Minireview

Biomarkers for Early Detection of Colon Cancer

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Abstract
There is an increasing demand for biomarkers in colon cancer for risk assessment, early detection, prognosis, and surrogate end points. A number of biomarkers have been identified for early detection of colon cancer, although the risk factors have not been identified extensively. The major advances in understanding colorectal cancer include the identification and the involvement of APC, p53, and Ki-ras in the development and progression of the disease, the identification of the aberrant crypt foci as an early preinvasive lesion, and its relation to the development of cancer. Detecting malignant neoplasms in the early stages offers clinical advantages; therefore, the National Cancer Institute has established an Early Detection Research Network. The emphasis of the network is on translational research and collaboration among scientists.

Introduction
The identification and characterization of the genetic changes in the malignant transformation process have progressed rapidly over the last three decades (1–4). The predominant changes include deletions, rearrangements, and mutations leading to either inactivation or activation of specific target genes (5–8). Two major classes of genes, oncogenes and suppressor genes, are involved. Oncogenes are activated or deacti-vated genes whose products normally promote cell growth. Products include peptide growth factors, growth factor receptors, signal transduction factors, tyrosine kinase, and transcription-regulatory proteins. More than 30 oncogenes have been mapped within the human karyotype. Tumor suppressor genes normally regulate cell growth. These genes typically produce nuclear-regulatory proteins, which interact with other regulatory proteins to alter their activity (9, 10). The frequent involvement of a limited number of oncogenes, e.g., c-myc (11–13) and ras (14–17), in a wide variety of tumor scenarios suggests, nevertheless, that the number of the most critical genes needed for the transformation process may be limited. This suggests that we might be able to develop common approaches to detect these cancers.

Recently, genes not related to the oncogenes or suppressor genes have been implicated in carcinogenesis (18, 19). These genes are usually referred to as the MMR² genes. Although they were found years ago in bacteria and yeast, investigators have discovered two human genes, hMSH2 and hMLH1, that are homologous with the yeast MMR genes (4, 20). In addition to carcinosmas of the colon, these genes have also been implicated in carcinomas occurring in other sites including the lung, urinary bladder, and ovary. These advances have fueled optimism that abnormal genes, whether acquired or inherited, may be used to assess the risk of cancer or to detect existing cancer early, even in its preclinical stages.

Knowledge of the biology of tumor progression therefore allows us to identify specific tests that are useful for early detection or screening. Molecular probes, for instance, could detect altered DNA shed into the feces (CRC), into sputum (lung cancer), and in exfoliated cells in bladder washings (bladder cancer). Correlation of the molecular alterations with demographic data, risk factors, environmental exposure, family history, and dietary history may provide important information on the etiology of cancer.

Molecular genetic alterations could also contribute toward the assessment of risk. Risk assessment is the search for risk factors that provide the earliest evidence for the risk of cancer in persons not diagnosed with the disease. Biomarkers that are predictive of risk usually trigger more aggressive interventions and surveillance. Individuals who test positive for any risk marker become candidates for an intervention or for surveillance. The earliest risk factors are probably the inherited genetic defects, which is well demonstrated in the case of CRC (21). APC and HNPCC are associated with germ-line mutations in the APC gene and mismatch repair genes, respectively (22). Large bowel cancers often arising at a young age are found in almost 100% of individuals who inherit APC gene mutations.

Biomarkers of Risk versus Early Detection

Markers of risk and markers of early detection share the same outcome, namely, the incidence of disease. However, markers of risk and markers for early detection differ in the degree of certainty they convey regarding the existence of cancer. A risk factor confers significantly less than 100% certainty of cancer within a specified time interval, whereas early detection markers confer close to 100% certainty of cancer. Risk markers indicate that cancer is more likely to occur within a specified time in persons with the marker than in the general population. Early detection markers indicate the existence of

²The abbreviations used are: MMR, mismatch repair; APC, adenomatous polyposis coli; HNPCC, hereditary nonpolyposis colorectal cancer; FAP, familial adenomatous polyposis; IGF, insulin-like growth factor; MSI, microsatellite instability; PCR-PIRA, PCR-primer-introduced restriction analysis; GI, gastrointestinal; L-DNA, long DNA; Tcf, T-cell factor; COX, cyclooxygenase; JPS, juvenile polyposis; PCNA, proliferating cell nuclear antigen; SEB, surrogate end point biomarker; NSAID, nonsteroidal anti-inflammatory drug; CRC, colorectal cancer.
cancer or that cancer will occur with nearly a 100% certainty within a specified time interval.

From a screening perspective, all surrogate outcomes in individuals not diagnosed with cancer are risk factors. Colonic polyps, for instance, are a surrogate end point for screening and a risk factor for colon cancer. At least three elements are necessary to use risk factors as surrogate outcomes in screening: (a) the risk factor and its detection method must be properly defined; (b) the definitive outcome of interest and a description on how to assess it should be indicated; and (c) knowledge of the strength and direction of the relationship between the surrogate outcome and the definitive outcome over a specified time interval should be known. For a risk factor to be a useful surrogate outcome, it must be strongly connected to the definitive outcome, and the probability and direction of the relationship must be known.

Several criteria must be met before biomarkers can serve as risk factors or as markers for early detection: (a) the biomarker must be differentially expressed in normal, premalignant or high-risk, and tumor tissue; (b) the marker and its assay must provide acceptable predictive accuracy for risk or for the presence of cancer; and (c) the variance of the detection tests and the intra- and interlaboratory variance must be known. For biomarkers to serve as surrogate endpoints in prevention interventions, it is necessary to satisfy additional criteria: (a) the marker must be a determinant of outcome; (b) the marker must be modulated by chemopreventive agents; and (c) modulation or elimination of the risk marker must correlate with a decrease in cancer incidence. Risk markers are usually used as surrogate outcomes to detect the effect of a prevention intervention more rapidly than waiting for the definitive outcome. These criteria can be tested and evaluated in animal models and in human tissue specimens. Biomarkers for colon cancer are most likely to be found in stool specimens.

**Biomarkers for Risk**

There are very few biomarkers for risk of colon cancer, such as colonic adenomas (23). Biomarkers of risk can be inherited or acquired. With the identification and characterization of inherited defective genes implicated in the etiology of CRC, it has now become possible to identify individuals predisposed to this disease through genetic testing. For this reason, widespread genetic screening will not substantially reduce the incidence of the disease unless additional genes are found. Additional genes may exist because the incidence of colon cancer is 3–4 times higher in families with a history of this disease than in the general population (24, 25). The risk of CRC begins to increase after the age of 40 years and rises sharply at the ages 50–55 years; the risk doubles with each succeeding decade, reaching a peak by age 75 years.

Models made using tissues removed from patients with hereditary CRC have indicated that hereditary CRCs follow an autosomal dominant pattern of inheritance. However, hereditary CRCs do not develop just because a single copy of a mutated gene is inherited (because the mutated gene is not sufficient by itself to cause the development of CRC). A somatic mutation must also occur in the remaining normal copy of the gene, and this mutation must occur in a cell that has the capability to proliferate. Therefore, a stem cell must have two mutated copies for the inherited gene (germ-line mutation and somatic mutation) to develop hereditary CRC. The interaction of germ-line mutations and other risk factors (such as diet) is probably due to the effects of risk factors on the induction of somatic mutations. On the other hand, FAP is a rare autosomal dominant condition (about 1 in 8000 individuals and 1% of CRC) in which patients by definition have more than 100 colorectal adenomas. FAP is associated with the development of CRC within 15 years after the adenomas begin to develop. Many of the genes and chromosomes involved in the development of CRC were identified by evaluating neoplastic and uninvolved tissues from patients with FAP (to identify chromosomal abnormalities), and the associated involved genes in patients and immediate family members with this condition. Because of genetic heterogeneity and the complexity of cancer, none of these defective genes by themselves or in combination seem to be fully predictive of cancer development. Thus, new potential biomarkers need to be investigated.

The diagnosis of HNPCC requires CRC development in at least two generations and in at least three different family members. Of the three affected family members, one must be a first-degree relative of at least two other involved family members, and at least one of the tumors must have developed in a family member before the age of 50 years. A large proportion of patients with HNPCC have genetic defects in enzymes associated with DNA MMR. Inactivation of the normal allele results in genomic instability manifested by ubiquitous somatic mutations in MSI.

A Western lifestyle, typical of North America, Europe, or Australia, is associated with a high risk of CRC. Kaaks et al. (26) recently conducted a case-control study of a cohort of 14,275 women in New York and observed that chronically high levels of circulating insulin and IGF-I associated with a Western lifestyle increased CRC risk, possibly by decreasing IGF-binding protein 1 and increasing the bioactivity of IGF-I. Insulin and IGF-I regulate energy metabolism, stimulate cell proliferation and anabolic processes as a function of available energy and elementary substrates (e.g., amino acids), and inhibit apoptosis. Overeating, a lack of physical activity, and obesity cause insulin resistance and hence tend to increase plasma insulin concentrations. Insulin, in turn, can increase the bioactivity of the IGF-I by inhibiting the synthesis of certain IGF-binding proteins (26).

Dietary pyrolysis product from overcooked meats may increase colorectal carcinogenesis. Dietary components that are postulated to reduce the risk of this cancer include high starch diets and high consumption of calcium and vitamins (A, B, C, D, E, β-carotene, and folate). These dietary components interact with colonic bacteria with bile acids as well as with intestinal mucin and dietary carcinogens (27).

**Biomarkers for Early Detection**

Colorectal cancer is the second most common cause of cancer death in the United States. Several studies have concluded that there is a higher incidence of CRC in persons with polyps. The term polyp is applied to any mucosal lesion that projects in the lumen of the GI tract (27). About 90% of preinvasive neoplastic lesions of the colorectum are polyps or
polyp precursors (aberrant crypt foci). However, several types of polyps are not neoplastic, such as hyperplastic polyps. Therefore, neoplastic polyps are often referred to more specifically as adenomas or adenomatous polyps. Nevertheless, the term polyp is used by some clinicians or investigators to refer to neoplastic lesions.

There is evidence that colon cancer progresses from normal tissue to adenoma and carcinoma through an accumulation of genetic alterations (4, 28, 29). These molecular genetic alterations in various types of adenomas and carcinomas offer the opportunity to detect specific tumor-related changes in DNA. In colon cancer, for example, delineation of various stages during tumor progression offers a window of opportunity to intervene in the process by detecting stage-specific molecular changes (such as change in gene expression and accumulation of mutations). To date, we know of mutations and loss of heterozygosity that affect oncogenes such as K-ras (16) and several tumor suppressor genes including p53 (10), MCC (mutated in CRC; Ref. 30), APC (31), and DCC (deleted in colon cancer; Ref. 32). Some of these genetic alterations are being tested for their potential to serve as clinical biomarkers of colon cancer development. In the following section, we discuss some potential biomarkers of colon cancer.

APC. Germ-line APC mutations are considered an early detection marker because nearly 100% of individuals with the mutation will develop colon cancer in the future. For example, RFLP of chromosome 5q21–22 (for loss of the APC gene) has been shown to be useful for premorbid diagnosis and counseling in FAP. Although APC is a rare disorder, which occurs in only 1 in 500 persons and accounts for less than 1% of all cases of colonic carcinoma, mutations in the APC gene occur in 60% of patients with colorectal carcinoma and are thought to be the earliest genetic abnormalities in the progression of colorectal carcinoma. The I1307K mutation was found in approximately 6% of the Ashkenazi Jewish population and is associated with elevated risk of CRC (22). PCR-based tests are used to detect these mutations (33).

MMR. Molecular research has identified a new family of genes, commonly known as MMR genes, that predispose individuals to colon cancer. A number of genes are involved in MMR, such as hMSH2, hMLH1, hPMS2, hMSH3, and hMSH6 (for references, see Ref. 27). For example, HNPPCC, popularly known as Lynch Syndrome (34, 35), is found in as many as 1 in 200 individuals in the Western world and has been the subject of intense study for the involvement of MMR genes. Mutations of these genes produce instability of microsatellite sequences. Loss of MMR leads to greatly elevated (100–1000-fold) frequencies of point mutations (mutator phenotype) and MSI frameshifts. MSI occurs in most CRCs from patients with HNPPCC and therefore has been used as a biomarker for the detection of the HNPPCC syndrome.

K-ras. More than 50% of patients with adenocarcinoma of the colon carry a mutant allele of K-ras genes. The high frequency of this mutation and its early appearance in colon cancer point to its potential to serve as a biomarker for early detection (3, 36, 37). Because an apparently satisfactory assay for ras mutations in feces has already been developed (3, 37), the focus is on sensitive detection of this molecular marker in colonic effluent samples that are routinely obtained before colonoscopy. More importantly, stool samples contain sufficient amounts of DNA for PCR amplification and represent the colonic cell spectrum (36, 38, 39). Among various methods that have been developed for facilitating the screening of point mutations in human genomic DNA, PCR-PIRA is of particular interest due to its practicality and short procedure allowing detection of point mutations by simple restriction enzyme digestion directly after PCR amplification. However, one limitation of the PCR-PIRA method is the absence of restriction sites in the region of detection; thus, creation of the recognition site in primers has been introduced. However, false positive results generated from undigested normal DNA sequence are always obtained. This effect is compounded when it is used to analyze mixed cell populations in paraffin-embedded sections. Assay of a mutant band generated from normal DNA by densitometric quantitation enabled the determination of background values and thereby eliminated false positive results. Samples with higher ratios between mutant and normal bands than the background one after the first PCR-PIRA would be subjected to the second PCR-PIRA to confirm the results.

p53. For p53, unless the mutation is inherited, the situation is somewhat different from that of ras, in that an acquired defect of p53 or loss of the wild-type allele generally tends to be a later event in colorectal carcinogenesis, probably occurring near the time of invasion (32). Data from the literature suggest that at least a subset of familial cases of GI malignancies, sarcomas, and breast carcinoma is associated with inherited p53 mutations (40–42) and that a family history with these cancers in the pedigree may identify a group of patients in whom this molecular risk factor could be detected and those patients logically pursued with periodic screening. Genomic DNA of 118 colon cancer patients was analyzed for mutation detection in exons 4–8 of the p53 gene and codons 12/13 and 61 of the K-ras gene by single-strand conformational polymorphism and direct sequencing (36). The production of p53 and K-ras proteins was studied by immunohistochemistry. The overall frequency of mutations in the p53 gene was 35%, but the true frequency appeared to be higher (up to 56%). In the K-ras gene, the mutation frequency (15%) was significantly lower than that reported for colon cancer. In the p53 gene, the mutation frequency increased significantly with patient age. In a high proportion of patients (14%), the rectal tumors contained small subclones of tumor cells that displayed extremely rare mutations at codons 110 and 232 of the p53 gene. Hot spot codon 175 mutations were significantly less common in rectal cancer than in cancer of the colon. p53 mutations were tested in DNA samples using PCR-based approaches.

Kinases. The family of protein tyrosine kinases includes many known oncogenes and growth factor receptors that have been implicated in the pathogenesis of human cancers (43–46). The tyrosine kinases exert their cellular growth control by phosphorylating tyrosine residues on proteins. The prototype tyrosine kinase, the product of pp60c-src oncogene, is an example of an intracellular protein kinase. This kinase has been shown to be activated at an early stage of colon tumorigenesis (47). Other protein tyrosine kinases are those that function as cell surface receptors, such as epidermal growth factor receptor and HER-2/neu. HER-2/neu has been shown to be associated with the earliest events in human breast carcinoma, as well as...
with the later stages of this disease (47). The focus of the study was on early detection by the following algorithm: tyrosine kinases expressed by (a) normal colonic mucosa, (b) colonic polyps (both tubular and villous adenomas), (c) primary colon cancers, and (d) metastatic colon cancer were identified and catalogued. A kinase that is expressed in both normal and neoplastic colonic epithelium would not be expected to have a role in tumorigenesis and would not be studied further. A kinase with higher expression levels in colon polyps or primary colon cancers than in normal colonic mucosa would be a potential biomarker for the development of colon cancer. Similarly, a marker expressed by villous adenomas, as opposed to tubular adenomas, would be another candidate for a marker of malignant progression, given the different malignant potential of these two precancerous lesions. Immunohistochemical approaches were applied to detect these markers in tissues, and immunoassays were used in serum samples.

**Serum Markers.** Several markers, including serum markers, have been used to detect early onset and the recurrence of colorectal tumors: (a) early detection of colorectal neoplasia by detection in feces of mutations in ras or p53 or of changes in microsatellites of DNA (37, 48); (b) targeting of carciinoembryonic antigens and TAG-72 for immunotherapy or for tumor vaccines; (c) use of markers for atypical mucins (e.g., TAG-72) and neuroendocrine markers (neuron-specific enolase or argyrophilic stains) in diagnosis to separate colorectal carcinoma and neuroendocrine markers (neuron-specific enolase or argyrophilic stains) in differentiation to separate colorectal carcinoma from carcinoid tumors; and (d) use of changes in proliferation and cytormorphometric parameters in pre- and postchepmmoprevention colorectal biopsies as SEBs. In practical use of biomarkers to identify colorectal tumors (which may have more aggressive clinical behavior), the first step would be to characterize the general expression of biomarkers throughout the development of colorectal tumors to correlate these differences independent of tumor stage. Monoclonal antibody B72.3 binds to TAG-72 antigen present in the serum of CRC patients. Petersen et al. (49) detected TAG-72 in 86% of serum samples using an enzyme immunoassay. Understanding the limitations in the detection of antigen expression in archival tissues is necessary to use biomarkers (for example, the sensitivity and specificities of antibodies to antigens in paraffin-embedded sections and the effects of tissue fixation and tissue processing on the detection of specific antigens). Failure to recognize these issues may result in false negative results, leading to inappropriate exclusion of patients from treatment or inappropriate diagnosis of protocols and to confounding comparisons of populations defined by these markers.

**L-DNA.** The high-integrity DNA or L-DNA has proven to be the most informative component marker of the stool DNA assay panel, and the marker has been found in 61% of colon cancers (3). Longer template DNA is an epigenetic phenomenon consistent with the known abrogation of apoptosis. DNA in normal stools would exist primarily in fragments (180–200 bp), whereas that of colon cancer patient should contain a subset of L-DNA arising from dysplastic cells. For the assay, the primers were designed to give products much longer than 200 bp, and thus only longer DNA was used as the template. Fragments as large as 2.4 kb have been observed by following this assay.

**β-Catenin.** An increase in cytoplasmic β-catenin levels and subsequent β-catenin/Tcf-lymphoid enhancer factor complex formation are believed to be important events in the early stage of colon carcinogenesis (4, 50–52). This complex binds to the DNA and results in overexpression of prostaglandin endoperoxide H synthase-2 (COX-2), induced by nitric oxide. Recently, Miyaki et al. (53) reported frequent mutations of the β-catenin and APC genes in primary colorectal tumors from patients with HNPCC. Their previous study detected a low frequency of APC gene mutation (21%) in colorectal tumors from HNPCC patients, in contrast to a high frequency of APC gene alteration (>70%) in non-HNPCC tumors. Because both β-catenin and APC gene mutations have recently been shown to activate the same signaling pathway, Miyaki’s group analyzed β-catenin mutation in HNPCC tumors. A notable frequency of β-catenin gene mutation (43%, 12 of 28) was found to occur in HNPCC colorectal tumors. However, β-catenin mutations were not detected in tumors with APC mutations. All β-catenin mutations detected in HNPCC tumors existed within the regulatory domain of β-catenin. Immunohistochemical staining of tumors with this mutation showed accumulation of β-catenin protein in nuclei. These and previous data from their laboratory suggest that activation of the β-catenin-Tcf signaling pathway, through either β-catenin or APC mutation, contributes to HNPCC carcinogenesis in approximately 65% of cases. β-Catenin is assayed in serum by immunological methods.

**bcl-2 Gene.** The product of the bcl-2 gene (Bcl-2) is a 26-kDa protein that inhibits apoptosis. Several studies have evaluated expression of Bcl-2 protein using immunohistochemistry (54–59). Expression of Bcl-2 in CRCs has been demonstrated as a favorable prognostic factor in Austrian (60) and German populations (58). However, such prognostic significance could not be seen in a large United States population with colorectal carcinomas, although the incidence of Bcl-2-expressing tumors was similar to that seen in the European studies. Results from other laboratories (61) indicated that patients with colorectal carcinomas that express Bcl-2 have a better clinical outcome than patients with CRCs that do not express Bcl-2 (62). The reasons for the reported differences in the prognostic importance of Bcl-2 are not known, but they may be due to geographic and/or ethnic/racial variations in the patient populations of the various studies.

**Proliferation in Aberrant Crypt Foci.** Proliferative cells are typically restricted to the lower two-thirds of the normal colorectal crypt. An increase in cellular proliferation has been observed in adenomas as compared with benign epithelium, with the highest proliferation seen in adenomas with high-grade dysplasia. In addition, a shift of proliferation toward the luminal surface of the epithelium occurs in adenomas as well as aberrant crypt foci. Increased proliferation as well as an upward shift of the proliferative component has also been observed in the “transitional” epithelium adjacent to adenomas or colorectal carcinomas. Furthermore, a shift or expansion of the proliferating zone toward the luminal surface has been reported in uninvolved colonic epithelium of patients with adenomatous polyps or colorectal carcinomas; however, a large study failed to demonstrate a difference in proliferation as measured by Ki-67 in the benign colorectal epithelium in patients with colorectal carcinomas as compared with controls (63). Although controversial, the increased proliferation observed in mucosa distant from CRC has been interpreted as evidence that the entire
colorectum may undergo specific premalignant changes, such as the field effect. Such changes in proliferation could be the result of stimulation by paracrine factors released by malignant cells or colonization of a large proportion of the colorectal epithelium by a clonal expansion. An increase in proliferation in the normal colorectal crypts of individuals with a high risk of CRC based on family history was demonstrated by Rooney et al. (64). Other investigators described a shift in proliferation from the base toward the surface in the intervening benign mucosa (in patients with FAP) (65). Another study demonstrated a shift in the proliferative cells toward the lumen in uninvolved colonic epithelium of FAP patients (66). Similarly, increased proliferative rates and an expansion of the proliferative zone to the luminal surface of the epithelium have been observed in the colonic epithelium of HNPCC patients (34, 67, 68).

**Cyclin D1.** Several studies have demonstrated increased expression of the cyclin D1 protein in 30–45% of CRCs when compared with uninvolved epithelium (69, 70). In addition, increased expression of cyclin D1 protein was observed in 34% of adenomatous polyps (69). Cyclin D1 is measured in serum by immunological assays.

**Cyclin E1.** Increased cyclin E1 levels were observed in colorectal carcinomas as compared with uninvolved colonic mucosa (in 90% of patients; Ref. 71). A direct association of increased cyclin E1 levels with the graded atypia within adenomas and with local invasiveness as well as proliferation potential of CRC (72, 73). Amplification of cyclin E1 has been reported in 9% of primary CRCs (73).

**SMAD4.** Woodford-Richens et al. (23) demonstrated allelic loss at SMAD4 in polyps from JPS patients and used fluorescent in situ hybridization to demonstrate clonal origin of the epithelium. Germ-line mutations in SMAD4 (DPC4) account for about one-third of this population. They found allele loss at the SMAD4 locus on 18q in polyps from JPS individuals with a germ-line SMAD4 mutation, showing that SMAD4 acts as a tumor suppressor gene in JPS polyps. These mutations were detected in DNA (isolated from cells) using PCR-based methods (4).

**Panel of Markers.** Ahlquist’s group (3, 74) has found that DNA released from cells that regularly slough into the stool can be used to identify cancer or precancerous polyps in the colon. They have developed a multitarget DNA assay panel that shows a remarkably higher sensitivity and specificity than traditional fecal blood tests (3). The multitarget DNA assay panel targets point mutations at any of 15 mutational hot spots on K-ras, p53, and APC genes; Bat-26, a microsatellite instability marker; and L-DNA. In the first pilot study on stools from 22 patients with CRC, 11 patients with adenomas (≥1 cm) and 28 colonoscopically normal controls revealed a sensitivity for cancer of 91% and a sensitivity for adenomas of 73% with a specificity of 100%. Thus, an assay of altered DNA holds promise as a stool screening approach for colorectal neoplasia. The putative biomarkers for colon cancer are shown in Table 1 and Fig. 1.

### Putative biomarkers for detection

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal location</th>
<th>Specimens</th>
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<tbody>
<tr>
<td>APC</td>
<td>5q21</td>
<td>Stool DNA</td>
</tr>
<tr>
<td>p53</td>
<td>17p13</td>
<td>Stool DNA, tissue</td>
</tr>
<tr>
<td>K-ras</td>
<td>12p</td>
<td>Stool DNA, colonic effluent, colonic DNA</td>
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<tr>
<td>CTNNB1</td>
<td>(−4% to 15%)</td>
<td>3p22</td>
</tr>
<tr>
<td>Src</td>
<td>(2%)</td>
<td>20q11</td>
</tr>
<tr>
<td>SMAD4</td>
<td>(16%)</td>
<td>18q21</td>
</tr>
<tr>
<td>SMAD2</td>
<td>(6%)</td>
<td>18q21</td>
</tr>
<tr>
<td>DCC</td>
<td>(3%)</td>
<td>18q21</td>
</tr>
<tr>
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<td>2p21</td>
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<td></td>
<td>2q31–33</td>
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<td>hMSH6</td>
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<td>2p21</td>
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<td>Ploidy</td>
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<td>Tissue</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Serum</td>
<td>Serum</td>
</tr>
<tr>
<td>CEA</td>
<td>Serum</td>
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*Numbers in parentheses represent prevalence of genetic mutations reported in Ref. 4.
Specimens listed are those in which the assay for mutations can be performed.

Biomarkers as Intermediate End Points in Colorectal Cancer Prevention

Although hyperproliferation has been postulated as a relatively early event in the pathway to colorectal carcinoma, it may not be a necessary step, and incorrect inferences may be drawn from a study that uses a proliferation marker as the sole surrogate end point for invasive cancer. An example of this is an adenoma recurrence trial at Dartmouth Medical School. Calcium had no effect on proliferation markers but showed a modest reduction in adenoma recurrence in the calcium arm (29). Alternatively, the effect of an intervention agent on hyperproliferation may be counteracted by its effect on another pathway, so there is no net effect on colon cancer. Surrogate end points must be a determinate of outcome.

The 36-kDa PCNA is an example of a colorectal cell proliferation marker. PCNA can be detected in cells in rectal mucosal crypts by immunohistochemical analysis. The PCNA-labeled cells are indicative of dividing/proliferating cells and mainly reflect DNA synthesis. Various indices of the total proportion of crypt cells that are labeled as well as indices reflecting the location of proliferating cells within the crypt can be determined.

Even when a marker meets these criteria when tested with one agent, it cannot be used with confidence in the evaluation of another agent because the active agent may affect both a pathway through the surrogate and a pathway through another marker. Even if the effect of the active agent on the alternative pathway does not offset its effect on the surrogate, the same cannot be said to be necessarily true for another agent. One could be more confident if the two agents under consideration operated through exactly the same pathway, but new agents, even those structurally similar to proven agents, have unanticipated actions and therefore have unintended consequences.

### Are Adenomas a Logical Surrogate End Point?

Adenomas satisfy the criteria for a surrogate end point for prevention studies and for early detection. They are a risk factor for colon cancer and a surrogate outcome for screening because...
most tumors appear to arise from polyps. Studies indicate that they may be modulated by chemopreventive agents, although it remains to be determined whether chemical modulation leads to a reduction in the incidence of colon cancer. Adenomas can be identified and removed through colonoscopy or sigmoidoscopy. Thus, as a risk factor, the polyp serves to initiate a preventive intervention. Although relatively common, the majority of adenomas do not progress to invasive cancer.

Nonsteroidal Chemopreventive Agents and Biomarkers

SEBs have become widely used in short-term cancer chemoprevention trials in place of cancer end points. Here we have discussed the criteria relevant to the selection and validation of SEBs for colon cancer risk and the use of SEBs in colon cancer chemoprevention trials. As with a number of other cancers, colon carcinogenesis is the result of a multistep process in which an increasing number of alterations, including specific gene mutations, occur as cells progress from a normal state to a precancerous state of increasing size and dysplasia to cancer and finally to metastatic disease. Ideally, a SEB would show differential expression between the various phases of colon carcinogenesis (i.e., normal, premalignant, and malignant tissues) and would be associated with risk of colon cancer. Some SEBs that do not meet these criteria may still be useful for demonstrating the effect of a particular agent. It is also necessary that a SEB be measured in tissues (or other sample material) accessible for multiple and sequential sampling and allow for the development of appropriate quality control procedures. Some SEBs must have the potential for modulation by chemopreventive agents. Validation of SEBs for use in chemoprevention studies requires that a relationship between the marker and subsequent risk of cancer be established. Also, the assay reliability and accuracy for each SEB must be determined adequately in well-designed prospective studies.

The epidemiological studies that have detected a 40–50% decrease in risk of CRC in individuals who regularly use aspirin and other NSAIDs such as celecoxib could not have included because of such regular NSAID use (29, 75–78). NSAIDs appear to act via induction of apoptosis, programmed cell death, as potential CRC chemopreventive agents. NSAIDs can alter the production of different metabolites of polyunsaturated fatty acids (linoleic and arachidonic acids) through effects on lipoxigenases and COXs. 15-Lipoxygenase-1 is the main enzyme for metabolizing colonic linoleic acid to 13-S-hydroxyoctadecadienoic acid, which induces apoptosis. Clinical trials with NSAIDs in patients with FAP have demonstrated that treatment with NSAIDs caused regression of preexisting adenomas. The surrogate end point in these studies was polyp burden (77).

Steinbach et al. (75) investigated the effect of celecoxib, a selective inhibitor of COX-2, on colorectal polyps in 77 patients with FAP. Patients underwent endoscopy at the beginning and end of the study. Six months of treatment resulted in a significant reduction in the number of colorectal polyps.

Several, but not all, epidemiological studies have reported a reduction in colon cancer incidence associated with the use of aspirin (75–78). Several cohort studies suggested a preventive effect of aspirin. Among a group of over 600,000 adults enrolled in an American Cancer Society study, mortality in regular users of aspirin was about 40% lower for cancers of the colon and rectum (78, 79). In a study of over 11,000 men and women in Sweden with rheumatoid arthritis (and presumably ingesting
NSAIDs), colon cancer incidence was 37% lower than predicted from cancer registry data, and rectal cancer was 28% lower than predicted from cancer registry data (80). In a report from the Health Professionals Follow-up Study of 47,000 males, regular use of aspirin (at least 2 times/week) was associated with a 30% overall reduction in CRC including a 50% reduction in advanced cases (81). In contrast to the results from cohort studies, the Physicians’ Health Study, which was a randomized controlled trial designed to test whether low-dose aspirin (325 mg of aspirin every other day) would reduce cardiovascular disease mortality, did not show a reduction in invasive cancers or adenomas after 4.5 years among the participants assigned to receive aspirin (82). The interpretation of the data is complicated by the possibility that prevalent tumors at the time of randomization could have been detected early and by the differences in age among the study populations (83, 84). Several studies conducted in a rigorous manner have demonstrated the effectiveness of sulindac in reducing the size and number of adenomas in familial polyposis (85, 86). The NSAID piroxicam, at a dose of 20 mg/day, reduced mean rectal prostaglandin concentration by 50% in individuals with a history of adenomas (87). In this study, mucosal prostaglandin concentration was measured as the end point. Different chemopreventive agents including sulindac sulfate used in clinical trials are shown in Table 2.

Preclinical efficacy studies have provided scientifically sound evidence as to how NSAIDs act to retard, block, or reverse colonic carcinogenesis. Equally exciting are opportunities for effective chemoprevention with selective COX-2 inhibitors in a variety of animal models of colon cancer. Selective COX-2 inhibitors such as celecoxib have been proven to be effective chemopreventive agents against colonic carcinogenesis with minimal GI toxicity. Similarly, the short-chain fatty acid butyrate, produced by microbial fermentation of dietary fiber in the large intestine, has been used to intervene in major pathways of colonic epithelial cell maturation, such as cell cycle arrest, lineage-specific differentiation, and apoptosis. β-Catenin and polyp burden were used as end point markers in these studies (76).

The potential for the use of NSAIDs as a primary prevention measure is intriguing. However, there are several unresolved issues that mitigate against making general recommendations for their use. These include a paucity of knowledge about the proper dose and duration for these agents. There is also concern about whether the potential preventive benefits would balance such long-term risks as GI ulceration and hemorrhagic shock for the average-risk individual (88).

### Future Direction

The National Cancer Institute has established a multi-institutional network, the Early Detection Research Network, to develop sensitive and specific tests for the earlier detection of cancer. The network links centers of expertise in tumor biology, diagnostic technologies, and clinical trial methodology in academia and industry to develop high-throughput assays suitable for clinical application. To date, the National Cancer Institute has funded 18 biomarker developmental laboratories, 8 clinical and epidemiological centers, 2 biomarker validation laboratories, and one data management and coordinating center. These laboratories/centers will cover a range of study designs, technology development, and innovative approaches from genomics to proteomics in pursuit of developing molecular, genetic, and biological markers for earlier cancer detection and for the identification of high-risk subjects.

### References


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**Table 2**  Chemopreventive agents used in clinical trials

<table>
<thead>
<tr>
<th>Nonsteroidal chemopreventive agent</th>
<th>End point</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celecoxib</td>
<td>Adenomas</td>
<td>75</td>
</tr>
<tr>
<td>Aspirin</td>
<td>Adenomas</td>
<td>29, 75–78, 81–84</td>
</tr>
<tr>
<td>Sulindac</td>
<td>Adenomas</td>
<td>85, 86</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>Mucosal prostaglandin</td>
<td>87</td>
</tr>
</tbody>
</table>


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Sudhir Srivastava, Mukesh Verma and Donald E. Henson


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