Convergence of Hormones, Inflammation, and Energy-Related Factors: A Novel Pathway of Cancer Etiology

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Abstract Colorectal cancer (CRC) is a multifactorial disease with several hypothesized etiologic factors including inflammatory processes; hormones such as estrogen, androgen, and insulin; and energy-related factors. We present evidence that integrates these elements in a pathway we call the convergence of hormones, inflammation, and energy-related factors (CHIEF). First, given the physiology of the gut, substantial epidemiologic and molecular data support the hypothesis that activation of innate immunity in the normal gut mucosa by various environmental agents (commensal bacteria, dietary antigens, mucosal irritants, pathogens) and endogenous factors such as estrogen, androgens, and insulin levels provokes basal inflammation as an underlying factor of the association of insulin, estrogen, and energy-related factors with CRC. Second, critical genes involved in this pathway, e.g., phosphatase tensin homologue on chromosome 10 (PTEN) and serine threonine kinase 11 (STK11)/LKB1, are tumor suppressor genes often mutated in intestinal cancer or CRC. Third, laboratory experiments show that cellular PTEN and STK11/LKB1 tumor suppressor enzymes are vulnerable to inactivation by redox-active species, especially chemically reactive lipid mediators of inflammation and redox stress. Epidemiologic data further support the underlying proposal that CHIEF comprises important elements of CRC risk. Although this discussion of the CHIEF pathway focuses on CRC, we believe that this pathway may play an important role in the etiology of other cancers as well.

Epidemiology is devoted to understanding the causes and distribution of diseases within populations. Cancer epidemiology has focused on associations between specific cancers and environmental, nutritional, behavioral, physiologic, and molecular and genetic factors. Observations from these studies have been central to our current understanding of the causes of cancer and our efforts to control cancer through public health policy, interventions, and translational research. Percival Pott's observation in 1775 that soot exposure led to a high rate of scrotal cancer in chimney sweeps likely gave birth to the concept of chemical carcinogenesis, which was validated in preclinical studies of 1918 proving that soot caused scrotal cancer in animals (the cancer link to polycyclic aromatic hydrocarbons in soot was made in 1938; ref. 1). Pott's observation led to important workplace measures for cancer prevention. The association between tobacco use and lung cancer is a more recent example of epidemiologic observations leading to public health measures to reduce cancer risk.

Not all associations between risk factors and cancer are as robust and singular as are the associations between chimney soot and scrotal cancer, or tobacco smoke and lung cancer. One of the challenges of contemporary epidemiology is to determine the common molecular processes through which risk factors operate. Epidemiologic observations of the present day emphasize the complexity of cancer etiology, comprising abundant data suggesting that cancers have multiple causes and usually both unique and overlapping risk factors. However, as in 1775, epidemiologic observations are crucial to our understanding of cancer etiology and continue to lay the foundations for cancer prevention and control.

In this review, we consider the associations of inflammation, hormones, and energy-related factors such as obesity, physical activity, and energy intake with colorectal cancer (CRC) and propose a mechanistic explanation for their convergent effects on colorectal carcinogenesis. We label our proposed pathway the convergence of hormones, inflammation, and energy-related factors (CHIEF) and provide epidemiologic and molecular evidence that supports the importance of this pathway to CRC. Although our discussion of the CHIEF pathway focuses on CRC, we believe that this pathway also may play an important role in the etiology of other cancers.

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The Gut: A Lymphoid Organ Routinely Exposed to Inflammatory Stimuli

Inflammation, a core component of the CHIEF pathway, is an underlying feature of physiologic and pathologic processes that operate throughout the gut, particularly the intestinal tract and colon. Many pathologic aspects of gut inflammation, e.g., colitis, are well established and well understood. By contrast, physiologic aspects of inflammation are unknown and often not acknowledged. Conventional sources of inflammation—infectious pathogens and tissue injury—occupy one end of a continuum of pathologic processes that inaugurate inflammation. They disrupt vascular integrity and cause plasma proteins and leukocytes to accumulate at an affected tissue site, where they annihilate pathogens and eventually help restore morphologic and histologic integrity. Toward the other end of the continuum, tissue stressors or malfunction, such as premalignant lesions including adenomatous polyps, can provoke an adaptive response sometimes called "parainflammation." This response may involve tissue-resident leukocytes (macrophages, eosinophils) and adjacent cells in the stroma (fibroblasts), and it is intermediate between the basal homeostatic state and a classic inflammatory response. Parainflammation is likely a major contributing factor to many modern human diseases including CRC. Parainflammation is inseparable from the role of the gut in human physiology.

The gut, particularly the large intestine, is one of the largest and most diversified lymphoid organs of the body (2) and is routinely exposed to immune and inflammatory stimuli. It contains gut-associated lymphoid tissues, mast cells, resident macrophages, lymphocytes, and eosinophils (3) in the lamina propria and submucosa of the intestine and colon (4), and specialized epithelial cells called M cells, which allow host immune cells to sample luminal antigens (5). All of these cells are close to the epithelial cells (colonocytes), suggesting that biochemical interactions between the stroma and epithelium could influence colorectal tumor progression. Basically, the gut mucosa and stroma are the first ramparts of a host's defense against swallowed matter, including bacteria, bacterial lipopolysaccarides, gut parasites, food antigens, particulates, chemical irritants, alcohol, dietary polyunsaturated fatty acids, carcinogens, and even certain drugs, which can activate innate and adaptive immune responses. Furthermore, anything that disrupts the mucosal barrier, e.g., ulcers or colorectal polyps (6), can expose the lamina propria to bacteria, exacerbate immune activation, and necessitate repair or replacement of cells at the affected site of the gut.

Once thought to be a host response against tumors, localized stromal inflammation may actually worsen cancer risk and progression (7–10). Eosinophils are inflammation-related leukocytes with a special capability to counter parasitic infections in the gut (3, 11), and mild-to-moderate eosinophilia occurs in the stroma of ~75% of colonic adenomas (12). Neutrophils, another inflammation-related group of leukocytes, are occasionally found in colorectal polyps (13). Eosinophils and neutrophils also can cause tissue remodeling and wound healing, not just tissue damage such as that associated with polyps. It is possible that eosinophils and neutrophils mediate both cell destruction and normalization and recovery from inflammation, and are potentially important modulators of intestinal tumorigenesis; they are the first inflammation cell types recruited in response to proinflammatory cytokines. Mast cells are then activated, stimulating the proinflammatory cytokines and chemokines such as the interleukin (IL) family, IFNs, and tumor necrosis factors (TNF; ref. 14). Macrophages derived from monocytes that have infiltrated the inflamed area provide a network of growth factors, cytokines, and prostaglandins.

The inflammatory loci are further influenced by interaction with epithelial and vascular endothelial cells and are closely linked with angiogenesis, which involves the growth of new blood vessels from preexisting vessels. Both angiogenesis and inflammation are hallmark features of tumorigenesis as well as other diseases such as Crohn's disease, diabetes, and obesity (14), and have related molecular events. Inflammatory cells that have infiltrated tissue are one of the factors that stimulate angiogenesis; the vascular endothelial growth factor (VEGF) is known to play an important role in angiogenesis (15). Without the recruitment of blood vessels during angiogenesis, tumors are limited in growth and in their ability to metastasize (16). In animal models, it has been shown that inflammatory cytokines such as IL-1α and IL-1 β are required for angiogenesis and tumor growth (17, 18), whereas cyclooxygenase (COX)-2 acts both as an proangiogenesis and proinflammatory agent (19, 20).

Inflammatory processes seem to be a key element in colorectal carcinogenesis. The intestine must carefully balance immunity, which protects us from harmful microbes, and tolerance, which permits interactions with harmless commensal bacteria and dietary antigens. Disruptions that tilt the balance toward immune activation/inflammation could facilitate tumor progression in the intestinal tract. The loss of balance between anti-inflammatory and proinflammatory mediators may precipitate or aggravate neoplastic transformation in the intestinal epithelium. This concept is best thought of as a continuum. Illustrating the mild end of the continuum are mice held in germ-free environments, where there are fewer bacterial threats and, presumably, reduced mucosal immune activation; they do not develop intestinal tumors (21). At the severe end of the continuum, persistent inflammation in the colon is a strong independent risk factor for CRC (7). Ulcerative colitis lasting >20 years increases the risk for CRC as much as do inherited cancer syndromes such as familial adenomatous polyposis (22). The initial inflammatory insult in the gastrointestinal (GI) tract may originate from diet and carcinogenic exposures that lead to a state of low-level inflammation. Disruptions in the delicate balance between harmful microbes and harmless commensal bacteria in the GI tract that enable immune activation/inflammation could facilitate multistep tumorigenesis in the intestinal tract (Fig. 1).

Molecular Support for CHIEF, the Pathway of Convergence

The importance of the convergence of inflammation with pathways that involve hormones and energy-related factors suggests the necessity to examine the broad spectrum of these interrelated pathways rather than each pathway in isolation. This integrated approach is the essence of the CHIEF pathway (Fig. 2). The LKB1 → AMP–activated protein kinase α → tuberous sclerosis 1&2 → mammalian target of rapamycin (mTOR) → ribosomal protein S6 kinase (S6K) component of

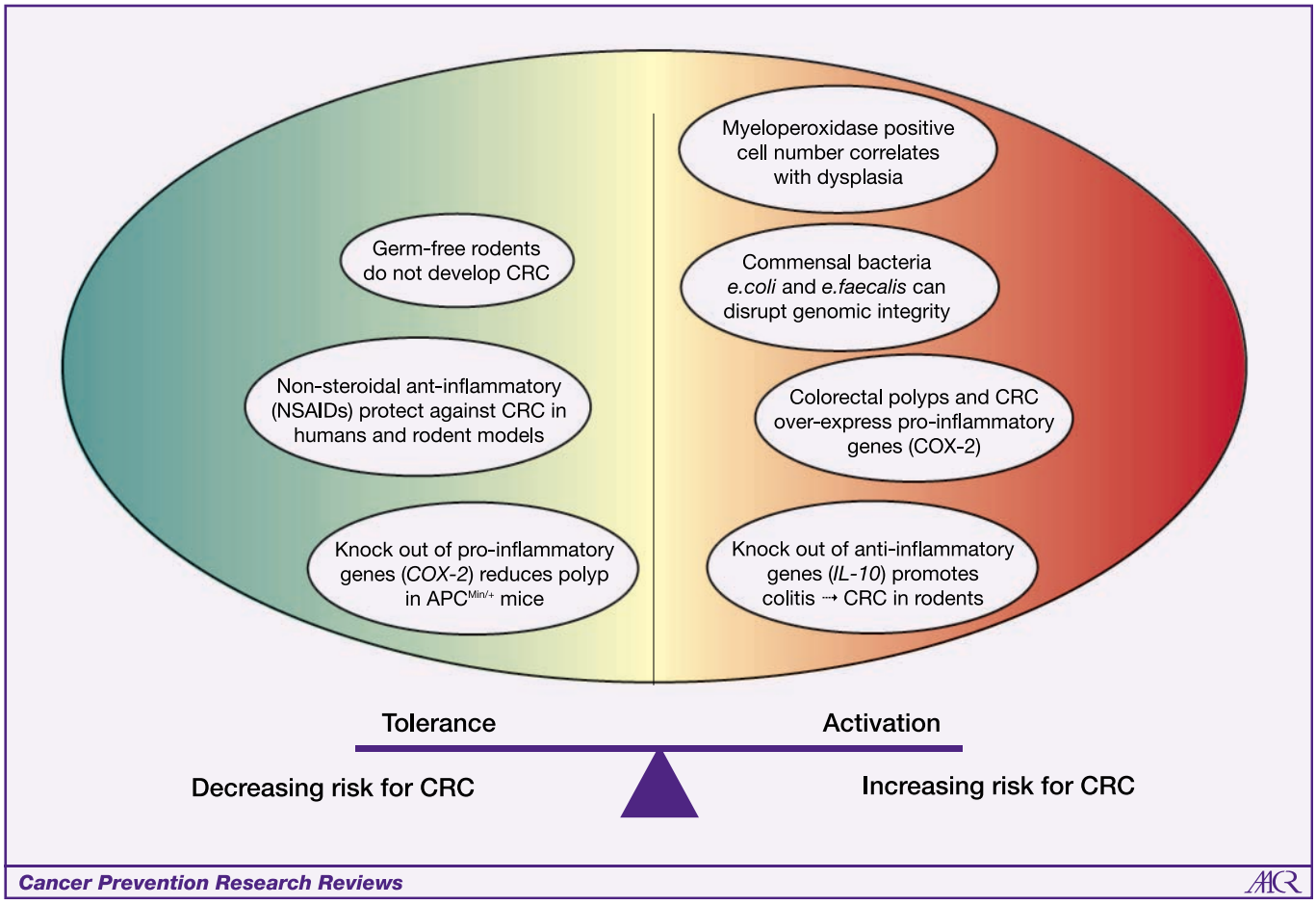

Fig 1. The balance between tolerance and activation of CRC risk. The intestine must carefully balance immunity, to protect us from harmful microbes, and tolerance, to permit interaction with harmless commensal bacteria and dietary antigens. Disruptions that tilt the balance toward immune activation/inflammation could facilitate multistage tumor progression in the intestinal tract.

our proposed pathway senses and responds to changes in cellular ATP levels, which may be governed in part by diet, physical exercise, or inflammation (23); therefore, this component integrates nutrient and insulin signaling. Cells with low ATP and excess AMP activate LKB (STK11) at the apex of this pathway (23–26) in repressing anabolic processes (ATP use) and enhancing catabolic processes (ATP generation). The signaling pathway senses and responds to changes in cellular energy balance (ATP levels; refs. 27, 28). LKB1 is a tumor suppressor that causes Peutz-Jeghers syndrome, an autosomal dominant disorder that often manifests as hamartomatous polyps that increase the risk of intestinal cancer. LKB is an AMPK kinase and a tumor suppressor and therefore is a prominent link between metabolic signaling pathways and cell proliferation and polarity pathways (28–31). In cells with excess AMP due to altered energy homeostasis, LKB1 phosphorylates AMP-dependent kinase (PRKAA1&2; refs. 24–27), which in turn phosphorylates proximal substrates like acetyl-CoA carboxylase and tuberous sclerosis 1&2. Sequential phosphorylation of distal substrates in this kinase cascade, such as mTOR and S6K (also known as RPS6KA1&2), ultimately represses anabolic processes (ATP use) and enhances catabolic processes (ATP generation). This process restores the system to normal energy homeostasis. Overall, LKB1 is a prominent, widely expressed AMPK kinase that governs whole-body insulin sensitivity via this signaling pathway (32, 33).

A different portion of the pathway that responds to insulin, estrogen, androgen, and certain proto-oncogene growth factors contains PTEN. PTEN is an unusual phosphatase enzyme which can preferentially remove phosphates from phosphatidyl inositol triphosphate 3, 4, and 5. Like LKB1, PTEN also has a dual role as a tumor suppressor and regulator of metabolic signaling. PTEN acts as a metabolic regulator by modulating the AKT1 kinase, which is downstream of the insulin receptor. This modulation ultimately affects the feedback inhibition processes [less insulin-receptor substrate (IRS) 1&2 transcription, more IRS1&2 phosphorylation] necessary to maintain normal insulin sensitivity. PTEN acts as a tumor suppressor by an analogous mechanism-negative regulation of the phosphatidyl inositol-3-kinase (PI3K)/AKT1 oncogenic signaling pathway. Loss of function from germ line mutations in the *PTEN* gene causes PTEN hamartoma tumor syndrome (34). This syndrome includes Cowden's disease, an autosomal dominant, complex disorder that manifests as malignant and benign (hamartomatous) lesions affecting breast, thyroid, uterus, brain, and mucocutaneous tissues. Because PTEN is both a tumor suppressor protein and a regulator of metabolic signaling, it is a candidate common factor in

the development both of some types of cancer and insulin resistance.

Cytokines also represent the inflammatory process in the CHIEF pathway because inflammation is initiated by the synthesis and secretion of proinflammatory cytokines (e.g., TNF and IL-6 in macrophages) in response to an inflammation-provoking insult. The increased production of cytokines and subsequent elevation in reactive oxygen and nitrogen species are recognized hallmarks of inflammation. This process is regulated by a negative-feedback mechanism and closely followed by the secretion of anti-inflammatory cytokines (e.g., IL-10). As seen in Crohn's disease and ulcerative colitis, the cellular antioxidant defense system is activated to limit the development of chronic inflammation, which gives rise to a much higher-than-normal cancer risk. The binding of proinflammatory cytokines to their receptors triggers the activation of NFκB, which in turn activates the expression of a wide variety of genes including cytokines and COX-2. The unchecked activation of NFκB/COX-2 frequently occurs in colon tumor cells.

NFκB is an important nuclear transcription factor that regulates a large number of cytokines and is critical for the regulation of tumorigenesis, cell proliferation, apoptosis, response to oxidative stress, and inflammation. It plays a critical role in diseases associated with dysregulated immune response and is an important regulator of insulin; inappropriate activation of NFκB has been linked to inflammatory events. TNFα can activate the inhibitor of κB kinase (IKK) complex and induce insulin resistance (35, 36) and is thought to be one of the most important promoters of inflammation because it induces activation of at least two of the relevant major signaling pathways (IKK and NFκB). The IKK complex is the key regulator of NFκB's transcriptional activity.

NFκB regulates the IL cytokines. IL-6 stimulates liver secretion of C-reactive protein, which is now considered to be an important biomarker for proinflammatory status in several diseases including cancer (37). Serum IL-6 is increased in many inflammatory diseases, but in contrast to other cytokines such as TNF, IL-6 can have both proinflammatory and antiinflammatory effects. Serum IL-6 has been shown to be elevated in several cancers, including prostate, bladder, colon, and breast cancer (38–40). IL-8 is a proinflammatory cytokine that primarily mediates the activation and migration of neutrophils into tissue from peripheral blood. IL-10 is an immunosuppressive cytokine and also is known as the cytokine synthesis inhibitory factor. Studies have shown that IL-10 may arrest chronic inflammatory responses in atherosclerosis (41) by responding to the secretion of proinflammatory cytokines such as IL-6 and TNF and by downregulating their activity. Circulating levels of IL-10 are elevated in obese women and decreased in metabolic syndrome (42, 43).

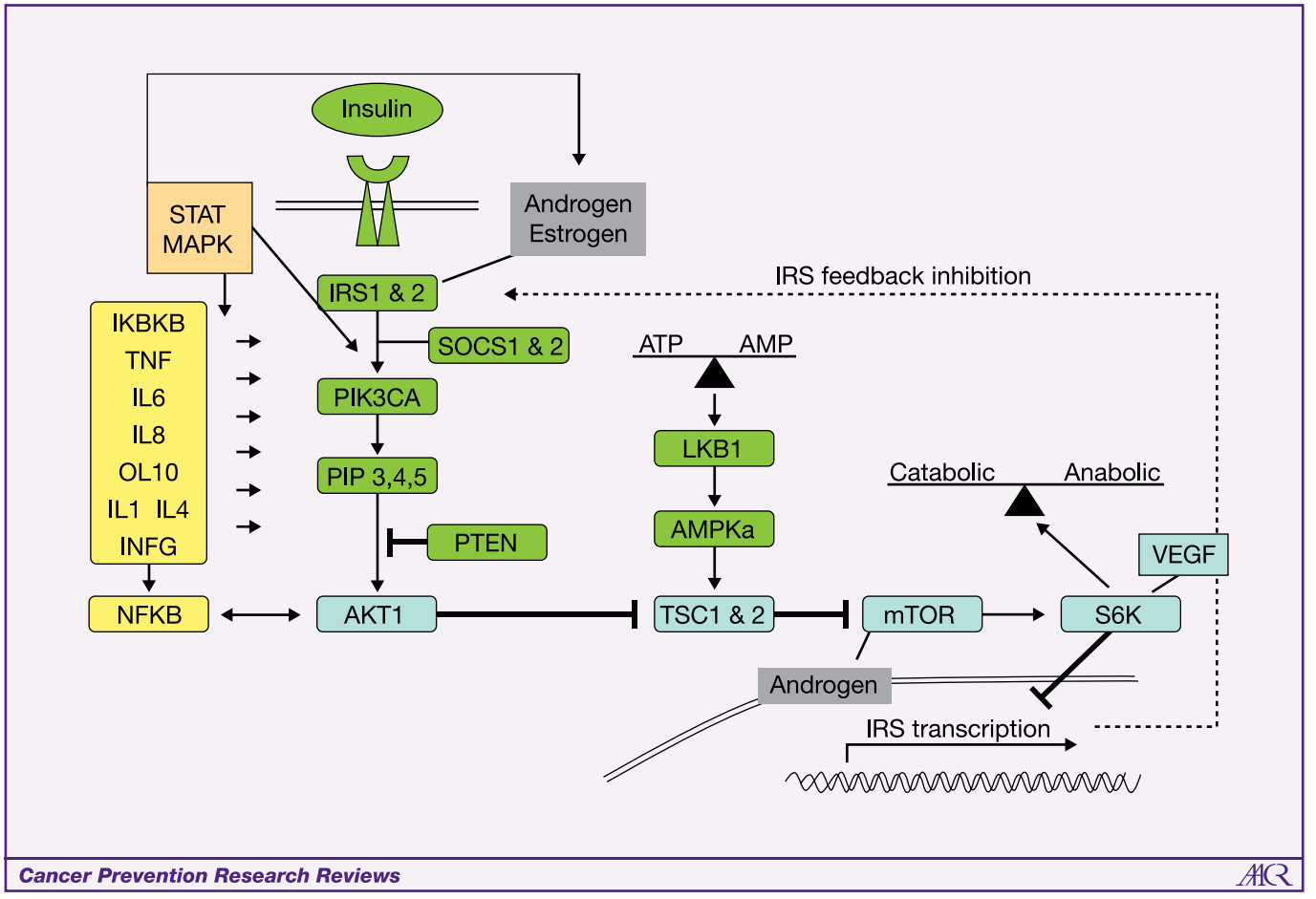

Fig 2. Convergent signaling pathways where inflammation and metabolic signaling intersect along the CHIEF pathway.

Other cytokines with a potential impact on CRC risk through inflammation-related mechanisms include IL-1 (both IL-1α and IL-1β), IL-4, and IFN-γ. IL-1α and IL-1β are potent mediators of inflammation and immunity and have been associated with IL-6 and TNF levels in several diseases thought to have an inflammation-response element in their etiology (44, 45). IL-1β is an important element in the angiogenesis and invasiveness of several tumor cells (18). IL-1 receptor A is responsible for regulating IL-1α and IL-1β. IL-4 protects endothelial cells from the cytotoxic effects of the PI3K/Akt signaling pathway (46) and inhibits NFκB and calcium signaling (47, 48). IL-4 may have a direct effect on intestinal epithelial cells and upregulates the expression of suppressors of cytokine signaling (49, 50). INF-γ is a proinflammatory cytokine that can activate the transcription factors NFκB and c-Jun N terminal kinase (JNK).

Steroid hormones including estrogen, androgen, and progesterone have been shown to have both anti-inflammatory and proinflammatory properties (51, 52) and, therefore, are components of an inflammation-related pathway. The receptors of the steroid hormones have been shown to interact with NFκB in an antagonist manner (52–54). However, studies also suggest that the influence of estrogen may depend on cell type and form of estrogen (51, 52, 55). Estrogen receptor (ER) β, the most abundant ER in the GI tract, has been shown to have a dramatically beneficial effect in the HLA-B27 transgenic rat model of inflammatory bowel disease (56). Estrogen also has been shown to repress IL-6 expression as well as IκB, potentially explaining its anti-inflammatory mechanism (52, 57). It has been hypothesized that ER-negative breast tumors may metastasize through activation of NFκB and IκB due to loss of ER function (52).

It is hypothesized that inflammation-related mechanisms are associated with insulin through the axis connecting IRS1&2 → PI3K → AKT1 → tuberous sclerosis 1&2 → mTOR→ S6K1&2. The *VEGF* gene may influence CRC development through its action on tumor cells and angiogenesis; VEGF also regulates S6K and IRS-1 and may play an important role in regulating cell growth signaling within this pathway (58). PTEN restrains insulin signaling via this pathway. Chemical inactivation of PTEN also predisposes to polyp formation and the attendant risk for cancer.

Signal transduction and activation of transcription (STAT) and mitogen-activated kinases (MAPK) are involved in both inflammation and metabolic signaling associated with hormones and energy-related factors. STAT protein family members are phosphorylated in response to cytokines and growth factors. Therefore, their involvement in convergent areas of multiple pathways makes them attractive candidate etiologic factors of CRC. STAT-1 is activated by NFκB, IFN-γ, IL-1, and IL-6 and mediates the expression of a variety of genes; STAT-1–dependent transcription of proinflammatory genes is regulated by IFN-γ–activated PI3K and mTOR pathways (59). Data also suggest that STAT-1 activation can contribute to maintaining and expanding the local inflammatory response in celiac disease and may therefore have implications for CRC (60). STAT-6 plays a central role in IL-4–mediated biological responses that involve TNFα, IL-8, and NFκB, and the STAT-6 pathway contributes to the hypercontractility of intestinal muscle in Crohn's disease (61). STAT-6 also is involved in signaling from the leptin receptor and may therefore be involved in obesity and energy balance (62).

MAPKs are a family of STKs that serve as an integration point for multiple biological signals and are involved in a variety of cellular processes such as proliferation, differentiation, and transcription regulation. JNK-1 or MAPK-8 is activated by TNFα and is necessary for apoptosis. NFκB is required to terminate JNK signaling. JNK-1 activity is elevated in obesity, and an absence of JNK-1 results in decreased adiposity and improved insulin sensitivity (63). P38MAPK (also known as MAPK-14) is activated by environmental stresses and proinflammatory cytokines. *TP53* is mutated in roughly half of CRCs and is one of the substrates of P38MAPK, suggesting a role in cell cycle regulation. P38MAPK plays a role in multiple pathways that include the release of VEGF and is involved in the PI3K pathway related to *IL6* gene expression.

Epidemiologic Support for the CHIEF Pathway

Inflammation

Given the physiologic properties of the gut, the molecular properties of the CHIEF pathway, and the relevant epidemiology literature, it is reasonable to hypothesize that inflammation is the underlying determinant of CRC risk because it mediates the risk associated with many other factors. There is intriguing epidemiologic evidence of inflammatory mediators interacting with various metabolic, hormonal, and energy-related factors to alter the risk of CRC, suggesting that they may be central to explaining observed inconsistencies in the literature surrounding CRC risk factors. As previously noted, several inflammatory conditions predispose to CRC.

Epidemiologic and clinical data consistently support an association between aspirin and other nonsteroidal anti-inflammatory drugs (NSAID) and a reduced risk of CRC (64), suggesting that inflammation-lowering factors reduce CRC risk. NSAIDs also have been shown to modulate CRC risk associated with diet, life-style, and genetic factors (65). In laboratory settings, high doses of salicylates (salts and esters of salicylic acid, which is similar but not identical to the active ingredient of aspirin) inhibit NFκB and its upstream activator IKKβ, which regulate immune cell differentiation and survival (36). A 2- to 3-fold increased risk of colon cancer has been associated in some studies with high levels of C-reactive protein, a marker of systemic inflammation (37).

Although limited, epidemiologic studies of polymorphisms of genes thought to be involved in inflammation-related pathways provide additional support for inflammatory processes as a component of CRC development. Polymorphisms in *IL8* have been associated with inflammation levels and CRC development (66). In a study using hospital-based controls (66), associations between CRC risk and inflammation-related polymorphisms included a 50% reduction associated with the Ala variant of P12A peroxisome proliferator-activated receptor γ (*PPAR-γ*), a 70% increase associated with the C allele for *IL6*, and a 30% reduction in risk associated with the A allele of *IL8*. Another population-based study of CRC showed that ibuprofen-type drugs significantly interacted with the P12A *PPAR-γ* polymorphism to alter rectal cancer risk (67). *SMAD7* single nucleotide polymorphisms have been shown to be associated with colon cancer risk in genome-wide association studies (68, 69); *SMAD7* is involved in inflammation-related

pathways and has been shown to modulate transforming growth factor β and wnt signaling (70). Confirmatory studies of the *SMAD7* association with colon cancer showed that aspirin and/or NSAID use modified the CRC risk associated with *SMAD7*.[5] Polymorphisms of *IFN-γ* have been shown to influence survival in patients with pancreatic cancer and breast cancer (71) and may be an important mediator of inflammation-related processes. Most inflammation-related genes, however, have not been examined for potential associations with CRC.

Hormones

Androgenic and estrogenic steroids and insulin-related hormones have been identified as key risk factors in the etiology of CRC. Women taking hormone replacement therapy have been shown to have a reduced CRC risk in both observational studies and clinical trials (72, 73); it seems that aspirin may modulate the risk associated with estrogen (74). Genetic polymorphisms in both the androgen receptor and *ER* genes are associated with altered CRC risk (75). Estrogen represses the production of IL-6 through an ER-dependent mechanism; serum levels of IL-6 increase following menopause in healthy women and with age in both men and women (76, 77).

An insulin-related pathway has been proposed as a unifying mechanism underlying several CRC risk factors including physical inactivity, obesity, and certain dietary factors such as glycemic index and dietary fiber (78, 79). Polymorphisms of genes associated with insulin-related pathways, such as *IRS1*, are associated with colon cancer, and these associations can be modified by aspirin use (80). Insulin-like growth factor (IGF) 1 (*IGF1*), IGF binding protein 3 (*IGFBP3*), *IRS2*, and IGF receptor 1 (*IGFR1*) polymorphisms have not been consistently associated with CRC risk. Polymorphisms of the transcription factor 7-like 2 (*TCF7L2*) gene have been associated with insulin sensitivity and diabetes, and *TCF7L2* is involved in the Wnt/β-catenin signaling pathway, all thought to be important factors in the etiology of colon cancer (81, 82). Folsom et al. (83) showed a significantly increased CRC risk in association with the T allele of the rs7903146 *TCF7L2* polymorphism. A significant interaction has been found between the rs7903146 *TCF7L2* polymorphism and recent use of aspirin/NSAIDs ($P_{interaction}$ = 0.001); an increased colon cancer risk associated with the T allele was restricted to people who had not recently used aspirin and/or NSAIDs [odds ratio, 1.65; 95% CI, 1.35-2.02) relative to recent (within 2 years before diagnosis) aspirin and/or NSAID users with the CC genotype; ref. 84]; and recent aspirin and/or NSAIDs use reduced colon cancer risk in people with the T allele (odds ratio, 0.78; 95% CI, 0.62-0.98) in a dose-response fashion (P = 0.03 for a linear trend across genotypes; ref. 84). These data provide additional support for aspirin and/or NSAIDs use, a crude potential surrogate for the inflammatory state of the GI tract, as an important mediator of hormone-related CRC risk.

The associations between insulin-related factors, including polymorphisms of genes in an insulin-related pathway, and CRC seem to be modified by aspirin use (80). Furthermore, salicylates have been shown to have a hypoglycemic effect (85–89), and high doses of salicylates can reverse hyperglycemia, hyperinsulinemia, and dyslipidemia by influencing insulin signaling (36). Epidemiologic studies show strong associations between systemic markers of inflammation (e.g., IL-6, TNF) and a heightened risk for obesity-related insulin resistance (90–93). Laboratory studies support a role for the inflammatory cytokine TNF and the NFκB signaling pathway in the association between obesity, chronic inflammation, and insulin resistance (94, 95). *TNF* has been reported to inhibit insulin-induced glucose uptake by targeting components of the insulin-signaling cascade, one component of which is IRS-1 (96–100). Aspirin has been shown to protect insulin-induced glucose uptake in TNF-treated adipocytes by blocking TNF effects on IRS1 (96).

Although steroid- and insulin-related hormonal factors seem to be important in CRC etiology, it also seems that they do not reflect isolated pathways to cancer development; rather, they reflect an interrelated hormone-related pathway that operates on an underlying state of inflammation. Given the recurring theme of effect modification by aspirin and/or NSAIDs, it seems that the inflammatory state of the GI tract may be an important determinant of the CRC risk associated with estrogen and androgen and insulin-related factors.

Energy-Related Factors

Energy-related factors include factors related to energy balance, diet, physical activity, and obesity. These factors also have been proposed as key factors related to insulin signaling (79). Epidemiologic data show inconsistent associations between energy intake and CRC risk, and between energy-contributing nutrients (such as dietary fat) and CRC risk (101). Although physical activity has been consistently associated with a reduced risk of colon cancer, its association with rectal cancer risk has been inconsistent (102). Overweight and obesity have been identified consistently as important risk factors for colon cancer among men, premenopausal women, and postmenopausal women taking hormone replacement therapy (103–106); data suggest that body mass index (BMI)–related colon cancer risk also is modulated by aspirin and/or NSAIDs (107).

Diet, physical inactivity, and obesity are associated with inflammation as well as with insulin signaling. The finding that high-fat meals increase the coabsorption of enteric bacterial lipopolysaccaride endotoxin, causing low-grade endotoxemia and postprandial inflammation (108), links diet to an inflammation pathway. Studies suggest that aspirin use may modify harmful effects of dietary fat, and it has been suggested that a lower CRC risk associated with dietary fat in more recent years may relate to higher aspirin use in the general population (109, 110), suggesting further that dietary fat effects on CRC risk may involve an inflammation-related mechanism. IL-6 is a "mytokine," or a cytokine produced in muscle, and is elevated in response to muscle contraction (111). It is significantly elevated with exercise and proceeds the appearance of other cytokines in the circulation. During exercise, IL-6 is thought to act in a hormone-like manner to mobilize extracellular substrates and/or augment substrate delivery (111). Therefore, inflammation-related mediators also may influence the effects of physical inactivity, a factor consistently associated with colon cancer risk.

Data on associations between body size and TNFα, IL-6, leptin, and adiponectin support obesity as an "inflammatory

[5] M.L. Slattery, J. Herrick, K. Curtin, et al., unpublished data.

state." Adipose tissue expresses mRNA for TNFα, and this expression is increased in healthy obese adults (112, 113). IL-6 is produced by the stroma of adipose tissue, and serum IL-6 levels are increased in healthy obese individuals, independently of age or menopause (112). It is estimated that about one third of circulating IL-6 in a healthy individual derives from adipose tissue (114). Qi et al. (115) studied associations between the genetic variability of *IL6* and long-term changes in or related to BMI among 2,255 healthy women and 980 healthy men from two prospective cohorts. The *IL6* haplotype 222211 (comprising rs2069827, rs1800797, rs1800795, rs1554606, rs2069861, and rs1818879; 1 codes the common allele and 2 codes the minor allele) was consistently and significantly associated with a greater waist circumference ($P = 0.009$ in men and 0.0003 in women) and baseline BMI ($P = 0.01$ in men and 0.046 in women) compared with the most common *IL6* haplotype, 111112. A 5-prime polymorphism, rs2069827, also was consistently associated with significantly higher early-adulthood BMI, baseline BMI, and waist circumference in men and women. Adipose tissue secretes adipokines such as adiponectin and leptin. As members of the IL-6 family of cytokines, leptin and its receptor are considered to be proinflammatory cytokines and to play a major role in modulating inflammation and immune response (116, 117). Epidemiologic data have shown a statistically significant interaction between recent use of aspirin and/or NSAIDs and leptin (*LEP*) polymorphisms and the risk of developing colon cancer (118).

Conclusions

The physiologic structure of the gut and supportive epidemiologic and molecular data led us to propose that basal immune activation—a repetitive, mild subclinical inflammation—is the underlying modulator of CRC risk and influences the CRC risk associated with insulin, estrogen, and energy-related factors. The CHIEF pathway integrates elements of angiogenesis, hormones, and energy-related factors with the underlying inflammatory state of the colon and rectum. Additional support for the importance of the CHIEF pathway comes from the fact that many of its genes are tumor suppressor genes, which frequently are mutated in CRC.

We believe that the CHIEF pathway of convergence resonates with other cancers including breast and possibly prostate cancer. Breast and prostate cancer risk assessments have revealed many of the same risk factors, with varying levels of risk and relative importance, as those identified for CRC. It is intriguing that obesity seems to increase colon cancer risk (119) and is associated with a reduced breast cancer risk (120) in premenopausal women. Hormone replacement therapy reduces the risk of colon cancer, whereas it increases the risk of breast cancer (73). Aspirin and NSAIDs reduce the risk of colon cancer, whereas their association with breast cancer risk is inconsistent (121–123). Inflammation-related factors may be pivotal for colon cancer risk, but the pivotal component of the pathway for breast cancer may be estrogen because the epidemiologic data suggest that menopausal status and estrogen are central effect modifiers of other risk factors. The pivotal pathway component is most likely determined by the underlying tissue structure. For CRC, we believe that the primary determinant of risk is inflammation. Given the close relationship of the key pathway elements, however, the ultimate pathway to carcinogenesis involves all of the elements of CHIEF.

Randomized clinical trials have reinforced the conclusions from over 40 observational studies that NSAIDs, which inhibit the COX enzymes, prevent colorectal adenomas as well as CRC (124, 125). An understanding of key elements in the CHIEF pathway and their role in the carcinogenic process promises to lead to practical approaches for both prevention and treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Lippman SM, Hawk ET. Cancer prevention: from 1727 to milestones of the past 100 years. Cancer Res 2009;69:5269–84.
2. Abreu-Martin MT, Targan SR. Regulation of immune responses of the intestinal mucosa. Crit Rev Immunol 1996;16:277–309.
3. Rothenberg ME, Mishra A, Brandt EB, Hogan SP. Gastrointestinal eosinophils. Immunol Rev 2001; 179:139–55.
4. Hunyady B, Mezey E, Palkovits M. Gastrointestinal immunology: cell types in the lamina propria-a morphological review. Acta Physiol Hung 2000;87: 305–28.
5. Corr SC, Gahan CC, Hill C. M-cells: origin, morphology and role in mucosal immunity and microbial pathogenesis. FEMS Immunol Med Microbiol 2008;52:2–12.
6. Soler AP, Miller RD, Laughlin KV, Carp NZ, Klurfeld DM, Mullin JM. Increased tight junctional permeability is associated with the development of colon cancer. Carcinogenesis 1999;20:1425–31.
7. Itzkowitz SH, Yio X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. Am J Physiol Gastrointest Liver Physiol 2004;287:G7–17.
8. Fitzpatrick FA. Inflammation, carcinogenesis and cancer. Int Immunopharmacol 2001;1:1651–67.
9. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860–7.
10. Clevers H. At the crossroads of inflammation and cancer. Cell 2004;118:671–4.
11. Kato M, Kephart GM, Morikawa A, Gleich GJ. Eosinophil infiltration and degranulation in normal human tissues: evidence for eosinophil degranulation in normal gastrointestinal tract. Int Arch Allergy Immunol 2001;125 Suppl 1:55–8.
12. Moezzi J, Gopalswamy N, Haas RJ, Jr., Markert RJ, Suryaprasad S, Bhutani MS. Stromal eosinophilia in colonic epithelial neoplasms. Am J Gastroenterol 2000;95:520–3.
13. Lotfi R, Lee JJ, Lotze MT. Eosinophilic granulocytes and damage-associated molecular pattern molecules (DAMPs): role in the inflammatory response within tumors. J Immunother 2007;30: 16–28.
14. Ono M. Molecular links between tumor angiogenesis and inflammation: inflammatory stimuli of macrophages and cancer cells as targets for therapeutic strategy. Cancer Sci 2008;99:1501–6.
15. Lohela M, Bry M, Tammela T, Alitalo K. VEGFs and receptors involved in angiogenesis versus lymphangiogenesis. Curr Opin Cell Biol 2009.
16. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature 2000;407:249–57.
17. Saijo Y, Tanaka M, Miki M, et al. Proinflammatory cytokine IL-1 β promotes tumor growth of Lewis lung carcinoma by induction of angiogenic factors: *in vivo* analysis of tumor-stromal interaction. J Immunol 2002;169:469–75.
18. Voronov E, Shouval DS, Krelin Y, et al. IL-1 is required for tumor invasiveness and angiogenesis. Proc Natl Acad Sci U S A 2003;100:2645–50.
19. Kuwano T, Nakao S, Yamamoto H, et al. Cyclooxygenase 2 is a key enzyme for inflammatory cytokine-induced angiogenesis. FASEB J 2004; 18:300–10.
20. Harris RE. Cyclooxygenase-2 (cox-2) and the inflammogenesis of cancer. Subcell Biochem 2007;42:93–126.
21. Sinicrope FA. Sporadic colorectal cancer: an infectious disease? Gastroenterology 2007;132:797–801.
22. Lashner BA. Colorectal cancer in ulcerative colitis patients: survival curves and surveillance. Cleve Clin J Med 1994;61:272–5.

23. Lizcano JM, Goransson O, Toth R, et al. LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. EMBO J 2004;23:833–43.
24. Woods A, Johnstone SR, Dickerson K, et al. LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. Curr Biol 2003;13:2004–8.
25. Hawley SA, Boudeau J, Reid JL, et al. Complexes between the LKB1 tumor suppressor, STRAD α/β and MO25 α/β are upstream kinases in the AMP-activated protein kinase cascade. J Biol 2003;2:28.
26. Shaw RJ, Kosmatka M, Bardeesy N, et al. The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. Proc Natl Acad Sci U S A 2004;101:3329–35.
27. Carling D. Ampk. Curr Biol 2004;14:R220.
28. Hardie DG. Minireview: the AMP-activated protein kinase cascade: the key sensor of cellular energy status. Endocrinology 2003;144:5179–83.
29. Kyriakis JM. At the crossroads: AMP-activated kinase and the LKB1 tumor suppressor link cell proliferation to metabolic regulation. J Biol 2003; 2:26.
30. Baas AF, Kuipers J, van der Wel NN, et al. Complete polarization of single intestinal epithelial cells upon activation of LKB1 by STRAD. Cell 2004;116: 457–66.
31. Corradetti MN, Inoki K, Bardeesy N, DePinho RA, Guan KL. Regulation of the TSC pathway by LKB1: evidence of a molecular link between tuberous sclerosis complex and Peutz-Jeghers syndrome. Genes Dev 2004;18:1533–8.
32. Carling D. The AMP-activated protein kinase cascade-a unifying system for energy control. Trends Biochem Sci 2004;29:18–24.
33. Viollet B, Andreelli F, Jorgensen SB, et al. Physiological role of AMP-activated protein kinase (AMPK): insights from knockout mouse models. Biochem Soc Trans 2003;31:216–9.
34. Pilarski R, Eng C. Will the real Cowden syndrome please stand up (again)? Expanding mutational and clinical spectra of the PTEN hamartoma tumour syndrome. J Med Genet 2004;41:323–6.
35. White MF. Insulin signaling in health and disease. Science 2003;302:1710–1.
36. Yuan M, Konstantopoulos N, Lee J, et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkβ. Science 2001;293:1673–7.
37. Erlinger TP, Platz EA, Rifai N, Helzlsouer KJ. C-reactive protein and the risk of incident colorectal cancer. JAMA 2004;291:585–90.
38. Bozcuk H, Uslu G, Samur M, et al. Tumour necrosis factor-α, interleukin-6, and fasting serum insulin correlate with clinical outcome in metastatic breast cancer patients treated with chemotherapy. Cytokine 2004;27:58–65.
39. Mantovani G, Maccio A, Madeddu C, et al. Quantitative evaluation of oxidative stress, chronic inflammatory indices and leptin in cancer patients: correlation with stage and performance status. Int J Cancer 2002;98:84–91.
40. Salgado R, Junius S, Benoy I, et al. Circulating interleukin-6 predicts survival in patients with metastatic breast cancer. Int J Cancer 2003; 103:642–6.
41. Terkeltaub RA. IL-10: an "immunologic scalpel" for atherosclerosis? Arterioscler Thromb Vasc Biol 1999;19:2823–5.
42. Esposito K, Marfella R, Giugliano D. Stress hyperglycemia, inflammation, and cardiovascular events. Diabetes Care 2003;26:1650–1.
43. Esposito K, Nappo F, Giugliano F, et al. Cytokine milieu tends toward inflammation in type 2 diabetes. Diabetes Care 2003;26:1647.
44. Watanabe E, Hirasawa H, Oda S, Matsuda K, Hatano M, Tokuhisa T. Extremely high interleukin-6 blood levels and outcome in the critically ill are associated with tumor necrosis factor- and interleukin-1-related gene polymorphisms. Crit Care Med 2005; 33:89–97; discussion 242–3.
45. Kasahara T, Yagisawa H, Yamashita K, Yamaguchi Y, Akiyama Y. IL1 induces proliferation and IL6 mRNA expression in a human astrocytoma cell line: positive and negative modulation by chorela toxin and cAMP. Biochem Biophys Res Commun 1990; 167:1242–8.
46. Grehan JF, Levay-Young BK, Fogelson JL, Francois-Bongarcon V, Benson BA, Dalmasso AP. IL-4 and IL-13 induce protection of porcine endothelial cells from killing by human complement and from apoptosis through activation of a phosphatidylinositide 3-kinase/akt pathway. J Immunol 2005;175: 1903–10.
47. Mangashetti LS, Khapli SM, Wani MR. IL-4 inhibits bone-resorbing activity of mature osteoclasts by affecting NF-{κ}B and Ca2+ signaling. J Immunol 2005;175:917–25.
48. Yamamoto T, Imoto S, Sekine Y, et al. Involvement of NF-[κ]B in TGF-[β]-mediated suppression of IL-4 signaling. Biochem Biophys Res Commun 2004;313:627–34.
49. Hebenstreit D, Luft P, Schmiedlechner A, et al. J Immunol 2003;171:5901–7.
50. Blanchard C, Durual S, Estienne M, et al. IL-4 and IL-13 up-regulate intestinal trefoil factor expression: requirement for STAT6 and *de novo* protein synthesis. J Immunol 2004;172:3775–83.
51. Nilsson BO. Modulation of the inflammatory response by estrogens with focus on the endothelium and its interactions with leukocytes. Inflamm Res 2007;56:269–73.
52. McKay LI, Cidlowski JA. Molecular control of immune/inflammatory responses: interactions between nuclear factor-κB and steroid receptor-signaling pathways. Endocr Rev 1999;20:435–59.
53. De Bosscher K, Vanden Berghe W, Haegeman G. Cross-talk between nuclear receptors and nuclear factor κB. Oncogene 2006;25:6868–86.
54. Chadwick CC, Chippari S, Matelan E, et al. Identification of pathway-selective estrogen receptor ligands that inhibit NF-κB transcriptional activity. Proc Natl Acad Sci U S A 2005;102: 2543–8.
55. Cerillo G, Rees A, Manchanda N, et al. The oestrogen receptor regulates NFκB and AP-1 activity in a cell-specific manner. J Steroid Biochem Mol Biol 1998;67:79–88.
56. Harris HA, Albert LM, Leathurby Y, et al. Evaluation of an estrogen receptor-β agonist in animal models of human disease. Endocrinology 2003; 144:4241–9.
57. Ray P, Ghosh SK, Zhang DH, Ray A. Repression of interleukin-6 gene expression by 17 β-estradiol: inhibition of the DNA-binding activity of the transcription factors NF-IL6 and NF-κB by the estrogen receptor. FEBS Lett 1997;409:79–85.
58. Watson CJ, Webb NJ, Bottomley MJ, Brenchley PE. Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production. Cytokine 2000;12:1232–5.
59. Kristof AS, Marks-Konczalik J, Billings E, Moss J. Stimulation of signal transducer and activator of transcription-1 (STAT1)-dependent gene transcription by lipopolysaccharide and interferon-{γ} is regulated by mammalian target of rapamycin 10.1074/jbc M301053200. J Biol Chem 2003; 278:33637–44.
60. Mazzarella G, MacDonald TT, Salvati VM, et al. Constitutive activation of the signal transducer and activator of transcription pathway in celiac disease lesions. Am J Pathol 2003;162:1845–55.
61. Klein W, Tromm A, Folwaczny C, et al. The G2964A polymorphism of the STAT6 gene in inflammatory bowel disease. Dig Liver Dis 2005;37: 159–161.
62. Ghilardi N, Ziegler S, Wiestner A, Stoffel R, Heim MH, Skoda RC. Defective STAT signaling by the leptin receptor in diabetic mice. Proc Natl Acad Sci U S A 1996;93:6231–5.
63. Onuma M, Bub JD, Rummel TL, Iwamoto Y. Prostate cancer cell-adipocyte interaction: leptin mediates androgen-independent prostate cancer cell proliferation through c-Jun NH2-terminal kinase 10.1074/jbc M304984200. J Biol Chem 2003;278:42660–7.
64. Collet JP, Sharpe C, Belzile E, Boivin JF, Hanley J, Abenhaim L. Colorectal cancer prevention by non-steroidal anti-inflammatory drugs: effects of dosage and timing. Br J Cancer 1999;81:62–8.
65. Camp NJ, Slattery ML. Classification tree analysis: a statistical tool to investigate risk factor interactions with an example for colon cancer (United States). Cancer Causes Control 2002;13:813–23.
66. Landi S, Moreno V, Gioia-Patricola L, et al. Association of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor α, NFKB1, and peroxisome proliferator-activated receptor γ with colorectal cancer. Cancer Res 2003; 63:3560–6.
67. Slattery ML, Murtaugh MA, Sweeney C, et al. PPARγ, energy balance, and associations with colon and rectal cancer. Nutr Cancer 2005;51: 155–61.
68. Broderick P, Carvajal-Carmona L, Pittman AM, et al. A genome-wide association study shows that common alleles of SMAD7 influence colorectal cancer risk. Nat Genet 2007;39:1315–7.
69. Tenesa A, Farrington SM, Prendergast JG, et al. Genome-wide association scan identifies a colorectal cancer susceptibility locus on 11q23 and replicates risk loci at 8q24 and 18q21. Nat Genet 2008.
70. ten Dijke P, Hill CS. New insights into TGF-β-Smad signalling. Trends Biochem Sci 2004;29: 265–73.
71. Wu JM, Bensen-Kennedy D, Miura Y, et al. The effects of interleukin 10 and interferon γ cytokine gene polymorphisms on survival after autologous bone marrow transplantation for patients with breast cancer. Biol Blood Marrow Transplant 2005;11:455–64.
72. Nanda K, Bastian LA, Hasselblad V, Simel DL. Hormone replacement therapy and the risk of colorectal cancer: a meta-analysis. Obstet Gynecol 1999;93:880–8.
73. Nelson HD, Humphrey LL, Nygren P, Teutsch SM, Allan JD. Postmenopausal hormone replacement therapy: scientific review. JAMA 2002;288: 872–81.
74. Slattery ML, Murtaugh MA, Quesenberry C, Caan BJ, Edwards S, Sweeney C. Changing population characteristics, effect-measure modification, and cancer risk factor identification. Epidemiol Perspect Innov 2007;4:10.
75. Slattery ML, Sweeney C, Murtaugh M, et al. Associations between ERα, ERβ, and AR genotypes and colon and rectal cancer. Cancer Epidemiol Biomarkers Prev 2005;14:2936–42.
76. Jiang XP, Yang DC, Elliott RL, Head JF. Reduction in serum IL-6 after vacination of breast cancer patients with tumour-associated antigens is related to estrogen receptor status. Cytokine 2000;12:458–65.
77. Morley JE, Baumgartner RN. Cytokine-related aging process. J Gerontol A Biol Sci Med Sci 2004;59:M924–9.
78. Giovannucci E. Insulin and colon cancer. Cancer Causes Control 1995;6:164–79.
79. Kaaks R. Nutrition, energy balance and colon cancer risk: the role of insulin and insulin-like growth factor-I. IARC Sci Publ 2002;156:289–93.
80. Slattery ML, Samowitz W, Hoffman M, Ma KN, Levin TR, Neuhausen S. Aspirin, NSAIDs, and colorectal cancer: possible involvement in an insulin-related pathway. Cancer Epidemiol Biomarkers Prev 2004;13:538–45.
81. Lyssenko V, Lupi R, Marchetti P, et al. Mechanisms by which common variants in the TCF7L2

gene increase risk of type 2 diabetes. J Clin Invest 2007;117:2155–63.

82. Wang J, Kuusisto J, Vanttinen M, et al. Variants of transcription factor 7-like 2 (TCF7L2) gene predict conversion to type 2 diabetes in the Finnish Diabetes Prevention Study and are associated with impaired glucose regulation and impaired insulin secretion. Diabetologia 2007;50:1192–200.

83. Folsom AR, Pankow JS, Peacock JM, Bielinski SJ, Heiss G, Boerwinkle E. Variation in TCF7L2 and increased risk of colon cancer: the Atherosclerosis Risk in Communities (ARIC) Study. Diabetes Care 2008;31:905–9.

84. Slattery ML, Folsom AR, Wolff R, Herrick J, Caan BJ, Potter JD. Transcription factor 7-like 2 polymorphism and colon cancer. Cancer Epidemiol Biomarkers Prev 2008;17:978–82.

85. Fang V, Foye WO, Robinson SM, Jenkins HJ. Hypoglycemic activity and chemical structure of the salicylates. J Pharm Sci 1968;57:2111–6.

86. Graef I, Gibbons DM. Salicylates and carbohydrate metabolism. Diabetes 1960;9:416–8.

87. Powell ED, Field RA. Studies on salicylates and complement in diabetes. Diabetes 1966;15:730–3.

88. McRae JR, Chen M, Robertson RP. Improvement of defective insulin responses to glucose, arginine, and β-adrenergic stimulation in diabetics by sodium salicylate. Adv Prostaglandin Thromboxane Res 1980;8:1287–9.

89. Baron SH. Salicylates as hypoglycemic agents. Diabetes Care 1982;5:64–71.

90. Festa A, D'Agostino R, Jr., Howard G, Mykkanen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). Circulation 2000;102:42–7.

91. Schmidt MI, Duncan BB, Sharrett AR, et al. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. Lancet 1999;353:1649–52.

92. Pradhan AD, Cook NR, Buring JE, Manson JE, Ridker PM. C-reactive protein is independently associated with fasting insulin in nondiabetic women. Arterioscler Thromb Vasc Biol 2003;23:650–5.

93. Kubaszek A, Pihlajamaki J, Komarovski V, et al. Promoter polymorphisms of the TNF-α (G-308A) and IL-6 (C-174G) genes predict the conversion from impaired glucose tolerance to type 2 diabetes: the Finnish Diabetes Prevention Study. Diabetes 2003;52:1872–6.

94. Xu H, Barnes GT, Yang Q, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003;112:1821–30.

95. Hirosumi J, Tuncman G, Chang L, et al. A central role for JNK in obesity and insulin resistance. Nature 2002;420:333–6.

96. Gao Z, Zuberi A, Quon MJ, Dong Z, Ye J. Aspirin inhibits serine phosphorylation of insulin receptor substrate 1 in tumor necrosis factor-treated cells through targeting multiple serine kinases. J Biol Chem 2003;278:24944–50.

97. Xu H, Hotamisligil GS. Signaling pathways utilized by tumor necrosis factor receptor 1 in adipocytes to suppress differentiation. FEBS Lett 2001;506:97–102.

98. Hotamisligil GS. The role of TNFα and TNF receptors in obesity and insulin resistance. J Intern Med 1999;245:621–5.

99. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α- and obesity-induced insulin resistance. Science 1996;271:665–8.

100. Hofmann C, Lorenz K, Braithwaite SS, et al. Altered gene expression for tumor necrosis factor-α and its receptors during drug and dietary modulation of insulin resistance. Endocrinology 1994;134:264–70.

101. Giovannucci E. Diet, body weight, and colorectal cancer: a summary of the epidemiologic evidence. J Womens Health (Larchmt) 2003;12:173–82.

102. Slattery ML. Physical activity and colorectal cancer. Sports Med 2004;34:239–52.

103. Caan BJ, Coates AO, Slattery ML, Potter JD, Quesenberry CP, Jr., Edwards SM. Body size and the risk of colon cancer in a large case-control study. Int J Obes Relat Metab Disord 1998;22:178–84.

104. Le Marchand L, Wilkens LR, Kolonel LN, Hankin JH, Lyu LC. Associations of sedentary lifestyle, obesity, smoking, alcohol use, and diabetes with the risk of colorectal cancer. Cancer Res 1997;57:4787–94.

105. Le Marchand L, Wilkens LR, Mi MP. Obesity in youth and middle age and risk of colorectal cancer in men. Cancer Causes Control 1992;3:349–54.

106. Giovannucci E, Ascherio A, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Physical activity, obesity, and risk for colon cancer and adenoma in men. Ann Intern Med 1995;122:327–34.

107. Slattery ML, Murtaugh MA, Quesenberry C, Caan BJ, Edwards S, Swenney C. Changing population characteristics, effect modification, and cancer risk factor identification. Epidemiol Perspect Innov 2007;4:10.

108. Erridge C, Attina T, Spickett CM, Webb DJ. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. Am J Clin Nutr 2007;86:1286–92.

109. Slattery ML, Benson J, Ma KN, Schaffer D, Potter JD. Trans-fatty acids and colon cancer. Nutr Cancer 2001;39:170–5.

110. Hartman TJ, Yu B, Albert PS, et al. Does nonsteroidal anti-inflammatory drug use modify the effect of a low-fat, high-fiber diet on recurrence of colorectal adenomas? Cancer Epidemiol Biomarkers Prev 2005;14:2359–65.

111. Pedersen BK. The anti-inflammatory effect of exercise: its role in diabetes and cardiovascular disease control. Essays Biochem 2006;42:105–17.

112. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. J Clin Invest 1995;95:2409–15.

113. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 1995;95:2111–9.

114. Mohamed-Ali V, Goodrick S, Rawesh A, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-α, *in vivo*. J Clin Endocrinol Metab 1997;82:4196–200.

115. Qi L, van Dam RM, Meigs JB, Manson JE, Hunter D, Hu FB. Genetic variation in IL6 gene and type 2 diabetes: tagging-SNP haplotype analysis in large-scale case-control study and meta-analysis. Hum Mol Genet 2006;15:1914–20.

116. Otero M, Lago R, Lago F, et al. Leptin, from fat to inflammation: old questions and new insights. FEBS Lett 2005;579:295–301.

117. Loffreda S, Yang SQ, Lin HZ, et al. Leptin regulates proinflammatory immune responses. FASEB J 1998;12:57–65.

118. Slattery ML, Wolff RK, Herrick J, Caan BJ, Potter JD. Leptin and leptin receptor genotypes and colon cancer: gene-gene and gene-lifestyle interactions. Int J Cancer 2008;122:1611–7.

119. Slattery ML, Ballard-Barbash R, Edwards S, Caan BJ, Potter JD. Body mass index and colon cancer: an evaluation of the modifying effects of estrogen (United States). Cancer Causes Control 2003;14:75–84.

120. Slattery ML, Sweeney C, Edwards S, et al. Body size, weight change, fat distribution and breast cancer risk in Hispanic and non-Hispanic white women. Breast Cancer Res Treat 2006.

121. Sandler RS. Aspirin and other nonsteroidal anti-inflammatory agents in the prevention of colorectal cancer. Important Adv Oncol 1996:123–37.

122. Egan KM, Stampfer MJ, Giovannucci E, Rosner BA, Colditz GA. Prospective study of regular aspirin use and the risk of breast cancer. J Natl Cancer Inst 1996;88:988–93.

123. Slattery M, Curtin K, Baumgartner R, et al. IL6, aspirin, non-steroidal anti-inflammatory drugs, and breast cancer risk in women living in the Southwestern United States. Cancer Epidemiol Biomarkers Prev 2007;16:1–9.

124. Bertagnolli MM, Eagle CJ, Zauber AG, et al. Celecoxib for the prevention of sporadic colorectal adenomas. N Engl J Med 2006;355:873–84.

125. Arber N, Eagle CJ, Spicak J, et al. Celecoxib for the prevention of colorectal adenomatous polyps. N Engl J Med 2006;355:885–95.