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RESEARCH ARTICLE

A REVIEW REPORT ON BIOMARKERS FOR DIAGNOSIS OF COLORECTAL CANCER

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ABSTRACT

Colorectal cancer (CRC) is the most common gastric intestinal malignancies and remain a prime death causing cancer worldwide. Although recent advancements in surgical and multimodal treatments, overall survival rates for advanced CRC patients remain poor. Molecular and metabolic processes that have been shown to contribute to phenotypic alterations favoring cancer include: gene mutation, angiogenesis, development of benign lesion, enhanced carcinoma proliferation. These occurrences may have a role in the onset and progression of cancer (carcinogenesis). A biomarker is a chemical that may be detected in blood, stool and tissue samples to allow pathological diseases like cancer to be identified. As a result, finding accurate and useful molecular biomarkers to help in the diagnosis and treatment of CRC would be advantageous. This review is based on the available information from published research papers related to different biomarkers that are used in the diagnosis of colorectal cancer also take a quick glimpse into the future.

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INTRODUCTION

Colorectal cancer (CRC) is the third leading cause of cancer death worldwide, with 2.2 mil- lion new cases and 1.1 million deaths expected in the next ten year (65). It affects 746,000 males (or 10% of all cancer cases) and 614,000 women (or 9.2% of all cancer cases) (21). The majorities of colorectal cancers are sporadic and are defined by a sequenced carcinogenesis process that entails the gradual accumulation of mutations over a 10 to 15 year period (6). This lengthy evolution interval allows for effective screening, early cancer identification, and excision of premalignant lesions (adenomas), resulting in lower incidence and death (60). Despite the possibility of early detection, 20–25% of CRC cases are identified at stage IV, when patients have already developed distant metastases and have a 5-year survival probability of just 10%. Regardless of all advancements in detection and treatment, CRC continues to be a serious public health problem globally, particularly in emerging nations with westernized lifestyle and an ageing population (5). The majority of CRC cases start with adenomatous polyps, which are precursor lesions (37).

Biomarkers are commonly believed to enhance the accuracy of CRC diagnosis. There are several biomarkers for CRC diagnosis. Changes in biomarker expression levels might be used to help diagnose CRC. Some biomarkers, such as TP53 and CEA, are now being utilized in clinical trials as diagnostic aids. Scientists are still looking for the "perfect" biomarker to achieve the highest level of accuracy in CRC diagnosis. Risk assessment might potentially be aided by genetic changes at the molecular level. The quest for risk variables that offer the first indication of cancer risk in those who haven't been diagnosed with the illness is known as risk assessment. Biomarkers that indicate risk generally result in more aggressive treatments and monitoring. Individuals who test positive for any risk marker become intervention or surveillance candidates. Inherited genetic abnormalities are most likely the first risk factors, as proven in the case of CRC. A good CRC biomarker should be simple to test quantitatively, highly specific and sensitive, dependable, and repeatable. It should also be able to distinguish between various risk groups and identify individuals who require a follow-up examination (endoscopic and radiologic investigations). These objectives should ideally be accomplished via a noninvasive and low-cost

approach based on readily available biological samples such as urine, breath, serum, or faeces (54). This review seeks to give an overview of recent advancements in the quest for CRC biomarkers that might be used in clinical settings to identify the disease.

Diagnostic Biomarkers

Blood Biomarkers

Carinoembryonic antigen (CEA): CRC-specific antigens in blood have been identified after extensive investigation. CEA and carbohydrate antigen 19-9 (CA19-9) are the only blood-based biomarkers available to monitor CRC patients. CEA is a glycoprotein with a high molecular weight that is present in embryonic tissue and colorectal cancers. It was developed in 1965 and is now the only tumour marker that may be used to track CRC recurrence. CEA levels over a certain threshold are thought to be a poor prognostic indicator for resectable CRC and are linked to cancer development (47). The sensitivity of this marker rises with tumour stage (34). Because high levels may be found in certain patients or in advanced illness, CEA for CRC has not been included as a viable screening tool. CA 19-9, on the other hand, is more selective and less sensitive in CRC, with an emphasis on pancreatic cancers (47). CEA, CA 19-9, CA 72-4, CA 125, and serum ferritin were compared to preoperative levels as well as pathological parameters in 279 CRC diagnosed patients in a recent research (34), and it was shown that utilizing all four markers combined is more effective than using just one. This combination might also be utilized to predict vascular invasion, tumour differentiation, and pTNM staging in CRC, as well as lymph node spread and brain invasion. Tissue polypeptide-specific antigen (TPS) and tissue polypeptide antigen (TPA), which are correlated with cytokeratins 8, 18, and 19 and in combination with CEA have a higher sensitivity in CRC patients' recurrence disease (64).

Circulating DNA: Circulating cell-free DNA (cfDNA) is a kind of cell-free nucleic acid that enters the circulation by apoptosis or necrosis in normal and malignant cells (36). The lengths of cfDNA strands vary depending on how they are produced. The cfDNA is released from apoptotic cells in healthy people, and the DNA fragments are roughly 180 bp length. Necrosis, on the other hand, releases cfDNA as much bigger pieces in tumour cells (36). As a consequence, quantitative examination of circulating cfDNAs using the ratio of longer to shorter DNA fragments, or assessing cfDNA integrity number during CRC diagnosis, yielded encouraging findings. The sensitivity and specificity of the qualitative PCR technique for determining cfDNA integrity were 73.08% and 97.27%, respectively (28). Later reported that cfDNA integrity was evaluated using an RT-PCR reaction with a sensitivity of 90% and specificity of 85% (20). Healthy people and CRC patients have different levels of cfDNA. CRC patients had serum cfDNA concentrations that were five times higher and plasma cfDNA concentrations that were 25 to 50 times higher than healthy controls (54). When released from apoptotic tumour cells, cfDNA fragments range in length from 185 to 200 bp, prompting the development of a novel potential biomarker based on the tiny fragments of cfDNA. A recent study of 4,105 individuals who had colonoscopy for varied reasons corroborated this. When comparing patients with colon cancers to native subjects, blood samples were taken and cell-free circulating nucleosomes containing a variety of epigenetic signals were examined, resulting in promising prediction models for CRC early detection (55) (58).

Genetic Alterations in cfDNA: The APC gene was the subject of early studies on point mutations in cfDNA (18). It was discovered that 8% of APC gene segments are altered, and that cfDNA detection was sensitive enough to detect remaining illness after surgical resection (18). Serum detection rates for the genes APC, KRAS, and TP53 were 30.4%, 34.0%, and 34.2%, respectively, according to another research (68).

APC: Adenomatous polyposis coli (APC) is a suppressor gene discovered in familial adenomatous polyposis (FAP) via genetic linkage research. Most spontaneous CRCs are also caused by mutated APC (41). APC inhibits the WNT signaling pathway and controls a variety of cell functions including motility and adhesion, transcriptional activation, and apoptosis (56). APC depletion is seen in 70% to 80% of individuals with CRC(38). A meta- analysis was conducted to look at the links between three APC polymorphisms (D1822V, E1317Q, and I1307K) with the risk of CRC (44). The findings revealed a weak link between E1317Q and the risk of CRC, particularly in adenomas. I1307K-variant carriers in Ashkenazi Jews had a substantially higher risk of CRC than I1307K wild-type carriers. There was no indication of heterogeneity across studies in this meta-analysis; however, all of the included investigations were case-control studies with a high risk of recall and selectionbias (44).

KRAS: The mutational status of the genes KRAS deserves extra attention in CRC because they might significantly influence the patient's response to anti-EGFR (Epidermal growth factor receptor) treatments, with somatic KRAS mutations accounting for 40% of cases. These mutations may be detected using a variety of molecular assays, with limits of detection ranging from 10–20% mutant allele for Sanger sequencing, approximately 5% for pyrosequencing and high-resolution melt (HRM) curve analysis, to 1–5% for qPCR assays. While direct sequencing remains the gold standard for detecting KRAS mutations, it is not widely used due to its inconvenient nature and lack of sensitivity (57).

Long noncoding RNAs: Long noncoding RNAs (lncRNAs), which are made up of more than 200 nucleotides but cannot be translated into protein, have been linked to epigenetic control, immunological responses, differentiation, and chromosomal dynamics (46). More than 150 human diseases have been linked to lncRNAs thus far, including colon cancer, breast cancer, leukemia, and psoriasis (12). Single or panel lncRNAs have been used in studies to test their diagnostic potential for CRC. The expression of circulating lncRNAs as a possible noninvasive diagnostic biomarker in CRC has received very little attention. Colorectal neoplasia differentially expressed-h (CRNDE-h) transcript expression was substantially increased in one study compared to healthy controls, with a sensitivity of 87% and specificity of 93% for diagnosing CRC (27). The combination of Combinatorial Code Analysis Tool (CCAT) and HOX transcript antisense RNA (HOTAIR) was examined in the other study. When compared to healthy controls, both lncRNAs were shown to be substantially elevated in CRC patients' plasma. With a sensitivity of 84.3% and specificity of 80.2%, the combination was shown to be more diagnostically useful. Furthermore, this combination proved efficient in detecting CRC at an early stage 4 (85%) (76). Serum hypoxia-inducible factor (HIF) 1alpha-antisense RNA 1 (HIF1A-AS1) was substantially

higher in 151 CRC patients compared to 160 healthy controls, and had a strong diagnostic capacity for CRC, with an AUC of 0.960 (95% CI, 0.940–0.980; P 0.001). Patients with high HIF1A-AS1 expression had a worse 5 years survival rate than those with low expression, suggesting that HIF1A-AS1 might be utilized as a diagnostic and prognostic biomarker for CRC (25).

Microsatellite: Microsatellites are DNA sequences that are repeated every 1–6 bp in coding and noncoding regions. Microsatellite instability (MSI) is the loss or insertion of microsatellite units, which results in cancer-related changes. MSI is seen in 15% of CRCs and is linked to DNA mismatch repair gene abnormalities. MSI has been linked to hereditary nonpolyposis CRC, despite the fact that most tumours with a high MSI level are sporadic (11). Most clinical laboratories now employ a panel of five mononucleotide markers (Bat-25, Bat- 26, NR-21, NR-24, and MONO-27) to identify MSI. MSI (also known as MSI-high) is defined as having more than 30% of unstable loci in a panel of mononucleotide and dinucleotide markers; MSI-low tumours have 10–29% of unstable loci in the panel. The absence of MMR protein expression in tumour tissue is frequently utilized as a surrogate test for MSI. Sporadic MSI CRC tumours are mainly seen in the proximal colon, show mucinous or signet ring histology, are poorly differentiated, have a high number of tumour infiltrating lymphocytes, and contain BRAF mutations (26).

BRAF: The immediate downstream effector of KRAS in the Ras/Raf/MAPK signaling pathway is BRAF, a serine/threonine kinase from the RAF gene family. BRAF gene mutations have been linked to the formation of CRC, and they are seen in 40–50% of sporadic MSI-high CRC (26). BRAF mutation status is a highly effective diagnostic tool for distinguishing between familial and sporadic CRC since they are missing in Lynch syndrome patients. The most frequent mutation found (V600E) is a missense mutation that causes a valine to glutamic acid change. In colorectal cancers, mutations in KRAS and BRAF are usually mutually exclusive (22).

Proteins: While CEA and CA19-9 have little diagnostic potential in CRC, other proteins have been investigated. Recently, 43 proteins were examined for their ability to differentiate between CRC patients and healthy people, with some encouraging results. MAPKAPK3 and ACVR2B showed 83.3% sensitivity and 73.9% specificity, making them the most reliable biomarkers to date. TIMP-1 was also evaluated as a single marker protein with a sensitivity of 42- 65% and a specificity of 95% (32)(7). Proteomic study of structural proteins found three more colon-specific antigens, CCSA-2, CCSA-3, and CCSA-4. As CRC diagnostic biomarkers, these proteins have shown to be extremely promising. The matrix metalloproteinase 9, S100A8, and S100A9 (26) have also been studied as potential diagnostic indicators. When compared to uninvolved epithelium, cyclin D1 protein expression was shown to be higher in 30–45% of CRCs in many investigations. In addition, cyclin D1 protein expression was found to be elevated in 34% of adenomatous polyps. Immunological tests are used to assess cytokine D1 levels in the blood(61).

TP53: As p53 mutations are the most often seen mutations in many forms of cancer, the tumour protein (TP)53 gene is the most extensively utilized tumour biomarker in diagnosing a possible tumour. Furthermore, most anti-p53 autoantibodies are generated in responseto a p53 mutation (43).

The p53 gene produces a protein product called p53 nuclear phosphoprotein in healthy cells. Anti-p53 antibodies, in contrast to p53 protein, are seldom seen in healthy people's blood (73). Due of the enhanced stability and half-life (several hours) of mutated p53 as compared to wild type p53 (20 minutes), nonfunctional protein accumulates (73).

RNAs in circulation: MicroRNAs (miRNAs) are tiny noncoding RNAs that control gene expression by binding to mRNA. They are 18–25 bp length. Many malignancies, including CRC, are thought to be linked to miRNAs, which function as oncogenes or tumour suppressor genes (TSGs)(50). MiRNAs have greater blood stability than mRNA because they are not degraded by endogenous RNase and are resistant to severe pH fluctuations. There is mounting evidence that alterations in miRNAs are linked to carcinogenesis and tumour progression (17). As a result, miRNAs might be useful biomarkers for early cancer diagnosis and therapeutic response prediction (65). Single or panels of miRNA have been used in studies to test their diagnostic potential for CRC. In one research a 69-gene miRNA signature panel in plasma was shown to be able to distinguish between CRC and healthy individuals (13). A panel of eight miRNAs (miR-532-3p, miR-331, miR-331, miR195, miR-17, miR142-3p, miR15b, miR532, and miR-652) was shown to reliably identify polyps in a small research (39).Another study found that a three-miRNA panel (miRNA 193a-3p, miR23a, and miR-338-5p) has 80 percent sensitivity, 84.4 percent specificity, and 83.3 percent accuracy in detecting CRC (74).

MiRNA isolated from CRC patients' serum or plasma is profiled first, and then verified using Real-time polymerase chain reaction (RT-qPCR). MiR-601 and miR-760 were validated on 90 CRC samples, 43 advanced adenoma (AA) samples, and 58 healthy controls after profiling of 742 miRNA on CRC samples and healthy controls (Table 1). With a sensitivity of 83.3% and a specificity of 69.1%, both miRNAs exhibited decreased expression in CRC and AA samples compared to healthy controls (70). Another study profiled 743 miRNA in CRC, AA, and healthy control samples, and then validated the results using 42 CRC, 40 AA, and 53 healthy control samples. With a sensitivity of 78.6% and specificity of 79.3%, a panel of six miRNA (miR-15b, miR-18a, miR-19a, miR-19b, miR-29a, and miR-335) was effectively differentiated between CRC samples and healthy controls. Furthermore, miR-18a had a sensitivity of 80% and a specificity of 80% in distinguishing between AA samples and healthy controls (24). Following the profiling of 380 miRNAs, another group proposed an study of eight-miRNA panel (miR-15b, miR-17, miR-142-3p, miR-195, miR-331, miR-532-5p, miR-532-3p, and miR-652) that was conformed on a cohort of 45 CRC, 16 AA, and 26 healthy individuals, and found to separate between AA and controls with a sensitivity of 88% and specificity of 64%. Different groups of miRNA with their sensitivity and specificity is given in table 1. The same group suggested a three panel miRNA (miR-431, miR-15b, and miR-139-3p) that has a sensitivity of 93% and a specificity of 74% in distinguishing between stage IV CRC and control samples (39). With a sensitivity of 90% and specificity of 95%, validated 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 up regulated and six (miR-124, miR-127-3p, miR-138, miR-143, miR-146a, and miR-222) (1)(23).

Table 1. Different groups of miRNA with their sensitivity and specificity

miRNA	Sensitivity	Specificity
miR-601 ↓, miR-760 ↓	83.3%	69.1%
miR-15b ↑, miR-18a ↑, miR-19a ↑, miR-19b ↑, miR-29a ↑, miR-335 ↑	78.6%	79.3%
miR-15b ↑, miR-17 ↑, miR-142-3p ↑, miR-195 ↑, miR-331 ↑, miR-532-5p ↑, miR-532-3p ↑, miR-652 ↑	88%	64%
miR-431 ↑, miR-15b ↑, miR-139-3p ↓	93%	74%
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%
miR-29a ↑, miR-92a ↑	83%	84.7%
miR-21 ↑, miR-92a ↑	68%	91.2%
miR-221 ↑	86%	41%

Stool biomarkers

DNA test: There has been a lot of research towards detecting CRC-specific DNA markers in faeces. Because these indicators are generated directly from tumour cells, they should have a better specificity. Human DNA accounts for less than 0.01% of total DNA in the faeces, with the remaining 99.99% coming from gut microbes or the food. As a result, identifying methylation or altered human DNA in the faeces is an essential diagnostic method for CRC (19). Recent study showed that a multitarget stool DNA test has greater sensitivity for the identification of advanced adenomas (42.4%) and CRC (92.3%) than FITs in a large trial with 9989 individuals (35). The screening capability of a multitarget stool test that identified KRAS mutations, methylation of NDRG4 and BMP3, measurement of actin as a reference gene for DNA amount, and immunochemical detection of haemoglobin was investigated in this cross-sectional research. Although this panel found more false positives than FITs, the screening utility of this test is promising since it detected polyps, including serrated adenomas, with high-grade dysplasia at a greater rate than FITs. Serrated adenomas are precursor lesions to serrated adenocarcinomas, a kind of colorectal cancer that makes up around 10% of all CRCs. BRAF mutations, MLH1 methylation, and CIMP are all linked to serrated adenocarcinomas (49).

Immunochemical Test: Using globin-specific antibodies, the Fecal Immunochemical Test (FIT) identifies human haemoglobin (2). When compared to guaiac based fecal occult blood test (gFOBT), FIT offers a few advantages, including the fact that it does not need any dietary restrictions and only takes one stool sample instead of three. Furthermore, it allows for both qualitative and quantitative findings to be obtained. FIT has a greater adherence rate and enables for improved identification of advanced adenomas (31), with sensitivity for CRC ranging from 69-100% and a specificity of 92-96% (64). The results on sensitivity and sensibility are contradictory. According to a research con- ducted, CRC detection accuracy was 95%, with a 79% sensitivity and a 94% specificity (42). A meta-analysis using colonoscopy as the reference diagnostic technique, on the other hand, found that FIT's CRC sensitivity and specificity are between 71% and 94% (52). According to current research, FIT sensitivity varies depending on the location of the tumour. As a result, FIT is more sensitive to lesions in the left colon than the right (64). In addition, habitual aspirin users had greater sensitivity rates than nonusers (14). Because globin is gradually destroyed as it travels through the gut, the FIT is unique to bleeding from the distal gastrointestinal tract. There are two types of FITs: a qualitative assay that requires visual interpretation and a quantitative test that analyses the sample automatically and calculates the quantity of haemoglobin present. Quantitative FITs, such as the OC-Sensa Micro (Eiken Chemical, Tokyo, Japan; sold in the US by Polymedco, Cort-land Manor, NY) and Insure (Enterix Inc., Quest Diagnostics Incorporated, Edison, NJ), have an advantage over qualitative assays because they eliminate observer variations in the interpretation of the results and have definitive cut-off levels, which improve reproducibility.

Microbiome: Few scientists have published new statistics in this edition of the journal thatare extremely important and give significant insight into the current state of colorectal cancer screening (75). Advanced studies of the human faecal microbiota, which emerge as adenoma progresses to colon carcinoma, have been shown to enhance colorectal cancer screening techniques, according to the scientists. The bacterial 16S rRNA gene was sequenced using the Illumina MiSeq sequencing technology, enabling for phylogenetic comparison and measurement of bacterial diversity. Importantly, the groups were examined separately, resulting in the discovery of bacterial operational taxonomic units that were enriched or reduced in healthy Vs adenoma clinical samples, healthy versus carcinoma clinical samples, and adenoma against carcinoma clinical samples. This showed that microbiome-based studies may detect the existence of precancerous and cancerous tumours.

Proteins: The specificity of faecal CRC diagnosis might be substantially improved by a new technique based on the detection of tumor-derived proteins. Although the majority of the protein markers examined thus far have been found in blood samples, proteins in faeces such as calprotectin and M2 pyruvate kinase among others have also been studied for diagnostic potential. Calprotectin, a calcium-binding protein found in granulocytes, macrophages, and epithelial cells, is found in granulocytes, macrophages, and epithelial cells. Calprotectin is a non- cancer protein marker whose level rises during intestinal inflammation and has been linked to inflammatory bowel disorders. As a result, it performs poorly, with reduced sensitivity (67% vs. 75%) and specificity (76% vs. 90%) for both CRC and precancerous lesions, as demonstrated in a Norwegian CRC screening experiment comprising 2,321 asymptomatic individuals (30). Studies on Fecal tumour M2 pyruvate kinase (M2-PK) have yielded mixed findings in terms of sensitivity in CRC, with results ranging from 68% to 85% for a threshold value of 4U/mL (64). A Chinese group is investigating the potential of integrating multiple protein markers in a biochip to identify CRC because no protein stool marker has shown to be completely accurate for CRC screening (67).

RNA test: The detection of RNA biomarkers in faeces has not been investigated as thoroughly as DNA biomarkers, owing to the fact that RNA is less stable in stool than DNA. The use of CRC tumor-specific RNA transcripts as stool biomarkers is now possible because to technological improvements in RNA preservation buffers. Single and multiple tumour mRNA transcripts, such as PTGS2 and MMP7, have been found to exhibit excellent specificity for CRC (63).

Despite the lack of data on miRNA expression in the stool compared to that in the blood, dysregulation of miRNA expression was found in the stool of CRC patients; miR-92a, miR- 21, miR144, miR-106a, miR17-92 cluster, and miR135 were up-regulated in CRC, while miR-143 and miR-145 were down-regulated in CRC (54). However, none of the miRNAs had enough predictive value to be used as a standalone CRC diagnostic test, and more research is needed to enhance miRNA diagnostic value by integrating several miRNAs (45).

Tissue biomarker

***β*-catenin:** *β*-catenin is a multifunctional protein that plays a role in cell adhesion as well as intracellular signaling, the latter of which is facilitated by *β*-activities catenin's via the Wnt signaling pathway (43)(72). The Wnt signaling pathway is activated, which increases the cytoplasmic pool of free *β*-catenin and, to a lesser degree, the nuclear pool, where it causes proliferation. As a result of mutant adenomatous polyposis coli (APC) or *β*-catenin, the Wnt signaling pathway is highly active in the majority of CRCs. Furthermore, increased Wnt signaling plays an important role in the development of CRC (69). Nuclear *β*-catenin may be detected immunohistochemically in the lack of functional APC, which is common in CRC (69)(16). Although *β*-catenin nuclear expression is not specific to CRC, it has been shown to be helpful as part of a diagnostic panel.

Cadherin 17 (CDH17): The rat liver and intestine were the first places where CDH17 was discovered. Later human studies found that it is only expressed in the small and large intestines, as well as a portion of the pancreatic duct. It's a peptide transporter for the intestine. However, only lately has its clinical use in the diagnosis of gastrointestinal cancers been identified (53). CDH17 has been suggested as a useful immunohistochemical marker for the identification of GI adenocarcinomas in a number of recent investigations. CDH17 is reported to be expressed in 96% to 100% of primary CRC and 100% of metastatic CRC (54). Other GI cancers, such as gastric, pancreatic, and biliary cancer, express CDH17, although it is seldom detected beyond the GI tract (10).

Caudal type homeobox 2 (CDX2): CDX2 is a homeobox protein involved in the control of normal cell development in the GI tract as well as tumour suppression in the colon. CRC may be caused by the loss of CDX2 expression (71). The epithelia of the normal small intestine, appendix, colon, and rectum, as well as the pancreatic centroacinar and intera acinar ductal cells, all express CDX2 (51). CDX2 deficiency has been linked to the development of human CRC. Aside from those with MSI, CRCs are always CDX2-positive (71). Indeed, a recent study in nude mice looked at the impact of restoring CDX2 expression on colon cancer cell survival, colony formation, cell cycle distribution, apoptosis, invasion ability, and xenograft tumour development (77).

Cytokeratins (CKs): CKs, like vimentin, desmin, neurofilament, and glial-filament, are members of the intermediate filaments family, which also includes vimentin, desmin, neurofilament, and glial-filament. When a physician wants to distinguish metastases from CRC, which are generally CK7-/CK20+, from other cancers, CK7 and CK20 come in handy(9). CK20 stains the normal gland cells and Merkel cells of the colonic mucosa nearly exclusively, while its expression is seldom detected in the urothelium or other mucosa (59). CK7, on the other hand, is commonly found in epithelia of the urine bladder and female genital tract, mesothelium, normal lung, and, on rare occasions, stomach and intestine normal glands. The majority of studies, however, believe that it does not exist in normal colonic mucosa (59). For detecting metastatic adenocarcinoma of uncertain original origin, CK staining patterns are one of the most useful methods. The CK7–/CK20+ pattern is a common technique for diagnosing metastatic CRC (8). When studying CRC development, CK20 and CK7 might be helpful. According to the find- ings, advanced CRCs were more likely to be CK20+/CK7+ than early-stage malignancies, which were primarily CK20+/CK7. As a result, CK7 expression may serve as a distinguishing factor in the development of CRC (29).

Mucins: Mucins are glycosylated proteins produced and expressed by many different organs, including the colon (4). Mucins can be divided into three categories:

Membrane-bound/transmembrane mucins, secreted (gel-forming) mucins, and soluble (non-gel-forming) mucins are the three types of mucins(4). Mucins protect epithelial cells from infections by trapping them on their surfaces (40). They have a role in cell signaling as well (40). As a result, they play a crucial role in cellular activities, particularly near the epithelial cell surface. MUC2 is the most widely released mucin in the intestines, and mice lacking the MUC2 gene (Muc2-/-) develop colon cancer on their own. Furthermore, MUC2 is down regulated in human CRC tissues, indicating that it has a tumour suppressor role. As a result, an increase in MUC2 can prevent and/or improve the prognosis of CRC. In turn, higher levels of MUC5AC and MUC6 expression are linked to a better prognosis in CRC patients. MUC3 expression has been found in both healthy and cancerous colons. In 84% of the instances, MUC3 was shown to be up regulated in clinical CRC samples. Among them, cytoplasmic and membrane localization were discovered in 91% and 38% of the instances, respectively(3).

MUC2, MUC5AC, MUC5B, MUC6, and MUC19, which code for secreted mucins, are all grouped on chromosome 11 and are apparently co-expressed. Furthermore, new research has looked at the expression of secreted mucins on chromosome 11 in a significant number of CRC cases, revealing that MUC5AC, MUC5B, and MUC6 over expression is linked to serrated forms of colonic glandular neoplasia. DNA hypermethylation, MSI, and BRAF somatic mutations are all linked to serrated CRC. Mucin genes also include a number of transcription factor sites, including Sp1, SP3, AP-1, NF-B, and CDX2 (3). The expression of MUC2, MUC5AC, and MUC6 is up regulated when this transcription factor is down regulated. Furthermore, Vincent et al investigated the epigenetic control of genes encoding mucins in chromosome 11p15 in different epithelial cancer cell lines, including esophageal, pancreatic, gastric, and colon cancer cell lines (66). They discovered that epigenetic alterations mostly influence MUC2 and MUCB genes, with MUC2 expression regulated by the repressive histone code and MUC5B expression controlled by methylation at CpG sites.

Special AT-rich sequence binding protein 2 (SATB2): SATB2 belongs to the nuclear matrix-associated transcription factors family, which functions as tissue-specific epigenetic regulators of gene regulation (53). Tumours used from nine cohorts (n = 1882) of patients with primary and metastatic CRCs to investigate the particular expression pattern of SATB2

in connection to a well-known biomarker of CRC, cytokeratin 20 (CK20). The findings revealed that 85% of the CRCs tested positive for SATB2, and 97% tested positive for both SATB2 and CK20(48). Increases in SATB2 caused by metals are one of the potential dangers. SATB2 expression is continuously increased in cells that have been exposed to carcinogenic metals such as nickel, arsenic, and chromium (VI)(15), suggesting that SATB2 induction is involved in metal carcinogenesis. When utilizing SATB2 to diagnose CRC, up regulation of SATB2 in metal- induced malignancies from organs other than the lower GI tract might lead to false-positive findings. Arsenic, for example, increased SATB2 expression in human bronchial epithelial cells (15). Because arsenic enhanced SATB2 expression in human bronchial epithelial cells (15) and as arsenic exposure induces lung cancer (33), arsenic-induced lung tumours are likely to have elevated SATB2 expression as well. In order to consider metal-induced malignancies when diagnosing CRC based on SATB2, studies should be undertaken to assess SATB2 expression in tumours from cohorts with occupational or high exposure to nickel, arsenic, and chromium (VI). Other CRC biomarkers that are not known to be influenced by metals, such as cadherin-17, should be utilized with SATB2; decreased MLH1 would not be a possibility because it is likewise suppressed by chromium (VI) exposure (62).

DISCUSSION

As of the huge number of biomarkers available in CRC, appropriate biomarker utilization is also essential. CRC carcinogenesis is triggered by a variety of reasons, both genetic and environmental, but the disease is often detected using genetic material obtained from different sources. The information gathered from various researchers led to the discussion of genetic factors. cfDNA shows greater sensitivity and selectivity, which can also be detected in the form of APC, various cancers involve lncRNAs, MSI is linked to around 15% of CRC, BRAF and KRAS go hand in hand and show greater detection, which is also linked to MSI and miRNA. To summarize, certain proteins and genes exhibit a higher level of detection than others, such as the CDH-17 and CDX2 genes, and the protein TP53.

CONCLUSION

CRC is a prevalent cancer that accounts for a considerable portion of cancer-related deaths. Because of the intricacy of colorectal carcinogenesis, survival outcomes differ from patient to patient. Thanks to break through in the study of genetics and a better knowledge of the process of carcinogenesis, the approach to patients with colorectal cancer has lately been drastically modified. The assessment of KRAS, BRAF, and MSI status has become an important part of therapy planning, particularly for individuals with metastatic illness. The final conclusion is that while there are several new ways for detecting CRC, there is still a need for a biomarker with full accuracy, which will undoubtedly be accessible in the near future.

AUTHOR CONTRIBUTIONS

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REFERENCES

1. Farid E Ahmed, Nancy C Amed, Paul W Vos, Chris Bonnerup, James N Atkins, Michelle Casey, Gerard J Nuovo, Wade Naziri, John E Wiley, and Ron R Allison. Diagnostic microrna markers to screen for sporadic human colon cancer in blood. *Cancer genomics & proteomics*, 9(4):179–192, 2012.
2. James E Allison, Callum G Fraser, Stephen P Halloran, and Graeme P Young. Pop- ulation screening for colorectal cancer means getting fit: the past, present, and future of colorectal cancer screening using the fecal immunochemical test for hemoglobin (fit). *Gut and liver*, 8(2):117, 2014.
3. Hussain Almasmoum. The roles of transmembrane mucins located on chromosome 7q22. 1 in colorectal cancer. *Cancer Management and Research*, 13:3271, 2021.
4. Mahefatiana Andrianifahanana, Nicolas Moniaux, and Surinder K Batra. Regulation of mucin expression: mechanistic aspects and implications for cancer and inflammatory diseases. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, 1765(2):189–222, 2006.
5. Melina Arnold, Mónica S Sierra, Mathieu Laversanne, Isabelle Soerjomataram, Ahmedin Jemal, and Freddie Bray. Global patterns and trends in colorectal cancer incidence and mortality. *Gut*, 66(4):683–691,2017.
6. Francisco Arvelo, Felipe Sojo, and Carlos Cotte. Biology of colorectal cancer. *Ecancer- medical science*, 9, 2015.
7. Ingrid Babel, Rodrigo Barderas, Ramon Diaz-Uriarte, Jorge Luis Mart´ınez- Torrecuadrada, Marta Sanchez-Carbayo, and J Ignacio Casal. Identification of tumor- associated autoantigens for the diagnosis of colorectal cancer in serum using high density protein microarrays. *Molecular & cellular proteomics*, 8(10):2382–2395, 2009.
8. Reyhan Bayrak, Hacer Haltas, and Sibel Yenidunya. The value of cdx2 and cytokeratins 7 and 20 expression in differentiating colorectal adenocarcinomas from extraintestinal gastrointestinal adenocarcinomas: cytokeratin 7-/20+ phenotype is more specific than cdx2 antibody. *Diagnostic pathology*, 7(1):1–11, 2012.
9. Reyhan Bayrak, Sibel Yenidünya, and Hacer Haltas. Cytokeratin 7 and cytokeratin 20 expression in colorectal adenocarcinomas. *Pathology-Research and Practice*, 207(3):156– 160, 2011.
10. Tingting Bian, Jinli Zhao, Jia Feng, Qing Zhang, Li Qian, Jian Liu, Daishan Jiang, Yifei Liu, and Jianguo Zhang. Combination of cadherin-17 and satb homeobox 2 serves as potential optimal makers for the differential diagnosis of pulmonary enteric adenocarcinoma and metastatic colorectal adenocarcinoma. *Oncotarget*, 8(38):63442, 2017.
11. C Richard Boland, Stephen N Thibodeau, Stanley R Hamilton, David Sidransky, James R Eshleman, Randall W Burt, Stephen J Meltzer, Miguel A Rodriguez-Bigas, Riccardo Fodde, G Nadia Ranzani, 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*, 58(22):5248–5257, 1998.
12. Geng Chen, Ziyun Wang, Dongqing Wang, Chengxiang Qiu, Mingxi Liu, Xing Chen, Qipeng Zhang, Guiying Yan, and Qinghua Cui. Lncrnadisease: a database for long- non-coding rna-associated diseases. *Nucleic acids research*, 41(D1):D983–D986, 2012.

13. Xi Chen, Yi Ba, Lijia Ma, Xing Cai, Yuan Yin, Kehui Wang, Jigang Guo, Yujing Zhang, Jiangning Chen, Xing Guo, et al. Characterization of micrornas in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell research*, 18(10):997–1006, 2008.
14. Yi-Yuan Chen, Tsung-Hsing Chen, Ming-Yao Su, Hsiao-Chen Ning, Chia-Jung Kuo, Wei-Pin Lin, Yu-Pin Ho, Chun-Jung Lin, Chen-Ming Hsu, Cheng-Tang Chiu, et al. Accuracy of immunochemical fecal occult blood test for detecting colorectal neoplasms in individuals undergoing health check-ups. *Advances in Digestive Medicine*, 1(3):74–79, 2014.
15. Hailey A Clancy, Hong Sun, Lisa Passantino, Thomas Kluz, Alexandra Muñoz, Jiri Zavadil, and Max Costa. Gene expression changes in human lung cells exposed to arsenic, chromium, nickel or vanadium indicate the first steps in cancer. *Metallomics*, 4(8):784–793, 2012.
16. Hans Clevers. Wnt/β-catenin signaling in development and disease. *Cell*, 127(3):469– 480, 2006.
17. Gianpiero Di Leva, Michela Garofalo, and Carlo M Croce. Micrornas in cancer. *Annual Review of Pathology: Mechanisms of Disease*, 9:287–314, 2014.
18. Frank Diehl, Meng Li, Devin Dressman, Yiping He, Dong Shen, Steve Szabo, Luis A Diaz, Steven N Goodman, Kerstin A David, Hartmut Juhl, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. *Proceedings of the National Academy of Sciences*, 102(45):16368–16373, 2005.
19. Frank Diehl, Kerstin Schmidt, Kristine H Durkee, Kent J Moore, Steve N Goodman, Anthony P Shuber, Kenneth W Kinzler, and Bert Vogelstein. Analysis of mutations in DNA isolated from plasma and stool of colorectal cancer patients. *Gastroenterology*, 135(2):489–498, 2008.
20. Dina El-Gayar, Nevine El-Abd, Noha Hassan, and Reem Ali. Increased free circulating dna integrity index as a serum biomarker in patients with colorectal carcinoma. *Asian Pacific Journal of Cancer Prevention*, 17(3):939–944, 2016.
21. Jacques Ferlay, Isabelle Soerjomataram, Rajesh Dikshit, Sultan Eser, Colin Mathers, Marise Rebelo, Donald Maxwell Parkin, David Forman, and Freddie Bray. Cancer incidence and mortality worldwide: sources, methods and major patterns in globocan 2012. *International journal of cancer*, 136(5):E359–E386, 2015.
22. Karin Fransén, Maria Klintenäs, Anna Österström, Jan Dimberg, Hans-Jürg Monstein, and Peter Söderkvist. Mutation analysis of the braf, araf and raf-1 genes in human colorectal adenocarcinomas. *Carcinogenesis*, 25(4):527–533, 2004.
23. Ganepola AP Ganepola, Joel Nizin, John R Rutledge, and David H Chang. Use of blood-based biomarkers for early diagnosis and surveillance of colorectal cancer. *World journal of gastrointestinal oncology*, 6(4):83, 2014.
24. María Dolores Giráldez, Juan José Lozano, Georgina Ramírez, Elizabeth Hijona, Luis Bujanda, Antoni Castells, and Meritxell Gironella. Circulating micrornas as biomarkers of colorectal cancer: results from a genome-wide profiling and validation study. *Clinical gastroenterology and hepatology*, 11(6):681–688, 2013.
25. Wanjun Gong, Ming Tian, Honggen Qiu, and Zhenhua Yang. Elevated serum level of lncrna-hif1a-as1 as a novel diagnostic predictor for worse prognosis in colorectal carcinoma. *Cancer Biomarkers*, 20(4):417–424, 2017.
26. Maria Gonzalez-Pons and Marcia Cruz-Correa. Colorectal cancer biomarkers: where are we now? *BioMed research international*, 2015,2015.
27. Lloyd D Graham, Susanne K Pedersen, Glenn S Brown, Thu Ho, Zena Kassir, Au- drey T Moynihan, Emma K Vizgoft, Robert Dunne, Letitia Pimlott, Graeme P Young, et al. Colorectal neoplasia differentially expressed (crnde), a novel gene with elevated expression in colorectal adenomas and adenocarcinomas. *Genes & cancer*, 2(8):829–840, 2011.
28. TB Hao, W Shi, XJ Shen, J Qi, XH Wu, Y Wu, YY Tang, and SQ Ju. Circulating cell- free dna in serum as a biomarker for diagnosis and prognostic prediction of colorectal cancer. *British journal of cancer*, 111(8):1482–1489, 2014.
29. Brenda Y Hernandez, Henry F Frierson Jr, Christopher A Moskaluk, Yu Jim Li, Limin Clegg, Timothy R Cote, Margaret E McCusker, Benjamin F Hankey, Brenda K Edwards, and Marc T Goodman. Ck20 and ck7 protein expression in colorectal cancer: demonstration of the utility of a population-based tissue microarray. *Human pathology*, 36(3):275–281, 2005.
30. Geir Hoff, Tom Grotmol, Espen Thiis-Evensen, Michael Bretthauer, G Gondal, and Morten H Vatn. Testing for faecal calprotectin (phical) in the norwegian colorectal cancer prevention trial on flexible sigmoidoscopy screening: comparison with an im- munochemical test for occult blood (flexsure obt). *Gut*, 53(9):1329–1333, 2004.
31. Lieke Hol, JA Wilschut, Marjolein van Ballegooijen, AJ Van Vuuren, Hans van der Valk, JCIY Reijerink, ACM van Der Togt, EJ Kuipers, JDF Habbema, and ME Van Leer- dam. Screening for colorectal cancer: random comparison of guaiac and immunochem- ical faecal occult blood testing at different cut-off levels. *British journal of cancer*, 100(7):1103–1110, 2009.
32. Mads N Holten-Andersen, Ib Jarle Christensen, Hans Jørgen Nielsen, Ross W Stephens, Vibeke Jensen, Ole Haagen Nielsen, Steen Sørensen, Jens Overgaard, Hans Lilja, Adrian Harris, et al. Total levels of tissue inhibitor of metalloproteinases 1 in plasma yield high diagnostic sensitivity and specificity in patients with colon cancer. *Clinical Cancer Research*, 8(1):156–164, 2002.
33. Young-Seoub Hong, Ki-Hoon Song, and Jin-Yong Chung. Health effects of chronic arsenic exposure. *Journal of preventive medicine and public health*, 47(5):245,2014.
34. Sabrina Hundt, Ulrike Haug, and Hermann Brenner. Blood markers for early detection of colorectal cancer: a systematic review. *Cancer Epidemiology and Prevention Biomarkers*, 16(10):1935–1953, 2007.
35. Sebastian Constantin Toma, Bogdan Silviu Ungureanu, Stefan Patrascu, Valeriu Surlin, and I Georgescu. Colorectal cancer biomarkers-a new trend in early diagnosis. *Current Health Sciences Journal*, 44(2):140, 2018.
36. Sabine Jahr, Hannes Hentze, Sabine Englisch, Dieter Hardt, Frank O Fackelmayer, Rolf- Dieter Hesch, and Rolf Knippers. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. *Cancer research*, 61(4):1659–1665, 2001.
37. JR Jass. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. *Histopathology*, 50(1):113–130, 2007.
38. Jin Jen, Steve M Powell, Nickolas Papadopoulos, Kelly J Smith, Stanley R Hamilton, Bert Vogelstein, and Kenneth W Kinzler. Molecular determinants of dysplasia in

colorectal lesions. *Cancer research*, 54(21):5523–5526, 1994.
39. Ziad Kanaan, Henry Roberts, M Robert Eichenberger, Adrian Billeter, Gairy Ocheretner, Jianmin Pan, Shesh N Rai, Jeffery Jorden, Anna Williford, and Susan Galandiuk. A plasma microrna panel for detection of colorectal adenomas: a step toward more precise screening for colorectal cancer. *Annals of surgery*, 258(3):400–408, 2013.
40. Sukhwinder Kaur, Sushil Kumar, Navneet Momi, Aaron R Sasson, and Surinder K Batra. Mucins in pancreatic cancer and its microenvironment. *Nature reviews Gastroenterology & hepatology*, 10(10):607–620, 2013.
41. Kenneth W Kinzler, Mef C Nilbert, Li-Kuo Su, Bert Vogelstein, Tracy M Bryan, Daniel B Levy, Kelly J Smith, Antonette C Preisinger, Philip Hedge, Douglas McK-echnie, et al. Identification of fap locus genes from chromosome 5q21. *Science*, 253(5020):661–665, 1991.
42. Jeffrey K Lee, Elizabeth G Liles, Stephen Bent, Theodore R Levin, and Douglas A Corley. Accuracy of fecal immunochemical tests for colorectal cancer: systematic review and meta-analysis. *Annals of internal medicine*, 160(3):171–181, 2014.
43. Arnold J Levine, Jamil Momand, and Cathy A Finlay. The p53 tumour suppressor gene. *Nature*, 351(6326):453–456, 1991.
44. Jing Liang, Chunqing Lin, Fulan Hu, Fan Wang, Lin Zhu, Xiaoping Yao, Yibaina Wang, and Yashuang Zhao. Apc polymorphisms and the risk of colorectal neoplasia: a huge review and meta-analysis. *American journal of epidemiology*, 177(11):1169–1179,2013.
45. Alexander Link, Francesc Balaguer, Yan Shen, Takeshi Nagasaka, Juan José Lozano, C Richard Boland, and Ajay Goel. Fecal micrornas as novel biomarkers for colon cancer screening. *Cancer Epidemiology and Prevention Biomarkers*, 19(7):1766–1774,2010.
46. Ming-Xi Liu, Xing Chen, Geng Chen, Qing-Hua Cui, and Gui-Ying Yan. A computational framework to infer human disease-associated long noncoding rnas. *PloS one*, 9(1):e84408, 2014.
47. Gershon Y Locker, Stanley Hamilton, Jules Harris, John M Jessup, Nancy Kemeny, John S Macdonald, Mark R Somerfield, Daniel F Hayes, and Robert C Bast Jr. Asco 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. *Journal of clinical oncology*, 24(33):5313–5327, 2006.
48. Kristina Magnusson, Meike de Wit, Donal J Brennan, Louis B Johnson, Sharon F McGee, Emma Lundberg, Kirsha Naicker, Rut Klinger, Caroline Kampf, Anna Asplund, et al. Satb2 in combination with cytokeratin 20 identifies over 95% of all colorectal carcinomas. *The American journal of surgical pathology*, 35(7):937–948, 2011.
49. MJ Mäkinen. Colorectal serrated adenocarcinoma. *Histopathology*, 50(1):131–150, 2007.
50. Patrick S Mitchell, Rachael K Parkin, Evan M Kroh, Brian R Fritz, Stacia K Wyman, Era L Pogosova-Agadjanyan, Amelia Peterson, Jennifer Noteboom, Kathy C O'Briant, April Allen, et al. Circulating micrornas as stable blood-based markers for cancer detection. *Proceedings of the National Academy of Sciences*, 105(30):10513–10518, 2008.
51. Christopher A Moskaluk, Hong Zhang, Steven M Powell, Lisa A Cerilli, Garret M Hampton, and Henry F Frierson. Cdx2 protein expression in normal and malignant human tissues: an immunohistochemical survey using tissue microarrays. *Modern pathology*, 16(9):913–919, 2003.
52. Maryam Mousavinezhad, Reza Majdzadeh, Ali Akbari Sari, Alireza Delavari, and Farideh Mohtasham. The effectiveness of fobt vs. fit: A meta-analysis on colorectal cancer screening test. *Medical journal of the Islamic Republic of Iran*, 30:366, 2016.
53. Taxiarchis Konstantinos Nikolouzakis, Loukia Vassilopoulou, Persefoni Fragkiadaki, Theodoros Mariolis Sapsakos, Georgios Z Papadakis, Demetrios A Spandidos, Aristides M Tsatsakis, and John Tsiaoussis. Improving diagnosis, prognosis and prediction by using biomarkers in crc patients. *Oncology reports*, 39(6):2455–2472, 2018.
54. Hyung-Hoon Oh and Young-Eun Joo. Novel biomarkers for the diagnosis and prognosis of colorectal cancer. *Intestinal research*, 18(2):168, 2020.
55. Susana Olmedillas-López, Dolores C García-Olmo, Mariano García-Arranz, Ramón Peiró-Pastor, Begoña Aguado, and Damián García-Olmo. Liquid biopsy by ngs: differential presence of exons (dpe) in cell-free DNA reveals different patterns in metastatic and nonmetastatic colorectal cancer. *Cancer medicine*, 7(5):1706–1716, 2018.
56. Nicole C Panarelli, Cecily P Vaughn, Wade S Samowitz, and Rhonda K Yantiss. Sporadic microsatellite instability-high colon cancers rarely display immunohistochem- ical evidence of wnt signaling activation. *The American journal of surgical pathology*, 39(3):313–317, 2015.
57. Siew-Wai Pang, Noel Jacques Awi, Subasri Armon, Wendy Wan-Dee Lim, John Seng- Hooi Low, Kaik-Boo Peh, Suat-Cheng Peh, and Sin-Yeang Teow. Current update of laboratory molecular diagnostics advancement in management of colorectal cancer (crc). *Diagnostics*, 10(1):9, 2020.
58. Simon Ladefoged Rasmussen, Henrik Bygum Krarup, Kåre Gotschalck Sunesen, Martin Berg Johansen, Mogens Tornby Stender, Inge Søkilde Pedersen, Poul Henning Madsen, and Ole Thorlacius-Ussing. The prognostic efficacy of cell-free dna hypermethyla- tion in colorectal cancer. *Oncotarget*, 9(6):7010, 2018.
59. Alberto Righi, Christine M Betts, Claudio Marchetti, Gianluca Marucci, Lucio Mon- tebugnoli, Carlo Prati, Leonardo HU Eusebi, Luciana Muzzi, Teresa Ragazzini, and Maria P Foschini. Merkel cells in the oral mucosa. *International journal of surgical pathology*, 14(3):206–211, 2006.
60. Mariann Rutka, Renáta Bor, Anita Bálint, Anna Fábián, Ágnes Milassin, Ferenc Nagy, Zoltan Szepes, Mónika Szűcs, László Tiszlavicz, Klaudia Farkas, et al. Diagnostic accuracy of five different fecal markers for the detection of precancerous and cancerous lesions of the colorectum. *Mediators of Inflammation*, 2016, 2016.
61. Sudhir Srivastava, Mukesh Verma, and Donald E Henson. Biomarkers for early detection of colon cancer. *Clinical Cancer Research*, 7(5):1118–1126, 2001.
62. Hong Sun, Harriet A Clancy, Thomas Kluz, Jiri Zavadil, and Max Costa. Comparison of gene expression profiles in chromate transformed beas-2b cells. *PloS one*, 6(3):e17982, 2011.
63. Tetsunari Takai, Shigeru Kanaoka, Ken-ichi Yoshida, Yasushi Hamaya, Mutsuhiro Ikuma, Naoyuki Miura, Haruhiko Sugimura, Masayoshi Kajimura, and Akira Hishida. Fecal cyclooxygenase 2 plus matrix metalloproteinase 7 mrna assays as a marker for colorectal cancer screening. *Cancer Epidemiology and Prevention Biomarkers*, 18(6):1888– 1893, 2009.

64. SEBASTIAN CONSTANTIN TOMA, BOGDAN SILVIU UNGUREANU, STEFAN PATRASCU, VALERIU SURLIN, and I Georgescu. Colorectal cancer biomarkers-a new trend in early diagnosis. *Current Health Sciences Journal*, 44(2):140, 2018.
65. Marco Vacante, Antonio Maria Borz`ı, Francesco Basile, and Antonio Biondi. Biomarkers in colorectal cancer: Current clinical utility and future perspectives. *World journal of clinical cases*, 6(15):869, 2018.
66. A Vincent, M Perrais, JL Desseyn, JP Aubert, P Pigny, and I Van Seuningen. Epigenetic regulation (dna methylation, histone modifications) of the 11p15 mucin genes (muc2, muc5ac, muc5b, muc6) in epithelial cancer cells. *Oncogene*, 26(45):6566–6576, 2007.
67. Hui-Peng Wang, Yang-Yun Wang, Jie Pan, Rong Cen, and Yuan-Kun Cai. Evaluation of specific fecal protein biochips for the diagnosis of colorectal cancer. *World Journal of Gastroenterology: WJG*, 20(5):1332, 2014.
68. Jaw-Yuan Wang, Jan-Sing Hsieh, Mei-Yin Chang, Tsung-Jen Huang, Fang-Ming Chen, Tian-Lu Cheng, Ketil Alexandersen, Yu-Sheng Huang, Wen-Shyong Tzou, and Shiu-Ru Lin. Molecular detection of apc, k-ras, and p53 mutations in the serum of colorectal cancer patients as circulating biomarkers. *World journal of surgery*, 28(7):721–726, 2004.
69. Ji-Lian Wang, Zhen Qi, Ye-Hua Li, Hong-Mei Zhao, Ye-Guang Chen, and Wei Fu. Tgfβ induced factor homeobox 1 promotes colorectal cancer development through activating wnt/β-catenin signaling. *Oncotarget*, 8(41):70214, 2017.
70. Qifeng Wang, Zhaohui Huang, Shujuan Ni, Xiuying Xiao, Qinghua Xu, Lisha Wang, Dan Huang, Cong Tan, Weiqi Sheng, and Xiang Du. Plasma mir-601 and mir-760 are novel biomarkers for the early detection of colorectal cancer. 2012.
71. Robert W Werling, Hadi Yaziji, Carlos E Bacchi, and Allen M Gown. Cdx2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. *The American journal of surgical pathology*, 27(3):303–310, 2003.
72. Karl Willert and Roel Nusse. β-catenin: a key mediator of wnt signaling. *Current opinion in genetics & development*, 8(1):95–102, 1998.
73. Min Wu, Chen Mao, Qing Chen, Xin-Wei Cu, and Wei-Seng Zhang. Serum p53 protein and anti-p53 antibodies are associated with increased cancer risk: a case–control study of 569 patients and 879 healthy controls. *Molecular biology reports*, 37(1):339–343, 2010.Fung Lin Yong, Chee Wei Law, and Chee Woon Wang. Potentiality of a triple microrna classifier: mir-193a-3p, mir-23a and mir-338-5p for early detection of colorectal cancer. *BMC cancer*, 13(1):1–10, 2013.
74. Joseph P Zackular, Mary AM Rogers, Mack T Ruffin, and Patrick D Schloss. The human gut microbiome as a screening tool for colorectal cancer. *Cancer prevention research*, 7(11):1112–1121, 2014.
75. Weimin Zhao, Mu Song, Jie Zhang, Mulati Kuerban, and Haijiang 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*, 8(11):14131, 2015.
76. Jianbao Zheng, Sai He, Jie Qi, Xiaolong Wang, Junhui Yu, Yunhua Wu, Qi Gao, Kai Wang, and Xuejun Sun. Targeted cdx2 expression inhibits aggressive phenotypes of colon cancer cells *in vitro* and *in vivo*. *International journal of oncology*, 51(2):478–488, 2017.

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