IMPROVED METHODS OF DETECTING COLORECTAL CANCER

FIELD OF THE INVENTION

The present invention is concerned with the diagnosis, staging and treatment of disease, in particular cancer and more specifically colorectal cancer. The invention relates to methods and kits for diagnosing colorectal cancer based upon detecting epigenetic modifications, typically in specific genes. The methods and kits may also permit the detection of blood in a fecal sample, with the combined tests proving particularly advantageous.

BACKGROUND OF THE INVENTION

Colorectal cancer (CRC) is a leading cause of cancer-related deaths worldwide, and is the second leading cause of cancer-related deaths in the United States. A patient's prognosis is good if the cancer is caught early, when the site of the cancer is confined to its site of origin. However, the cure rates fall once the cancer has spread. Most colon cancers arise from conventional adenomatous polyps (conventional adenoma-to-carcinoma sequence), while some colon cancers appear to arise from the recently recognized serrated adenomatous polyp (serrated adenoma-to-carcinoma theory). Because conventional adenomas and serrated adenomas are usually asymptomatic, mass screening of asymptomatic patients has become the cornerstone for detecting and eliminating these precursor lesions to reduce the risk of colon cancer.

A number of different screening methods for CRC are available. Procedures such as digital rectal examination (DRE); colonoscopy or sigmoidoscopy are highly invasive, painful and can cause a great deal of patient discomfort. Other less invasive screening tests include fecal occult blood test (FOBT); fecal immunochemical test (FIT); barium enema with air contrast; virtual colonoscopy; biopsy (e.g., CT guided needle biopsy); and imaging techniques (e.g., ultrasound, CT scan, PET scan, and MRI).

Colonoscopy has become the primary screening test for CRC because of its high sensitivity and specificity, and the ability to perform polypectomy. While sensitive and specific, the procedure is invasive, costly, has limited availability and includes certain risks such as induction of infection and perforation of the bowel.

A commonly used and less expensive way of screening for CRC is a fecal occult blood test (FOBT), which tests for the presence of blood in faeces. The presence of haemoglobin as a representative blood protein in faeces is an indicator of intestinal bleeding, which is frequently associated with CRC. However, since occult in a fecal sample could be indicative of a variety of gastrointestinal disorders, further medical testing such as colonoscopy remains necessary to identify colorectal cancer.

Fecal occult blood tests fall primarily into two categories, tests based on the use of chromogenic chemical reagents such as gum guaiac and immunochemical tests. The chemically based guaiac methods determine the presence of occult blood by the detection of the perioxidase activity of the hemoglobin in the blood present in the faecal sample. They require catalysis of peroxide into oxygen and water, and the subsequent oxidation of a colorless dye (most often into a colored form). However, peroxidase activity is also found in meats and vegetables. In order to produce accurate results, these tests require restriction of the intake of certain foods, drugs, vitamins, and other substances prior to and during the sample collection period. The sensitivity of the most commonly used guaiac FOBT (Hemoccult) is approximately 50%. Despite a specificity of 98%, the positive predictive value for FOBT is low. Methods of detecting occult blood based on porphyrin (heme and protpoporphyrin IX) analysis or immunologic tests using anti-hemoglobin antibodies improve on these results. Immunochemical tests (FIT or iFOBT) that use anti-hemoglobin antibodies specific for human blood in extracts from stool do not require dietary restrictions; however, they are more complicated and more expensive than peroxidase-based tests. In addition, human hemoglobin in fecal samples degrades with time, resulting in a loss of antigenicity which can produce false negative results. Reported sensitivity of these immunologic tests varies widely but is typically 60-80% depending on the population tested. Specificity is estimated to be ~98%. Because of the intermittent nature of colorectal bleeding, the sensitivity of FOBT and FIT is directly proportional to the number of samples taken and the frequency of testing.

Recent developments in testing look specifically for mutations in DNA characteristic of colorectal neoplasia that are detectable in exfoliated epithelial cells in the stool (Pignone, et al., 2002; Ahlquist, et al., 2002). While neoplastic bleeding is intermittent, epithelial shedding is continual, potentially making stool-based DNA testing (i.e., also known as fecal DNA [f-DNA] and stool DNA [sDNA]) testing more sensitive than other methods.

Early studies of molecular feacal screening primarily focused on single mutations. Gene mutations in P53, K-ras, and BAT 26, for instance, have been linked to colorectal cancer and remain detectable in feacal samples. Colorectal neoplasms are varied in nature and no single mutation has been identified as being expressed universally. For this reason, multiple target assay panels (MTAP) are preferably used. PreGen-Plus™ (EXACT Sciences Corporation, Maynard, MA; Laboratory Corporation of America, Burlington, NC) is a single test that identifies the presence of 23 different microsatellite (MSI) mutations known to be associated with CRC, including mutations in BAT-26. Additionally, 21 other point mutations in other genes associated with CRC are included in this test: APC, K-ras, and p53. This test is further designed to detect long DNA fragments, which have been specifically associated with cells called non-apoptotic colonocytes, which are common in CRC. While this test is more sensitive than fecal occult blood testing, it is not as sensitive as colonoscopy and will miss about half of cancers in an average risk group of people without symptoms.

Increased DNA methylation is an epigenetic alteration that is common in human cancers and is often associated with transcriptional silencing. Aberrantly methylated DNA has also been proposed as a potential tumor marker for CRC detection. Genes such as vimentin, which are transcriptionally silent in normal epithelium, have been considered as targets for cancer-associated aberrant methylation and for use as cancer markers (JNCI Journal of the National Cancer Institute 2005 97(15):1124-1132). A combined assay utilizing hypermethylated vimentin gene (hV) and a two site DNA integrity assay (DY), demonstrated a sensitivity of 88% for CRC with a specificity of 82% (Am J Gastroenterol. 2008 Nov;103(11):2862-70). Further, ColoSure® is a single marker laboratory developed, stool based DNA test. This method examines DNA in exfoliated colon cells for cancer-associated aberrant methylation of the vimentin gene and reaches a sensitivity range of 72-77% and a specificity range of 83-94% in average risk individuals.

Protein tests provide an alternative method for detecting CRC. Tests assessing the presence of tumor-derived enzymes such as M2 pyruvate kinase (M2-PK), and/or proteins such as calprotectin, carcinoembryonic antigen (CEA), tissue inhibitor of metalloproteinase-1 (TIMP-1) and S100 calcium binding protein A12 (S100A12) have been described. A diagnosis of colorectal cancer using a combination of fecal occult blood and novel fecal protein markers S100A12 and TIMP-1 has been described in Clin

Gastroenterol Hepatol. 2008 Oct;6(10):1122-8. Dimeric isoenzyme of pyruvate kinase, M2-PK, expressed by tumor cells, has as well been proposed as a screening tool for CRC. The performance of fecal M2-PK has been evaluated with IFOBT and colonoscopy in Am J Gastroenterol. 2008 Jun;103(6):1496-504. Compared to immunochemical FOBTs, TuM2-PK does not have supplemental value for screening for CRC because of a lower sensitivity and specificity (Eur J Gastroenterol Hepatol. 2007 Oct;19(10):878-82)

Although combined assays for detecting CRC have been described, their approach targets either multiple protein markers or either multiple DNA alterations. To date, immunochemical tests and DNA tests for CRC detection have been evaluated and compared on a separate basis only.

EP0308227 describes a chemical fecal occult blood test employing a guaiac matrix.

EP0032782 describes a method for the detection of haemoglobin or decomposition products of haemoglobin in feces by means of an immunological reaction by using an antibody specific for human haemoglobin.

US7288413 describes methods that combine a chemical fecal occult blood test and an immunochemical fecal occult blood test.

WO 04/092709 concerns a fecal blood test involving the dispersement of a dye in toilet water.

EP0817968 describes several suitable stool collecting and testing methods and devices.

WO 05/017207 discloses that the vimentin gene can be a common target for methylation and epigenetic gene silencing in colon neoplasia, and may function as a candidate tumor suppressor gene.

WO 2008/084219 relates to detection of colorectal cancer based upon determining methylation of a number of different genes, including panels of genes.

WO 2006/113671 and WO 2008/010975 describe methylation markers relevant to colorectal cancer.

SUMMARY OF THE INVENTION

The invention provides a method of detecting a predisposition to, or the incidence of, colorectal cancer in a faecal sample comprising:

(a) detecting the presence of blood in the faecal sample, wherein detection of the presence of blood is indicative of a predisposition to, or the incidence of, colorectal cancer,

(b) detecting an epigenetic modification in the DNA contained within the faecal sample, wherein detection of the epigenetic modification is indicative of a predisposition to, or the incidence of, colorectal cancer

and based upon a positive result obtained in either (a) or (b) or in both (a) and (b) detecting a predisposition to, or the incidence of, colorectal cancer.

Also described herein is a method of sample processing, prior to carrying out a method of the invention, comprising removing a portion of a collected faecal sample and adding the removed portion of the sample to a buffer which prevents denaturation or degradation of blood proteins found in the sample.

The invention also provides a method of detecting a predisposition to, or the incidence of, colorectal cancer in a sample comprising detecting an epigenetic modification in a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1, wherein detection of the epigenetic modification in at least one of the genes in the panel is indicative of a predisposition to, or the incidence of, colorectal cancer.

The invention also provides a method of detecting a predisposition to, or the incidence of, cancer (and in particular colorectal cancer) in a sample comprising detecting an epigenetic modification in at least one gene selected from LAMA1 and CDO1, wherein detection of the epigenetic modification in the at least one gene is indicative of a predisposition to, or the incidence of, cancer (and in particular colorectal cancer).

The invention also relates to a method of detecting a predisposition to, or the incidence of, colorectal cancer (in particular in a faecal sample) comprising detecting an epigenetic

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