miR-143 and miR-145 were found deregulated in CRC tissue. In two of the plasma-based investigations, MiR-92a was shown to be significantly higher in CRC patients, and in one of the tissue-based studies, MiR-92a was found to be significantly raised in CRC tissue [126].

MiR 106a, which is located on Xq26.2, is up-regulated and exhibits carcinogenic activity in humans. When compared to noncancerous controls, miR-106a was shown to be up-regulated in colon adenoma tissues. MiR-106a expression levels are much greater in stomach malignant tissues than in noncancerous tissues [127]. In the current investigation, RNA sequencing was used for whole-transcriptome analysis in colorectal cancer (CRC) and normal mucosa (NM). This Identified 27 up-regulated and 22 down-regulated long non-coding RNAs (lncRNAs) in CRC, which we confirmed using the Cancer Genome Atlas (TCGA) dataset. Up-regulation of four lncRNAs termed colorectal cancer-associated lncRNA [128]. MiRNAs bind to their gene promoters directly. MiR-224 has recently been found to activate Wnt/-catenin signaling and direct beta-catenin nuclear translocation in CRC. Wnt/-catenin-mediated cell metastasis and proliferation are inhibited by miR-224 knockdown. Furthermore, miR-101 overexpression in CRC lowers beta-catenin nuclear localization, which hinders cancer stem cell-related gene production. As a result, pharmacological restoration of miR-101 may be considered a unique treatment option for preventing CRC recurrence [129]. The aberrant DNA methylation of miR-34a in the feces was also found effective for CRC identification, showing 63 methylated samples out of a total of 82 while only 2 out of 40 healthy samples. In total, 170 different miRNAs were shown highly expressed in CRC tumor tissue versus normal colonic mucosa. Another 110 miRNAs have been discovered to be up-regulated [46].

6.2. Non-Coding RNAs

NcRNAs are classified according to their length: short ncRNAs with fewer than 200 nucleotides and long ncRNAs with over 200 nucleotides [128]. Long non-coding RNAs (lncRNA) are non-translated RNA transcripts that constitute 68% of the human transcriptome. They may take part in cellular processes such as mRNA regulation, protein stability, structural organization, and epigenetic regulation [11]. The fact that circulating ncRNAs are stable in feces, blood plasma, and serum allows for the development of novel approaches to use circulating ncRNAs as early diagnostic biomarkers of CRC [46]. There could be several undiscovered lncRNAs that perform essential roles in human cancers. The role of lncRNAs and their deregulation in colorectal cancer development is yet unknown [128].

6.3. Circular RNAs

After microRNAs and lncRNAs, circular RNAs (circRNAs) are the third most important part of the non-coding RNA family and are involved in splicing and transcription [48]. Furthermore, because circRNAs have organ selectivity, they can be used as indicators for cancer. CircRNAs are abnormally expressed in tumor tissues, according to a recent study, and several circRNAs have been identified as possible biomarkers [48]. CircRNAs are studied as specific targets for CRC diagnostic and prognostic detection. When CRC tissues were compared to normal tissues, 11 circRNAs were up-regulated and 28 were down-regulated among the 39 circRNAs in tumor tissues. The ratio of circRNA in tumors was lower than in controls [46, 130].

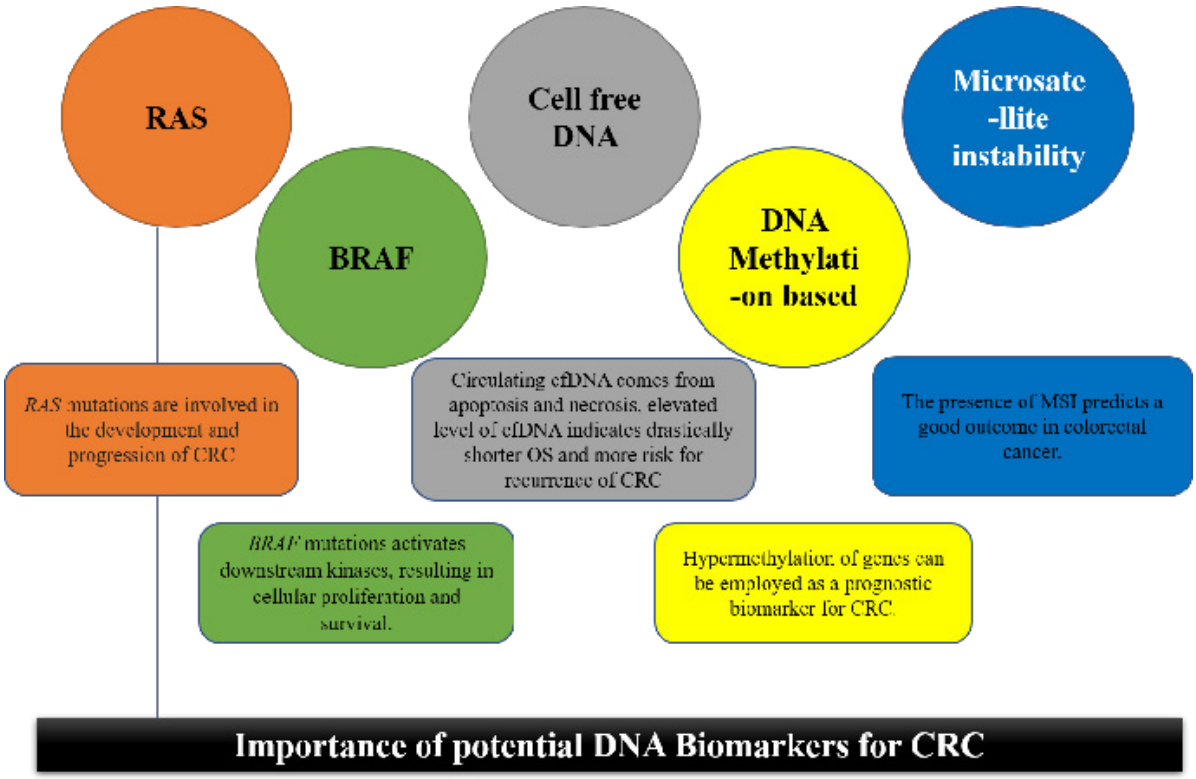

Figure 2: Importance of Potential DNA Biomarkers in Colorectal cancer: This figure gives brief idea of significance of 5 DNA Biomarkers including RAS, BRAF, Cell free DNA (cfDNA), DNA methylation-based biomarkers, and Microsatellite instability (MSI) [44, 82, 84, 114-117].
***Here, OS is abbreviation of Overall survival**

Table 1: A brief overview of the roles of potent DNA Biomarkers for CRC

DNA Biomarkers	Role in Colorectal Cancer	References
RAS	• KRAS affects the epidermal growth factor receptor downstream signaling pathway. • RAS-GTPase-activating proteins and Mutations in RAS and KRAS are key causes of CRC.	[50-53] [55-58]
BRAF	• They are prognostic biomarkers of CRC.	[71-74]
Cell free DNA	• cfDNA acts as a good biomarker, when the level of cfDNA increases, it points outs severely shorter survival and a high threat of recurrence of CRC.	[84]
DNA methylation-based biomarkers	• improve the current diagnosis, screening, prognosis and treatment prediction. • DNA methylation of biomarkers can be identified and quantified several technologies like genome-wide screening approaches and gene or locus specific high-resolution examination. • Related to genomic instability and tumor formation, the level of DNA methylation can detect CRC.	[118] [119] [90]
Microsatellite Instability (MSI)	• Several MSI biomarkers are found, BAT25 and NR-21 biomarkers are more beneficial due to their enhanced instability as compared to the other biomarkers.	[111]

6.4. Piwi-interacting RNA (PiRNAs)

Piwi-interacting RNA (PiRNAs) is the largest class of RNA molecules with 24-31 nucleotides and is involved in the post-transcriptional silencing of repeat-derived transcripts. They are found to have a significant role in cancer biogenesis e.g., piR-651 has greater expression levels in CRC tissues, so PiRNAs can act as biomarkers for the screening of CRC [47]. 14 miRNAs were found to be particularly expressed in CRC patients. MiR-92a and miR-29a differentiated CRC from adenoma and controls. MiR-21 and miR-106a were shown to be over-expressed in CRC patients as compared to adenomas or healthy people. MiR-143 and miR145, contrarily, were found to be downregulated in CRC patients. MiR-92a levels were greater in polyp patients compared to controls [123].

As CRC develops, EGFR, a therapeutic and prognostic biomarker, and c-Met (also known as tyrosine-protein kinase Met), a prognostic biomarker, are shown to be down-regulated. C-Met is regulated by miR-34, showing that different miRNAs may be implicated in different cancer types there were various protein biomarkers that were altered by multiple miRNAs and were classified as CRC biomarkers [47]. MiR-21 deregulation is widespread during the early stages of the adenoma-carcinoma sequence; and is highly expressed and released by cancer cells. MiR 155, miR-106a, and miR-20a are promising individual diagnostic biomarkers in CRC. MiR-601 and miR-760 were downregulated in CRC plasma samples and could be used as markers to distinguish CRC patients and healthy controls plasma samples [46]. A 460 KRAS mutation has been linked to the deregulation of many miRNAs that target genes involved in apoptosis and proliferation. Furthermore, a colon miRNA profile consisting of eleven over-expressed and eight under-expressed miRNAs may be implicated in stem cell differentiation regulation and hence present novel cancer stem cell therapeutic targets [120]. MiRNAs have properties that make them suitable for gene therapy, miRNA expression is often altered in cancerous cells, and targeting miRNA expression may affect cancer phenotype. Some miRNAs have tumor silencer activities and may be down-regulated in CRC cells. MiRNAs may one day be used as novel prognostic and diagnostic tools [123]. **Figure 3,** given below summarizes the significance of above reviewed potent RNA biomarkers. Moreover, **Table 2** given below summarizes an overview of the roles of potent RNA Biomarkers for CRC.

7. Conclusion

In conclusion, we found that CRC is prevalent cancer accompanying increased cancer-related deaths. Many biomarkers possess the huge capability for characterizing CRC and signifying proper diagnosis and treatment options for patients having different molecular, genetic, and epigenetic alterations. Numerous DNA, and RNA-based sensitive and specific biomarkers in stool, blood and other biological fluids have been identified for better diagnosis and treatment. Biomarkers can significantly enhance the choice of treatment approaches for CRC patients. They lead towards precision medicine and tailored treatments offering clinical and patient satisfactoriness. This review directs further studies to distinguish those biomarkers which could offer an inexpensive and non-invasive diagnosis of CRC; as well as upcoming studies should be focused on the recognition of a group of appropriate prognostic biomarkers and the characterization of predictive biomarkers which will guide for choosing the most suitable treatment approach. Imminent researchers should concentrate on all these aspects.

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Author Contributions

The idea was conceived by MJI, other authors performed the critical literature review and wrote the first draft of the manuscript which was finalized by MJI.

Conflict Of Interest

None to declare.

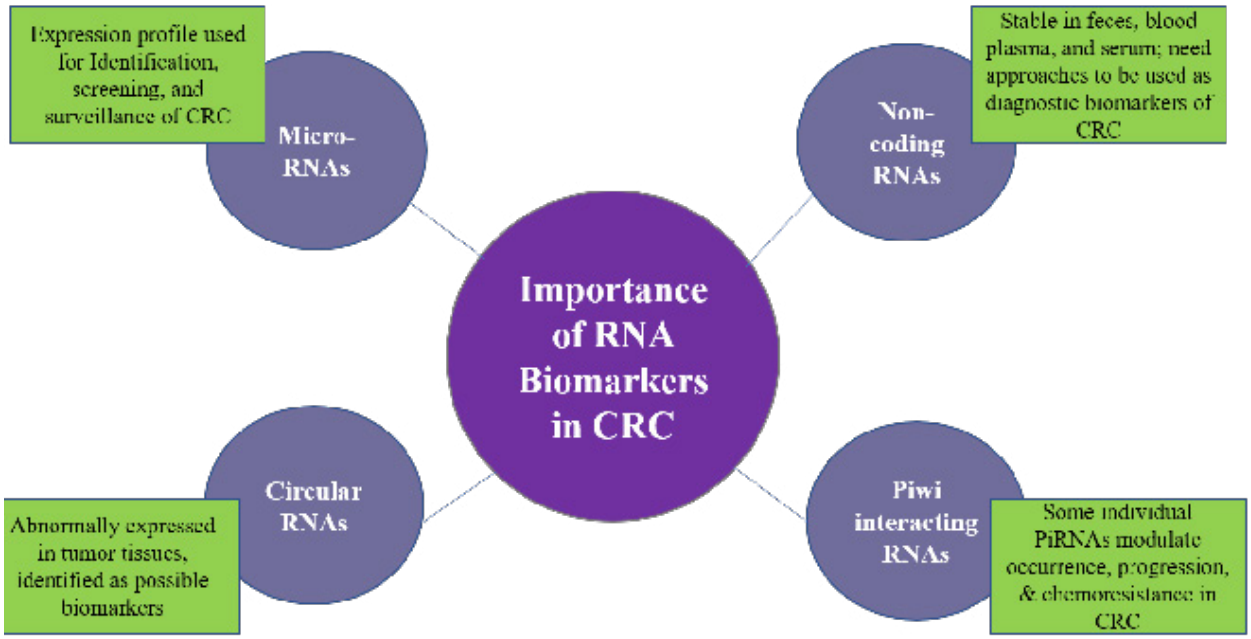

Figure 3: Importance of some Potent RNA Biomarkers in Colorectal cancer: This figure gives brief idea of significance of 4 classes of RNA Biomarkers including microRNAs (MiRNAs), non-coding RNA (ncRNAs), Circular RNAs (circRNAs), and Piwi-interacting RNAs (PiRNAs) [46, 48, 125, 131].

Table 2: A brief overview of the roles of potent RNA Biomarkers for CRC

RNA Biomarkers	Role in Colorectal Cancer	References
MicroRNAs	• Change in expression of specific miRNAs lead to CRC.	[121, 125]
Non-Coding RNAs	• The circulating ncRNAs are early diagnostic biomarkers of CRC. • LncRNAs regulate the differentiation, apoptosis, proliferation, invasion and metastasis of colorectal cancer.	[46] [132]
Circular RNAs	• CircRNAs are abnormally expressed in tumor tissues acting as diagnostic and prognostic biomarkers.	[46, 48, 130]
Piwi-interacting RNA	• They hold a substantial role in cancer biogenesis e.g., piR-651 shows higher expression levels in CRC tissues, subsequently PiRNAs can act as biomarkers for CRC.	[47]

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