REVIEW ARTICLE

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Blood-Based Biomarkers for Prognosis and Diagnosis of Colorectal Cancer

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ABSTRACT: Colorectal cancer (CRC) is a disease that mostly affects developing countries with its high incidence and mortality rates. Global burden of CRC is increasing, and early diagnosis of the disease affects the survival rate of patients. Current screening methods such as colonoscopy are inadequate because of invasive, high costs and a low participation rate. It is important to find a non-invasive, low cost and easily accessible test for CRC screening. Because of their high sensitivity and specificity, blood-based biomarkers have potential for early diagnosis and prognosis of CRC. The purpose of this review is to summarize candidate and currently used diagnostic and prognostic biomarkers for blood samples in patients with CRC.

Keywords: Biomarker, Circulating tumor cells, Colorectal cancer, Colonoscopy, miRNA.

INTRODUCTION

According to Cancer Statistics in 2023, colorectal cancer (CRC) is one of the major reasons of morbidity and mortality. It is the third most frequent cancer and the second most common cause of death in men and women (Siegel et al., 2023). It is expected that 2.2 million new cases of CRC will be diagnosed in the developing countries by 2030 (Arnold et al., 2016). The survival rate of patients in CRC increases when the early detection of the disease (Hauptman and Glavač, 2017). Although 5-year survival rate in patients with CRC is nearly 90% at early diagnosis, this rate for cases with distant metastases is only 12.5% (Yoruker et al., 2016).

Colonoscopy is a gold standard method for the detection of CRC with high sensitivity and specificity and is the most used confirmation methods by clinicians. Although colonoscopy is advantageous with its high performance, it is an invasive and high cost method. Therefore, it is crucial to determine an easily detectable, and non-invasive biomarker in blood for the early-stage CRC detection. Detection of biomarkers in circulation blood could ensure the most useful test for CRC (Hauptman and Glavač, 2017). The main aim of this review is to provide a summary of the presently known blood based biomarkers that can be used for screening colorectal carcinogenesis.

Currently used CRC screening tests

Various tests are recommended for colorectal carcinoma screening, such as blood screening in the stool, immunochemical tests, and endoscopic /radioscopic imaging techniques. Fecal blood tests are useful because they are non-invasive, low cost and not require a bowel preparation. However, these tests may indicate high false-positive results in CRC detecting and needs a confirmatory colonoscopy for positive results. Flexible sigmoidoscopy is a small-scale colonoscopy which is applied to investigate the lower 1/3 of the colon, and can be performed without sedation (Ganepola et al., 2014). Disadvantages of this test is not detecting abnormalities in the upper part of the colon. Besides, it has small risk of tearing and perforation.

Colonoscopy is a gold standard for CRC detection, which is used to examine the entire colon. Also, it has some advantages such as take biopsies and remove polyps. Although colonoscopy is the highest performance for CRC screening, there are various risks including bleeding, tearing or perforation (Ganepola et al., 2014). Additionally, colonoscopy cannot detect some polyps, cancer, and non-polypoid lesions due to variations in polyp size and requires bowel preparation and sedation (Pons and Correa, 2015).

Each method has advantages and disadvantages in CRC screening, so it is crucial to identify a non-invasive marker that can minimize the disadvantages. The ideal blood-based marker should have several properties: It can be easily performed; it should be noninvasive and safe; and It should have high selectivity and sensitivity (Pons and Correa, 2015). Detection of specific molecular indicators in blood samples or any body fluids would provide the most practical diagnostic tool for CRC. These molecular indicators, including DNA, RNA and protein, can be found in serum or plasma.

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Circulating tumor cells (CTCs)

Circulating tumor cells (CTCs) are present in the bloodstream and are associated with the various types of cancers, including breast, renal, colon, gastric, pancreas, lung, liver and prostate. The epithelial-mesenchymal transition (EMT) plays a critical role in many metastatic cancers by reducing the adheren junction of cells (Vu and Datta, 2017). Prognostic value of CTCs undergoing EMT in CRC has been proven (Yokobori et al., 2013)

CellSearch system is an FDA-approved method for CTC detection, and it is used only for a few types of cancer such as breast, prostate and colorectal cancers (Millner et al., 2013). However, it is hard to identify CTCs as a prognostic and diagnostic tool due to their low concentrations in blood. Although the number of CTCs in circulation blood is lower in early-stage cancers, it is between 1-10 cells per 10 mL of blood (Alix-Panabières and Pantel, 2014). Other challenge for detecting CTCs is that many capture methods do not allow cell viability (Millner et al., 2013). Further studies are needed to prove clinical validity of CTCs as novel biomarkers.

BLOOD BASED DNA MARKERS

Cell-free DNAs (cfDNAs) are non−cell−bound nucleic acids in the bloodstream of patients with cancer. Although cfDNA was firstly found in blood in the nineteen-forties (Mandel and Metais, 1948), the importance of cfDNA as a prognostic and diagnostic biomarker recognized in the twenty-first century (Thierry et al., 2016). cfDNAs are released from apoptotic and necrotic tumor cells or living tumor cells.

Some studies have evaluated the serum/plasma levels of cfDNA in CRC. Leon et al. (1977) found that cancer patients had higher plasma cfDNA levels than healthy individuals. In another study showed that the levels of cfDNA in plasma were significantly higher in CRC patients (Frattini et al., 2006). Besides measuring total cfDNA, there are several other methods to evaluate cfDNA, including cfDNA integrity, DNA methylation and microsatellite alterations.

cfDNA Integrity

cfDNA fragments can be produced by various cell types and can vary in length when derived by tumor cells. Several studies aimed to reveal whether the integrity of cfDNA in serum or plasma could be used as a screening tool of colorectal carcinogenesis. There are different findings showing increased (Umetani et al., 2016; Leszinski et al., 2014) or decreased (Mouliere et al., 2011; Mead et al., 2011) cfDNA fragmentation in CRC.

A recent study showed that cfDNA that analyzed from CRC patients was found highly fragmented compared to healthy controls (Mouliere et al., 2014). Hao et al. (2014) tested higher cfDNA integrity in recurrent compared with primary CRC. This report also indicates that both integrity and concentration of cfDNA can be used as a biomarker for CRC.

DNA Methylation

DNA methylation occurs through the covalent addition of methyl groups to the 5-carbon positions on cytosines in CpG dinucleotides and is associated with the early stages of tumorigenesis (Wang et al., 2014). Aberrant DNA methylation that can contribute to carcinogenesis via DNA hypermethylation and hypomethylation is one of the most investigated epigenetic alteration. Transcriptional silencing of the target gene, including tumor suppressor genes and DNA repair genes occurs via hypermethylation. Hypomethylation is a loss of methylation and leading to chromosome instability.

SEPT9 gene evaluated as a methylation marker and has shown 90% sensitivity and 88% specificity (Warran et al., 2011). In a genom scale study, two potential methylation markers, THBD and C9orf50, were identified for early CRC detection (Lange et al., 2012). Other blood based DNA methylation markers such as *NEUROG1, p16, APC, hMLH1, HLTF*, and *RUNX3* have also been evaluated.(Board et al., 2008; Herbst et al., 2011; Tan et al., 2007)

Microsatellite Alterations

Microsatellites are repeated short DNA sequences in lenght of 1-6 bp that can be found in coding or noncoding regions. Microsatellite instability (MSI) is an alteration in highly repetitive DNA sequences due to insertion or deletion in genes. Alterations in microsatellites occur via the mutations in the DNA mismatch repair genes.

CRC tumors with MSI are located in proximal colon and occurs sporadically (Iacopetta et al., 2010). There is growing evidence that MSI can be a useful prognostic biomarker for CRC. However, clinical usefulness is not clear for this purpose and additional studies are needed.

BLOOD BASED RNA MARKERS

Searching biomarkers in human bodily fluids is difficult because of the instability of RNA molecules. In blood, RNase degrades RNA rapidly and affects its stability. However, recent studies have reported that RNA is stable outside the cell (Ganepola et al., 2014) and RNA expression is different between normal and cancer cells (Cortez et al., 2011). Different types

of RNA molecules including mRNA, miRNA, and lncRNA can be detected in blood. Most investigations have focused on detecting these molecules for CRC screening.

mRNA Markers

The mRNA can be a useful marker that can be isolated from plasma, serum or peripheral blood cells. Several techniques are used for detecting mRNA expression, such as microarrays, quantitative real-time PCR (qRT-PCR), and next-generation sequencing. Tsouma et al. (2010) performed multiplex RT-qPCR technology in peripheral blood cells to determine the disease stage and overall survival in patients with CRC. Three genes *CEA, CK20* and *EGFR* were showed to have the clinical importance in CRC. De primo et al. (2003) isolated RNA from peripheral blood cells and applied microarray-based mRNA expression profiling. In this study, some transcripts are demonstrated to be a potential biomarker of drug treatment.

GeneNews Ltd. developed a blood based risk-assessment test from peripheral blood cells. Seven-gene panel (*ANXA3, CLEC4D, LMNB1, PRRG4, TNFAIP6, VNN1* and *IL2RB*) was used, and the panel was selected from 196-gene expression profile of 112 CRC patients and 120 controls. This panel was validated using 202 CRC patients and 208 controls from the Canadian population and reported 72% sensitivity and 70% specificity (Marshall et al., 2010). The test was also confirmed in 99 CRC cases and 111 healthy controls from the Malaysian population. They reported sensitivity of 61% and specificity of 77% (Yip et al., 2010).

miRNA Markers

microRNAs are 18-25 bp long, single stranded and noncoding RNA. microRNAs were first discovered by Ambros and Ruvkun in 1993. The number of miRNAs that discovered have increased enormously since then. They regulate gene expression by binding to specific complementary sites at the 3′ untranslated regions of target mRNAs and cause translational repression or gene silencing (Carter et al., 2017). miRNAs have critical function in almost every biological process such as differentiation, migration, cell proliferation, and apoptosis (Lee and Dutta, 2009). They may also affect oncogenes and tumor suppressors, and dysregulated miRNA expression have been studied an assortment of cancers including colorectal cancer (Carter et al., 2017; Krutovskikh and Herzeg, 2010; Slack, 2013).

In recent years, studies that focused on circulating blood miRNA have accumulated because of their high stability in bloodstream. miR- 17-3p and miR-92 was found significantly higher in CRC patients with 89% sensitivity and 70% specificity (Ng et al., 2009). Cheng et al. (2011) studied three miRNAs named miR-21, -92, and -141 in 102 CRC patients and healthy individuals from the United States population. They also confirmed their results using 156 CRC patients and matched healthy donors from the Chinese population, and found miR-141 higher in stage IV CRC with 90.9% sensitivity and 77.1% specificity. Several miRNAs as a prognostic marker in colorectal cancer have also been investigated. miR-200c has been found at higher levels in serum and is related with lymph metastasis and cancer recurrence (Toiyama et al.,2014). Carter et al. (2017) studied miR-21 to detect their role as a prognostic marker. miR-21 is one of the unique miRNA, and may be a useful marker in several cancers including CRC (Wang and Zhang, 2012). It was reported that a panel of eight miRNAs including miR-532-3p, miR-331, miR- 195, miR-17, miR-142-3p, miR-15b, miR-532, and miR-652 could separate patients from healthy individuals (Kanaan et al., 2013). Another panel of three-miRNA (miR-431, miR-15b, and miR-139-3p) could be used to separate stage Ⅳ CRC cases from controls with 93% sensitivity and 74% specificity. Although the screening panels are promising, further studies are needed to determine their clinical benefits as diagnostic and prognostic markers.

LncRNA Markers

Long non-coding RNAs which are also known as lncRNA are frequently transcribed longer than 200 nucleotides. It was emphasized that lncRNAs associated with different types of cancer and can be a potential marker in circulation blood (Zhao et al., 2015). LncRNA H19 is highly expressed in plasma of gastric cancer patients when compared to controls (Zhao et al., 2015). The expression levels of CRNDE-h were reported significantly upregulated in patients with CRC than healthy controls. It was effective to identify CRC with a specificity of 93% and a sensitivity of 87% (Graham et al., 2011). It was shown that CCAT2 was highly overexpressed in CRC, and has the potential as a prognostic and diagnostic marker (Ling et al., 2013) Zhao et al. (2015) demonstrated that the combination of plasma CCAT1 and HOTAIR had a high diagnostic value of CRC with 84.3% sensitivity and 80.2% specificity.

BLOOD BASED PROTEIN MARKERS

Proteins that are secreted or leaking from cancer cells into the bloodstream, referred as cancer secretome, are promising biomarkers and could be detectable in blood or other biofluids (Visser et al., 2013). Common assays to identify and verify protein markers are ELISA, Western blot, chromatographic and mass spectrometric (MS) technologies. Also, new technologies, such as MALDI/MS, SELDI-TOF/MS and LC-MS/MS can be used to identify biomarkers in different types of biofluids and to fractionate the particular protein molecules. Many potential protein markers have been reported for CRC. However, there are only two protein markers having clinical utilities for CRC, these are CEA and CA 19-9 (Hauptman and Glavač, 2017). A protein marker, TIMP-1, has been evaluated for the early identification of CRC with high sensitivity and specificity (Holten et al., 2002). In a proteomic study, 6 new candidate biomarkers (CD79B, DDR1, EFNA4, FLRT2, LTA4H and NCR1) were discovered as

circulating proteins for CRC risk (Holten et al., 2002). Further researchs are needed to validate all of the proposed protein biomarkers to be used as clinically diagnostic tools in CRC.

CONCLUSION

Finding a molecular marker in circulation blood that can be used for screening of CRC could be an alternative assay to colonoscopy. There are many publications reporting miRNA markers having the greatest potential with high sensitivity and specificity. Some of them are discussed in this review. On the other hand, lncRNA and DNAmethylation analyses showed a high potential as CRC screening tests as well. Future studies are needed to prove the reliability of these blood based biomarkers in CRC.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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