Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field

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Molecular Pathologic Epidemiology of Colorectal Neoplasia: An Emerging Transdisciplinary and Interdisciplinary Field

Shuji Ogino, **Andrew T. Chan**, **Charles S. Fuchs**, and **Edward Giovannucci**
Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA USA (SO); Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA USA (SO, CSF); Cancer Epidemiology Program and Gastrointestinal Malignancies Program, Dana-Farber/Harvard Cancer Center, Boston, MA USA (SO, ATC, CSF, EG); Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA USA (ATC); Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA USA (ATC, CSF, EG); Departments of Epidemiology and Nutrition, Harvard School of Public Health, Boston, MA USA (EG)

Abstract

Colorectal cancer is a complex disease resulting from somatic genetic and epigenetic alterations, including locus-specific CpG island methylation and global DNA or LINE-1 hypomethylation. Global molecular characteristics such as microsatellite instability (MSI), CpG island methylator phenotype (CIMP), global DNA hypomethylation, and chromosomal instability cause alterations of gene function in a genome-wide scale. Activation of oncogenes including *KRAS, BRAF* and *PIK3CA* affects intracellular signaling pathways and has been associated with CIMP and MSI. Traditional epidemiology research has investigated various factors in relation to an overall risk of colon and/or rectal cancer. However, colorectal cancers comprise a heterogeneous group of diseases with different sets of genetic and epigenetic alterations. To better understand how a particular exposure influences the carcinogenic process, somatic molecular changes and tumor biomarkers have been studied in relation to the exposure of interest. Moreover, an investigation of interactive effects of tumor molecular changes and the exposures of interest on tumor behavior (prognosis or clinical outcome) can lead to a better understanding of tumor molecular changes, which may be prognostic or predictive tissue biomarkers. These new research efforts represent "*Molecular Pathologic Epidemiology*", which is a multidisciplinary field of investigations of the interrelationship between exogenous and endogenous (e.g., genetic) factors, tumoral molecular signatures and tumor progression. Furthermore, integrating genome-wide association studies (GWAS) with molecular pathologic investigation is a promising area. Examining the relationship between susceptibility alleles identified by GWAS and specific molecular alterations can help elucidate the function of these alleles and provide insights into whether susceptibility alleles are truly causal. Although there are challenges, molecular pathologic epidemiology has unique strengths, and can provide insights into the pathogenic process and help optimize personalized prevention and therapy. In this review, we overview this relatively new field of research and discuss measures to overcome challenges and move this field forward.

Correspondence to: Shuji Ogino, MD, PhD, MS(Epidemiology), Center for Molecular Oncologic Pathology, Dana-Farber Cancer Institute, Brigham and Women's Hospital, Harvard Medical School, 44 Binney St., Room JF-215C, Boston, MA 02115 USA, Telephone: +1-617-632-3978; Fax: +1-617-582-8558, shuji_ogino@dfci.harvard.edu.

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Introduction to Molecular Pathologic Epidemiology

Molecular pathologic epidemiology, the concept of which has been consolidated by Ogino and Stampfer, [1] is a relatively new field of epidemiology based on molecular classification of cancer. In molecular pathologic epidemiology, a known or suspected etiologic factor is examined in relation to a specific somatic molecular change, in order to gain insights into the carcinogenic mechanism.[1] In recent years, there is a new direction of this field where we examine an interactive effect of tumoral molecular features and a lifestyle or other exposure factor on tumor behavior (prognosis or clinical outcome).[2] In this review, we focus on colorectal neoplasia, overview current status of molecular pathologic epidemiology, describe various challenges in this field, and propose future directions.

Molecular Classification of Colorectal Cancer

Colorectal cancer is a disease which is characterized by uncontrolled growth of colorectal epithelial cells. According to the multistep carcinogenesis theory, [3,4] colorectal epithelial cells accumulate a number of molecular changes and eventually become fully malignant cells. Genetic and epigenetic events during the carcinogenesis process differ considerably from tumor to tumor. Thus, colorectal cancer is not a single disease. Rather, colorectal cancer encompasses a heterogeneous complex of diseases with different sets of genetic and epigenetic alterations. Essentially, each tumor arises and behaves in a unique fashion that is unlikely to be exactly recapitulated by any other tumor.[5]

We typically classify colorectal cancers into categories according to a well-defined molecular feature (e.g., microsatellite instability, MSI-high vs. microsatellite stability, MSS), because substantial evidence suggests that tumors with similar characteristics (e.g., MSI-high) have arisen by similar mechanisms and will behave in a similar fashion.[5] Thus, the major purposes of molecular classification are: 1) to predict natural history (i.e., prognosis); 2) to predict response or resistance to a certain treatment or intervention; and 3) to examine the relationship between a certain etiologic factor (i.e., lifestyle, environmental or genetic) and a molecular subtype, so that we can provide evidence for causality and optimize preventive strategies.

For any marker for molecular classification, we need to consider two key points. The first question is whether a given molecular feature reflects genome-wide changes. For example, MSI, chromosomal instability (CIN), the CpG island methylator phenotype (CIMP), and global DNA hypomethylation reflect genome-wide or epigenome-wide aberrations. Because these molecular features often confound the relationship between a locus-specific change and an exposure or outcome of interest, it is important to consider potential confounding by these genome-wide features whenever one examines locus-specific changes. The second question is whether a given molecular change has by itself driven cancer initiation or progression, or is simply linked to other important molecular events. For example, loss of heterozygosity (LOH) events may not by itself cause tumor progression; rather, underlying genomic instability (i.e., CIN) or functional loss of important genes within the lost chromosomal segment may cause tumor progression. Nevertheless, even if a given molecular change is consequential rather than causal, the change not only can be a good

surrogate marker of a certain cancer pathway, but also may ultimately become a driver in later steps of tumor progression.

Emergence and Evolution of Molecular Pathologic Epidemiology

Traditional epidemiology research has investigated lifestyle, environmental or genetic factors that might increase or decrease risk of developing colorectal cancer.[6,7] The weight of the evidence, in conjunction with results from in vitro and animal models or human experimental trials, can lead to particular factors being widely considered to be etiologically linked to cancer. Etiologic factors which have been implicated in colorectal carcinogenesis include red and processed meat, excess alcohol intake, deficiency of B and D vitamins, obesity, physical inactivity, diabetes mellitus, smoking, family history of colorectal cancer, inflammatory bowel diseases, among others.[8] More recently, the field of molecular epidemiology has evolved since 1990s, encompassing genome-wide association studies (GWAS) since 2000s.[9,10] Molecular epidemiology refers to a specialized field of epidemiology where investigators examine genetic and molecular variation in a population and its interaction with dietary, lifestyle or environmental factors, to find clues to plausible causative links between etiologic factors and diseases. However, the mechanisms with which plausible etiologic factors influence the carcinogenic process remain largely speculative.

In traditional molecular pathology, investigators examine molecular characteristics in tumor cells to better understand carcinogenic processes and tumor cell behavior. In the last two decades, our knowledge on somatic molecular alterations in the carcinogenic process has substantially improved.[5,11–16] As illustrated in Figure 1, these two approaches, epidemiology and molecular pathology, have converged to improve our understanding of how certain exposures influence carcinogenesis by examining molecular pathologic marks of tumor initiation or progression, in relation to the exposures of interest.[1] This represents a relatively new field of scientific investigation, which has been coined "*Molecular Pathologic Epidemiology*".[1] If a specific lifestyle or dietary factor can prevent the occurrence of a specific somatic molecular change, it would add considerable scientific basis to such a preventive strategy. Specificity of the association for a certain molecular change provides further evidence for a causal relationship. For an individual who has a susceptibility to a specific somatic molecular change, we may be able to develop a personalized preventive strategy, which targets specific molecules or pathways.

Table 1 is a comprehensive list of molecular pathologic epidemiology studies on colorectal neoplasia.[17–45][46–88][89–101][102–151] One challenge is that, despite a number of studies on some topics (e.g., one-carbon metabolism gene polymorphisms and epigenetic changes), generalisable confirmed findings are uncommon. We discuss possible reasons and various challenges in a later section. Nonetheless, there have been observations confirmed by notable independent studies: a case-control study by the Slattery et al.'s group[114] and a prospective cohort study by Iowa Women's Health Study (IWHS)[78] have independently shown that cigarette smoking is associated with CIMP-positive tumor, and with *BRAF*-mutated tumor. As another example, the association between obesity and microsatellite stable (MSS) tumor has been demonstrated by three independent case-control studies, including the Slattery et al.'s group, [123] North Carolina Colon Cancer Study (NCCCS), [116] and Colon Cancer Family Registry (CCFR).[35] With regard to germline genetic variants and molecular changes, *MLH1* rs1800734 promoter SNP has been associated with MSI-high tumors in three independent case-case and case-control studies, [38,108,115] and *MGMT* rs16906252 promoter SNP has been associated with *MGMT* promoter methylation and loss of expression in colorectal cancer[94] and normal colorectal mucosa and peripheral blood cells in individuals without cancer.[152,153] These consistent data across different

studies increase validity of each other's findings and support etiologic roles of cigarette smoking, obesity and germline variants in specific pathways of colorectal carcinogenesis. Ultimately, our understanding of these specific neoplasia pathways will clarify areas for disease intervention.

Recently, GWAS have identified a number of candidate susceptibility loci for colorectal cancer.[9,10] Currently, a significant limitation in interpreting GWAS results is our limited understanding of the functional relevance of risk alleles identified by GWAS. As a promising future direction, a molecular pathologic epidemiology approach can be used to validate findings of GWAS in certain ways. First, if a candidate cancer susceptibility variant is hypothesized to regulate expression of a nearby gene, the relationship between the variant and gene expression in tumor tissue can be examined.[59] Second, if a candidate variant is hypothesized to cause a genetic or epigenetic alteration in a critical pathway, the relationship between the variant and tumoral molecular alterations in the particular pathway can be examined.[134] Specificity of the relationship between the variant and the tumor molecular alterations will provide additional evidence to support a causal effect of the putative cancer susceptibility allele.

Additional examples of studies and findings on three specific areas (energy balance, inflammation, and one-carbon metabolism) will be discussed in later sections because these have been particularly active areas of investigations.

Study Design in Molecular Pathologic Epidemiology

Figure 2 illustrates three basic approaches to investigate the relationship between an exposure (e.g., smoking) and a tumor molecular change (e.g., *KRAS* mutation). A fourth approach, an interventional cohort study (not illustrated in Figure 2) is a gold standard; however, to date no interventional molecular pathologic epidemiology data have been published.

The first approach is a "case-case" approach (Figure 2A), where tumors are classified into subtypes according to a molecular feature, and then distributions of an exposure variable of interest among different subtypes are compared. For example, if it is hypothesized that smoking causes *KRAS* mutation, one may expect to observe that *KRAS*-mutated cancer patients contain a higher fraction of smokers than *KRAS*-wild-type cancer patients. A limitation of this approach is that it is not possible to obtain information on distribution of an exposure variable among the background population that has given rise to the cancer cases. Thus, the direction of any association cannot be determined; if there is a positive association between smoking and *KRAS*-mutated tumors (i.e., a negative association between smoking and *KRAS*-wild-type tumors), it is uncertain whether smoking protects against *KRAS*-wild-type tumors, or smoking causes *KRAS*-mutated tumors.

The second approach is a case-control study (Figure 2B), where non-cancer control subjects should ideally be randomly sampled from the background population that has given rise to the cancer cases. In traditional cancer epidemiology, distributions of an exposure of interest between cases and controls are compared. In molecular pathologic epidemiology, one can compare distributions of a given exposure between cancer cases with a specific molecular alteration (e.g., *KRAS* mutation), cancer cases without the alteration, and controls. If the exposure has caused the particular alteration, it is expected to see a higher fraction of exposed individuals among cancer cases with the alteration but not among cancer cases without the alteration, compared to controls. Nevertheless, case-control approaches in molecular pathologic epidemiology face the same inherent limitations of traditional case-control studies. Such caveats include recall bias, differential selection bias between cases

and controls, among others. One advantage of a case-control design over a prospective cohort design is its relative ease to recruit a large number of colorectal cancer cases. Important examples of case-control studies include Colon Cancer Family Registry (CCFR), [26, 34, 35, 39, [56], [66], [76], [77], [79], 80, 106, 107, 154] a population-based case-control study of colorectal cancer by Slattery et al., [41–45, 113–115, 123–139, 155, 156] and the Molecular Epidemiology of Colorectal Cancer Study (MECCS) in northern Israel.[59, 110, 111, 157–159]

The third approach is a prospective cohort study (Figure 2C), which is less prone to potential bias related to case-case and case-control designs. A nested case-control design, a case-case design within a prospective cohort study, and a case-cohort design[160] are derivatives of prospective cohort studies. In molecular pathologic epidemiology, investigators examine the incidence rates of cancer with a specific alteration (e.g., *KRAS* mutation) in exposed vs. unexposed individuals, as well as the incidence rates of cancer without the specific alteration in exposed vs. unexposed individuals. If the exposure causes the particular alteration, one would expect to see a higher incidence rate of cancer with the alteration in exposed individuals than in unexposed individuals, and similar incidence rates of cancer without the alteration between the exposed and unexposed groups. In molecular pathologic epidemiology of colorectal cancer, to date, seven prospective cohort studies have published substantial data: European Prospective Investigation into Cancer and Nutrition (EPIC), [89, 103, 161–164] the Health Professionals Follow-up Study (HPFS), [2, 18–25, 36, [61], [65], [72], [91]–101, 118, 119, 121] the Iowa Women's Health Study (IWHS), [78, 165, 166] the Melbourne Collaborative Cohort Study (MCCS), [53, 167–169] the Netherlands Cohort Study (NLCS), [28–33, 46–48, [64], [82]–[84], 141, 144, 146, 147] the Northern Sweden Health and Disease Study (NSHDS), [142, 170, 171] and the Nurses' Health Study (NHS).[2, 18–25, 36, [61], [65], [72], [91]–[101], 117–119, 121, 172] Prospective cohort studies require substantial amounts of participants, follow-up time and funding support, and substantial efforts of researchers and other personnel. Therefore, judicious utilization of the existing resource of prospective cohort studies is a cost effective approach.

Interactive Effect of Exposure and Tumoral Feature on Tumor Aggressiveness: New Direction of Molecular Pathologic Epidemiology

As a new direction of molecular pathologic epidemiology, our group has started examining how lifestyle or genetic factors interact with tumor molecular features to influence tumor cell behavior (prognosis or clinical outcome). Table 2 lists studies on interactive prognostic effects of lifestyle or genetic factors and tumoral features in colorectal cancer.[2,18–21,57,[72],[92],[93],95–101,173–178] In traditional molecular pathology, investigators examine tumoral molecular characteristics to better predict prognosis and response to specific treatments.[11] In addition to tumoral molecular features, lifestyle, environmental or genetic factors likely influence tumor cell behavior through the tumor microenvironment. Lifestyle factors (e.g., physical activity or smoking) or genetic factors (e.g., SNPs or family history) have been shown to influence clinical outcome of colorectal cancer patients.[168,179–185] To better understand how a certain lifestyle, environmental or genetic factor influences tumor cell behavior, it is of interest to examine interactive prognostic effects of the lifestyle, environmental or genetic factor and tumoral molecular features. If a particular exposure is associated with worse outcome only among patients with a specific tumoral molecular change, but not among those without the molecular change, this provides evidence that the exposure factor might influence tumor aggressiveness through that molecular change or pathway. We will discuss specific examples in the following sections.

Interactive Prognostic Effects of Obesity, Physical Activity and Tumoral Changes

Studies have shown that obesity is associated with worse survival of colon cancer patients.[168,186–189] However, how obesity affects clinical outcome of cancer patients remains largely unknown. In 2008, our group started a new direction of molecular pathologic epidemiology, to examine an interactive prognostic effect of obesity (prediagnosis body mass index, BMI) and FASN (fatty acid synthase) expression in colon cancer.[2] We found that the adverse prognostic effect of obesity was present in patients with FASN-positive colon cancers, but not in patients with FASN-negative colon cancers.[2] These data suggest that excessive energy present in obese patients may contribute to growth and proliferation of tumor cells with FASN activation.[2] This study has opened new opportunities for investigating how lifestyle factors affect tumor cell behavior through cellular molecules. In traditional epidemiology, investigators examine the relationship between an exposure factor (e.g., obesity) and survival of cancer patients regardless of tumor molecular subtype; thus, mechanistic hypotheses remain speculative. For example, it is hypothesized that obesity increases tumor aggressiveness potentially through a certain cellular molecule such as FASN. Without analysis of FASN in tumor, the hypothesis still remains speculative. In molecular pathologic epidemiology, we can specifically test the hypothesis by examining the relations between obesity and patient survival in tumor FASN-positive cases and between obesity and patient survival in tumor FASN-negative cases.[2] If the hypothesis is true, we expect to observe the significant obesity/survival relationship in FASN-positive cases, but not in FASN-negative cases.[2]

Our subsequent investigations have found that a number of other tumor molecular changes interact with prediagnosis BMI to modify tumor aggressiveness.[96,98,99] Those tumor changes include STMN1 expression, [96] CDKN1A (p21) expression, [99] and CDKN1B (p27) cellular localization, [98] all of which have been linked to energy balance and related signal transduction pathways.[190–193] In addition, our analysis on interactive prognostic effects of physical activity and tumor markers have revealed that postdiagnosis physical activity is beneficial only in patients with CDKN1B-nuclear-positive colon cancers, but not in patients with CDKN1B-altered or lost colon cancers.[174] These results collectively provide evidence for tumor-host interactions (energy balance status and tumor molecular alterations) that influence tumor cell behavior.

Inflammation and Carcinogenesis

Epidemiological studies have shown that regular use of aspirin or non-steroidal anti-inflammatory drug (NSAID) is associated with decreased risks of colorectal cancer and adenomas.[194–203] Randomized trials have confirmed that regular use of aspirin[204–206] or other inhibitors of PTGS2 (prostaglandin endoperoxide synthase 2, cyclooxygenase-2, COX-2)[207–209] decreases risk of developing colorectal adenomas. Experimental evidence suggests an important role of PTGS2 in colorectal carcinogenesis.[210–212] Thus, it is hypothesized that PTGS2 (COX-2) inhibitors may prevent colorectal tumor through inhibition of PTGS2. Molecular pathologic epidemiology research has provided further insights on mechanisms of cancer preventive effect of PTGS2 inhibition. Utilizing the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS), we found that regular aspirin use decreases risk of cancers with PTGS2 (COX-2) overexpression, but not that of cancers without PTGS2 overexpression.[36] This specific inverse association between aspirin use and incidence of PTGS2-positive cancer provides further evidence for the carcinogenic role of PTGS2 (COX-2), and for the role of PTGS2 (COX-2) inhibitors in cancer prevention.

We have also shown that PTGS2 (COX-2) overexpression is associated with aggressive tumor behavior, [176] and that regular aspirin use after colorectal cancer diagnosis significantly decreases mortality in patients with PTGS2-positive cancers, but not in patients with PTGS2-negative cancers.[173] This specificity of the relation between aspirin use and low mortality in PTGS2-expressing cases provides additional evidence for the role of PTGS2 inhibition in prevention of cancer progression.

One-Carbon Metabolism, Germline Variants, and Somatic Epigenetic Changes

Colorectal cancer is a complex disease resulting from both genetic and epigenetic alterations, including abnormal DNA methylation patterns.[213,214] DNA hypomethylation at LINE-1 repetitive elements has been associated with poor prognosis in colon cancer.[177] LINE-1 hypomethylation may provide alternative promoter activation, [215] and contribute to non-coding RNA expression that regulates expression of many genes.[216,217] Retrotransposons activated by DNA hypomethylation may transpose themselves throughout the genome, leading to gene disruptions[218] and chromosomal instability (CIN).[219,220] In addition, there exists a specific tumor phenotype – the CpG island methylator phenotype (CIMP), characterized by propensity for widespread CpG island hypermethylation.[221] High degree of CIMP (CIMP-high) is a distinct phenotype, [5,15,222–225] and the most common cause of microsatellite instability (MSI) in colorectal cancer through epigenetic inactivation of a mismatch repair gene *MLH1*.[226–230] Independent of MSI, CIMP-high is associated with older age, female gender, proximal tumor location, [228,231,232] high tumor grade, signet ring cells, [233] *BRAF* mutation, [228,231,232] wild-type *TP53*, [228,234] inactive PTGS2 (COX-2), [234] inactive CTNNB1 (β-catenin), [235] loss of CDKN1B (p27), [236] high-level LINE-1 methylation, [231,237] stable chromosomes, [238,239] and expression of DNMT3B, [175] CDKN1A (p21), [240] and SIRT1.[92] Thus, CIMP status is a potential confounder for many locus-specific tumor variables.[5] Moreover, the relationship between *KRAS* mutation and another type of CIMP {"CIMP-low", [5,231,241–245] "CIMP2", [246] and "intermediate-methylation epigenotype"[247]} has been demonstrated. Importantly, different CIMP subtypes appear to show different locus-specific methylation patterns.[231,244,246–248] Accumulating evidence suggests that CIMP-high colorectal cancers arise through the "serrated pathway", [249–259] which has substantial implications in studies on colorectal polyps and adenomas, because of potential differences in detection rates, removal rates and natural histories between conventional and serrated precursor lesions. The elucidation of mechanisms of epigenetic aberrations will improve our understanding of the carcinogenic process.

One-carbon metabolism is considered to play major roles in DNA synthesis and methylation reactions.[260] In most epidemiological studies, low folate intake has been associated with higher risks of colorectal cancer[261–266] and adenoma.[266–269] However, results from randomized clinical trials of folic acid supplementation among individuals with a prior history of colorectal adenomas have been disappointing. A meta-analysis of these randomized trials[270] has demonstrated that folic acid supplementation does not decrease adenoma recurrence risk after short-term follow-up. In fact, one randomized trial[271,272] suggested a potential tumor-promoting effect of folic acid supplementation. Thus, there has been much controversy on dietary folate, folic acid fortification/supplementation and risks of colorectal neoplasia.[270,272–274] Examining molecular changes in tumor cells in relation to folate intake may provide additional insights on the possible link between one-carbon metabolism and carcinogenesis.

Folate deficiency is associated with an increase in de novo DNA methyltransferase activity.[275,276] Altered levels of folate metabolites and intermediates are associated with aberrant

DNA methylation patterns.[70,277] The *MTHFR* rs1801131 polymorphism (codon 429) has been associated with colon cancer with the CpG island methylator phenotype (CIMP) in case-control and case-case studies, [41,61] although another case-cohort study has not confirmed this finding.[48] Notably, the latter case-cohort study has shown that the *MTR* rs1805087 polymorphism is inversely associated with CIMP in men.[48] Collectively, genetic variations in one-carbon metabolism pathways may play roles in epigenetic events during carcinogenesis.

With regard to global DNA methylation level, experimental data support a link between folate level and cellular DNA methylation level.[278–280] In our prospective cohort studies, subjects reporting low folate intake experienced an increased risk of colon cancer with global DNA (LINE-1) hypomethylation, but folate intake had no influence on a risk of LINE-1 hypermethylated cancer.[119] In a randomized, double-blinded, placebo-controlled trial, folic acid supplementation was inversely associated with global DNA hypomethylation in normal colon mucosa.[281] However, in the Aspirin/Folate Polyp Prevention Trial, there was no significant influence on LINE-1 methylation in normal colon mucosa by folic acid supplementation.[282]

Besides influence of one-carbon nutrients, local DNA sequence context may influence assembly of a methylation reaction machinery and locus-specific DNA methylation. Studies have shown that *cis*-acting elements cause allele-specific methylation in the mammalian genome.[283–286] Thus, germline variations in putative *cis*-acting elements may influence epigenetic status; such examples include *MLH1* rs1800734 promoter SNP, [38,108,115] and *MGMT* rs16906252 promoter SNP.[94,152,153]

Challenges in Molecular Pathologic Epidemiology

Although molecular pathologic epidemiology is a very promising field, a number of challenges exist. Molecular pathologic epidemiology research has the same set of inherent limitations as traditional epidemiology research and pathology research, including those related to bias (e.g., selection bias, recall bias, measurement errors, and misclassification), confounding, generalisability and causal inference. In addition, there are other issues specific to molecular pathologic epidemiology. Many of the issues have previously been discussed.[287–289] In this section, we systematically discuss various issues specific to molecular pathologic epidemiology and propose measures to overcome those issues.

1. Selection bias

Since we can analyze only a finite number of cases, controls, or cohort participants, selection bias is a universal issue. The use of cancer cases in one or a few hospitals may be a source of selection bias since patients have selected the one or few hospitals based on referral or their own preference. To decrease bias due to differential hospital selection by patients, a large population-based investigation or multicenter investigation is desirable. To minimize selection bias, it is necessary to make the best effort to retrieve enough tissue materials from as many hospitals and pathology laboratories as possible.

In molecular pathologic epidemiology, a tumor tissue retrieval rate is almost inevitably less than 100%.[156,290] Patient and disease characteristics may influence the tissue retrieval rate. Specimen availability may be related to tumor size and patient outcome;[291] this may be especially true in colorectal adenomas. A large epidemiological study has shown that tumor tissue retrieval rates in early-stage intramucosal cancer and advanced stage IV cancer are lower compared to stage I-III cancers.[156] Nonetheless, both case-control and prospective cohort studies have shown that demographic features and dietary and other

exposure factors are similar between cases with tumor tissue analyzed and those without available tumor tissue.[36,156]

Another source of selection bias is treatment before surgical resection of tumor. While this has not been a major issue in colon cancer, treatment prior to surgical resection of rectal cancer is now common. First, selection of patients for treatment is likely nonrandom and influenced by many factors. Second, treatment before surgery can eliminate most or all tumor cells in resection specimens in some patients, while treatment is ineffective in other patients. Thus, availability of ample tumor cells is determined by treatment effect which is likely influenced by tumor molecular characteristics. Third, treatment itself can introduce molecular changes which may not naturally occur. Thus, if treatment is administered before surgical resection, it is recommended to collect tumor specimens that were taken prior to such treatment.

2. Sample size

In studies on tumor prognostic markers, a frequent problem is using inappropriate sample sizes that are too small to conduct robust statistical analysis and draw meaningful conclusions.[292] Small sample sizes lead to a number of problems including a large variation of an effect estimate with wide confidence limits, random and nonrandom selection bias, and publication bias. Publication bias refers to a phenomenon that studies with null findings have a higher likelihood of being unwritten and unpublished compared to studies with “significant” findings. In the published literature, small underpowered studies with “significant” findings have been over-represented, relative to small underpowered studies with null findings. In a meta-analysis of *TP53* alterations and head and neck cancer outcome, [293] not only publication bias, but also selective presentations of data in many small studies appear to be a serious problem that can lead to biased and misleading conclusions.

In molecular pathologic epidemiology, sample size is a substantial issue. Even when a parent study is large-scale, any given molecular pathologic epidemiology study requires multiple exclusions based on availability of tumor tissue materials and valid assay results. In molecular pathologic epidemiology, by definition, a subset analysis for different outcomes (a molecular change present vs. absent) is performed. A sample size for a smaller subset may not be large enough to provide adequate statistical power. Population-based studies have shown that molecular subtyping is often skewed: *BRAF* mutation (10–15% mutated vs. 85–90% wild-type), [53,228,294,295] *PIK3CA* mutation (15–20% mutated vs. 80–85% wild-type), [296,297] *NRAS* mutation (2% mutated vs. 98% wild-type), [65] MSI (15% high vs. 85% low/MSS), [35,64,126,231] *KRAS* mutation (35–40% mutated vs. 60–65% wild-type), [30,242,298] or CIMP (10–20% high vs. 80–90% low/negative;[170,243,294,299] or 15–30% positive vs. 70–85% negative[53,64,228]). Therefore, for any future cancer epidemiology research, one should design a study as large as possible, because tumor molecular subtyping is increasingly common in cancer epidemiology.

3. Measurement error and misclassification

In addition to measurement error and misclassification in exposure variables and covariates, nontrivial measurement error and misclassification may be present in an outcome variable, i.e., tumor molecular subtyping. This particular combination (i.e., measurement errors and misclassification in both exposure and outcome assessments) is a unique challenge in molecular pathologic epidemiology.

Tumor molecular and immunohistochemical assays should be validated and monitored for its precision and accuracy. In immunohistochemical analysis, it is possible to observe a

correlative error between two completely unrelated proteins because of the presence of poor quality tissue specimens, which fail to react with any specific antibody leading to false negative results.[5] Thus, in such poor quality cases, negativity of one protein tends to coincide with negativity of another protein even with the absence of any true association. Since those cases with poor quality materials are inevitably present in large-scale epidemiology studies, one should be cautious when interpreting an apparent positive correlation between two proteins by immunohistochemistry assays.[5] The presence of internal control in tumor tissue may solve this problem to some extent.

To decrease run-to-run variability in immunohistochemical assays, the use of tissue microarray (TMA) is recommended.[289] All cases in the same TMA slide can be processed and treated in a similar manner during immunostaining. We recommend inclusion in TMA of normal tissue adjacent to tumor tissue from the same individual whenever normal tissue is available. Normal colon mucosa may serve as an internal control. Tissue cores can be separately taken from tumor edge and center and labeled as such. Because TMA is cost efficient for a large-scale study, any epidemiology study or clinical trial should consider TMA for immunohistochemical evaluations of expression of multiple proteins.

4. Multiple hypothesis testing

Multiple hypothesis testing is a common issue in epidemiology, and is even more problematic in molecular pathologic epidemiology. By definition, molecular pathologic epidemiology involves subset analyses on tumor subtypes, which exacerbate the potential for false positive findings due to multiple hypothesis testing.[1] If one crosses a wide range of lifestyle and other exposure variables with a variety of molecular changes, the likelihood for a nominally significant chance finding is high.[1] In this post-genomic era, we can potentially generate a countless number of hypotheses as we have already experienced in GWAS.[300–302] False positive findings can potentially confuse the literature, scientific field, and clinical practice.[303] If a higher significance level is required, then we require to have a large sample size.

An important question is whether the molecular pathologic epidemiology approach should be hypothesis-driven or exploratory as GWAS. If the former is the case, how can we prioritize various hypotheses to allocate our limited resource? If the latter is the case, how can we make formal rules of statistical significance and validation of findings? The border between hypothesis-driven research and exploratory research may not be distinct in molecular pathologic epidemiology. For example, a proposed link between smoking and MSI-high, CIMP-high or *BRAF*-mutated colon cancers may be regarded as either exploratory or hypothesis-based. Where do we draw a line between hypothesis testing vs. exploration? At the very least, initial few studies examining the relationship between a certain exposure and a specific molecular change should be regarded as exploratory and hypothesis-generating. Any generated hypothesis needs to be validated by independent datasets.

We acknowledge that any novel hypothesis could at first result from a fortuitous discovery by multiple hypothesis testing. If we successfully implement proper measures, the pace of our new discovery can be much faster than before. To generate and test new hypotheses, validate new findings, solidify new knowledge, and implement new public health recommendations and measures, we should develop an optimal and standardized way of streamlining the sequence of discoveries and validation in molecular pathologic epidemiology.

5. Generalisability

All issues mentioned above affect generalisability of study findings. Many findings by molecular pathologic epidemiology studies (as shown in Tables 1 and 2) are yet to be validated in other independent datasets. It is challenging since there is a wide variety in study designs and populations, and differences in tumor molecular assays add further diversity between different studies. On the other hand, because of the presence of such enormous heterogeneity between different studies, consistent findings across different studies can be regarded as generalisable findings.

6. Direct causation of molecular changes vs. selective advantage

Although molecular pathologic epidemiology illuminates carcinogenic mechanisms, it still needs experimental data to confirm a causal relationship. There still remains a question whether an exposure of interest can either directly or indirectly cause a specific molecular change, or create a specific environment which can provide selective advantage for clonal expansion of a cell with a specific molecular change. Tumor molecular alterations may not only represent the interactions of carcinogens with DNA repair mechanisms or epigenetic machinery, but also reflect the tissue-specific selection of those alterations that provide pre-malignant and malignant cells with a clonal growth advantage.

7. How we can examine the process of tumor progression in observational molecular pathologic epidemiology

Since some molecular changes have been known to occur early (e.g., *APC* loss, *KRAS* mutation), etiologic factors which appear to cause those early events can be considered to contribute to tumor initiation/progression early in the carcinogenic process. Another way is to analyze colorectal polyp/adenoma and colorectal cancer within the same population, and investigate how an exposure of interest is related to somatic molecular events in cancer and precursor lesions.

8. Multidisciplinary research environment and cross-training and education

Molecular pathologic epidemiology is transdisciplinary and interdisciplinary by nature (see Stokols et al.[304] for the definitions of transdisciplinarity and interdisciplinarity). It requires expertise of diverse fields including, at least, epidemiology, biostatistics, pathology, and oncology. Therefore, collaborative environment is essential, and cross training and education are extremely useful to advance this interdisciplinary area of science. Especially, training in epidemiology and biostatistics during pathology training is very beneficial.[305] Increasing needs and trend for team science rather than solo science have been well documented.[304,306,307]

Future Direction and Concluding Remarks

“Molecular Pathologic Epidemiology” is a relatively new, evolving field of epidemiology which is designed to elucidate how various exposures affect initiation, transformation and progression of neoplasia.[1] A new direction of molecular pathologic epidemiology is to investigate interactive effects of dietary or lifestyle exposures and tumoral molecular features on tumor behavior (prognosis or clinical outcome), so that one can attribute the effects of dietary or lifestyle variables to a specific molecular subtype of cancer.[2] A number of hurdles must be overcome because of unique and new challenges which we have not faced in traditional epidemiology research. To overcome those issues, it is necessary to coordinate research effort around the world and to possibly formulate a system where one can discover and validate new findings. As a result, molecular pathologic epidemiology research will continue to provide profound insights on carcinogenic process and help us optimize prevention and treatment strategies.

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Abbreviations

BMI	body mass index
CCFR	Colon Cancer Family Registry
CI	confidence interval
CIMP	CpG island methylator phenotype
CIN	chromosomal instability
EPIC	European Prospective Investigation into Cancer and Nutrition
GWAS	genome-wide association study
HPFS	Health Professionals Follow-up Study
HR	hazard ratio
KPMCP-UT-MN	Kaiser Permanente Medical Care Program of Northern California, the state of Utah and the Twin City Metropolitan area of Minnesota (the M Slattery group's case-control study)
LOH	loss of heterozygosity
MCCS	Melbourne Collaborative Cohort Study
MSI	microsatellite instability
MECCS	Molecular Epidemiology of Colorectal Cancer Study (northern Israel)
MSI-H	microsatellite instability-high
MSI-L	microsatellite instability-low
MSS	microsatellite stability
NCCCS	North Carolina Colon Cancer Study
NHS	Nurses' Health Study
NLCS	The Netherlands Cohort Study
NSAID	non-steroidal anti-inflammatory drug
NSHDS	Northern Sweden Health and Disease Study
OR	odds ratio
PTGS2	prostaglandin endoperoxide synthase 2 (cyclooxygenase-2, COX-2)
RCT	randomized, placebo-controlled trial
SEER	Surveillance Epidemiology, and End Results
SNP	single nucleotide polymorphism
WBFT	Wheat Bran Fiber Trial

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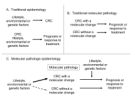

Figure 1.
Illustration of traditional epidemiology (A), traditional molecular pathology (B), and molecular pathologic epidemiology (C). Note that molecular pathology plays a central role in molecular pathologic epidemiology. Molecular pathologic epidemiology addresses a question whether a particular exposure factor is associated with a specific molecular change in colorectal cancer (C, left side), as well as a question whether a specific molecular change can interact with a particular exposure factor to affect tumor cell behavior (C, right side). The latter represents a new direction of molecular pathologic epidemiology where results can provide additional insights on mechanism of how the tumoral molecular change and the exposure factor of interest influence tumor cell behavior. CRC, colorectal cancer.

Figure 2.
Comparison of a case-case study design (A), a case-control study design (B) and a prospective cohort study design (C). Smoking status is used as an example of an exposure variable, and *KRAS* mutation status in colorectal cancer as an outcome variable. See detailed explanations in text. CRC, colorectal cancer.

Table 1

Molecular pathologic epidemiology studies on possible etiologic factors and molecular changes in colorectal neoplasia

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Exposure variables	Potential modifiable factors	Outcome variables	Main findings on modifiable (or genetic) factors and tumoral molecular changes
Case-case studies									
[17]	Arain	2010	Case-case	CC	194 CC (63 interval CC), 0 non-cancer controls	Colonoscopy within 5 years prior to diagnosis of CC (interval cancer)		CIMP, MSI in CC	Colonoscopy within 5 years prior to diagnosis of CC is associated with CIMP and MSI in CC.
[18]	Baba	2009	Case-case (in PCS)	CRC	NHS, HPFS. 621 CRC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		CDX2 expression in CRC	BMI or family history of CRC is not significantly associated with loss of CDX2 expression in CRC.
[19]	Baba	2009	Case-case (in PCS)	CRC	NHS, HPFS. 517 CRC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		AURKA expression in CRC	BMI or family history of CRC is not significantly associated with AURKA expression in CRC.
[20]	Baba	2010	Case-case (in PCS)	CRC	NHS, HPFS. 516 CRC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		HIF1A, EPAS1 (HIF-2A) expression in CRC	BMI or family history of CRC is not significantly associated with HIF1A or EPAS1 expression in CRC.
[21]	Baba	2010	Case-case (in PCS)	CRC	NHS, HPFS. 731 CRC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		PTGER2 (prostaglandin EP2 receptor) expression in CRC	BMI or family history of CRC is not significantly associated with PTGER2 expression in CRC.
[22]	Baba	2010	Case-case (in PCS)	CRC	NHS, HPFS. 869 CRC, 0 non-cancer controls	BMI (prediagnosis), family hisotry of CRC, smoking		LINE-1 methylation in CRC	Family history of CRC may be associated with LINE-1 hypomethylation in CRC. LINE-1 extreme hypomethylators are associated with young age of onset.
[23]	Baba	2010	Case-case (in PCS)	CRC	NHS, HPFS. 1105 CRC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		*IGF2* differentially methylated region-0 (DMR0) hypomethylation in CRC	Family history of CRC is associated with *IGF2* DMR0 hypomethylation.
[24]	Baba	2010	Case-case (in PCS)	CRC	NHS, HPFS. 718 CRC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		phospho-PRKA (AMPK) expression in CRC	Family history of CRC or BMI is not associated with phospho-PRKA (AMPK) expression in CRC.
[25]	Baba	2010	Case-case (in PCS)	CRC	NHS, HPFS. 717 CRC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		phospho-AKT expression in CRC	Family history of CRC or BMI is not associated with phospho-AKT expression in CRC.
[26]	Bapat	2009	Case-case	CRC	CCFR.3143 CRC	Family history of CRC and endometrial cancer		MSI in CRC	Family history of CRC and endometrial cancer is associated with MSI-high in CRC. Familial risk associated with MSI-high CRC is primarily driven by the Amsterdam criteria patients.
[30]	Brink	2003	Case-case (in PCS)	CRC	NLCS.737 CRC, 0 non-cancer controls	Family history of CRC		*KRAS* mutation in CRC	Family history of CRC is not associtade with *KRAS* mutation in CRC

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Exposure variables	Potential modifiable factors	Outcome variables	Main findings on modifiable (or genetic) factors and tumoral molecular changes
[37]	Chang	2007	Case-case	CRC	195 CRC	*MTHFR* codon 222 SNP, plasma folate		MSI, aneuploidy in CRC	*MTHFR* codon 222 variant is associated with MSI-H CRC. Plasma folate is lower in aneuploid MSS CRC than in diploid MSS CRC.
[38]	Chen	2007	Case-case	CRC	387 CRC	*MLH1* SNPs		*MLH1* methylation in CRC	*MLH1* rs1800734 (−93G>A) SNP is associated with *MLH1* methylation in CRC.
[40]	Clarizia	2006	Case-case	CRC	105 CRC, 0 non-cancer controls	*MTHFR* codon 222 SNP		MSI, methylation in *MLH1, CDKN2A, MGMT, DAPK1*, p14 in CRC	*MTHFR* codon 222 SNP variant is associated with MSI-H in CRC.
[52]	Eaton	2005	Case-case	CC	NCCCS.486 CC, 0 non-cancer controls	*MTHFR* SNPs	Dietary and supplement folate	MSI in CRC	Among high folate intake group (≥400 ug/day), the presence of either *MTHFR* SNP variant is associated with MSS.
[54]	Fernandez-Peralta	2010	Case-case	CRC	143 CRC	*MTHFR* SNPs		MSI, LOH at *APC, DCC, TP53, MLH1, MSH2*, mutation in *KRAS, BAX, TGFBR2* in CRC	None of molecular feature in CRC is differentially related to *MTHFR* SNP with certainty.
[55]	Ferraz	2004	Case-case	CRC	165 CRC, 0 non-cancer controls	*GSTM1, GSTT1, GSTP1, NAT2* genotypes		*KRAS, TP53* mutations in CRC	*GSTT1* or *GSTP1* SNPs may be associated with *KRAS* or *TP53* mutations in CRC.
[57]	Firestein	2010	Case-case(in PCS)	CRC	470 CRC, 0 non-cancer controls	BMI (prediagnosis)		CDK8 expression in CRC	Female sex is associated with CDK8 expression in CRC. BMI (prediagnosis) is not associated with CDK8 expression in CRC.
[58]	Gonzalo	2010	Case-case	CRC	82 CRC patients (37 synchronous CRC patients, 4 metachronous CRC patients)	Tumor synchronicity/metachronicity		Methylation in *MGMT, CDKN2A, SFRP1, TMEFF2, HS3ST2, RASSF1, GATA4* in CRC	Tumor synchronicity/metachronicity is associated with methylation in *MGMT* and *RASSF1* in CRC.
[59]	Gruber	2007	Case-case	CRC	MECCS (northern Israel). 133 CRC	SNP rs10505477 in 8q24		mRNA expression of genes in 8q24 in CRC	SNP rs10505477 is not associated with any difference in expression of examined genes in 8q24.
[60]	Hansen	2010	Case-case	CRC	109 CRC, 0 non-cancer controls	*KDR* SNPs		Microvessel density (assessed by immunohisto-chemistry for ENG and CD34) in CRC	*KDR* rs2305948 SNP T variant is associated with high microvessel density.
[61]	Hazra	2010	Case-case (in PCS)	CRC	NHS, HPFS.182 CRC, 0 non-cancer controls	SNPs in one-carbon metabolism genes		CIMP, LINE-1 methylation in CRC	*MTHFR* rs1801131 (codon 429) and *TCN2* rs1801198 SNP variants are associated with CIMP-high in CRC.
[62]	Huang	2009	Case-case	CRC	151 CRC, 0 non-cancer controls	*NAT2* genotypes		KRAS mutation in CRC	*NAT2* genotype may be associated with *KRAS* mutation in CRC in female.
[65]	Irahara	2010	Case-case (in PCS)	CRC	NHS, HPFS. 225 CRC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		*NRAS* mutation in CRC	There is no association between BMI or family history of CRC and *NRAS* mutation in CRC.

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Exposure variables	Potential modifiable factors	Outcome variables	Main findings on modifiable (or genetic) factors and tumoral molecular changes
[67]	Jensen	2008	Case-case	CRC	130 CRC, 0 non-cancer controls	Plasma homocysteine		MSI in CRC	MSI-H cases show higher plasma homocysteine level than MSS cases.
[68]	Kang	2008	Case-case	CRC	188 CRC	p14 (*CDKN2A*/ARF) SNPs		p14 methylation in CRC	p14 promoter SNP haplotype is associated with p14 methylation in CRC.
[70]	Kawakami	2003	Case-case	CRC	103 CRC, 0 non-cancer controls	*TYMS*, *MTHFR*, *MTR*, *CBS* genotypes		5, 10-methylene-tetrahydrofolate, tetrahydrofolate, methylation in *MLH1*, *TIMP3*, p14 (CDKN2A/ARF), *CDKN2A*, MINT-2, *DAPK*, *APC* in CRC	*MTHFR* rs1801133 SNP (codon 222) with decreased 5,10-methylene-tetrahydrofolate and tetrahydrofolate contents in CRC. homozygous variant is associated
[71]	Konishi	2009	Case-case	CRC	97 CRC patients (28 synchronous CRC patients)	Tumor synchronicity		Methylation in MINT-1, MINT-2, MINT-31, *MLH1*, *CDKN2A*, p14, *MGMT*, *ESR1* in CRC	Synchronous CRC is associated with higher methylation levels at p14 methylation level at MINT-31 in CRC. (*CDKN2A*/ARF) and *MGMT* and lower
[72]	Kure	2009	Case-case (in PCS)	CRC	NHS, HPFS. 619 CRC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		VDR expression in CRC	BMI or family history of CRC is not significantly associated with VDR expression in CRC.
[74]	Langerod	2002	Case-case	CRC	162 CRC, 0 controls	*TP53* codon 72 SNP		*TP53* mutation in CRC	*TP53* codon 72 SNP is not related to *TP53* mutation in CRC, but to *TP53* mutation in breast cancer (N=390).
[79]	Lindor	2010	Case-case	CRC	CCFR. 789 CRC	Parent of origin family history of CRC		MSI in CRC	Among overall CRC cases, HNPCC, or MSS cancer cases, family history of CRC in father is associated with lower age of onset of CRC in daughters than in family history of CRC in mother, but no such difference in age of onset is present among affected sons.
[81]	Lubbe	2009	Case-case	CRC	NSCCG. 488 CRC	Family history of CRC in first degree relatives		MSI in CRC	Family history of CRC in first degree relatives is associated with MSI-H CRC.
[84]	Luchtenborg	2005	Case-case (in PCS)	CRC	NLCS. 656 CRC	Family history of CRC		*APC*, *KRAS* mutation, MLH1 loss in CRC	*APC*, *KRAS* or MLH1 alteration is not associated with family history of CRC.
[85]	Martinez	1999	Case-case	CRA	WBFT. 678 CRA, 0 non-cancer controls	Various nutrients, alcohol, family history, aspirin use, smoking, BMI, physical activity, hormone use		*KRAS* mutation in CRA	Folate intake is inversely associated with *KRAS* mutation in CRA.
[86]	Mas	2007	Case-case	CRC	120 CRC, 0 con-cancer controls	Various nutrients		*CDKN2A* (p16), p14, *MLH1* methylation in CRC	Patents with *CDKN2A* methylation consumed less folate, vitamin A, vitamin B1, potassium and iron. Patients with p14 or *MLH1* methylation consumed less vitamin A.
[87]	Mokarram	2008	Case-case	CC	151 CC, 0 non-cancer controls	Folate, vitamin B12 in serum	*MTHFR* rs1801133 SNP	Methylation in *MLH1*, *MSH2*, *CDKN2A* (p16) in CC	Relation between folate/B12 and methylation in CC may be modified by *MTHFR* rs1801133 (codon 222) SNP.

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Exposure variables	Potential modifiable factors	Outcome variables	Main findings on modifiable (or genetic) factors and tumoral molecular changes
[89]	Naguib	2010	Case-case (in PCS)	CRC	EPIC-Norfolk Study. 186 CRC, 0 non-cancer controls	BMI, smoking, physical activity, HRT, alcohol, dietary factors		*KRAS* and *BRAF* mutations in CRC	*KRAS*-mutated tumors are associated with higher white meat consumption, compared to *KRAS*-wild-type tumors.
[91]	Nosho	2009	Case-case (in PCS)	CRC	NHS, HPFS. 863 CRC, 0 non-cancer controls	Tumor synchronicity	BMI, family history of CRC	MSI, CIMP, LINE-1 methylation, 18q LOH, *KRAS*, *BRAF*, *PIK3CA* mutation, expression of TP53, CTNNB1, CDKN1A (p21), CDKN1B (p27), CCND1, FASN, PTGS2 (COX-2) in CRC	Tumor synchronicity is associated with CIMP-high, MSI-H, and *BRAF* mutation. There is no significant modifying effect by BMI or family history of CRC.
[92]	Nosho	2009	Case-case (in PCS)	CRC	NHS, HPFS. 485 CRC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		SIRT1 expression in CRC	BMI or family history of CRC is not significantly associated with SIRT1 expression in CRC.
[93]	Nosho	2009	Case-case (in PCS)	CRC	NHS, HPFS. 766 CRC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		JC virus T antigen expression in CRC	Family history of CRC may be inversely associated with JC virus T antigen expression in CRC.
[94]	Ogino	2007	Case-case (in PCS)	CRC	NHS, HPFS. 182 CRC, 0 non-cancer controls	*MGMT* SNPs		*MGMT* methylation, CIMP, MSI, 18q LOH, *KRAS*, *BRAF* mutation in CRC	*MGMT* rs16906252 SNP variant is associated with *MGMT* methylation (adjusted OR=18; 95% CI, 6.2–52) and loss of expression.
[2]	Ogino	2008	Case-case (in PCS)	CC	NHS, HPFS. 623 CC, 0 non-cancer controls	BMI (prediagnosis)		FASN expression in CRC	There is an inverse relation between BMI and FASN expression in CRC.
[95]	Ogino	2009	Case-case (in PCS)	CRC	NHS, HPFS. 470 CRC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		PPARG expression in CRC	There is no relation between BMI or family history of CRC and PPARG expression in CRC.
[96]	Ogino	2009	Case-case (in PCS)	CRC	NHS, HPFS. 546 CRC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		STMN1 expression in CRC	There is no relation between BMI or family history of CRC and STMN1 expression in CRC.
[97]	Ogino	2009	Case-case (in PCS)	CC	NHS, HPFS. 450 CC, 0 non-cancer controls	BMI (prediagnosis)		*PIK3CA* mutation in CC	There is no relation between BMI and *PIK3CA* mutation in CC.
[98]	Ogino	2009	Case-case (in PCS)	CC	NHS, HPFS. 630 CC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		CDKN1B (p27) localization in CC	There is no relation between BMI or family history of CRC and CDKN1B (p27) localization in CC.
[99]	Ogino	2009	Case-case (in PCS)	CC	NHS, HPFS. 647 CC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		CDKN1A (p21) expression in CC	There is no relation between BMI or family history of CRC and CDKN1A (p21) expression in CC.
[100]	Ogino	2009	Case-case (in PCS)	CC	NHS, HPFS. 602 CC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		CCND1 (cyclin D1) expression in CC	There is no relation between BMI or family history of CRC and CCND1 expression in CC.
[101]	Ogino	2009	Case-case (in PCS)	CRC	NHS, HPFS. 555 CRC, 0 non-cancer controls	BMI (prediagnosis), family history of CRC		18q LOH in CRC	Obesity (prediagnosis) is associated with 18q LOH in CRC.

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Exposure variables	Potential modifiable factors	Outcome variables	Main findings on modifiable (or genetic) factors and tumoral molecular changes
[102]	Oyama	2004	Case-case	CRC	194 CRC, 0 non-cancer controls	*MTHFR* SNPs		Methylation in *CDKN2A, MLH1, TIMP3*, p14 in CRC	*MTHFR* codon 429 SNP variant is associated with *CDKN2A* methylation in CRC.
[103]	Park	2010	Case-case (in PCS)	CRC	EPIC-Norfolk Study. 185 CRC, 0 non-cancer controls	Dietary factors, family history, BMI, physical activity, smoking		*TP53* mutation in CRC	There is a positive relation between meat intake and *TP53* mutation in Duke's stage C and D cases, while there is a positive relation between meat intake and wild-type *TP53* in Duke's stage A and B cases.
[104]	Paz	2002	Case-case	CRC	118 CRC, 0 non-cancer controls	Genotypes of one-carbon metabolism genes		Methylation in *CDKN2A*, p14, *MLH1, MGMT, APC, STK11, DAPK1, GSTP1, BRCA1, RARB, CDH1, RASSF1* in CRC	Results on all cancers (CRC, breast presented, and CRC-specific results are not presented. cancers, and lung cancers) are
[109]	Ricciardiello	2003	Case-case	CRA	70 CRA	Family history of CC		MSI, *MLH1* methylation, expression of MLH1 and MSH2 in CRA	Family history of CC is associated with *MLH1* methylation and loss of MLH1 in CRA.
[110]	Rozek	2008	Case-case	CRC	MECCS (northern Isreal). 82 CRC	*CDX2* SNPs and haplotypes		CDX2 mRNA expression in CRC	*CDX2* SNPs or haplotypes are not associated with CDX2 mRNA expression in CRC.
[112]	Samowitz	1995	Case-case	CC	188 CC	Family history of CRC, *GSTM1* genotype		MSI in CC	Family history of CRC or *GSTM1* genotype is not associated with MSI in CC.
[121]	Shima	2010	Case-case (in PCS)	CRC	HPFS, NHS. 902 CRC, 0 non-cancer controls	BMI, family history of CRC		*CDKN2A* (p16) promoter methylation, loss of CDKN2A in CRC	There is no relation between BMI or family history of CRC and *CDKN2A* methylation (or loss of expression) in CRC.
[122]	Sinicrope	2010	Case-case	CC	7 colon cancer adjuvant therapy trials. 2222 CC, 0 non-cancer controls	BMI		Mismatch repair protein loss (or MSI-H) in CC	High BMI is inversely associated with MSI in CC.
[141]	van Engeland	2003	Case-case	CRC	NLCS. 121 CRCs,0 non-cancer controls.	Various nutrients, alcohol		Methylation in *APC, CDKN2A* (p16), p14, *MLH1, MGMT, RASSF1A* in CRC	Folate and alcohol intake may be associated with promoter hypermethylation in CRC.
[143]	Ward	2004	Case-case	CRC	547 CRC	Family history of CRC, family history of any cancer		CIMP in CRC	CIMP in CRC is not associated with family history of CRC or any cancer.
[149]	Wu	2001	Case-case	CC	Los Angeles County Cancer Surveillance Program (a part of SEER). 276 CC, 0 non-cancer controls	Smoking, red meat cooking practice		MSI in CC	Certain red meat cooking (well-doing) and heterocyclic amine score are associated with MSI-H CC. Smoking is associated with MSI-H CC.
[150]	Wu	2010	Case-case	CC	Los Angeles County Cancer Surveillance Program (a part of SEER). 280 CC	Hormone therapy		*ESR1, ESR2, PGR, CDKN2A, MGMT, MYOD1, MLH1* methylation in CC	There may be an inverse association between hormone therapy and *ESR1* methylation in CC.
Case-cohort studies									
[28]	Bongaerts	2006	Case-cohort study	CRC	NLCS. 4076 subcohort, 578 CRC	Alcohol intake		*KRAS* mutation in CRC	Alcohol intake does not influence *KRAS* mutation in CRC.

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Exposure variables	Potential modifiable factors	Outcome variables	Main findings on modifiable (or genetic) factors and tumoral molecular changes
[29]	Bongaerts	2007	Case-cohort study	CRC	NLCS. 4076 subcohort, 573 CRC	Alcohol intake		*KRAS*, *APC* mutation, TP53 expression, MLH1 loss in CRC	Alcohol intake does not influence *KRAS*, *APC* mutation, TP53 or MLH1 alteration in CRC.
[31]	Brink	2004	Case-cohort study	CRC	NLCS. 2948 subcohort, 608 CRC	Various fat components		*KRAS* mutation in CRC	High intake of polyunsaturated fat is associated with risk of *KRAS*-mutated CC.
[32]	Brink	2005	Case-cohort study	CRC	NLCS. 2948 subcohort, 608 CRC	Meat consumption		*KRAS* mutation in CRC	There may be an inverse association between pork consumption and *KRAS* wild-type CRC.
[33]	Brink	2005	Case-cohort study	CRC	NLCS. 3048 subcohort, 330 CRC	Various nutrients		*KRAS* mutation in CRC	Folate intake is associated with lower risk of *KRAS*-mutated CRC in men, but not in women.
[46]	de Vogel	2006	Case-cohort study	CRC	NLCS. 4343 subcohort. 547 CRC with APC data	Various nutrients		*APC* mutation in CRC	Folate may influence the occurrence of *APC* mutation in CRC.
[47]	de Vogel	2008	Case-cohort study	CRC	NLCS. 4059 subcohort. 648 CRC	Various nutrients		*MLH1* methylation, MLH1 expression, MSI, *BRAF* mutation in CRC	Among men, folate intake may increase risk of *BRAF*-mutated CRC, and vitamin B6 may increase risk of *MLH1* methylated CRC.
[48]	de Vogel	2009	Case-cohort study	CRC	NLCS. 4774 subcohort. 373 CRC	SNPs in folate enzyme genes metabolizing		CIMP, *MLH1* methylation, MSI in CRC	*MTR* rs1805087 (A2756G) SNP is inversely associated with CIMP in men.
[64]	Hughes	2009	Case-cohort study	CRC	NLCS. 4650 subcohort, 662 CRC	Hunger in adolescence and young adulthood		CIMP, MSI in CRC	Exposure to hunger in young age is associated with decreased risk of CIMP+ CRC, but not associated with CIMP-negative CRC.
[82]	Luchtenborg	2005	Case-cohort study	CRC	NLCS. Subcohort 2948. 588 CRC	Meat and fish consumption		*APC* mutation, MLH1 loss in CRC	Beef consumption is associated with risk of CC without *APC* mutation.
[83]	Luchtenborg	2005	Case-cohort study	CRC	NLCS. Subcohort 2948. 650 CRC	Smoking	*GSTM1*, *GSTT1* genotypes	*APC* mutation, MLH1 loss in CRC	Smoking increases risk of *APC*-WT CRC, and there is no modifying effect of *GSTM1* or *GSTT1* genotypes.
[144]	Wark	2005	Case-cohort study	CRC	NLCS. 3048 subcohort, 441 CC	Fruits, vegetable consumption		MLH1 loss in CC	Fruits consumption decrease risk of MLH1-lost CC, but not that of MLH1-expressing CC.
[146]	Weijenberg	2007	Case-cohort study	CRC	NLCS. 2948 subcohort, 531 CRC	Various fat components		*APC*, *KRAS* mutation, MLH1 loss in CRC	High intakes of polyunsaturated fatty acid and linoleic acid increase risk of KRAS-mutated CC.
[147]	Weijenberg	2008	Case-cohort study	CRC	NLCS. 4083 subcohort, 428 CRC	Smoking		*KRAS* mutation in CRC	Effect of smoking on CRC risk is not different according to *KRAS* mutational status.
Case-control studies (CCS)									
[27]	Bautista	1997	CCS	CRC	106 CRC, 295 controls	Various nutrients		*KRAS* mutation in CRC	Monounsaturated fat is inversely associated with *KRAS* wild-type CRC compared to controls, but no such association is present for *KRAS*-mutated CRC.

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Exposure variables	Potential modifiable factors	Outcome variables	Main findings on modifiable (or genetic) factors and tumoral molecular changes
[34]	Campbell	2009	CCS	CC	KPMCP-UT-MN. 1211 CC, 1972 controls	SNPs in *MLH1*, *MSH6*	Smoking, dietary pattern	MSI in CC	Smoking does not modify MSI-H CC risk that is conferred by *MLH1* rs1800734 (−93G>A) SNP.
[35]	Campbell	2010	CCS	CRC	CCFR. 1250 CRC, 1880 controls (unaffected siblings)	BMI, BMI at age 20, weight gain		MSI in CRC	Obesity is associated with MSS CRC risk, but not with MSI-H CRC risk.
[39]	Chia	2006	CCS	CRC	CCFR. 1792 CRC, 1501 controls.	Smoking, NSAIDs		MSI in CRC	Smoking is associated with increased risk of MSI-H CRC, but not strongly with that of MSI-L/MSS CRC.
[41]	Curtin	2007	CCS	CC	KPMCP-UT-MN. 916 CC, 1972 controls	SNPs in one-carbon metabolism genes	One-carbon nutrients, alcohol, dietary pattern	CIMP in CC	*MTHFR* rs1801131 (codon 429) SNP may interact with alcohol intake and dietary pattern to modify CIMP+ CC risk.
[42]	Curtin	2007	CCS	CC	KPMCP-UT-MN. 1206 CC, 1962 controls	*TYMS* SNPs		MSI, *TP53*, *KRAS* mutations in CC	*TYMS* SNPs are not differentially associated with CC by MSI, *TP53* or *KRAS* status.
[43]	Curtin	2009	CCS	CC	KPMCP-UT-MN. 1604 CC, 1969 controls	SNPs in base excision repair genes	Smoking	MSI, CIMP, mutations in *BRAF*, *KRAS*, *TP53* in CC	There is no significant effect modification by smoking status.
[44]	Curtin	2009	CCS	CC	KPMCP-UT-MN. 1048 CC, 1964 controls	*MSH6* rs1042821 SNP	Alcohol intake, age, family history	CIMP, MSI, *BRAF* mutation in CC	*MSH6* rs1042821 SNP is associated with CIMP+ CC, and this relation is not modified by alcohol intake, age at diagnosis or family history.
[45]	Curtin	2009	CCS	Rectal cancer	KPMCP-UT-MN. 750 rectal cancers, 1201 controls	*GSTM1*, *NAT2* genotypes	Smoking	MSI, CIMP, *TP53*, *KRAS*, *BRAF* mutation in rectal cancers	Smoking is associated with CIMP, *TP53*, *BRAF* mutation in rectal cancer.
[49]	Diergaarde	2003	CCS	CC	Population-based case-control study in The Netherlands. 176 CC, 249 controls	Smoking		*KRAS*, *TP53*, *APC* mutations, MSI in CC	Smoking may be associated with transversion mutations and with *TP53*-negative CC.
[50]	Diergaarde	2003	CCS	CC	Population-based case-control study in The Netherlands. 184 CC, 259 controls	Various food and nutrients		MSI, *MLH1* methylation, expression of MLH1 and MSH2 in CC	Red meat intake may differentially modify CC risk stratified by MSI status.
[51]	Diergaarde	2003	CCS	CC	Population-based case-control study in The Netherlands. 184 CC, 259 controls	Various food and nutrients		*APC* mutation in CC	Alcohol intake may differentially modify CC risk stratified by *APC* mutation status.
[56]	Figueiredo	2010	CCS	CRC	CCFR. 1200 CRC, 1880 matched unaffected sibling controls.	*FOLR1*, *FPGS*, *GGH*, *SLC19A1* SNPs	Dietary one-carbon nutrients	MSI in CRC	CRC risks associated with any SNP do not significantly differ by MSI status.
[63]	Hubner	2007	CCS	CRC	NSCCG. 1649 CRC, 2692 non-cancer controls	*MTHFR* rs1801133 SNP		MSI in CRC	*MTHFR* rs1801133 SNP (codon 222) variant is associated with MSI-H CRC compared to controls, but not with MSS CRC.

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Exposure variables	Potential modifiable factors	Outcome variables	Main findings on modifiable (or genetic) factors and tumoral molecular changes
[66]	Jacobs	2010	CCS	CRC	CCFR. 1182 CRC, 1880 matched unaffected sibling controls.	SNPs in *RXRA*, *CASR*		MSI in CRC	RXRA SNP rs12004589 is associated with MSI-high cancer, but not with MSS/MSI-low cancer.
[69]	Karpinski	2010	CCS	CRC	186 CRC, 140 con-cancer controls	*MTHFR*, *TYMS*, *DNMT3B* genotypes		CIMP in CRC	Compared to controls, DNMT3B - 283T>C SNP is associated inversely with CIMP+ CRC, but not with CIMP− CRC.
[73]	Lafuente	2000	CCS	CRC	117 CRC, 296 controls	*NQO1* SNP		*KRAS* mutation in CRC	*KRAS* codon 12 mutations are associated with *NQO* C609T SNP.
[75]	Laso	2004	CCS	CRC	117 CRC, 296 controls	Micro-nutrients		*KRAS* mutation in CRC	*KRAS* codon 12 mutations are associated with lower intake of vitamin A, B1, D and iron than controls.
[76]	Levine	2010	CCS	CRC	CCFR. 1133 CRC, 1787 controls (unaffected siblings)	*MTHFR* SNPs		MSI in CRC	*MTHFR* rs1801133 (codon 222) SNP variant is associated with a decreased risk of MSI-L/MSS CRC.
[77]	Levine	2010	CCS	CRC	CCFR. 1185 CRC, 1787? controls (unaffected siblings)	SNPs of one-carbon metabolism genes	Folate and multi-vitamin supplement use, dietary folate, family history of CRC	MSI in CRC	SNPs of one-carbon metabolism genes are not associated with CRC differently by MSI status.
[80]	Lindor	2010	CCS	CRC	CCFR. 940 CRC, 940 controls	Smoking, *SERPINA1* SNP		MSI in CRC	Smoking is associated with MSI-H CRC in patients ≥age 50.
[88]	Naghibalhos saini	2010	CCS	CRC	151 CRC, 231 controls	*MTHFR* SNPs (rs1801133, rs1801131)		MSI in CRC	There is no significant difference in risks associated with *MTHFR* SNPs between MSI and MSS cancers.
[90]	Newcomb	2007	CCS	CRC	Cancer Surveillance System (a part of SEER). 311 CRC, 1062 controls	Exogenous hormone use		MSI in CRC	The relation between hormone use and CRC risk does not differ by MSI status.
[105]	Plaschke	2003	CCS	CRC	287 CRC, 346 controls	*MTHFR* SNPs		MSI in CRC	*MTHFR* SNPs are not associated with MSI-H CRC.
[106]	Poynter	2009	CCS	CRC	CCFR. Case-unaffected sibling design. 1564 CRC, 4486 controls	Smoking, alcohol		MSI in CRC	Smoking is associated with increased risk of MSI-H CRC (OR, 1.94; 95% CI, 1.09–3.46). Alcohol intake is associated with increased risk of MSI-L CRC (OR, 1.85; 95% CI, 1.06–3.24).
[107]	Poynter	2010	CCS	CRC	CCFR. Case-unaffected sibling design. 1200 CRC, 1880 controls	*VDR*, *GC* SNPs		MSI in CRC	*GC* rs222029, rs222016 and rs16847039 SNPs are associated with lower risk of MSI-H CRC, but not associated with MSS CRC.
[108]	Raptis	2007	CCS	CRC	766 CRC, 1098 controls	*MLH1*, *MSH2* SNPs		MSI in CRC	*MLH1* rs1800734 (−93G>A) SNP is associated with MSI-H CRC.
[111]	Rozek	2010	CCS	CRC	MECCS (northern Isreal). 1297 CRC, 2019 matched controls	Ethnicity, smoking, family history of CRC		*BRAF* mutation in CRC	Men who smoked are more likely to have *BRAF*-mutated tumor than women who never smoked.

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Exposure variables	Potential modifiable factors	Outcome variables	Main findings on modifiable (or genetic) factors and tumoral molecular changes
[113]	Samowitz	2006	CCS	CC	KPMCP-UT-MN. 1510 CC, 1981 controls	*IRS1*, *IRS2*, *IGF1*, *IGFBP3* SNPs		MSI, *KRAS*, *TP53* mutations in CC	*IRS1* G972R SNP is associated with MSI CC.
[114]	Samowitz	2006	CCS	CC	KPMCP-UT-MN. 1315 CC, 2392 controls	Smoking		CIMP, *BRAF* mutation in CC	Smoking ≥20 cigarettes/day is associated with CIMP+ (OR, 2.06; 95% CI, 1.43–2.97) and *BRAF* mutation (OR, 3.16; 95% CI, 1.80–5.54) compared to controls, but smoking is not associated with CIMP-negative or BRAF-WT.
[115]	Samowitz	2008	CCS	CC	KPMCP-UT-MN. 795 CC, 1968 controls	*MLH1* rs1800734 SNP		MSI, CIMP, *BRAF* mutation in CC	*MLH1* rs1800734 (−93G>A) SNP is associated with CIMP, *MLH1* methylation and *BRAF* mutation in MSI-H CC.
[116]	Satia	2005	CCS	CC	NCCCS. 486 CC, 1048 controls	Various food and nutriens, BMI, smoking, physical activity, family history, NSAIDs, vitamin mineral supplements		MSI in CC	No dietary factor is differentially related to MSI-H compared to MSI-L/MSS CC.
[120]	Shannon	2002	CCS	CRC	456 CRC, 1207 controls	*MTHFR* SNP, *CBS* polymorphisms		MSI in CRC	*CBS* 844ins68 variant is inversely associated with MSI in proximal CRC.
[123]	Slattery	2000	CCS	CC	KPMCP-UT-MN. 1510 CC, 2410 controls	BMI, physical activity, smoking, aspirin, NSAIDs		MSI in CC	Among both men and women, cigarette smoking is associated with MSI but not with MSS.
[124]	Slattery	2000	CCS	CC	KPMCP-UT-MN. 1428 CC, 2410 controls	Various food and nutrients		*KRAS* mutation in CC	Low cruciferous vegetable intake may be differentially associated with *KRAS* mutation vs. WT (p=0.01).
[125]	Slattery	2001	CCS	CC	KPMCP-UT-MN. 1428 CC, 2410 controls	BMI, dietary pattern, physical activity, smoking, aspirin, NSAIDs		*KRAS* mutation in CC	Among men, but not women, low physical activity is associated with *KRAS* mutation but not with *KRAS*-WT.
[126]	Slattery	2001	CCS	CC	KPMCP-UT-MN. 1510 CC, 2410 controls	Various food and nutrients		MSI in CC	Alcohol intake may increase MSI cancer risk.
[127]	Slattery	2001	CCS	CC	KPMCP-UT-MN. 1510 CC, 2410 controls	Oral contraceptive use, Estrogen replacement, number of pregnancies, BMI, physical activity		MSI in CC	Estrogen exposure in women may decrease MSI cancer risk.
[128]	Slattery	2002	CCS	CC	KPMCP-UT-MN. 1457 CC, 2410 controls	Family history of CRC		MSI, *KRAS*, *TP53* mutations in CC	Family history of CRC is not differentially associated with CC risk by MSI, *KRAS* or *TP53* status.
[129]	Slattery	2002	CCS	CC	KPMCP-UT-MN. 1458 CC, 2410 controls	Diet, physical activity, BMI, smoking, aspirin/NSAIDs use,		*TP53* mutation in CC	Western dietary pattern, red meat, and high glycemic load are associated with *TP53* mutation in CC.
[130]	Slattery	2002	CCS	CC	KPMCP-UT-MN. 1344 CC, 1958 controls	*GSTM1*, *NAT2* genotypes	Smoking	MSI, *TP53*, *KRAS* mutation in CC	*GSTM1* genotype and smoking may interact to influence occurrence of *KRAS* mutation in CC.
[131]	Slattery	2006	CCS	CRC	KPMCP-UT-MN. 1577 CC (unknown number for	*PPARG* P12A SNP		MSI, *TP53*, *KRAS* mutations in CC	It is unknown whether *PPARG* SNP differentially relates to CC risk by MSI, *TP53* or *KRAS* status.

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Exposure variables	Potential modifiable factors	Outcome variables	Main findings on modifiable (or genetic) factors and tumoral molecular changes
					molecular data), 1971 controls				
[132]	Slattery	2007	CCS	CC	KPMCP-UT-MN. 1154 CC, 2410 controls	BMI, nutrients, physical activity, smoking, aspirin, NSAIDs		MSI, CIMP, *BRAF* mutation in CC	Obesity is associated with CIMP-negative, but not CIMP+ (OR, 2.0; 95% CI, 1.5–2.6). Among MSI-high tumors, high alcohol intake is associated with BRAF-WT (OR 2.2; 95% CI, 1.2–3.7), but not among MSS tumors.
[133]	Slattery	2009	CCS	CC	KPMCP-UT-MN. 1375 CC, 2014 controls	Polymorphisms in various genes		MSI, CIMP, *KRAS*, *TP53* mutations in CC	Variants of insulin-related genes are associated with CIMP and MSI-H CC, especially among aspirin users.
[134]	Slattery	2010	CCS	CC	KPMCP-UT-MN. 1198 CC, 1987 controls	*SMAD7* SNPs (rs4939827, rs12953717, rs4464148)		MSI, CIMP, *KRAS*, *TP53* mutations in CC	*SMAD7* SNPs were not differentially associated with CC risk by MSI, CIMP, *KRAS* or TP53 status.
[135]	Slattery	2010	CCS	Rectal cancer	KPMCP-UT-MN. 750 rectal cancers, 1205 controls	Diet, physical activity, body size		CIMP, *TP53* mutation, *KRAS* mutation in rectal cancer	Certain dietary factors and physical activity are associated with CIMP, *TP53* mutation or *KRAS* mutation in rectal cancer. However, no comparison (CIMP+ vs. CIMP−; *TP53* mutation vs. WT; *KRAS* mutation vs. WT) is performed.
[136]	Slattery	2010	CCS	Rectal cancer	KPMCP-UT-MN. 750 rectal cancers, 1250 controls	Calcium, vitamin D, *VDR* genotypes		CIMP, *TP53* mutation, *KRAS* mutation in rectal cancer	Vitamin D intake and certain *VDR* genotypes are associated with certain *TP53* mutations in rectal cancer
[137]	Slattery	2010	CCS	Rectal cancer	KPMCP-UT-MN. 337 rectal cancers, 1192 controls	Alcohol intake		CIMP, *TP53* mutation, *KRAS* mutation in rectal cancer	Recent high beer consumption is associated with *TP53* mutation in rectal cancer. However, no comparison (*TP53* mutation vs. WT) is performed.
[138]	Slattery		CCS	CRC	KPMCP-UT-MN. 794 CRC, 1956 controls	SNPs in metabolic signaling pathway genes (*MTOR, PTEN, STK11, PRKAA1, PRKAG2, TSC1, TSC2, PIK3CA, AKT1*)		CIMP, MSI, *KRAS* mutation, *TP53* mutation in CRC	*PRKAA1* SNP (rs461404) is inversely associated with CIMP, and *PRKAA1* SNP rs13167906 is positively associated with CIMP. However, no comparison (CIMP+ vs. CIMP−; MSI-high vs. MSS; *TP53* mutation vs. WT; *KRAS* mutation vs. WT) is performed.
[139]	Urlich	2005	CCS	CC	KPMCP-UT-MN. 1248 CC, 1972 controls	*MTHFR* SNPs		*TP53*, *KRAS* mutations in CC	*MTHFR* codon 222 SNP variant is inversely associated with G>A mutations at CpG sites in *TP53* in CC.
[140]	van den Donk	2007	CCS	CRA	POLIER study. 149 CRA, 286 controls	Various nutrients, *MTHFR* codon 222 SNP		Methylation in *APC, CDKN2A* (p16), p14, *MLH1, MGMT, RASSF1A* in CRC	Folate intake may increase risk of adenoma without promoter methylation.
[145]	Wark	2006	CCS	CRA	534 CRA, 709 controls	Smoking, various food and nutrients		*KRAS* mutation in CRA	Smoking may increase risk of *KRAS*-WT CRA, but not that of *KRAS*-mutated CRA.

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Exposure variables	Potential modifiable factors	Outcome variables	Main findings on modifiable (or genetic) factors and tumoral molecular changes
[151]	Yang	2000	CCS	CRC	161 CRC, 191 controls	*SERPINA1* (A1AT) SNP	smoking	MSI in CRC	*SERPINA1* SNP variant and smoking may synergistically increase risk of MSI-H CRC.
Nested case-control study									
[142]	Van Guelpen	2010	Nested CCS (in PCS)	CRC	NSHDS. 190 CRC, 380 (?) controls	Plasma folate, vitamin B12, homocysteine; *MTHFR* SNPs		CIMP, MSI, *BRAF* mutation in CRC	*MTHFR* rs1801131 (codon 429) SNP variant may be associated with CIMP-negative CRC, but not with CIMP-high or CIMP-low CRC.
Prospective cohort studies (PCS)									
[36]	Chan	2007	PCS	CRC	NHS, HPFS. 13,0274 participants, 636 CRC	Aspirin		PTGS2 (COX-2) expression in CRC	Aspirin is associated with decreased risk of PTGS2 (COX-2)-positive CRC, but not with PTGS2-negative CRC.
[53]	English	2008	PCS	CRC	MCCS. 41528 participants, 582 CRC	Ethnicity (Anglo-Celtic vs. southern European origins)		CIMP, *BRAF* mutation in CRC	Southern European origin is associated with lower risk for CIMP+ or *BRAF*-mutated CRC, but not with risk for CIMP-negative or *BRAF*-wild-type CRC.
[78]	Limsui	2010	PCS	CRC	IWHS. 37,399 participants, 540 CRC	Smoking		MSI, CIMP, *BRAF* mutation in CRC	Smoking increases risks of MSI-high cancer, CIMP-high cancer, and *BRAF*-mutated cancer, but not MSS-low/MSS, non-CIMP-high or *BRAF*-wild-type cancer.
[117]	Schernhammer	2008	PCS	CC	NHS. 88,691 participants, 399 CC	Dietary one-carbon nutrients, alcohol		TP53 expression in CC	Folate intake decreases TP53-positive CC risk, but not TP53-negative CC risk.
[118]	Schernhammer	2008	PCS	CC	NHS, HPFS. 136,062 participants, 669 CC	Dietary one-carbon nutrients, alcohol		MSI, *KRAS* mutation in CC	CC risk does not significantly differ by MSI or *KRAS* mutation status.
[119]	Schernhammer	2010	PCS	CC	NHS, HPFS. 136,054 participants, 609 CC	Dietary one-carbon nutrients, alcohol		LINE-1 methylation level in CC	Folate intake decreases LINE-1 hypomethylated CC risk, but not LINE-1 hypermethylated CC risk.
[148]	Wish	2010	PCS	CRC	4337 at-risk first degree relatives of 552 index CRC patients in the Newfoundland Cancer Registry	MSI, *BRAF* mutation in CRC		CRC events in first degree relatives	Compared to family members of patients with MSS *BRAF*-wild-type CRC, family members of patients with MSI-high *BRAF*-mutated CRC and those with MSS *BRAF*-mutated CRC show higher risks of developing CRC.

Official gene and protein symbols are described in the HUGO-Gene Nomenclature Committee (HGNC) website (www.genenames.org). Studies with less than 100 cases with tumor tissue data are not listed, except for studies on rarely examined exposures or outcome. Studies on etiologically well-known types (polyposis syndromes, hereditary nonpolyposis colorectal cancer, inflammatory bowel disease-associated CRC) are not listed.

*Sample size is based on cases with available tumor tissue data.

Abbreviations: A1AT, alpha-1-antitrypsin; BMI, body mass index; CC, colon cancer; CCFR, Colon Cancer Family Registry; CCS, case-control study; CGH, comparative genomic hybridization; CI, confidence interval; CIMP, CpG island methylator phenotype; COX-2, cyclooxygenase 2; CRA, colorectal adenoma; CRC, colorectal cancer; DMR, differentially methylated region; EPIC, European Prospective Investigation into Cancer and Nutrition; HPFS, Health Professionals Follow-up Study; HR, hazard ratio; HRT, hormone replacement therapy; IWHS, Iowa Women's Health Study; KPMCP-UT-MN, Kaiser Permanente Medical Care Program of Northern California, the state of Utah and the Twin City Metropolitan area of Minnesota (the M. Slattery group's case-control study); LOH, loss of heterozygosity; MCCS, Melbourne Collaborative Cohort Study; MECCS, Molecular Epidemiology of Colorectal Cancer Study; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite stability; NCCCS, North Carolina Colon Cancer Study; NHS, Nurses' Health Study; NLCS, The Netherlands Cohort Study; NSAID, non-steroidal anti-inflammatory drug; NSCCG, National Study of Colorectal Cancer Genetics (UK); NSHDS, Northern Sweden Health and Disease Study; OR, odds ratio; RR,

incidence rate ratio; PCS, prospective cohort study; PTGS2, prostaglandin endoperoxide synthase 2; RCT, randomized, placebo-controlled trial; SEER, Surveillance Epidemiology, and End Results; SERPINA1, serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1; SNP, single nucleotide polymorphism; WBFT, Wheat Bran Fiber Trial; WT, wild-type.

Table 2

Molecular pathologic epidemiology studies to examine interactive prognostic effects of lifestyle or other etiologic factors and tumoral somatic changes in colorectal cancer.

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Tumoral feature	Hypothetical potential effect modifiers	Exploratory potential effect modifiers	Clinical outcome (number of events)	Findings
[18]	Baba	2009	PCS	CRC (stage I–IV)	NHS, HPFS. 598 CRC	CDX2 expression in CRC		Sex, age, BMI (prediagnosis), family history of CRC, tumor location, stage, grade, CIN, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA* mutation, expression of TP53, CDKN1A (p21), CCND1, CTNNB1, PTGS2 (COX-2)	CRC-specific survival (156 events), overall survival (255 events)	Loss of CDX2 expression is associated with poor prognosis among patients with family history of CRC, but not those without family history of CRC.
[19]	Baba	2009	PCS	CRC (stage I–IV)	NHS, HPFS. 487 CRC	AURKA (Aurora-A) expression in CRC		Sex, age, BMI (prediagnosis), family history of CRC, tumor location, stage, grade, CIN, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA* mutation, expression of TP53, CDKN1A (p21), CCND1, CTNNB1, PTGS2 (COX-2), FASN	CRC-specific survival (124 events), overall survival (216 events)	AURKA expression in CRC is not associated with prognosis and there is no interaction between AURKA and any of the covariates.
[20]	Baba	2010	PCS	CRC (stage I–IV)	NHS, HPFS. 731 CRC	HIF1A, EPAS1 (HIF-2A) expression in CRC		Sex, age, BMI (prediagnosis), family history of CRC, tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA* mutation, expression of	CRC-specific survival (221 events), overall survival (344 events)	HIF1A expression in CRC is associated with poor prognosis, and its prognostic effect is consistent across any stratum of the covariates. EPAS1 (HIF-2A) expression in CRC is not associated with prognosis and

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Tumoral feature	Hypothetical potential effect modifiers	Exploratory potential effect modifiers	Clinical outcome (number of events)	Findings
								TP53, PTGS2 (COX-2)		there is no interaction between EPAS1 and any of the covariates.
[21]	Baba	2010	PCS	CRC (stage I–IV)	NHS, HPFS. 491 CRC	PTGER2 (prostaglandin EP2 receptor) expression in CRC	MSI, PTGS2 (COX-2) in CRC	Sex, age, BMI (prediagnosis), family history of CRC, tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA* mutation, expression of TP53, CTNNB1	CRC-specific survival (139 events), overall survival (235 events)	PTGER2 expression in CRC is not associated with prognosis, and there is no interaction between PTGER2 and any of the covariates.
[23]	Baba	2010	PCS	CRC (stage I–IV)	NHS, HPFS. 1033 CRC	*IGF2* DMR0 hypomethylation in CRC		Sex, age, BMI (prediagnosis), family history of CRC, tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA* mutation	CRC-specific survival (292 events), overall survival (494 events)	*IGF2* DMR0 hypomethylation in CRC is associated with poor prognosis, and there is no interaction between *IGF2* DMR0 hypomethylation and any of the covariates.
[24]	Baba	2010	PCS	CRC (stage I–IV)	NHS, HPFS. 718 CRC	Phosphorylated PRKA (AMPK) expression in CRC	Phosphorylated MAPK3/1 (ERK) expression in CRC	Sex, age, BMI (prediagnosis), family history of CRC, tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA* mutation, expression of TP53, FASN	CRC-specific survival (194 events), overall survival (306 events)	There is a significant interactive prognostic effect between p-PRKA (p-AMPK) and p-MAPK3/1 in CRC. p-PRKA expression is associated with good prognosis in p-MAPK3/1-positive cases, but not in p-MAPK3/1-negative cases.

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Tumoral feature	Hypothetical potential effect modifiers	Exploratory potential effect modifiers	Clinical outcome (number of events)	Findings
[25]	Baba	2010	PCS	CRC (stage I–IV)	NHS, HPFS. 717 CRC	Phosphorylated AKT expression in CRC	*PIK3CA* mutation in CRC	Sex, age, BMI (prediagnosis), family history of CRC, tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF* mutation, expression of TP53, FASN	CRC-specific survival (210 events), overall survival (341 events)	p-AKT expression in CRC is associated with good prognosis, and there is no interaction between p-AKT and any of the covariates.
[173]	Chan	2009	PCS	CRC (stage I–III)	NHS, HPFS. 459 CRC	PTGS2 (COX-2) expression in CRC	Aspirin use (post-diagnosis)		CRC-specific survival (65 events), overall survival (167 events)	Aspirin decreases mortality of patients with PTGS2 (COX-2)-positive CRC, but not those with PTGS2-negative CRC.
[57]	Fierstein	2010	PCS	CRC (stage I–IV)	NHS, HPFS. 452 CRC	CDK8 expression in CRC	CTNNB1 in CRC	Sex, age, BMI (prediagnosis), tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA* mutation, expression of TP53, CDKN1A (p21), CDKN1B (p27), CCND1, PTGS2 (COX-2), FASN	CRC-specific survival (116 events), overall survival (202 events)	CDK8 expression in CC is associated with poor prognosis and there is no interaction between CDK8 and any of the covariates.
[72]	Kure	2009	PCS	CRC (stage I–IV)	NHS, HPFS. 599 CRC	VDR expression in CRC		Sex, age, BMI (prediagnosis), family history of CRC, tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA* mutation, expression of TP53, CTNNB1,	CRC-specific survival (158 events), overall survival (260 events)	VDR expression in CRC is not associated with prognosis and there is no interaction between VDR and any of the covariates.

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Tumoral feature	Hypothetical potential effect modifiers	Exploratory potential effect modifiers	Clinical outcome (number of events)	Findings
								CDKN1A (p21), PTGS2 (COX-2)		
[174]	Meyerhardt	2009	PCS	CC (stage I–III)	NHS, HPFS. 484 CRC	*KRAS*, *PIK3CA* mutation, expression of TP53, CDKN1A (p21), CDKN1B (p27), FASN	Physical activity (post-diagnosis)		CC-specific survival (50 events), overall survival (152 events)	Beneficial prognostic effect of physical activity may be limited to patients with CDKN1B (p27) nuclear+ CC.
[92]	Nosho	2009	PCS	CRC (stage I–IV)	NHS, HPFS. 456 CRC	SIRT1 expression in CRC	BMI (pre-diagnosis)	Sex, age, family history of CRC, tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA* mutation, expression of TP53, CTNNB1, FASN, PTGS2 (COX-2)	CRC-specific survival (116 events), overall survival (200 events)	SIRT1 expression in CRC is not associated with prognosis and there is no interaction between SIRT1 and any of the covariates.
[93]	Nosho	2009	PCS	CRC (stage I–IV)	NHS, HPFS. 708 CRC	JC virus T antigen expression in CRC		Sex, age, BMI (prediagnosis), family history of CRC, tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA* mutation, expression of TP53, CTNNB1, FASN, PTGS2 (COX-2)	CRC-specific survival (182 events), overall survival (300 events)	JC virus T antigen expression in CRC is not associated with prognosis and there is no interaction between JC virus T antigen and any of the covariates.
[175]	Nosho	2009	PCS	CRC (stage I–IV)	NHS, HPFS. 733 CRC	DNMT3B expression in CRC	CIMP in CRC	Sex, age, tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA* mutation, expression of TP53, CTNNB1	CRC-specific survival (191 events), overall survival (313 events)	DNMT3B expression in CRC is not associated with prognosis and there is no interaction between DNMT3B and any of the covariates.

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Tumoral feature	Hypothetical potential effect modifiers	Exploratory potential effect modifiers	Clinical outcome (number of events)	Findings
[2]	Ogino	2008	PCS	CC (stage I–IV)	NHS, HPFS. 647 CC	FASN expression in CC	BMI (pre-diagnosis)	Sex, age, tumor location, stage, grade, MSI, CIMP, *KRAS*, *BRAF* mutation, TP53 expression	CC-specific survival (160 events), overall survival (279 events)	High prediagnosis BMI increases mortality of patients with FASN+ CC, but not those with FASN-negative CC. Beneficial prognostic effect of FASN+ is limited to patients with non-obese prediagnosis BMI.
[176]	Ogino	2008	PCS	CC (stage I–IV)	NHS, HPFS. 662 CC	PTGS2 (COX-2) expression in CC	TP53 expression, MSI in CRC	Sex, age, tumor location, stage, grade, CIMP, *KRAS*, *BRAF* mutation	CC-specific survival (163 events), overall survival (283 events)	The adverse prognostic effect of PTGS2 (COX-2) is especially apparent in TP53-negative CC.
[177]	Ogino	2008	PCS	CC (stage I–IV)	NHS, HPFS. 643 CC	LINE-1 methylation in CC		Sex, age, tumor location, stage, grade, MSI, CIMP, TP53 expression, *KRAS*, *BRAF* mutation	CC-specific survival (160 events), overall survival (276 events)	The adverse prognostic effect of LINE-1 hypomethylation is consistent across any stratum of potential effect modifiers.
[95]	Ogino	2009	PCS	CRC (stage I–IV)	NHS, HPFS. 470 CRC	PPARG expression in CRC	BMI (pre-diagnosis)	Sex, age, family history of CRC, tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA* mutation, expression of TP53, CDKN1A (p21), CDKN1B (p27), CCND1, FASN, PTGS2 (COX-2), CTNNB1	CRC-specific survival (118 events), overall survival (199 events)	PPARG expression is associated with good prognosis, and its effect is not modified by any of the covariates.

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Tumoral feature	Hypothetical potential effect modifiers	Exploratory potential effect modifiers	Clinical outcome (number of events)	Findings
[96]	Ogino	2009	PCS	CRC (stage I–IV)	NHS, HPFS. 546 CRC	STMN1 expression in CRC	BMI (pre-diagnosis)	Sex, age, family history of CRC, tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA* mutation, expression of TP53, CDKN1A (p21), CDKN1B (p27), CCND1, FASN, PTGS2 (COX-2)	CRC-specific survival (147 events), overall survival (236 events)	Obesity (prediagnosis) increases mortality of patients with STMN1+ CRC, but not those with STMN1-negative CRC. The beneficial prognostic effect of STMN1+ is limited to patients with non-obese prediagnosis BMI.
[97]	Ogino	2009	PCS	CC (stage I–III)	NHS, HPFS. 450 CC	*PIK3CA* mutation in CC	BMI (pre-diagnosis), *KRAS* mutation in CRC	Sex, age, tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *BRAF*, expression of TP53	CC-specific survival (66 events), overall survival (152 events)	*PIK3CA* mutation in CC is associated with poor prognosis, and its adverse effect may be limited to patients with *KRAS*-WT tumors.
[178]	Ogino	2009		CC (stage III)	Inter-group trial CALGB 89803. 508 CC	*KRAS* mutation in CC		Sex, age, BMI, tumor location, stage, performance status, clinical bowel obstruction, bowel perforation, treatment arm, MSI in CC.	Disease-free survival (196 events), recurrence-free survival (180 events), overall survival (149 events)	*KRAS* mutation is not associated with clinical outcome. There is no interaction between *KRAS* and any of the covariates.
[98]	Ogino	2009	PCS	CC (stage I–IV)	NHS, HPFS. 630 CC	CDKN1B (p27) localization in CC	BMI (pre-diagnosis)	Sex, age, family history of CRC, tumor location, stage, grade, MSI, CIMP, *KRAS*, *BRAF*, *PIK3CA* mutation, expression of TP53, CDKN1A (p21), CCND1,	CC-specific survival (160 events), overall survival (272 events)	Obesity (prediagnosis) increases mortality of patients with CDKN1B (p27) nuclear+ CC, but not those with CDKN1B-altered CC. The beneficial prognostic effect

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Tumoral feature	Hypothetical potential effect modifiers	Exploratory potential effect modifiers	Clinical outcome (number of events)	Findings
								CTNNB1 (β-catenin), FASN, PTGS2 (COX-2)		of CDKN1B alteration is limited to obese patients (prediagnosis).
[99]	Ogino	2009	PCS	CC (stage I–IV)	NHS, HPFS. 647 CC	CDKN1A (p21) expression in CC	BMI (pre-diagnosis)	Sex, age, family history of CRC, tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA* mutation, expression of TP53, CCND1	CC-specific survival (162 events), overall survival (279 events)	Obesity (prediagnosis) increases mortality of patients with CDKN1A (p21) expressing CC, but not those with CDKN1A-lost CC. CDKN1A loss is associated with good prognosis in patients 60 years old or older, but with poor prognosis in patients younger than 60 years.
[100]	Ogino	2009	PCS	CC (stage I–IV)	NHS, HPFS. 602 CC	CCND1 (cyclin D1) expression in CC	MSI in CRC	Sex, age, BMI (prediagnosis), family history of CRC, tumor location, stage, grade, CIMP, *KRAS*, *BRAF*, mutation, expression of TP53, CDKN1A, CDKN1B, PTGS2 (COX-2), FASN	CC-specific survival (153 events), overall survival (259 events)	The beneficial prognostic effect of CCND1 expression in CC may be limited to MSI-low/MSS CC.
[101]	Ogino	2009	PCS	CC (stage I–IV)	NHS, HPFS. 532 CRC	18q LOH in CRC		Sex, age, BMI (prediagnosis), family history of CRC, tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA*, mutation, expression of TP53, CTNNB1, JC virus T antigen	CRC-specific survival (155 events), overall survival (239 events)	18q LOH in CRC is not associated with prognosis. There is no interaction between 18q LOH and any of the covariates.

Ref.	First author	Year	Study design	Tissue specimens	Study cohort, sample sizes (N)* and notes	Tumoral feature	Hypothetical potential effect modifiers	Exploratory potential effect modifiers	Clinical outcome (number of events)	Findings
[121]	Shima	2010	PCS	CRC (stage I–IV)	NHS, HPFS. 902 CRC	*CDKN2A* (p16) promoter methylation, loss of CDKN2A		Sex, age, BMI (prediagnosis), family history of CRC, tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA*, mutation, expression of TP53, CDKN1A, CDKN1B, CCND1, CTNNB1, PTGS2, FASN.	CRC-specific survival (235 events), overall survival (409 events)	*CDKN2A* promoter methylation (or loss of expression) in CRC is not associated with prognosis. There is no interaction between *CDKN2A* and any of the covariates.

Only studies with >300 tumor cases (generally with >100 events) are listed. To examine interactions with adequate statistical power, a sample size of at least 300 cases is necessary.

Official gene and protein symbols are described in the HUGO-Gene Nomenclature Committee (HGNC) website (www.genenames.org).

* Sample size is based on tumor tissue data available cases.

Abbreviations: BMI, body mass index; CALGB, Cancer and Leukemia Group B; CC, colon cancer; CIMP, CpG island methylator phenotype; CIN, chromosomal instability; COX-2, cyclooxygenase 2; CRC, colorectal cancer; DMR, differentially methylated region; HPFS, Health Professionals Follow-up Study; HR, hazard ratio; LOH, loss of heterozygosity; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite stability; NHS, Nurses' Health Study; NLCS, The Netherlands Cohort Study; PCS, prospective cohort study; PTGS2, prostaglandin endoperoxide synthase 2; WT, wild-type.