The early detection of colorectal cancer with effective screening is essential for reduction of cancer-specific mortality. The addition of fecal DNA testing in the armamentarium of screening methods already in clinical use launches a new era in the noninvasive part of colorectal cancer screening and emanates from a large number of previous and ongoing clinical investigations and technological advancements. In this review, we discuss the molecular rational and most important genetic alterations hallmarking the early colorectal carcinogenesis process. Also, representative DNA targets-markers and key aspects of their testing at the clinical level in comparison or/and association with other screening methods are described. Finally, a critical view of the strengths and limitations of fecal DNA tests is provided, along with anticipated barriers and suggestions for further exploitation of their use.

Key words: Colorectal cancer; Screening; Fecal DNA; Cologuard®; Adenoma

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Abstract

The early detection of colorectal cancer with effective screening is essential for reduction of cancer-specific mortality. The addition of fecal DNA testing in the armamentarium of screening methods already in clinical use launches a new era in the noninvasive part of colorectal cancer screening and emanates from a large number of previous and ongoing clinical investigations and technological advancements. In this review, we discuss the molecular rational and most important genetic alterations hallmarking the early colorectal carcinogenesis process. Also, representative DNA targets-markers and key aspects of their testing at the clinical level in comparison or/and association with other screening methods are described. Finally, a critical view of the strengths and limitations of fecal DNA tests is provided, along with anticipated barriers and suggestions for further exploitation of their use.

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Core tip: The molecular DNA targets from genetic and epigenetic alterations hallmarking colorectal carcinogenesis are reviewed here in the context of fecal testing. Also, comparison with other screening methods in terms of limitations, advantages and future perspectives of fecal DNA tests are discussed.
INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in men and women and accounts for 8% of all cancer-related deaths[1]. The incidence of CRC varies within different geographic locations and racial/ethnic groups. These differences may be related with different dietary and environmental exposures in association with a different genotype-driven susceptibility[2]. Screening for CRC plays a key role in reduction of CRC-related mortality, and the observed decline in the incidence of CRC since the mid-1980s is a striking proof of this effect, along with changes in risk factors[3].

CRC screening may be divided into two main categories: (1) biological sample-based tests, including fecal, blood and urine tests, as well as (2) colon structure-based and image-based tests, including flexible sigmoidoscopy, CT colonoscopy and double-contrast barium enema[4,5]. Stool-based tests, including guaiac-based fecal occult blood test (g-FOBT), and the newer ones, fecal immunochemical test (FIT) and stool DNA test are already included in the American Cancer Society recommendations for CRC screening[6].

Molecular Rationale for Fecal DNA Testing

The detection of altered DNA from cancerous and precancerous lesions of the colonic mucosa is based on the natural exfoliation of these cells and is further facilitated by their high degree of "integrity" compared to DNA from stools of healthy patients. Accumulating data on key mutations occurring during the early stages of colon carcinogenesis including K-Ras, adenoma polyposis coli (APC), and p53, as well as epigenetic changes such as microsatellite instability (MSI), has guided the targeted development of clinically relevant detection tests[4].

The genetic heterogeneity of CRC is essentially the reason underlying the concept of targeting multiple DNA markers. K-Ras encodes a RAS family protein which is a GTPase involved in many downstream signal transduction pathways[6]. The mutation is found in 13%-95% of CRC patients and is one of the initial mutations in colon carcinogenesis[6]. APC is an important tumor suppressor gene product involved in the Wnt/β-catenin signaling pathway, which in turn is a transcription regulator of several growth-controlling genes, including the oncogene MYC[7]. Thus it is not surprising that mutation or inactivation of the APC protein is a driver of inherited (familial adenomatous polyposis) and sporadic forms of CRC, occurring in the early stages of transition from adenoma to carcinoma[7]. Another tumor suppressor gene, p53 is found deleted or mutated in 30%-60% of CRC tumors[8]. Given its critical role in cell cycle control, apoptosis, and DNA damage response, p53 aberrations ultimately promote the development of increased genomic instability which facilitates transformation of colorectal adenomas to cancer[7].

MSI is a condition of genetic hypermutability within tandem repeats of short nucleotide sequences, the microsatellites, that results from impaired DNA mismatch repair (MMR) and is a frequent event in cancers, including 15% of all CRC[9]. The most common cause of sporadic MSI is epigenetic silencing of MMR genes, such as MLH1 due to promoter hypermethylation[7] and there are several MSI markers (BAT25, BAT26, D2S123, D5S346, and D17S2720) for detection of MSI with polymerase chain reaction. The clinical relevance of MSI lies in the fact that patients with MSI positive tumors have better prognosis and longer overall survival compared with non-MSI tumors[9].

Epigenetic methylation of gene promoters is a central mechanism that can promote carcinogenesis in the appropriate context and several preclinical studies have identified hypermethylated genes in stool samples from CRC patients, which are strikingly un-methylated in normal epithelial cells[10]. Characteristic examples include the genes secreted frizzled-related protein (SFRP), vimentin, MGMT, FBN1, and p16[7]. In addition, the panel of methylated genes varies depending on the different stages of carcinogenesis, involving (1) SLC5A8, SFRP1, SFRP2, CDH13, CRBP1, RUNX3, MINT1 and MINT31 from normal colon mucosa to aberrant crypt focus formation; (2) p14, HLF, ITGA4, p16, CDH1, and ESR1 from aberrant crypt focus to adenoma formation; and (3) TIMP3, CXCL12, ID4, and IRF8 from adenoma to carcinoma formation and metastatic progression of CRC[7].

Clinical Studies of Fecal DNA Tests

An important limiting factor for developing a screening stool test with high sensitivity is the fact that only 0.01% of total fecal DNA is human and the tumor DNA is only a small percentage of the former[10].

K-RAS was the first gene tested for mutations in feces from CRC patients[11-13]. A comparative study assessed gFOBT and a fecal DNA test analyzing a panel of 21 gene mutations[14]. Imperiale et al[14] concluded that the multitarget fecal DNA test detected more invasive cancers plus adenomas with high-grade dysplasia than did gFOBT (40.8% vs 14.1%) without compromising specificity (94.4% vs 95.2%). In a blinded, multicenter, case-control study, with cases including CRC, advanced adenoma (AA), or sessile serrated adenoma ≥ 1 cm (SSA), an automated multitarget stool DNA assay was able to detect AA with high-grade dysplasia with 83% sensitivity[15]. Another blinded, multicenter, case-control study assessing a similar panel of DNA markers identified 85% of patients with CRC and 54% with AA,
without sensitivity differences based on location, but with tumor size affecting detection rates\[^{16}\].

More recently, Imperiale et al\[^{17}\] reported their results from comparison of fecal DNA to FIT in a huge patient population who had a complete screening colonoscopy (n = 9989). The sensitivity of fecal DNA test including evaluation of KRAS mutations, aberrant NDRG4 and BMP3 methylation, B-actin and a hemoglobin assay was superior to that of FIT (92.3% vs 73.8%). However, in addition to a lower specificity of fecal DNA and the lack of comparison with repeated FIT applications over time, a far higher number of patients (n = 689) were excluded due to problematic fecal DNA.
CURRENT STATUS OF FECAL DNA TESTING (COLOGUARD®)

The United States Food and Drug Administration has recently approved Cologuard® (Exact Sciences Corporation, Madison, WI, United States), a multitarget stool DNA test in CRC screening[39]. The frequency of interval testing was determined to be every 3 years with adequate Medicare coverage[40]. Cologuard® incorporates molecular assays for aberrantly methylated BMP3 and NDRG4 gene promoter regions, mutant KRAS and β-actin as well as an immunochemical assay for human hemoglobin. It is based on the recent study of Imperiale et al[17] which showed a significantly better sensitivity for cancer detection compared to FIT. Further laboratory-based processing of the samples is necessary, entailing amplification and detection with the use of Quantitative Allele-specific Real-time Target and Signal Amplification (QuARTSTM) technology[41].

FUTURE PERSPECTIVES FOR FECAL DNA SCREENING TESTS

The combined use of screening tests would likely maximize the benefits of different biomarkers for early detection of CRC and adenomas. However, synchronous implementation of these tests in a mass screening program would not fulfill the cost-effectiveness requirement for clinical use.

Thus, there is a need for prospectively designed, systematic evaluations of the most promising fecal tests in a well-defined, large-scale screening population, with standardized sample collection, processing, and storage. This assessment should be combined with sigmoidoscopy or colonoscopy screening and ideally involve repeated testing and longitudinal monitoring of the screened population[19]. Another parameter that merits prospective evaluation is the clinical significance of fecal DNA-positive results in patients with negative colonoscopy results[40].

In the future, Imperiale and colleagues plan to “take this work forward by conducting a post-approval study, which will inform the important issue of test interval, that is, how often does the test need to be repeated”. They will also conduct computer simulation studies that will inform comparative effectiveness and cost-effectiveness relative to other screening tests and strategies[42].

Given the high sensitivity for CRC that is unaffected by tumor location and its superior sensitivity over FIT for detection of SSA and AA with greatest risk of progression, Cologuard® may be a good candidate for interval testing after initial colonoscopy. For the same reason, in cases of poor preparation or incomplete colonoscopy, it might represent a convenient follow-up screening test alternative to repeat colonoscopy or other CT colonography, particularly for those patients who are either unable or unwilling to undergo repeat bowel preparation and invasive endoscopy[40].

In an expanding view, fecal DNA testing could be implemented as a screening in CRC predisposing conditions, such as inflammatory bowel disease, playing a role complementary to colonoscopy for early dysplasia detection and surveillance[40,43]. A relevant multicenter validation study has recently been initiated (Government-registered Trial: NCT01819766) and its results are eagerly awaited.

Finally, technological advancements in detection assays of small fragment DNA from stool may render the identification of altered DNA shed from upper GI pre-cancerous and malignant lesions feasible[44-46].

Discussion of screening tests involving non-DNA (e.g., miRNA, miRNA) or non-fecal origin (e.g., blood, urine) biomarkers was beyond the scope of this review. However, it is reasonable to assume that fecal shedding of tumor DNA is an earlier event compared to inner tissue and bloodstream invasion, and is also directly related to the natural, constant process of luminal colonic mucosa exfoliation; thus rendering fecal testing more timely sensitive for the purpose of screening.

Collectively, the accumulation of experience from clinical use of Cologuard® and the numerous ongoing studies on a plethora of biomarkers, as well as further technological advancement of colonoscopy with the full-spectrum endoscopy[47] are expected to further elucidate and expand the landscape of CRC screening research in the coming years, with the hope of further reducing CRC-specific mortality through earlier and accurate detection of pre-cancerous lesions.

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