Hindawi
Gastroenterology Research and Practice
Volume 2017, Article ID 2195361, 11 pages
https://doi.org/10.1155/2017/2195361

Review Article

Colorectal Cancer Blood-Based Biomarkers

Nina Hauptman and Damjan Glavač

Department of Molecular Genetics, Institute of Pathology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Correspondence should be addressed to Nina Hauptman; nina.hauptman@mf.uni-lj.si

Received 14 April 2017; Revised 16 July 2017; Accepted 13 September 2017; Published 25 September 2017

Academic Editor: Wenhao Weng

Mortality and morbidity associated with colorectal cancer (CRC) are increasing globally, partly due to lack of early detection of the disease. The screening is usually performed with colonoscopy, which is invasive and unpleasant, discouraging participation in the screening. As a source of noninvasive and easily accessible biomarkers, liquid biopsies are emerging. Blood-based biomarkers have the potential as diagnostic and prognostic tool in CRC. Early stage detection of CRC with high sensitivity and specificity would likely lead to higher participation in the screening test. It would also improve the prognosis of the disease and improve the recurrence risk. In this review, we summarize the potential biomarkers for early detection and monitoring of CRC.

1. Introduction

Colorectal cancer (CRC) is a malignant neoplasm of the colon, rectum, and appendix and is a major health burden, causing one-third of cancer-related deaths [1]. The time of diagnosis greatly influences the overall survival rate of patients with CRC. Five-year survival rates are estimated to be between 85 and 90% for patients with localized cancer to colon or rectum. Survival decreases significantly for patients with distant metastasis, with 5-year survival of only 12.5%. Appendix cancers exhibit higher survival rates in all stages [1]. The number of cases of CRC has decreased due to advances in screening and diagnostic methods [2].

CRC develops as a result of genetic and epigenetic alterations, as well as environmental factors. CRC occurs as familial, inherited, and sporadic disease, where less than 10% reported cases are inherited CRCs. Lynch syndrome, familial adenomatous polyposis (FAP), and Peutz-Jeghers syndrome are inherited diseases which have a predisposition to progress to CRC. Familial form of CRC accounts for 25% of all cases. The largest group is the sporadic form of CRC, which accounts for 70% of all CRCs. With this form of CRC etiological, dietary and environmental factors are connected [3].

CRC is a heterogeneous disease, arising through different molecular mechanisms. Currently, three major molecular mechanisms through which CRC carcinogenesis can evolve are chromosomal instability (CIN), microsatellite instability (MSI), and the CpG island methylator phenotype (CIMP). Imbalances in chromosome number and a loss of heterozygosity (LOH) are characteristic for CIN molecular subtype [3]. Mutations in mismatch repair genes, such as *MLH1*, *MSH2*, and *MSH6*, are characteristic for MSI. MSI molecular subtype is related to inherit CRC. CIMP is found in sporadic cases of CRC and is characterized by aberrant methylation of tumor suppressor genes silencing them.

The survival of CRC affected patients depends highly on early detection [4]. The main screening approaches include direct structural examinations and fecal tests. Fecal tests are used to detect blood in the stool, which can be detected with hemoglobin test or immunohistochemistry. Fecal test is not specific for CRC and direct structural exam, such as flexible sigmoidoscopy and colonoscopy, must succeed fecal test. The structural examinations are invasive procedures and reduce the willingness of a patient to participate in CRC screening.

Improving the patients' prognosis, treatment response prediction, and recurrence risk would be enabled with reliable biomarkers for early detection of CRC. Carcinoembryonic antigen (CEA) is the most widely used biomarker for CRC in the clinical setting but has limited sensitivity and specificity [5]. In recent years, "liquid biopsies" are

becoming recognized as sources of novel blood-based biomarkers. Detection of biomarkers in a patient blood sample would provide the most practical screening tool. The advantages of detecting a biomarker in blood or other bodily fluids include minimal invasiveness and easy accessibility. In this review, we summarize the currently known circulating biomarkers that may potentially be used for the early detection of CRC and monitoring the prognosis of CRC patients. These biomarkers include circulating tumor cells, DNA, RNA, and proteins [6].

2. Currently Available Blood-Based Tests

Extensive efforts have been made to identify blood-based markers for the early detection of CRC. Most of the candidate markers have been evaluated in clinical settings and are mostly detected in advanced stages of CRC. Noninvasive blood-based tests promise to improve screening and reduce mortality of this malignancy. The most widely used blood-based CRC biomarker is carcinoembryonic antigen (CEA), although it has proved to be a valuable tool for patient monitoring.

2.1. Carcinoembryonic Antigen. Blood-based CRC-specific antigens are a topic extensively researched. However, there are only two biomarkers available to monitor CRC patients, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9). CEA is a high molecular weight glycoprotein, discovered in 1965, and found in embryonic tissue and colorectal tumors. It is a biomarker to monitor CRC recurrence. CEA elevated levels are a poor prognostic factor for resectable CRC correlating to cancer progression [7]. The sensitivity of this marker increases with tumor stage [8], while levels decrease after tumor resection. High levels of CEA in blood are not specific for CRC but can also include inflammatory bowel disease, pancreatitis, liver disease, or other malignancies. Interestingly, elevated levels of CEA are detected in advanced stages of CRC in a fraction of all CRC patients; therefore, CEA levels are not an effective method of screening.

The CA19-9 antigen is less sensitive and specific for CRC compared to CEA, but it is a marker available to detect pancreaticobiliary malignancies. CEA is still the antigen of choice to use as a prognostic marker after diagnosis and to monitor disease progression.

Tissue polypeptide-specific antigen (TPS) and tissue polypeptide antigen (TPA) which detects the fragments of cytokeratins 8, 18, and 19 lack the sensitivity and specificity for CRC screening. Most of the studies report increased levels of TPA and TPS in the metastatic stage of CRC. Some studies suggested that the combination of TPA and CEA has greater sensitivity and can identify recurrence in CRC patients [9–12].

3. Circulating Tumor Cells

Circulating tumor cells (CTCs) are found in peripheral blood and are tumor cells that have separated from primary tumor or metastasis. CTCs are found in almost all cancers, such as colon, breast, prostate, lung, ovary, pancreas, liver, gastric, esophageal, renal, bladder, thyroid, nasopharyngeal, and melanoma. CTCs are usually not found in patients without cancer and can usually be detected before the metastasis occurs [13, 14]. Although it was discovered that up to 1 million tumor cells enter the circulation per gram of tumor tissue, most of the cells undergo apoptosis induced by cell detachment [15–21]. A small portion of cells might form micrometastasis, most of which disappear and few cells progress to form macroscopic tumors [17, 20]. The growth requires the attachment of tumor cells to the vascular endothelium, where cancer cell aggregation can occur at sites of attachment [22, 23]. The epithelial-mesenchymal transition (EMT) may also be a key step for CTCs to spread and form metastasis [24–30]. In breast cancer and prostate cancer, CTC analysis found expressed genes related to EMT; also, CTCs were commonly associated with metastatic disease [31]. Tumor cells also interact with platelets, which also can contribute to mesenchymal transition leading to metastasis [32]. Whether EMT is necessary for metastasis development is not clear, since cell invasion can occur in the absence of EMT [33, 34]. A recent report supports evidence that a small subset of CTCs is able to induce metastasis, where the cells express epithelial cell adhesion molecule (EpCAM), CD44, CD47, and MET [35]. Further research is needed to answer these questions fully.

Genomic analyses of single cells from primary and metastatic tumors are genetically diverse [36, 37]. Mutations occurring in *KRAS*, *BRAF*, and *PIK3CA* in single CTCs are heterogeneous among CTCs from the same patient. Identifying different populations of CTCs is important for therapy response [38, 39]. Furthermore, CTCs have different characteristics from those cells that were able to form a metastasis [40].

4. Innovative Blood-Based Tests

Patients with diagnosed advanced stage of CRC have a poor prognosis. The key to reduce morbidity and mortality associated with this disease is early detection. Availability of noninvasive, blood-based biomarkers would increase screening and tumor detection at earlier and more treatable stage. With technological advances and more comprehensive understanding of the molecular mechanism contributing to colorectal carcinogenesis, intense effort has been put into identifying CRC biomarkers detectable in blood. Circulating nucleic acids, proteins, and tumor cells have been evaluated as CRC diagnostic tools with promising levels of sensitivity and specificity. However, a new blood-based biomarker for CRC has not yet been implemented in the clinic.

4.1. Circulating DNA-Based Biomarkers. Cell-free DNA (cfDNA) was found in blood over 60 years ago [41]. Recently, cfDNA is opening a new field of potential biomarkers; however, high levels of cfDNA are observed also in patients with inflammatory and autoimmune diseases, besides cancer [29, 42]. Cell-free DNA is found in serum or plasma, and it originates from apoptotic and necrotic cells, although release of DNA from living cells can also occur [43].

Quantifying serum or plasma cfDNA has been shown to be significantly elevated in CRC patients. The levels decreased over the course of follow-up and disease-free patients. Increased levels of cfDNA were observed in patients with cancer recurrence or metastasis [44]. Recent studies confirmed that levels of cfDNA in CRC patients are significantly higher than those in healthy subjects. Serum levels of cfDNA also increase with tumor stage and fluctuate during chemotherapy [45, 46]. Quantification of cfDNA can be a useful tool for monitoring of CRC patients. However, circulating cfDNA amount is influenced by various issues. Amount of cfDNA was observed to be higher in serum than in plasma due to white blood cells clotting in serum. This means that plasma has a higher amount of tumor cfDNA, since extraction of cfDNA from serum might contain also wild-type DNA [47, 48]. Another thing is that circulating cfDNA is unstable, with a half-life ranging from 15 minutes to several hours [49]. Therefore, the amount of circulating cfDNA does not provide consistent results.

Other characteristics of cfDNA can be assessed to obtain more information, such as cfDNA integrity, point mutations, microsatellite alterations, and hypermethylation of gene promoters.

4.2. cfDNA Integrity. cfDNA is uniformly truncated when released from apoptotic cells; when released from necrotic tumorous cells, it varies in size from 185 to 200 bp long fragments [43, 50]. The integrity of cfDNA is measured as a ratio of long fragments to short fragments of cfDNA. CRC studies report different findings: some report decreased cfDNA fragmentation [51, 52], while others increased cfDNA fragmentation [53, 54]. Although reports are inconsistent, the recent study describes significantly less fragmented cfDNA in metastatic CRC compared to primary CRC [55]. This is promising information for using integrity of cfDNA as a monitoring tool for evaluation of progression of CRC.

4.3. Microsatellite Alterations. Microsatellites are repeated 1–6 bp units of DNA sequence in coding or noncoding regions. Microsatellite instability (MSI) is deletion or insertion of microsatellite units, which causes alterations associated with cancer. MSI in CRC is detected in 15% of all CRCs and is associated with defects in DNA mismatch repair genes. MSI is associated with hereditary nonpolyposis CRC, although most cancers with high level of MSI are sporadic [56]. Cancers with high level of MSI also show increased resistance to chemotherapeutic agents [57]. Many studies focused on detection of MSI in cfDNA [58, 59], where MSI markers were detected in cfDNA in 35% of patients with CRC; however, the detection rate varies from 0 to 60% in studies.

4.4. Genetic Alterations in cfDNA. Early research on point mutations of cfDNA focused on *APC* gene [60, 61]. It was shown that 8% of APC gene fragments are mutated and that detection of cfDNA was sensitive enough to identify residual disease following surgical resection [61]. Another study showed that serum detection rates of genes APC, KRAS, and TP53 were 30.4%, 34.0%, and 34.2%, respectively [62]. Usually, the mutations are unique for each patient, which prevents the development of panel covering all the somatic mutations at a low cost [63].

4.5. Aberrant DNA Methylation. Methylation of DNA occurs when a methyl group is covalently added to 5-carbon position on cytosine in CpG dinucleotides. Both DNA hypermethylation and hypomethylation can contribute to carcinogenesis. DNA hypermethylation usually occurs in CpG islands and refers to methylation of normally unmethylated regions. Result of hypermethylation is gene silencing, affecting DNA repair genes and tumor suppressor genes [64]. Hypomethylation is a loss of methylation compared to healthy methylation pattern and results in genomic instability [65]. Both hyper- and hypomethylation were observed in the early stages of carcinogenesis [66–68]; with advanced technologies that enable detection of genome-wide methylation, the promise of early stage methylation biomarkers has arisen [69]. The plasma and serum of CRC patients have been studied for aberrant methylation patterns for diagnostic and prognostic use. The most known is the *SEPT9* gene, encoding a guanosine triphosphate enzyme. Validation of *SEPT9* methylation biomarker has shown that the sensitivity is 90% and the specificity is 88% [70]. Currently, three commercial tests based on *SEPT9* methylation for CRC screening are available: Epi proColon 2.0 (Epigenomics), ColoVantage™ (Quest Diagnostic), and RealTime ms9 (Abbott).

Epi proColon has developed from version 1.0 to 2.0 where the new version has fewer handling steps, shorter time to result, and increased clinical performance compared to the first generation test. From a report on a recent trial, the test has detected 48.2% of cancers, with a specificity of 91.5%, and sensitivity for advanced cancers was 11.2% [71]. Further studies are needed to discover limitations of these assays and improve their sensitivity and specificity [67]. Also, other genes have been reported to be highly specific and sensitive cfDNA CRC biomarkers, such as *APC*, *CDKN2A*/*P16h*, *MLH1*, *ALX4*, *TMEFF2*, *NGFR*, *FRP2*, *NEUROG1*, *TPEF*/*HPP1*, and *RUNX3* [72–74]. Beside diagnostic, also prognostic biomarkers are needed, such as *HLTF* gene. Methylation of HLTF was reported to strongly correlate with tumor size, metastatic disease, and tumor stage; furthermore, it was associated with disease recurrence [75, 76]. Other genes with prognostic value include *HPP1* and *DFNA5* [76, 77]. Combination of these markers and further validation is needed to bring these biomarkers to clinical practice [78].

4.6. Circulating RNA-Based Biomarkers. In blood, RNA molecules are exposed to RNase, which rapidly degrade RNAs, making the search for RNA-based biomarkers a difficult task. Cell-free RNAs have been detected in many bodily fluids, including serum/plasma, saliva, cerebrospinal fluid, synovial fluid, tear fluid, amniotic fluid, and urine [79]. In blood, mRNA, miRNA, and lncRNA can be detected.

4.7. mRNA Markers. mRNA cancer biomarkers have been studied by many groups. mRNA that was analyzed was obtained from peripheral blood cells or from either serum

Table 1: Potential blood miRNA biomarkers (adapted from [85]).

miRNA	Sensitivity	Specificity	Distinguish AA	Differentiate	Validation cohort (CRC/AA/healthy)	Reference
miR-601 ↓, miR-760 ↓	83.3%	69.1%	Yes	CRC from controls; AA from controls	90/43/58	[95]
miR-15b ↑, miR-18a ↑, miR-19a ↑, miR-19b ↑, miR-29a ↑, miR-335 ↑	78.6%	79.3%	Yes	CRC from controls; AA from controls	42/40/53	[96]
miR-15b ↑, miR-17 ↑, miR-142-3p ↑, miR-195 ↑, miR-331 ↑, miR-532-5p ↑, miR-532-3p ↑, miR-652 ↑	88%	64%		AA from controls	45/16/26	[97]
miR-431 ↑, miR-15b ↑, miR-139-3p ↓	93%	74%		Stage IV CRC from controls	45/16/26	[97]
miR-7 ↑, miR-17-3p ↑, miR-20a ↑, miR-21 ↑, miR-92a ↑, miR-183 ↑, miR-196a ↑, miR-214 ↑, miR-124 ↓, miR-127-3p ↓, miR-138 ↓, miR-143 ↓, miR-146a ↓, miR-222 ↓	90%	95%		CRC from controls		[98]
miR-29a ↑, miR-92a ↑	83%	84.7%		CRC from controls	120/37/59	[99]
miR-21 ↑, miR-92a ↑	68%	91.2%		CRC from controls	200/50/80	[100]
miR-221 ↑	86%	41%		CRC from controls	103/0/37	[101]

or plasma [80]. It was demonstrated that detection of tumor mRNA is possible in the serum of malignant melanoma patients [81]. Another study employed multiplex RT-qPCR to determine the expression of CEA, cytokeratin 20, and epidermal growth factor receptor (EGFR) on RNA obtained from peripheral blood [82]. Other studies include survivin and telomerase reverse transcriptase (TERT) [83, 84]. These studies have shown that these molecules have diagnostic and prognostic value [85]. However, this research usually remained at the pilot stage. There is, however, one blood-based, ColonSentry used as a risk assessment test. The panel includes seven genes (*ANXA3*, *CLEC4D*, *LMNB1*, *PRRG4*, *TNFAIP6*, *VNN1*, and *IL2RB*) and is tested on RNA extracted from peripheral blood [86]. The genes were selected from expression profiling of 196 genes performed on 112 CRC patients and 120 controls. This panel was validated several times. The Canadian population of 202 CRC patients and 208 controls had a sensitivity of 72% and specificity of 70%. The Malaysian population of 99 CRC patients and 111 controls had a sensitivity of 61% and specificity of 77% [87]. The test was ultimately verified with 727 CRC patients of all stages, although it appears that high-risk individuals were not included in the test.

4.8. MicroRNAs. MicroRNAs (miRNAs) belong to the group of noncoding RNAs. They are 18–25 bp long and bind to the 3′ UTR part of mRNA with their complementary sequence, which results in translational silencing or repression of genes. They play important roles in many biological processes, such as cell proliferation, differentiation, and apoptosis [88]. Many miRNA genes are located at chromosomal regions that undergo deletions, amplifications, or translocations, making their expression patterns aberrant. Deregulated expression of miRNA was reported in many cancers, also in CRC, where they can act as tumor suppressors or oncogenes, depending on their target genes [89–91].

miRNA express higher stability in blood, compared to mRNA, due to their protection from degradation by endogenous RNase activity and resistance to extreme pH values [92]. miRNA stability is achieved due to their binding to high-density lipoproteins and their inclusion in exosomes [93]. Since their stability and evidence of their deregulation in cancers, miRNAs are promising as noninvasive biomarkers in cancer [94].

miRNA extracted from serum or plasma of CRC patients are first profiled and subsequently validated with RT-qPCR. Profiling of 742 miRNA on CRC samples and healthy controls resulted in validation of miR-601 and miR-760 on 90 CRC samples, 43 advanced adenoma (AA) samples, and 58 healthy controls (Table 1). Both miRNA had a lower expression in CRC and AA samples compared to healthy controls with a sensitivity of 83.3% and specificity of 69.1% [95]. Another research group performed profiling of 743 miRNA on CRC, AA, and healthy control samples, followed by validation with 42 CRC, 40 AA, and 53 healthy control samples. A panel of six miRNA (miR-15b, miR-18a, miR-19a, miR-19b, miR-29a, and miR-335) was successfully distinguished between CRC samples and healthy controls with the sensitivity of 78.6% and specificity of 79.3%. Furthermore, miR-18a was able to distinguish between AA samples and healthy controls with a sensitivity of 80% and specificity of 80% [96]. After profiling of 380 miRNA, another research group proposed a panel of eight miRNAs (miR-15b, miR-17, miR-142-3p, miR-195, miR-331, miR-532-5p, miR-532-3p, and miR-652), which was validated on a cohort of 45 CRC, 16 AA, and 26 healthy individuals, and found to distinguish between AA and controls with a sensitivity of 88% and specificity of 64%. The same group proposed a three-panel miRNA (miR-431, miR-15b, and miR-139-3p), which can distinguish between stage IV CRC and control samples with a sensitivity of 93% and specificity of 74% [97]. Ahmed et al.

reported to validate a set of fifteen miRNA, of which nine (miR-7, miR-17-3p, miR-20a, miR-21, miR-92a, miR-183, miR-196a, and miR-214) were upregulated and six (miR-124, miR-127-3p, miR-138, miR-143, miR-146a, and miR-222) were downregulated in CRC patients' plasma and tissue with the sensitivity of 90% and specificity of 95% [98].

Some research groups have selected their candidate miRNA from literature. One study confirms miR-29a and miR-92a on 120 CRC, 37 AA, and 59 healthy individuals to differentiate between CRC and healthy individuals with a sensitivity of 83% and specificity of 84.7% [99]. Liu et al. reported miR-92a and miR-21 to be upregulated in CRC patients compared to controls with a sensitivity of 68% and specificity of 91.2%, validated on 200 CRC patients, 50 AA patients, and 80 healthy individuals [100]. Pu et al. found upregulated miR-221 in CRC with a sensitivity of 86% and specificity of 41%, validated on a cohort of 103 CRC and 37 healthy controls [101].

Studies with proposed miRNA panels are summarized in Table 1. Most of the studies focus on early stage detection in CRC, and some also include AA patients. In these studies, sensitivity ranges from 78% to 93%, and specificity from 41% to 95%. Also, some miRNAs were proposed in several panels, such as miR-15b, miR-17-3p, miR-18a, miR-20a, miR-21, miR-29a, and miR-92a. However, not all of these miRNA biomarkers were confirmed by other researchers. This could be due to patient population, using different endogenous controls, or different instrumentation. Nevertheless, these sets of miRNA could be further validated and evaluated for potential biomarkers.

4.9. Long Noncoding RNAs. Serum or plasma detected lncRNAs can be a potential biomarker in different types of tumors. One of the first reports on circulating lncRNA associated with a type of tumor was HULC, which expression was found to be upregulated in patients with hepatocellular carcinoma [102]. The plasma of gastric cancer patients has highly expressed lncRNA H19, compared to normal controls; its levels were observed to decrease after tumor resection [103]. For gastric cancer, a combination of UCA1/CUDR, LSINCT-5, and PTENP1 was reported to be of diagnostic value [104]. In esophageal squamous cell carcinoma, lncRNA POU3F3 expression in serum combined with plasma levels of squamous cell carcinoma antigen improves screening efficiency for early detection [105]. Only few reports were published on the expression of circulating lncRNAs as potential noninvasive diagnostic biomarker in CRC. In one, the expression levels of CRNDE-h transcript were significantly upregulated than in healthy controls, with a sensitivity of 87% and specificity of 93% for diagnosing CRC [106]. The other studied the combination of CCAT and HOTAIR. Both lncRNAs were found significantly upregulated in plasma of CRC patients, compared to normal controls. The combination proved to be of greater diagnostic value, with a sensitivity of 84.3% and specificity of 80.2%. More importantly, this combination was effective to detect CRC at an early stage (85%) [107].

5. Colitis-Associated Colorectal Cancer

Patients with extensive chronic ulcerative colitis (UC) have also increased risk for colorectal cancer. In these patients, surveillance is colonoscopy looking for dysplasia (precancer) or cancer. Colitis-associated cancer (CAC) is different from sporadic CRC. The cancer risk in patients with CAC depends on UC duration, severity of inflammation, and extent of disease [108, 109]. This method is cost-effective and able to reduce mortality and cancer incidence, but exposed to pathologist subjective evaluation [110, 111]. To increase clinical value, a molecular biomarker would be beneficial to the management of UC patients. UC is a disease undergoing cycles of inflammation and tissue repair, which results in oxidative stress, increasing reactive oxidative species (ROS) [112–115]. Accumulation of ROS causes damage to DNA, proteins, and lipids, initiating tumor. CAC progresses in steps, which are negative dysplasia, indefinite for dysplasia, low-grade dysplasia, high-grade dysplasia, and cancer [116]. Molecular alterations in UC are detectable prior to dysplasia, such as telomere shortening, chromosomal and microsatellite instability, aneuploidy, and loss of p53 [117–125]. Tumor suppressor gene p53 harbors an important early event in CAC, which occurs in 47%–85% [126, 127]. Another mechanism in CAC is the release of proinflammatory cytokines that are released by inflammation [128, 129]. Chronic inflammation releases cytokines which are involved in all stages of cancer, including initiation and progression of tumor, angiogenesis, and metastasis. The molecular link between inflammation and carcinogenesis is NF-κB. Activated NF-κB upregulates the expression of many proinflammatory molecules, such as adhesion molecules, cytokines, TNF-α, and IL-6, which play a critical role in tumor development and progression of colitis-associated cancer [127].

CAC molecular alterations are different from those of sporadic CRC. Alterations in APC occur early in sporadic CRC and are considered a late event in CAC progression or are not present at all. Mutations in p53 appear as early event in CAC, prior to dysplasia, while in CRC mutations in p53 are a late event. This suggests that pathways from CAC and CRC are different. Interestingly, it was observed that patients with UC have genomically abnormal nondysplastic mucosa, while having a neoplasia elsewhere in the colon [119, 130]. Furthermore, it was established that molecular alterations occurring in these UC patients are expression of proteins, genomic instability in repetitive DNA, and mitochondrial dysfunction, which are not present in dysplasia-free UC patients and provide the basis for development of biomarkers for diagnostic and therapeutic advances in CAC [119, 120, 131–133].

CAC seems to be driven largely by damage related to inflammation. Many biomarkers are being researched to facilitate the development and discriminate subtypes of patients with inflammatory bowel disease (IBD-ulcerative colitis versus Crohn's disease) and biomarkers for differentiating between UC patients who develop dysplasia and those who do not. Blood is easily accessible and is ideal for diagnostic purposes; moreover, it provides a wide variety of material

(DNA, RNA, proteins, cells, etc.). Apart from enzyme-linked immunosorbent assays (ELISA), modified ELISA and targeted proteomics are emerging, facilitating analysis of low abundance proteins. Levels of p53 in the serum of UC patients were found higher when compared to normal controls. Elevated levels were found in 8/13 UC patients with CRC [134]. Although alteration in p53 is an early event in UC tumorigenesis, detection of p53 protein in serum showed moderate sensitivity, limiting its use as a neoplastic marker for high-risk patients.

5.1. miRNA in Peripheral Blood of UC Patients. Several studies were reported, where miRNA levels in peripheral blood of UC patients were studied. One study found that six miRNAs were significantly upregulated compared to normal controls, and those were miR-16, miR-21, miR-28-5p, miR-151-5p, miR-155, and miR-199a-5p, where miR-155 exhibited the highest expression of those six [135]. Another study found that 12 miRNAs were significantly upregulated in UC patients compared to normal controls, while miR-505 was downregulated in UC patients [136]. Attempts were also made to analyze miRNAs from different hematologic fractions, and seven miRNAs derived from platelets were found to be upregulated (miR-188-5p, miR-378, miR-422a, miR-500, miR-501-5p, miR769-5p, and miR874) [137]. This confirms that also miRNAs derived from platelets have the potential of becoming biomarkers in pathogenesis of UC.

6. Conclusion

Noninvasive screening of CRC could be achieved by utilizing blood-based biomarkers, which would enable early detection of the disease. They could also be used as a monitoring tool for recurrence or response to therapy. There are many studies that provided the data on blood-based markers useful for diagnosis and prognosis of CRC, including DNA, RNA, and protein-based biomarkers. Methylated septin 9, mutations in cell-free DNA, and several miRNAs extracted from plasma or serum show promising results towards the noninvasive tests for CRC. However, further validation of sensitivity and specificity is needed, to ensure the reproducibility of the results.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

Nina Hauptman acknowledges Slovenian Research Agency for support through Project Z3-6797.

References

[1] R. Siegel, C. Desantis, and A. Jemal, "Colorectal cancer statistics, 2014," *CA: A Cancer Journal for Clinicians*, vol. 64, no. 2, pp. 104–117, 2014.

[2] R. A. Smith, D. Manassaram-Baptiste, D. Brooks et al., "Cancer screening in the United States, 2015: a review of current American cancer society guidelines and current issues in cancer screening," *CA: A Cancer Journal for Clinicians*, vol. 65, no. 1, pp. 30–54, 2015.

[3] M. S. Pino and D. C. Chung, "The chromosomal instability pathway in colon cancer," *Gastroenterology*, vol. 138, no. 6, pp. 2059–2072, 2010.

[4] J. B. Rawson and B. Bapat, "Epigenetic biomarkers in colorectal cancer diagnostics," *Expert Review of Molecular Diagnostics*, vol. 12, no. 5, pp. 499–509, 2012.

[5] K. F. Newton, W. Newman, and J. Hill, "Review of biomarkers in colorectal cancer," *Colorectal Disease*, vol. 14, no. 1, pp. 3–17, 2012.

[6] E. E. Yoruker, S. Holdenrieder, and U. Gezer, "Blood-based biomarkers for diagnosis, prognosis and treatment of colorectal cancer," *Clinica Chimica Acta*, vol. 455, pp. 26–32, 2016.

[7] G. Y. Locker, S. Hamilton, J. Harris et al., "ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer," *Journal of Clinical Oncology*, vol. 24, no. 33, pp. 5313–5327, 2006.

[8] S. Hundt, U. Haug, and H. Brenner, "Blood markers for early detection of colorectal cancer: a systematic review," *Cancer Epidemiology, Biomarkers & Prevention*, vol. 16, no. 10, pp. 1935–1953, 2007.

[9] A. Nicolini, P. Ferrari, M. J. Duffy et al., "Intensive risk-adjusted follow-up with the CEA, TPA, CA19.9, and CA72.4 tumor marker panel and abdominal ultrasonography to diagnose operable colorectal cancer recurrences: effect on survival," *Archives of Surgery*, vol. 145, no. 12, pp. 1177–1183, 2010.

[10] M. Levy, V. Visokai, L. Lipska, and O. Topolcan, "Tumor markers in staging and prognosis of colorectal carcinoma," *Neoplasma*, vol. 55, no. 2, pp. 138–142, 2008.

[11] P. Bystrom, Å. Berglund, P. Nygren et al., "Evaluation of predictive markers for patients with advanced colorectal cancer," *Acta Oncologica*, vol. 51, no. 7, pp. 849–859, 2012.

[12] S. Holdenrieder, P. Stieber, V. Liska et al., "Cytokeratin serum biomarkers in patients with colorectal cancer," *Anticancer Research*, vol. 32, no. 5, pp. 1971–1976, 2012.

[13] W. J. Allard, J. Matera, M. C. Miller et al., "Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases," *Clinical Cancer Research*, vol. 10, no. 20, pp. 6897–6904, 2004.

[14] D. Glaves, "Correlation between circulating cancer cells and incidence of metastases," *British Journal of Cancer*, vol. 48, no. 5, pp. 665–673, 1983.

[15] Y. S. Chang, E. di Tomaso, D. M. McDonald, R. Jones, R. K. Jain, and L. L. Munn, "Mosaic blood vessels in tumors: frequency of cancer cells in contact with flowing blood," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 26, pp. 14608–14613, 2000.

[16] L. Weiss, E. Mayhew, D. G. Rapp, and J. C. Holmes, "Metastatic inefficiency in mice bearing B16 melanomas," *British Journal of Cancer*, vol. 45, no. 1, pp. 44–53, 1982.

[17] K. J. Luzzi, I. C. MacDonald, E. E. Schmidt et al., "Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early

micrometastases," *The American Journal of Pathology*, vol. 153, no. 3, pp. 865–873, 1998.

[18] C. W. Wong, A. Lee, L. Shientag et al., "Apoptosis: an early event in metastatic inefficiency," *Cancer Research*, vol. 61, no. 1, pp. 333–338, 2001.

[19] L. Weiss, U. Nannmark, B. R. Johansson, and U. Bagge, "Lethal deformation of cancer cells in the microcirculation: a potential rate regulator of hematogenous metastasis," *International Journal of Cancer*, vol. 50, no. 1, pp. 103–107, 1992.

[20] E. Barbera-Guillem and L. Weiss, "Cancer-cell traffic in the liver. III. Lethal deformation of B16 melanoma cells in liver sinusoids," *International Journal of Cancer*, vol. 54, no. 5, pp. 880–884, 1993.

[21] O. Berezovskaya, A. D. Schimmer, A. B. Glinskii et al., "Increased expression of apoptosis inhibitor protein XIAP contributes to anoikis resistance of circulating human prostate cancer metastasis precursor cells," *Cancer Research*, vol. 65, no. 6, pp. 2378–2386, 2005.

[22] A. B. Al-Mehdi, K. Tozawa, A. B. Fisher, L. Shientag, A. Lee, and R. J. Muschel, "Intravascular origin of metastasis from the proliferation of endothelium-attached tumor cells: a new model for metastasis," *Nature Medicine*, vol. 6, no. 1, pp. 100–102, 2000.

[23] V. V. Glinsky, G. V. Glinsky, O. V. Glinskii et al., "Intravascular metastatic cancer cell homotypic aggregation at the sites of primary attachment to the endothelium," *Cancer Research*, vol. 63, no. 13, pp. 3805–3811, 2003.

[24] G. Kallergi, M. A. Papadaki, E. Politaki, D. Mavroudis, V. Georgoulias, and S. Agelaki, "Epithelial to mesenchymal transition markers expressed in circulating tumour cells of early and metastatic breast cancer patients," *Breast Cancer Research*, vol. 13, no. 3, article R59, 2011.

[25] S. Kasimir-Bauer, O. Hoffmann, D. Wallwiener, R. Kimmig, and T. Fehm, "Expression of stem cell and epithelial-mesenchymal transition markers in primary breast cancer patients with circulating tumor cells," *Breast Cancer Research*, vol. 14, no. 1, p. R15, 2012.

[26] B. Aktas, M. Tewes, T. Fehm, S. Hauch, R. Kimmig, and S. Kasimir-Bauer, "Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients," *Breast Cancer Research*, vol. 11, no. 4, article R46, 2009.

[27] A. Lecharpentier, P. Vielh, P. Perez-Moreno, D. Planchard, J. C. Soria, and F. Farace, "Detection of circulating tumour cells with a hybrid (epithelial/mesenchymal) phenotype in patients with metastatic non-small cell lung cancer," *British Journal of Cancer*, vol. 105, no. 9, pp. 1338–1341, 2011.

[28] C. Raimondi, A. Gradilone, G. Naso et al., "Epithelial-mesenchymal transition and stemness features in circulating tumor cells from breast cancer patients," *Breast Cancer Research and Treatment*, vol. 130, no. 2, pp. 449–455, 2011.

[29] A. J. Armstrong, M. S. Marengo, S. Oltean et al., "Circulating tumor cells from patients with advanced prostate and breast cancer display both epithelial and mesenchymal markers," *Molecular Cancer Research*, vol. 9, no. 8, pp. 997–1007, 2011.

[30] M. Yu, A. Bardia, B. S. Wittner et al., "Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition," *Science*, vol. 339, no. 6119, pp. 580–584, 2013.

[31] C. L. Chen, D. Mahalingam, P. Osmulski et al., "Single-cell analysis of circulating tumor cells identifies cumulative expression patterns of EMT-related genes in metastatic prostate cancer," *Prostate*, vol. 73, no. 8, pp. 813–826, 2013.

[32] M. Labelle, S. Begum, and R. O. Hynes, "Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis," *Cancer Cell*, vol. 20, no. 5, pp. 576–590, 2011.

[33] T. Tsuji, S. Ibaragi, and G. F. Hu, "Epithelial-mesenchymal transition and cell cooperativity in metastasis," *Cancer Research*, vol. 69, no. 18, pp. 7135–7139, 2009.

[34] A. Wicki, F. Lehembre, N. Wick, B. Hantusch, D. Kerjaschki, and G. Christofori, "Tumor invasion in the absence of epithelial-mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton," *Cancer Cell*, vol. 9, no. 4, pp. 261–272, 2006.

[35] I. Baccelli, A. Schneeweiss, S. Riethdorf et al., "Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay," *Nature Biotechnology*, vol. 31, no. 6, pp. 539–544, 2013.

[36] N. Navin, J. Kendall, J. Troge et al., "Tumour evolution inferred by single-cell sequencing," *Nature*, vol. 472, no. 7341, pp. 90–94, 2011.

[37] M. Gerlinger, A. J. Rowan, S. Horswell et al., "Intratumor heterogeneity and branched evolution revealed by multiregion sequencing," *The New England Journal of Medicine*, vol. 366, no. 10, pp. 883–892, 2012.

[38] C. Gasch, T. Bauernhofer, M. Pichler et al., "Heterogeneity of epidermal growth factor receptor status and mutations of KRAS/PIK3CA in circulating tumor cells of patients with colorectal cancer," *Clinical Chemistry*, vol. 59, no. 1, pp. 252–260, 2013.

[39] R. Nadal, A. Fernandez, P. Sanchez-Rovira et al., "Biomarkers characterization of circulating tumour cells in breast cancer patients," *Breast Cancer Research*, vol. 14, no. 3, article R71, 2012.

[40] F. L. Carvalho, B. W. Simons, E. S. Antonarakis et al., "Tumorigenic potential of circulating prostate tumor cells," *Oncotarget*, vol. 4, no. 3, pp. 413–421, 2013.

[41] P. Mandel and P. Metais, "Les acides nucleiques du plasma sanguin chez l'homme," *Comptes Rendus des Seances de la Societe de Biologie et de Ses Filiales*, vol. 142, no. 3-4, pp. 241–243, 1948.

[42] X. Y. Zhong, I. von Mühlenen, Y. Li et al., "Increased concentrations of antibody-bound circulatory cell-free DNA in rheumatoid arthritis," *Clinical Chemistry*, vol. 53, no. 9, pp. 1609–1614, 2007.

[43] S. Jahr, H. Hentze, S. Englisch et al., "DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells," *Cancer Research*, vol. 61, no. 4, pp. 1659–1665, 2001.

[44] M. Frattini, G. Gallino, S. Signoroni et al., "Quantitative analysis of plasma DNA in colorectal cancer patients: a novel prognostic tool," *Annals of the New York Academy of Sciences*, vol. 1075, pp. 185–190, 2006.

[45] L. Boni, E. Cassinotti, M. Canziani, G. Dionigi, F. Rovera, and R. Dionigi, "Free circulating DNA as possible tumour marker in colorectal cancer," *Surgical Oncology*, vol. 16, Supplement 1, pp. S29–S31, 2007.

[46] H. Schwarzenbach, J. Stoehlmacher, K. Pantel, and E. Goekkurt, "Detection and monitoring of cell-free DNA in blood of patients with colorectal cancer," *Annals of the New York Academy of Sciences*, vol. 1137, pp. 190–196, 2008.

[47] T. H. Lee, L. Montalvo, V. Chrebtow, and M. P. Busch, "Quantitation of genomic DNA in plasma and serum samples: higher concentrations of genomic DNA found in serum than in plasma," *Transfusion*, vol. 41, no. 2, pp. 276–282, 2001.

[48] K. C. Chan, S. W. Yeung, W. B. Lui, T. H. Rainer, and Y. M. Lo, "Effects of preanalytical factors on the molecular size of cell-free DNA in blood," *Clinical Chemistry*, vol. 51, no. 4, pp. 781–4, 2005.

[49] M. Fleischhacker and B. Schmidt, "Circulating nucleic acids (CNAs) and cancer—a survey," *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, vol. 1775, no. 1, pp. 181–232, 2007.

[50] J. Yu, G. Gu, and S. Ju, "Recent advances in clinical applications of circulating cell-free DNA integrity," *Laboratoriums Medizin*, vol. 45, no. 1, pp. 6–11, 2014.

[51] N. Umetani, J. Kim, S. Hiramatsu et al., "Increased integrity of free circulating DNA in sera of patients with colorectal or periampullary cancer: direct quantitative PCR for ALU repeats," *Clinical Chemistry*, vol. 52, no. 6, pp. 1062–1069, 2006.

[52] G. Leszinski, J. Lehner, U. Gezer, and S. Holdenrieder, "Increased DNA integrity in colorectal cancer," *In Vivo*, vol. 28, no. 3, pp. 299–303, 2014.

[53] F. Mouliere, B. Robert, E. Arnau Peyrotte et al., "High fragmentation characterizes tumour-derived circulating DNA," *PLoS One*, vol. 6, no. 9, article e23418, 2011.

[54] R. Mead, M. Duku, P. Bhandari, and I. A. Cree, "Circulating tumour markers can define patients with normal colons, benign polyps, and cancers," *British Journal of Cancer*, vol. 105, no. 2, pp. 239–245, 2011.

[55] T. B. Hao, W. Shi, X. J. Shen et al., "Circulating cell-free DNA in serum as a biomarker for diagnosis and prognostic prediction of colorectal cancer," *British Journal of Cancer*, vol. 111, no. 8, pp. 1482–1489, 2014.

[56] C. R. Boland, S. N. Thibodeau, S. R. Hamilton et al., "A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer," *Cancer Research*, vol. 58, no. 22, pp. 5248–5257, 1998.

[57] G. P. Kim, L. H. Colangelo, H. S. Wieand et al., "Prognostic and predictive roles of high-degree microsatellite instability in colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project Collaborative Study," *Journal of Clinical Oncology*, vol. 25, no. 7, pp. 767–772, 2007.

[58] B. Taback, S. Saha, and D. S. Hoon, "Comparative analysis of mesenteric and peripheral blood circulating tumor DNA in colorectal cancer patients," *Annals of the New York Academy of Sciences*, vol. 1075, pp. 197–203, 2006.

[59] I. Lazarev, L. Leibovitch, D. Czeiger et al., "Cell-free DNA blood levels in colorectal cancer patients do not correlate with mismatch repair-proficiency," *In Vivo*, vol. 28, no. 3, pp. 349–354, 2014.

[60] E. Ling, A. Fich, S. Man, M. Wolfson, R. Mikhailowsky, and S. A. Lamprecht, "Detection of tumor mutant APC DNA in plasma of patients with sporadic colorectal cancer," *In Vivo*, vol. 14, no. 4, pp. 543–546, 2000.

[61] F. Diehl, M. Li, D. Dressman et al., "Detection and quantification of mutations in the plasma of patients with colorectal tumors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 45, pp. 16368–16373, 2005.

[62] J. Y. Wang, J. S. Hsieh, M. Y. Chang et al., "Molecular detection of APC, K-ras, and p53 mutations in the serum of colorectal cancer patients as circulating biomarkers," *World Journal of Surgery*, vol. 28, no. 7, pp. 721–726, 2004.

[63] S. Ito, K. Hibi, H. Nakayama et al., "Detection of tumor DNA in serum of colorectal cancer patients," *Japanese Journal of Cancer Research*, vol. 93, no. 11, pp. 1266–1269, 2002.

[64] F. M. Selaru, S. David, S. J. Meltzer, and J. P. Hamilton, "Epigenetic events in gastrointestinal cancer," *The American Journal of Gastroenterology*, vol. 104, no. 8, pp. 1910–1912, 2009.

[65] M. Ehrlich, "DNA hypomethylation in cancer cells," *Epigenomics*, vol. 1, no. 2, pp. 239–259, 2009.

[66] W. P. Mu, J. Wang, Q. Niu, N. Shi, and H. F. Lian, "Clinical significance and association of RUNX3 hypermethylation frequency with colorectal cancer: a meta-analysis," *OncoTargets and Therapy*, vol. 7, pp. 1237–1245, 2014.

[67] Y. Wang, Y. Long, Y. Xu et al., "Prognostic and predictive value of CpG island methylator phenotype in patients with locally advanced nonmetastatic sporadic colorectal cancer," *Gastroenterology Research and Practice*, vol. 2014, Article ID 436985, 7 pages, 2014.

[68] A. Benard, E. C. Zeestraten, I. J. Goossens-Beumer et al., "DNA methylation of apoptosis genes in rectal cancer predicts patient survival and tumor recurrence," *Apoptosis*, vol. 19, no. 11, pp. 1581–1593, 2014.

[69] D. Deng, Z. Liu, and Y. Du, "Epigenetic alterations as cancer diagnostic, prognostic, and predictive biomarkers," *Advances in Genetics*, vol. 71, pp. 125–176, 2010.

[70] J. D. Warren, W. Xiong, A. M. Bunker et al., "Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer," *BMC Medicine*, vol. 9, p. 133, 2011.

[71] T. R. Church, M. Wandell, C. Lofton-Day et al., "Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer," *Gut*, vol. 63, no. 2, pp. 317–325, 2014.

[72] A. Herbst, K. Rahmig, P. Stieber et al., "Methylation of NEUROG1 in serum is a sensitive marker for the detection of early colorectal cancer," *The American Journal of Gastroenterology*, vol. 106, no. 6, pp. 1110–1118, 2011.

[73] H. Suzuki, E. Yamamoto, R. Maruyama, T. Niinuma, and M. Kai, "Biological significance of the CpG island methylator phenotype," *Biochemical and Biophysical Research Communications*, vol. 455, no. 1-2, pp. 35–42, 2014.

[74] S. H. Tan, H. Ida, Q. C. Lau et al., "Detection of promoter hypermethylation in serum samples of cancer patients by methylation-specific polymerase chain reaction for tumour suppressor genes including RUNX3," *Oncology Reports*, vol. 18, no. 5, pp. 1225–1230, 2007.

[75] A. Herbst, M. Wallner, K. Rahmig et al., "Methylation of helicase-like transcription factor in serum of patients with colorectal cancer is an independent predictor of disease recurrence," *European Journal of Gastroenterology & Hepatology*, vol. 21, no. 5, pp. 565–569, 2009.

[76] M. Wallner, A. Herbst, A. Behrens et al., "Methylation of serum DNA is an independent prognostic marker in colorectal cancer," *Clinical Cancer Research*, vol. 12, no. 24, pp. 7347–7352, 2006.

[77] M. S. Kim, X. Chang, K. Yamashita et al., "Aberrant promoter methylation and tumor suppressive activity of the DFNA5 gene in colorectal carcinoma," *Oncogene*, vol. 27, no. 25, pp. 3624–3634, 2008.

[78] D. A. Ahlquist, W. R. Taylor, D. W. Mahoney et al., "The stool DNA test is more accurate than the plasma septin 9 test in detecting colorectal neoplasia," *Clinical Gastroenterology and Hepatology*, vol. 10, no. 3, pp. 272–277.e1, 2012.

[79] J. A. Weber, D. H. Baxter, S. Zhang et al., "The microRNA spectrum in 12 body fluids," *Clinical Chemistry*, vol. 56, no. 11, pp. 1733–1741, 2010.

[80] D. H. Chang, J. R. Rutledge, A. A. Patel, B. G. Heerdt, L. H. Augenlicht, and R. J. Korst, "The effect of lung cancer on cytokine expression in peripheral blood mononuclear cells," *PLoS One*, vol. 8, no. 6, article e64456, 2013.

[81] M. S. Kopreski, F. A. Benko, L. W. Kwak, and C. D. Gocke, "Detection of tumor messenger RNA in the serum of patients with malignant melanoma," *Clinical Cancer Research*, vol. 5, no. 8, pp. 1961–1965, 1999.

[82] A. Tsouma, C. Aggeli, P. Lembessis et al., "Multiplex RT-PCR-based detections of CEA, CK20 and EGFR in colorectal cancer patients," *World Journal of Gastroenterology*, vol. 16, no. 47, pp. 5965–5974, 2010.

[83] D. Xu, X. F. Li, S. Zheng, and W. Z. Jiang, "Quantitative real-time RT-PCR detection for CEA, CK20 and CK19 mRNA in peripheral blood of colorectal cancer patients," *Journal of Zhejiang University Science. B*, vol. 7, no. 6, pp. 445–451, 2006.

[84] C. Shen, L. Hu, L. Xia, and Y. Li, "Quantitative real-time RT-PCR detection for survivin, CK20 and CEA in peripheral blood of colorectal cancer patients," *Japanese Journal of Clinical Oncology*, vol. 38, no. 11, pp. 770–776, 2008.

[85] G. A. Ganepola, J. Nizin, J. R. Rutledge, and D. H. Chang, "Use of blood-based biomarkers for early diagnosis and surveillance of colorectal cancer," *World Journal of Gastrointestinal Oncology*, vol. 6, no. 4, pp. 83–97, 2014.

[86] K. W. Marshall, S. Mohr, F. E. Khettabi et al., "A blood-based biomarker panel for stratifying current risk for colorectal cancer," *International Journal of Cancer*, vol. 126, no. 5, pp. 1177–1186, 2010.

[87] K. T. Yip, P. K. Das, D. Suria, C. R. Lim, G. H. Ng, and C. C. Liew, "A case-controlled validation study of a blood-based seven-gene biomarker panel for colorectal cancer in Malaysia," *Journal of Experimental & Clinical Cancer Research*, vol. 29, p. 128, 2010.

[88] M. A. Cortez, C. Bueso-Ramos, J. Ferdin, G. Lopez-Berestein, A. K. Sood, and G. A. Calin, "MicroRNAs in body fluids—the mix of hormones and biomarkers," *Nature Reviews Clinical Oncology*, vol. 8, no. 8, pp. 467–477, 2011.

[89] C. M. Croce, "Causes and consequences of microRNA dysregulation in cancer," *Nature Reviews Genetics*, vol. 10, no. 10, pp. 704–714, 2009.

[90] A. L. Kasinski and F. J. Slack, "MicroRNAs en route to the clinic: progress in validating and targeting microRNAs for cancer therapy," *Nature Reviews Cancer*, vol. 11, no. 12, pp. 849–864, 2011.

[91] F. J. Slack, "MicroRNAs regulate expression of oncogenes," *Clinical Chemistry*, vol. 59, no. 1, pp. 325-326, 2013.

[92] P. S. Mitchell, R. K. Parkin, E. M. Kroh et al., "Circulating microRNAs as stable blood-based markers for cancer detection," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 30, pp. 10513–10518, 2008.

[93] K. C. Vickers, B. T. Palmisano, B. M. Shoucri, R. D. Shamburek, and A. T. Remaley, "MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins," *Nature Cell Biology*, vol. 13, no. 4, pp. 423–433, 2011.

[94] F. Berger and M. F. Reiser, "Micro-RNAs as potential new molecular biomarkers in oncology: have they reached relevance for the clinical imaging sciences?," *Theranostics*, vol. 3, no. 12, pp. 943–952, 2013.

[95] Q. Wang, Z. Huang, S. Ni et al., "Plasma miR-601 and miR-760 are novel biomarkers for the early detection of colorectal cancer," *PLoS One*, vol. 7, no. 9, article e44398, 2012.

[96] M. D. Giraldez, J. J. Lozano, G. Ramírez et al., "Circulating microRNAs as biomarkers of colorectal cancer: results from a genome-wide profiling and validation study," *Clinical Gastroenterology and Hepatology*, vol. 11, no. 6, pp. 681–688.e3, 2013.

[97] Z. Kanaan, H. Roberts, M. R. Eichenberger et al., "A plasma microRNA panel for detection of colorectal adenomas," *Annals of Surgery*, vol. 258, no. 3, pp. 400–408, 2013.

[98] F. E. Ahmed, N. C. Amed, P. W. Vos et al., "Diagnostic microRNA markers to screen for sporadic human colon cancer in blood," *Cancer Genomics Proteomics*, vol. 9, no. 4, pp. 179–192, 2012.

[99] Z. Huang, D. Huang, S. Ni, Z. Peng, W. Sheng, and X. Du, "Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer," *International Journal of Cancer*, vol. 127, no. 1, pp. 118–126, 2010.

[100] G. H. Liu, Z. G. Zhou, R. Chen et al., "Serum miR-21 and miR-92a as biomarkers in the diagnosis and prognosis of colorectal cancer," *Tumour Biology*, vol. 34, no. 4, pp. 2175–2181, 2013.

[101] X. X. Pu, G. L. Huang, H. Q. Guo et al., "Circulating miR-221 directly amplified from plasma is a potential diagnostic and prognostic marker of colorectal cancer and is correlated with p53 expression," *Journal of Gastroenterology and Hepatology*, vol. 25, no. 10, pp. 1674–1680, 2010.

[102] K. Panzitt, M. M. Tschernatsch, C. Guelly et al., "Characterization of HULC, a novel gene with striking up-regulation in hepatocellular carcinoma, as noncoding RNA," *Gastroenterology*, vol. 132, no. 1, pp. 330–342, 2007.

[103] T. Arita, D. Ichikawa, H. Konishi et al., "Circulating long non-coding RNAs in plasma of patients with gastric cancer," *Anticancer Research*, vol. 33, no. 8, pp. 3185–3193, 2013.

[104] L. Dong, P. Qi, M. D. Xu et al., "Circulating CUDR, LSINCT-5 and PTENP1 long noncoding RNAs in sera distinguish patients with gastric cancer from healthy controls," *International Journal of Cancer*, vol. 137, no. 5, pp. 1128–1135, 2015.

[105] Y. S. Tong, X. W. Wang, X. L. Zhou et al., "Identification of the long non-coding RNA POU3F3 in plasma as a novel biomarker for diagnosis of esophageal squamous cell carcinoma," *Molecular Cancer*, vol. 14, p. 3, 2015.

[106] L. D. Graham, S. K. Pedersen, G. S. Brown et al., "Colorectal neoplasia differentially expressed (CRNDE), a novel gene

with elevated expression in colorectal adenomas and adenocarcinomas," *Genes & Cancer*, vol. 2, no. 8, pp. 829–840, 2011.

[107] W. Zhao, M. Song, J. Zhang, M. Kuerban, and H. Wang, "Combined identification of long non-coding RNA CCAT1 and HOTAIR in serum as an effective screening for colorectal carcinoma," *International Journal of Clinical and Experimental Pathology*, vol. 8, no. 11, pp. 14131–14140, 2015.

[108] M. Rutter, B. Saunders, K. Wilkinson et al., "Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis," *Gastroenterology*, vol. 126, no. 2, pp. 451–459, 2004.

[109] J. A. Eaden, K. R. Abrams, and J. F. Mayberry, "The risk of colorectal cancer in ulcerative colitis: a meta-analysis," *Gut*, vol. 48, no. 4, pp. 526–535, 2001.

[110] P. Prior, S. N. Gyde, J. C. Macartney, H. Thompson, J. A. Waterhouse, and R. N. Allan, "Cancer morbidity in ulcerative colitis," *Gut*, vol. 23, no. 6, pp. 490–497, 1982.

[111] R. H. Riddell, H. Goldman, D. F. Ransohoff et al., "Dysplasia in inflammatory bowel disease: standardized classification with provisional clinical applications," *Human Pathology*, vol. 14, no. 11, pp. 931–968, 1983.

[112] E. M. Ellis, "Reactive carbonyls and oxidative stress: potential for therapeutic intervention," *Pharmacology & Therapeutics*, vol. 115, no. 1, pp. 13–24, 2007.

[113] A. Keshavarzian, S. Sedghi, J. Kanofsky et al., "Excessive production of reactive oxygen metabolites by inflamed colon: analysis by chemiluminescence probe," *Gastroenterology*, vol. 103, no. 1, pp. 177–185, 1992.

[114] A. Keshavarzian, A. Banan, A. Farhadi et al., "Increases in free radicals and cytoskeletal protein oxidation and nitration in the colon of patients with inflammatory bowel disease," *Gut*, vol. 52, no. 5, pp. 720–728, 2003.

[115] A. Roessner, D. Kuester, P. Malfertheiner, and R. Schneider-Stock, "Oxidative stress in ulcerative colitis-associated carcinogenesis," *Pathology, Research and Practice*, vol. 204, no. 7, pp. 511–524, 2008.

[116] A. Bressenot, V. Cahn, S. Danese, and L. Peyrin-Biroulet, "Microscopic features of colorectal neoplasia in inflammatory bowel diseases," *World Journal of Gastroenterology*, vol. 20, no. 12, pp. 3164–3172, 2014.

[117] T. A. Brentnall, D. A. Crispin, P. S. Rabinovitch et al., "Mutations in the p53 gene: an early marker of neoplastic progression in ulcerative colitis," *Gastroenterology*, vol. 107, no. 2, pp. 369–378, 1994.

[118] T. A. Brentnall, D. A. Crispin, M. P. Bronner et al., "Microsatellite instability in non-neoplastic mucosa from patients with chronic ulcerative colitis," *Cancer Research*, vol. 56, no. 6, pp. 1237–1240, 1996.

[119] R. Chen, P. S. Rabinovitch, D. A. Crispin et al., "DNA fingerprinting abnormalities can distinguish ulcerative colitis patients with dysplasia and cancer from those who are dysplasia/cancer-free," *The American Journal of Pathology*, vol. 162, no. 2, pp. 665–672, 2003.

[120] R. Chen, P. S. Rabinovitch, D. A. Crispin, M. J. Emond, M. P. Bronner, and T. A. Brentnall, "The initiation of colon cancer in a chronic inflammatory setting," *Carcinogenesis*, vol. 26, no. 9, pp. 1513–9, 2005.

[121] R. Chen, M. P. Bronner, D. A. Crispin, P. S. Rabinovitch, and T. A. Brentnall, "Characterization of genomic instability in ulcerative colitis neoplasia leads to discovery of putative tumor suppressor regions," *Cancer Genetics and Cytogenetics*, vol. 162, no. 2, pp. 99–106, 2005.

[122] L. A. Lai, R. A. Risques, M. P. Bronner et al., "Pan-colonic field defects are detected by CGH in the colons of UC patients with dysplasia/cancer," *Cancer Letters*, vol. 320, no. 2, pp. 180–188, 2012.

[123] J. N. O'Sullivan, M. P. Bronner, T. A. Brentnall et al., "Chromosomal instability in ulcerative colitis is related to telomere shortening," *Nature Genetics*, vol. 32, no. 2, pp. 280–284, 2002.

[124] P. S. Rabinovitch, S. Dziadon, T. A. Brentnall et al., "Pancolonic chromosomal instability precedes dysplasia and cancer in ulcerative colitis," *Cancer Research*, vol. 59, no. 20, pp. 5148–5153, 1999.

[125] R. A. Risques, L. A. Lai, C. Himmetoglu et al., "Ulcerative colitis-associated colorectal cancer arises in a field of short telomeres, senescence, and inflammation," *Cancer Research*, vol. 71, no. 5, pp. 1669–1679, 2011.

[126] S. H. Itzkowitz, "Molecular biology of dysplasia and cancer in inflammatory bowel disease," *Gastroenterology Clinics of North America*, vol. 35, no. 3, pp. 553–571, 2006.

[127] P. M. O'Connor, T. K. Lapointe, P. L. Beck, and A. G. Buret, "Mechanisms by which inflammation may increase intestinal cancer risk in inflammatory bowel disease," *Inflammatory Bowel Diseases*, vol. 16, no. 8, pp. 1411–1420, 2010.

[128] S. H. Itzkowitz and X. Yio, "Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation," *American Journal of Physiology Gastrointestinal and Liver Physiology*, vol. 287, no. 1, pp. G7–17, 2004.

[129] J. Terzic, S. Grivennikov, E. Karin, and M. Karin, "Inflammation and colon cancer," *Gastroenterology*, vol. 138, no. 6, pp. 2101–2114.e5, 2010.

[130] J. B. Kisiel, M. M. Garrity-Park, W. R. Taylor, T. C. Smyrk, and D. A. Ahlquist, "Methylated eyes absent 4 (EYA4) gene promotor in non-neoplastic mucosa of ulcerative colitis patients with colorectal cancer: evidence for a field effect," *Inflammatory Bowel Diseases*, vol. 19, no. 10, pp. 2079–2083, 2013.

[131] T. A. Brentnall, S. Pan, M. P. Bronner et al., "Proteins that underlie neoplastic progression of ulcerative colitis," *Proteomics Clinical Applications*, vol. 3, no. 11, p. 1326, 2009.

[132] D. May, S. Pan, D. A. Crispin et al., "Investigating neoplastic progression of ulcerative colitis with label-free comparative proteomics," *Journal of Proteome Research*, vol. 10, no. 1, pp. 200–209, 2011.

[133] J. J. Salk, S. J. Salipante, R. A. Risques et al., "Clonal expansions in ulcerative colitis identify patients with neoplasia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 49, pp. 20871–20876, 2009.

[134] S. Yoshizawa, K. Matsuoka, N. Inoue et al., "Clinical significance of serum p53 antibodies in patients with ulcerative colitis and its carcinogenesis," *Inflammatory Bowel Diseases*, vol. 13, no. 7, pp. 865–873, 2007.

[135] A. Paraskevi, G. Theodoropoulos, I. Papaconstantinou, G. Mantzaris, N. Nikiteas, and M. Gazouli, "Circulating microRNA in inflammatory bowel disease," *Journal of Crohn's & Colitis*, vol. 6, no. 9, pp. 900–904, 2012.

[136] F. Wu, N. J. Guo, H. Tian et al., "Peripheral blood microRNAs distinguish active ulcerative colitis and Crohn's disease," *Inflammatory Bowel Diseases*, vol. 17, no. 1, pp. 241–250, 2011.

[137] R. Duttagupta, S. DiRienzo, R. Jiang et al., "Genome-wide maps of circulating miRNA biomarkers for ulcerative colitis," *PLoS One*, vol. 7, no. 2, article e31241, 2012.