Research Article

Microarray Analyses of Genes Differentially Expressed by Diet (Black Beans and Soy Flour) during Azoxymethane-Induced Colon Carcinogenesis in Rats

Elizabeth A. Rondini and Maurice R. Bennink

Department of Food Science and Human Nutrition, 106 GM Trout Building, Michigan State University, East Lansing, MI 48824, USA

Correspondence should be addressed to Maurice R. Bennink, mbennink@anr.msu.edu

Received 26 May 2011; Accepted 25 October 2011

Academic Editor: H. K. Biesalski

Copyright © 2012 E. A. Rondini and M. R. Bennink. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

We previously demonstrated that black bean (BB) and soy flour (SF)-based diets inhibit azoxymethane (AOM)-induced colon cancer. The objective of this study was to identify genes altered by carcinogen treatment in normal-appearing colonic mucosa and those attenuated by bean feeding. Ninety-five male F344 rats were fed control (AIN) diets upon arrival. At 4 and 5 weeks, rats were injected with AOM (15 mg/kg) or saline and one week later administered an AIN, BB-, or SF-based diet. Rats were sacrificed after 31 weeks, and microarrays were conducted on RNA isolated from the distal colonic mucosa. AOM treatment induced a number of genes involved in immunity, including several MHC II-associated antigens and innate defense genes (RatNP-3, Lyz2, Pla2g2a).

BB- and SF-fed rats exhibited a higher expression of genes involved in energy metabolism and water and sodium absorption and lower expression of innate (RatNP-3, Pla2g2a, Tlr4, Dmbt1) and cell cycle-associated (Cdc2, Ccnb1, Top2a) genes. Genes involved in the extracellular matrix (Col1a1, Fn1) and innate immunity (RatNP-3, Pla2g2a) were induced by AOM in all diets, but to a lower extent in bean-fed animals. This profile suggests beans inhibit colon carcinogenesis by modulating cellular kinetics and reducing inflammation, potentially by preserving mucosal barrier function.

1. Introduction

Colorectal cancer (CRC) is one of the most common neoplasms afflicting industrialized societies [1]. In 2008, there were 609,051 deaths due to colorectal cancer worldwide, with 50,640 cases in the United States alone [1]. Both genetic and environmental exposures have been implicated in the etiology of CRC, and it has been estimated that up to 75% of cases may be preventable by adequate diets and regular exercise [2–4]. Consumption of diets low in red meat and alcohol and high in vegetables and cereal grains is generally associated with a decreased risk of developing CRC [4–6]. Additionally, populations consuming higher intakes of legumes (peas, beans, lentils, peanuts) are reported to have a lower risk of [6–12] and mortality from CRC [13].

It has long been known that dietary patterns modulate the incidence and mortality of colorectal cancer [2, 3], however, identification of specific mechanisms has been limited. The azoxymethane- (AOM-) induced colon cancer model in rodents has been utilized extensively to examine dietary influences on colon cancer. Tumors develop almost exclusively in the colon, primarily in the distal region, similar to the distribution observed in humans from high-risk areas. Additionally, many of the common genetic and pathogenic changes contributing to human colon cancers are also observed during AOM-induced colon carcinogenesis [14–19]. Although Apc mutations are infrequently detected [20], mutations in GSK-3β phosphorylation consensus sites on β-catenin are present in up to 77% of AOM-induced colon cancers as well as in early preneoplastic lesions [17, 21–23]. These sites are important for downregulation of β-catenin by ubiquitination and result in stabilization and nuclear localization of the protein [23]. Activating mutations on codon 12 and 13 of the k-ras gene [14, 15], upregulation of
cyclo-oxygenase 2 (COX-2), and inducible nitric oxide synthase (iNOS), as well as alterations in transforming growth factor β (TGF-β) signaling, are also common features to both human and AOM-induced colon cancers [24–29]. Using this model, experiments conducted in our laboratory [30–33] and by others [34–36] have demonstrated the potential of bean-based diets to inhibit AOM-induced colon cancer. For example, Hughes et al. [35] and Hangen and Bennink [32] found that rats fed dry beans (pinto, navy, or black beans) had a 50–57% lower incidence of colon cancer than rats fed a casein-based diet. Similarly, in a series of experiments, Bennink et al. [30, 31, 33] reported a significant reduction in colon tumor incidence and tumor burden in rats fed defatted soy flour compared to casein-fed control animals.

The purpose of the current investigation was to elucidate cellular mechanisms underlying colon cancer inhibition by beans in vivo. Microarrays were performed on mRNA isolated from distal colonic epithelial cells of saline and AOM-injected F344 rats fed either a casein (AIN), black bean (BB), or defatted soy flour (SF) diet for 31 weeks. We chose to focus on the distal segment because most tumors develop in this area using standard protocol (15 mg/kg AOM) and there is evidence for site-specific effects of food constituents on tumorigenesis [37]. It was anticipated that genes most important to dietary suppression of colon cancer would be similarly affected by black beans and soy flour and have altered expression (increased or decreased) that corroborated tumor incidence. The profile of genes altered in this experiment suggests aberrant activation of innate and adaptive immune responses are permissive for colon carcinogenesis, whereas inhibition of tumor promotion by bean feeding is associated with modulation of genes involved in crypt cell homeostasis, innate defense, and extracellular matrix components.

2. Materials and Methods

2.1. Animal Care and Experimental Diets. This study was conducted in conformity with the regulatory guidelines of the Michigan State University Institutional Animal Care and Use Committee. Ninety-five male Fischer (F344) rats were obtained from Harlan Sprague-Dawley (Indianapolis, IL) at 3 weeks of age and housed in plastic cages (2-3 rats/cage) obtained from Harlan Sprague-Dawley (Indianapolis, IL) and matched to have similar nutrient : energy ratios (1) high nitrogen casein (AIN), (2) black beans (BB), or (3) defatted soy flour (SF) (Archer Daniels Midland; Decatur, IL) and matched to have similar nutrient : energy ratios (Table 1). Black beans were soaked overnight in distilled water, cooked in a steam jacket kettle for 30 minutes, dried at 58°C, and then finely ground to pass through a 1.6 mm diameter screen prior to mixing with other diet ingredients. All diets contained approximately 18.9% (wt/wt) total protein, 11.3% dietary fiber, and 16.7% fat (wt/wt). Casein and tryptophan were added to black bean diet and methionine was added to all diets to increase the amino acid score to >90%. Lard, corn, and soybean oil were added to all diets, adjusted so the total saturated (SFA) : monounsaturated (MUFA) : polyunsaturated (PUFA) fatty acid ratios were 1.0 : 1.2 : 1.1, respectively.

2.2. Experimental Design. Upon arrival, animals were fed the control (AIN) diet and allowed one week to acclimatize to new conditions. At 4 and 5 weeks of age, rats received subcutaneous injections (100 μL) of either 15 mg/kg of azoxymethane (AOM) prepared in saline (n = 75; Ash Stevens, Detroit, MI) or saline (saline, n = 20). Animals were fed the control (AIN) diet until one week after the second injection, when they were randomized by weight to either continue on the control (AIN) diet or to be fed one of the experimental diets (BB or SF). At 36 weeks of age, animals were sacrificed by CO2 inhalation and exsanguination, and the colon was immediately excised, opened longitudinally, and rinsed briefly in tap water to remove debris. Macroscopic tumors, when present, were excised and stored at −80°C to be analyzed as a separate part of this study. The colon was then transected into proximal and distal segments and epithelial cells were collected by gently scraping normal-appearing mucosa from the distal half of the colon (excluding the lowermost 1 cm) with a glass slide. Samples were snap frozen and stored at −80°C until RNA extraction could be performed.

2.3. Microarray Target Preparation and Hybridization. Affymetrix RU34A rat genome chips (Santa Clara, CA) were used in this experiment. For total RNA isolation, the distal colonic mucosa was homogenized using a Tekmar homogenizer in TRIzol reagent containing RNase-free glycerogen according to the manufacturer’s instructions (Gibco, Carlsbad, CA). After RNA extraction, samples were cleaned with RNeasy minicolumns (Qiagen, Valencia, CA), quantified using a UV spectrophotometer (A260/A280), and the quality of RNA assessed by agarose-formaldehyde gel electrophoresis. Only high quality RNA was used in subsequent steps.

Biotinylated cRNA was prepared in accordance with instructions supplied in the GeneChip Expression Manual (Affymetrix, Santa Clara, CA). Double-stranded cDNA was synthesized from 10μg of total RNA, pooled from 2–4 animals/treatment, using T7-(dT)24 primers containing a T7 RNA polymerase promoter site (ProliGo, Boulder, CO) and the Superscript II system (Invitrogen, Carlsbad, CA). Biotinylated cRNA was prepared using the Enzo BioArray HighYield RNA Transcript Labeling Kit (Affymetrix, Santa Clara, CA) and then purified with RNeasy minicolumns. Approximately 15μg cRNA was fragmented at 94°C for 35 minutes and hybridized to RU34A rat genome chips for 16 hours at 45°C. Following hybridization, arrays were washed and stained with a streptavidin-phycocerinythin conjugate on an Affymetrix Fluidics station according to standard protocol. Processed arrays were scanned at 570 nm using a Hewlett Packard GeneArray Scanner.
2.4. Confirmation of Gene Changes by Quantitative Reverse Transcriptase PCR (qRT-PCR). Select genes were confirmed using qRT-PCR. Gene-specific primers for cell division cycle 2 (Cdc2), cyclin B1 (Ccnb1), topoisomerase II alpha (Top2A), group II alpha secretory phospholipase A2 (Pla2g2a), fibronectin 1 (Fbn1), collagen, type I, alpha 1 (Col1a1), rat neutrophil (NP) defensin 3 (RatNP-3), aquaporin 8 (Aqp8), and 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (Hmgs2) were designed with the Primer Express 2.0 program (Applied Biosystems, Foster City, CA). β-actin was used as an internal control. The sequences of the primer pairs used are available in Supplementary Table 1 available at doi:10.1155/2012/351796. Single-stranded (ss) cDNA was synthesized from 2.5 μg of total RNA using T7-(dT)24 primers (Proligo, Boulder, CO) and the Superscript II system (Invitrogen, Carlsbad, CA). Reverse transcription was performed in a thermocycler following the Superscript first strand synthesis protocol (Invitrogen, Carlsbad, CA).

Quantitative determination of gene expression was performed with the ABI Prism7000 (Perkin Elmer Corp., Foster City, CA) using the SYBR Green Universal Master Mix (Applied Biosystems, Foster City, CA). The reaction mixture (25 μL total volume) contained 20 ng ss cDNA, 12.5 μL SYBR Green Universal Master mix, and 10.5 μL of diluted primers (300–600 nM). The real-time cycle conditions were as follows: PCR initial activation step at 95°C for 15 min and a total of 40 cycles for melting (95°C, 15 s) and annealing/extension (60°C, 1 min). All assays were performed in duplicate, using 3–4 samples per group (representing 9–12 animals/group) and relative fold-changes were quantified using the comparative CT (ΔΔCT) method (User bulletin number 2, Applied Biosystems, Foster City, CA).

2.5. Statistical Analyses. Data for weight gain, microarrays, and qRT-PCR were analyzed using the General Linear Models procedure of SAS (SAS Institute, Cary, NC, Version 7.0). When statistical differences were detected with the F statistic, individual comparisons were made using the least significant difference (LSD) method. Tumor incidence data were analyzed with a χ² test using the Proc Freq procedure in SAS. Prior to statistical analyses, fluorescence intensity data from microarrays were globally scaled to a target intensity of 500 in Affymetrix Microarray Suite, Version 5.0 to control within-chip variations. Globally scaled data were then imported into GeneSpring (Silicon Genetics, Inc., Redwood City, CA, Version 6.0) for normalization and filtering. All chips were normalized to the median intensity of a set of invariant genes whose expression across all conditions (injection type, diet) after global scaling showed less than a 30% coefficient of variation (CV). A filtering step excluded genes not considered “Present” or “Marginal” in at least 42% of the individual samples. An additional filtering step limited the genes further to those exhibiting greater than 1.3 or less than 0.7 fold-change difference between diets or injection type. Normalized expression values were exported then analyzed using the GLM procedure of SAS. When present, duplicate transcripts were averaged prior to statistical analysis.

Differentially expressed transcripts (P < 0.05) were broadly grouped into categories based on known gene ontologies and biological functions reported in the literature. Gene ontologies were retrieved using the Affymetrix NetAffx Analysis Center (http://www.affymetrix.com/analysis/index.affx) and DAVID 6.7 functional analysis tool [39, 40]. Data are presented as mean fold-change differences standardized to the AIN (control) group for diet-dependent differences or

### Table 1: Nutrient composition of experimental diets.

| Ingredient               | AIN            | g/100 g diet | Black bean | Soy flour |
|--------------------------|----------------|--------------|------------|-----------|
| Casein                   | 20             | 2.7          | —          | —         |
| Black bean flour         | —              | 74           | —          | —         |
| Defatted soy flour       | —              | —            | 34         | —         |
| Cornstarch               | 45             | —            | 36         | —         |
| Sucrose                  | 1.8            | 1.8          | 1.8        | 1.8       |
| Total lipid              | 17             | 17           | 17         | 17        |
| Total fiber in diet      | 11             | 11           | 11         | 11        |
| Mineral mix              | 3.9            | 3.9          | 3.9        | 3.9       |
| Vitamin mix              | 1.1            | 1.1          | 1.1        | 1.1       |
| Methionine               | 0.33           | 0.40         | 0.33       | —         |
| Tryptophan               | —              | 0.004        | —          | —         |
| Calcium carbonate        | 0.25           | 0.25         | 0.25       | —         |
| Choline bitartrate       | 0.28           | 0.28         | 0.28       | —         |
| Tert-butylhydroquinone   | 0.002          | 0.002        | 0.002      | —         |

1 Nutrient compositions were calculated from the USDA nutrient database and Reeves [38].
2 Total lipid content in diets calculated based on natural occurring lipids and added fat. The SFA:MUFA:PUFA composition of all diets was 1:1.2:1.
3 Total fiber content (11.25%) in all diets based on the amount present from individual dietary components as well as added fiber (cellulose).
3. Results

3.1. Weight Gain and Tumor Incidence. There were no significant effects of diet (AIN, BB, SF) or injection regime (AOM versus saline) on body weight gain. The total weight gain (g, LSM ± SEM) of rats while on experimental diets was AIN = 304 ± 7.4, BB = 287 ± 8.3, SF = 299 ± 8.2. There was a significant effect of diet on tumor incidence (P = 0.03). As previously established, bean-based diets inhibited tumor incidence by ~60% compared to rats fed the control diet (Figure 1).

3.2. Biological Classification of Gene Changes in Distal Colonic Mucosa during AOM-Induced Carcinogenesis. Among the 8799 genes and ESTs present on the rat genome UG34A array, a total of 155 transcripts were significantly affected by injection regime (AOM versus saline), 257 by dietary treatment (AIN, BB, SF), and 5 were affected by both (P < 0.05). Transcripts differentially expressed by either carcinogen or diet were broadly classified into one of twelve functional categories and results are depicted in Figure 2.

3.3. Genes Differentially Expressed by Carcinogen (AOM) in Distal Colonic Mucosa. A total of 108 transcripts were higher and 47 lower in the colon of rats injected with carcinogen (AOM) compared to saline-injected controls (P < 0.05). As shown in Figure 2, a majority (55%) of transcripts affected were associated with immune, defense, inflammation (n = 19, 12%), signal transduction (n = 15, 10%), other (n = 17, 11%), or protein processing, synthesis, degradation (n = 20, 13%). Genes involved in antigen presentation (RT1-Ba, RT1-Da, RT1-Dm1, RT1-M3-1, RT1-Da), immune, defense, inflammation (RatNPl-3, Lyz2, Pla2g2a), and components of the extracellular matrix (Col1a1, Fn1, Col3a1) were among those most highly induced by AOM-treatment (Table 2). Several of these genes have previously been shown to be overexpressed in carcinogen-induced colon cancer [41] and inflammatory conditions of the colon [42]. Several ribosomal proteins, including components of the 40S ribosomal protein subunit (Rps7, Rps15, Rps17, Rps9, Rps4x) and the 60S unit (Rpl37, Rpl4, Rpl36a) were also moderately induced by carcinogen compared to saline-injected controls.

3.4. Genes Differentially Affected by Dietary Treatment in Distal Colonic Mucosa. Compared to the AIN diet, feeding rats black beans (BB) significantly affected 188 genes (102 upregulated, 86 downregulated), and soy flour (SF) affected 140 genes (97 upregulated, 43 downregulated). Fifty three genes were significantly coinduced and 34 corepressed by BB and SF, representing 34% of gene changes, although an additional 24% showed the same direction of change. A majority of known transcripts affected by dietary treatment (68%) fell into one of five categories including other (n = 44, 17%), enzymes (n = 33, 13%), energy metabolism (n = 30, 12%), cell cycle, cell growth and maintenance, and apoptosis (n = 24, 9.3%), channel, transporter, carrier proteins (n = 22, 8.6%), and signal transduction (n = 22, 8.6%) see Figure 2.

A select listing of transcripts similarly affected the colon of bean-fed compared to casein-fed rats is presented in Table 3. As shown, bean-based diets coinduced a number of genes involved in fatty acid metabolism and gluconeogenesis (Hmgcs2, Aldob, Pck1, Ech1), electron transport, oxido-reduction, detoxification (Cyp4b1, Gstm5, Gstm1, Cyp27a1, Prdx6), and solute, ion transport (Aqp8, Scnn1g, Slc12a7, Scl5a1, Scl6a1). Among genes corepressed by beans included those involved in cell cycle (Top2a, Ccnb1, Cdk2), fatty acid desaturation (Scd1, Scd5), extracellular matrix (Col1a1, Fn1), immune, defense, inflammation (Dmbtl, Pla2g2a, RatNPl-3, Tlr4, Cxcl14), and nucleic acid binding, transcription regulation (Egr1, Egr2).

The relative expression of select genes (Cdk2, Ccnb1, Top2a, Hmgcs2, and Aqp8) was further evaluated by qRT-PCR. As shown in Figure 3, the mRNA for Cdk2, Ccnb1, Top2a were all significantly lower, whereas Hmgcs2 and Aqp8 were higher in the colon of BB- and SF-fed rats compared to controls (AIN P < 0.05). The direction of change was generally consistent with those obtained using microarrays (Table 3).

3.5. Genes Affected by Both Dietary Treatment and Carcinogen (AOM) in Distal Colonic Mucosa. Five genes were found to be influenced both by diet and carcinogen and are presented in Table 4. Transcripts for phospholipase A2, group IIA (Pla2g2a), rat neutrophil defensin 3 (RatNPl-3), collagen, type I, alpha 1 (Col1a1), and fibronectin 1 (Fn1) were all induced by carcinogen treatment, but to a lower extent in rats fed either BB or SF. Changes in expression of these genes were further evaluated by qRT-PCR and results presented in Figure 4. In accordance with microarray data, there were significant main effects for both diet (P < 0.05) and carcinogen (P < 0.05) treatment for each gene examined. Generally, expression was lowest in bean-fed rats but increased in all
Figure 2: Functional classification of genes significantly altered by carcinogen (AOM) and by dietary treatment in the distal colon mucosa of rats detected by microarrays. A total of 155 genes were altered by carcinogen (AOM) and 257 by dietary treatment (AIN versus BB versus SF, P < 0.05).

diets with carcinogen injections. Rats injected with AOM and fed the control (AIN) diet had the highest overall expression level coinciding with the higher tumor incidence observed in these animals. Somatostatin 2 receptor was also influenced by both diet and carcinogen treatment, being basally higher in bean-fed animals and decreasing in all groups following carcinogen administration (Table 4).

4. Discussion

The focus of the current research was to identify potential cellular and molecular events underlying suppression of tumorogenesis by beans using a highly relevant animal model of colon cancer. We profiled global changes in gene expression affected by AOM treatment in normal-appearing colon mucosa to determine early events permissive for tumor formation and whether these changes could be attenuated by dietary treatment. Although not a primary focus of this study, tumor incidence was also assessed. As previously demonstrated, both BB- [32] and SF-fed [30, 31, 43] rats developed significantly fewer tumors overall, confirming that these diets inhibit experimental colon carcinogenesis. In the study by Hangen and Bennink [32], however, black and navy bean-fed rats ate less and as a result had significantly lower body weights at termination of the study. Because of the inverse association between energy restriction and tumorogenesis, the tryptophan and methionine content in the black bean diets was adjusted to raise the amino acid score comparable to that of the AIN and SF diets. As a result, no significant differences in final weight gain were detected, indicating that black beans inhibit tumorogenesis by a mechanism other than energy restriction.

We found that AOM treatment most notably affected genes involved in innate defense and immunity. For example, the antimicrobial genes lysozyme, group IIA phospholipase A2 (Pla2g2a; sPLA2), and neutrophil (NP) defensin 3 (RatNP-3) were approximately 2-fold higher than in saline-injected controls. Several major histocompatibility class (MHC) II-associated antigens as well as CD74, the class II MHC-associated invariant chain, were also induced in the colon of AOM-injected rats. Epithelial cells, activated dendritic cells, and/or macrophages underlying the intestinal cell layer can function as antigen-presenting cells [44–48], and specific upregulation of these genes implies that carcinogen treatment alters immune responsiveness to luminal and/or bacterial antigens. Additionally, CD74, aside from its
Table 2: Select genes significantly affected by carcinogen (AOM) treatment in the distal colonic epithelium of male F344 rats.

| Gene symbol | Gene title | AOM (fold change) |
|-------------|------------|-------------------|
| **(I) Extracellular matrix, cell adhesion, cytoskeleton** | | |
| Fn1 | fibronectin 1 | 1.7 |
| COL1A1 | collagen, type I, alpha 1 | 1.6 |
| Vim | vimentin | 1.5 |
| THMB10 | thymosin, beta 10 | 1.3 |
| COL3A1 | collagen, type III, alpha 1 | 1.3 |
| **(II) Immune, defense, inflammation, stress** | | |
| PLA2G2A | phospholipase A2, group IIA (platelets, synovial fluid) | 2.4 |
| CD74 | CD74 molecule, major histocompatibility complex, class II invariant chain | 2.1 |
| Lyz2 | lysozyme 2 | 2.1 |
| RT1-Da1 | RT1 class II, locus Da1 | 2.0 |
| RT1-DMb | RT1 class II, locus DMb | 1.7 |
| RatNP-3 | defensin RatNP-3 precursor | 1.7 |
| RT1-Da | histocompatibility 2, class II antigen E alpha | 1.6 |
| RT1-Ba | RT1 class II, locus Ba | 1.5 |
| Cxcl13 | chemokine (C-X-C motif) ligand 13 | 1.5 |
| RT1-Bb | RT1 class II, locus Bb | 1.5 |
| CCL2 | chemokine (C-C motif) ligand 2 | 1.4 |
| MIF | macrophage migration inhibitory factor | 1.3 |
| RT1-M3-1 | RT1 class Ib, locus M3, gene 1 | 1.3 |
| IRF7 | interferon regulatory factor 7 | 1.3 |
| CXCR4 | chemokine (C-X-C motif) receptor 4 | 1.3 |
| IL15 | interleukin 15 | 0.6 |
| **(III) Protein processing, synthesis, degradation** | | |
| RPS7 | ribosomal protein S7 | 1.4 |
| RPS15 | ribosomal protein S15 | 1.4 |
| PFBN2 | prefoldin subunit 2 | 1.4 |
| HSPC1 | heat shock protein 1 (chaperonin 10) | 1.3 |
| RPL37 | ribosomal protein L37 | 1.3 |
| RPL4 | ribosomal protein L4 | 1.3 |
| PEMB4 | proteasome (prosome, macropain) subunit, beta type 4 | 1.3 |
| RPL3L | ribosomal protein L3-like | 1.3 |
| RPS4X | ribosomal protein S4, X-linked | 1.3 |
| RPS17 | ribosomal protein S17 | 1.3 |
| RPS36AL | ribosomal protein L36a-like | 1.3 |
| RPS9 | ribosomal protein S9 | 1.3 |

Data expressed as mean-fold change normalized to saline-injected animals (n = 12/group). All genes presented were significantly altered compared to saline-injected animals (P < 0.05).

Classical antigen transporting role, has also been reported to bind macrophage inhibitory factor (MIF), leading to nuclear factor kappa B (NFκB) activation, inhibition of p53 phosphorylation, and cell proliferation [49, 50]. Another cluster of genes influenced by AOM treatment involved moderate induction of several ribosomal proteins. RNA and protein synthesis decrease as cells terminally differentiate [51], and enhanced presence of ribosomal protein transcripts is consistent with findings from other studies in the colon of rats susceptible to PhIP-induced colon cancer [52] and in animals during aging [53].

Chronic inflammation creates an environment permissive to carcinogenesis through enhanced production of lipid mediators, cytokines, and chemokines that influence cell proliferation and apoptosis [54–58]. Additionally generation of reactive oxygen and nitrogen species either directly or through activation of phagocytic cells can lead to oxidative damage of DNA [55, 59, 60]. The mucosal barrier, comprised of goblet cell-derived mucin and reinforced by tight junctions normally protects the epithelium and limits activation of immune cells within the lamina propria [61–64]. Dysfunctions in one or more components of the mucosal barrier...
Table 3: Genes similarly affected by bean-feeding (BB and SF) in the distal colonic epithelium of male F344 rats.

| Gene symbol | Gene title | BB    | SF    |
|-------------|------------|-------|-------|
| **(I) Cell cycle, cell growth and maintenance, apoptosis** |
| Ceacam1     | carcinoembryonic antigen-related cell adhesion molecule 1 | 2.3*  | 1.9*  |
| Rb1         | retinoblastoma 1 | 2.2*  | 2.2*  |
| Gadd45a     | growth arrest and DNA-damage-inducible, alpha | 1.5*  | 1.5*  |
| Bax         | Bcl2-associated X protein | 1.3   | 1.4*  |
| Egln3       | EGL nine homolog 3 (C. elegans) | 0.67* | 0.79  |
| Wfcl1       | WAP four-disulfide core domain 1 | 0.67* | 0.50* |
| Ccnb1       | cyclin B1 | 0.66* | 0.85  |
| Rfc4        | replication factor C (activator 1) 4 | 0.59* | 1.1   |
| Cdc2        | cell division cycle 2, G1 to S and G2 to M | 0.58* | 0.83  |
| Top2a       | topoisomerase (DNA) II alpha | 0.57* | 0.85  |
| Bub1b       | budding uninhibited by benzimidazoles 1 homolog, beta (S. cerevisiae) | 0.49* | 0.60* |
| **(II) Channel, transporters, & carriers** |
| Aqp8        | aquaporin 8 | 2.7*  | 2.9*  |
| Scnn1g      | sodium channel, nonvoltage-gated 1 gamma | 2.2*  | 2.0*  |
| Apoa1       | apolipoprotein A-I | 1.6   | 2.4*  |
| Slc12a7     | solute carrier family 12 (potassium/chloride transporters), member 7 | 1.6*  | 1.4*  |
| Slc5a1      | solute carrier family 5 (sodium/glucose cotransporter), member 1 | 1.5*  | 1.3   |
| Slc16a1     | solute carrier family 16, member 1 (monocarboxylic acid transporter 1) | 1.5*  | 1.5*  |
| Atp1a1      | ATPase, Na+/K+ transporting, alpha 1 polypeptide | 1.4*  | 1.3*  |
| Lgals9      | lectin, galactoside-binding, soluble, 9 | 1.3*  | 1.3*  |
| Kcnk1       | potassium channel, subfamily K, member 1 | 1.3*  | 1.3*  |
| Skol1a1     | solute carrier organic anion transporter family, member 1a1 | 0.68  | 0.35* |
| Slc16a6     | solute carrier family 16, member 6 (monocarboxylic acid transporter 7) | 0.61* | 0.82  |
| Fabp5       | fatty acid binding protein 5, epidermal | 0.48* | 0.64* |
| Ttpa        | tocoopherol (alpha) transfer protein | 0.47* | 0.66* |
| **(III) Electron transport, oxidoreductase, detoxification** |
| Cyp4b1      | cytochrome P450, family 4, subfamily b, polypeptide 1 | 3.6*  | 2.2*  |
| Gstm5       | glutathione S-transferase, mu 5 | 1.5*  | 1.5*  |
| Gstm1       | glutathione S-transferase mu 1 | 1.4*  | 1.3   |
| Cyp27a1     | cytochrome P450, family 27, subfamily a, polypeptide 1 | 1.4*  | 1.5*  |
| Prdx6       | peroxiredoxin 6 | 1.4   | 1.5*  |
| Cyp2d1      | cytochrome P450, family 2, subfamily d, polypeptide 1 | 1.4*  | 1.4*  |
| Por         | P450 (cytochrome) oxidoreductase | 1.4*  | 1.4*  |
| Cyb5a       | cytochrome b5 type A (microsomal) | 1.4*  | 1.3*  |
| Tst         | thiosulfate sulfurtransferase, mitochondrial | 1.3*  | 1.3*  |
| **(IV) Energy metabolism** |
| Hmgs2       | 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 | 2.2*  | 2.1*  |
| Aldob       | aldolase B, fructose-bisphosphate | 2.1*  | 1.8*  |
| Pck1        | phosphoenolpyruvate carboxykinase 1 (soluble) | 2.0*  | 1.8*  |
| Ech1        | enoyl coenzyme A hydratase 1, peroxisomal | 1.6*  | 1.6*  |
| Glul        | glutamate-ammonia ligase | 1.4*  | 1.2*  |
| Hadhb       | hydroxacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit | 1.3*  | 1.3*  |
| Cbr1        | carbonyl reductase 1 | 1.2   | 1.5*  |
| Gene symbol | Gene title                                                                 | BB | SF |
|------------|----------------------------------------------------------------------------|----|----|
| *Hadha*    | hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit | 1.2* | 1.3* |
| *Pfkp*     | phosphofructokinase, platelet                                             | 0.67* | 0.76* |
| *Gpd2*     | glycerol-3-phosphate dehydrogenase 2, mitochondrial                      | 0.67* | 0.82* |
| *Acsl4*    | acyl-CoA synthetase long-chain family member 4                            | 0.64* | 0.80* |
| *Pyy*      | peptide YY                                                                | 0.64* | 0.72* |
| *Gcg*      | glucagon                                                                  | 0.57* | 0.81* |
| *Scd*      | stearoyl-CoA desaturase (delta-9-desaturase)                              | 0.53* | 0.72* |
| *Scd1*     | stearoyl-Coenzyme A desaturase 1                                          | 0.47* | 0.65* |

(V) Extracellular matrix, cell adhesion, cytoskeleton

| Gene symbol | Gene title                                                                 | BB | SF |
|------------|----------------------------------------------------------------------------|----|----|
| *Sdc1*     | syndecan 1                                                                 | 1.3* | 1.3* |
| *Sparc*    | secreted protein, acidic, cysteine-rich (osteonectin)                      | 1.0 | 0.48* |
| *Fn1*      | fibronectin 1                                                              | 0.66* | 0.61* |
| *Tubb5*    | tubulin, beta 5                                                            | 0.65* | 0.92* |
| *Colla1*   | collagen, type I, alpha 1                                                  | 0.53* | 0.52* |

(VI) Immune, defense, inflammation, stress

| Gene symbol | Gene title                                                                 | BB | SF |
|------------|----------------------------------------------------------------------------|----|----|
| *Hspa1a*   | heat shock 70 kD protein 1A                                                | 1.9* | 1.4 |
| *RT1-EC2*  | RT1 class Ib, locus Aw2                                                   | 1.5* | 1.4* |
| *Tlr4*     | toll-like receptor 4                                                      | 0.78* | 0.68* |
| *Mif*      | macrophage migration inhibitory factor                                    | 0.71* | 0.88 |
| *Dmnt1*    | deleted in malignant brain tumors 1                                    | 0.64* | 0.74* |
| *Pla2g2a*  | phospholipase A2, group IIA (platelets, synovial fluid)                    | 0.50* | 0.39* |
| *RatNP-3*  | defensin RatNP-3 precursor                                                | 0.48* | 0.69* |
| *Cxcl14*   | chemokine (C-X-C motif) ligand 14                                         | 0.41* | 0.48* |

(VII) Nucleic acid binding, transcription regulation

| Gene symbol | Gene title                                                                 | BB | SF |
|------------|----------------------------------------------------------------------------|----|----|
| *Nr1d2*    | nuclear receptor subfamily 1, group D, member 2                            | 2.2* | 1.3 |
| *Nfib*     | nuclear factor I/B                                                        | 1.5* | 1.4* |
| *Vdr*      | vitamin D (1,25-dihydroxyvitamin D3) receptor                            | 1.4 | 1.6* |
| *Syt4*     | synaptotagmin IV                                                          | 0.82 | 0.65* |
| *Egr2*     | early growth response 2                                                   | 0.77* | 0.60* |
| *Nr4a2*    | nuclear receptor subfamily 4, group A, member 2                            | 0.70* | 0.75* |
| *Egr1*     | early growth response 1                                                   | 0.50* | 0.66* |

(VIII) Signal transduction

| Gene symbol | Gene title                                                                 | BB | SF |
|------------|----------------------------------------------------------------------------|----|----|
| *Gchfr*    | GTP cyclohydrolase I feedback regulator                                   | 1.9* | 1.8* |
| *Ppm1b*    | protein phosphatase 1B, magnesium dependent, beta isoform                 | 1.6* | 1.4 |
| *Mapk14*   | mitogen activated protein kinase 14                                       | 1.3* | 1.4* |
| *Gucy2c*   | guanylate cyclase 2C                                                      | 1.3* | 1.4* |
| *P2ry2*    | purinergic receptor P2Y, G-protein coupled 2                              | 0.81 | 0.61* |
| *Ptpp3*    | protein tyrosine phosphatase, non-receptor type 3                         | 0.79* | 0.73* |
| *P2ry6*    | pyrimidinergic receptor P2Y, G-protein coupled 6                           | 0.69* | 0.68* |
| *Pld1*     | phospholipase D1                                                          | 0.68* | 0.85 |
| *Ptpp18*   | protein tyrosine phosphatase, non-receptor type 18                         | 0.63* | 0.83* |
| *Ptpo*     | protein tyrosine phosphatase, receptor type, O                            | 0.62* | 0.72* |
| *Fzd1*     | frizzled homolog 1 (Drosophila)                                           | 0.62* | 0.74* |

>Data expressed as mean-fold change normalized to the AIN group (n = 8/group). * denotes significant difference compared to the control (AIN) diet (P < 0.05).
Figure 3: qRT-PCR analysis of genes for (a) Cdc2, (b) Ccnb1, (c) Top2a, (d) Hmgcs2, and (e) Aqp8 in the distal colonic mucosa of rats fed either an AIN (control), black bean- (BB-), or soy flour- (SF-) based diet. Results were normalized to the housekeeping gene β-Actin and are presented as relative fold-changes (LSM ± SEM) standardized to the AIN (control) diet. *Denotes significance compared to AIN controls; + denotes significance between BB and SF-fed animals ($P<0.05$). Abbreviations: Cdc2, cell division cycle 2; Ccnb1, cyclin B1; Top2a, topoisomerase II alpha; Hmgcs2, 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2; Aqp8, Aquaporin 8.
Table 4: Genes significantly affected both by carcinogen (AOM) and dietary treatment in the distal colonic epithelium of male F344 rats.

| Gene symbol | Gene title              | Saline-treated | AOM-treated |
|-------------|-------------------------|----------------|-------------|
|             |                         | AIN BB SF AIN BB SF |              |
| Pla2g2a     | phospholipase A2, group IIA | 1.0 0.64 0.33 2.5 0.97 1.2 |              |
| RatNP-3     | NP defensin 3           | 1.0 0.44 0.70 1.7 0.78 1.0 |              |
| Colla1      | collagen, type I, alpha 1 | 1.0 0.67 0.59 2.3 0.94 1.0 |              |
| Fn1         | fibronectin 1           | 1.0 0.72 0.77 2.0 1.2 1.0 |              |
| Sstr2       | somatostatin receptor 2 | 1.0 1.4 1.7 0.69 1.1 1.2 |              |

Data expressed as mean fold-change differences standardized to the AIN (saline-injected) group (n = 4/group). There were significant main effects for injection type (saline versus AOM) and dietary treatment (AIN versus BB or SF) for each gene listed (P < 0.05).

Figure 4: Relative fold-changes in (a) Pla2g2a, (b) RatNP-3, (c) Colla1, and (d) Fn1 detected with qRT-PCR. Results were normalized to the housekeeping gene β-Actin and are presented as mean fold-changes (LSM ± SEM) relative to the AIN(saline-injected) group. There were significant main effects for both diet and carcinogen for each gene presented (P < 0.05). * Denotes significant effect of diet compared to AIN controls; δ denotes significant effect of carcinogen treatment compared to saline controls (P < 0.05). Abbreviations: BB, black bean; SF, soy flour; Pla2g2a, phospholipase A2, group IIA; RatNP-3, rat neutrophil defensin 3; Colla1, collagen, type I, alpha 1; Fn1, fibronectin 1.
have been implicated in the pathogenesis of inflammatory bowel diseases (IBD) [65–67] as well as in CRC [68–72].

For example, increased intestinal permeability and altered structure of tight junction proteins precedes relapse in individuals with IBD [73]. Additionally, Soler et al. [70] noted defects in tight-junction permeability in normal mucosa and in colon tumors from carcinogen-treated animals, and the surface epithelial cells of aberrant crypt foci are reported to be deficient in mature goblet cells, have altered mucin composition, and contain irregular microvilli [74]. Alterations in one or more components of the mucosal barrier would support our findings of higher immune responsive genes observed in carcinogen treated animals and is likely an early and permissive event in promotion of colon carcinogenesis.

Because both black beans and soy flour reduced tumorigenesis to a similar extent in this study, we next evaluated genes that were either co-induced or suppressed compared to AIN-fed animals. We identified several transcripts associated with proliferation and apoptosis to be regulated in a diet-dependent manner. An interesting finding was a 2-fold higher expression of Ceacam1 in rats fed either BB or SF. Ceacam1 encodes a cell-surface glycoprotein expressed in the differentiated cell compartment of colonic crypts and expression correlates positively with normal rates of apoptosis [75, 76]. A tumor suppressive function for Ceacam1 has been suggested due to loss of expression in hyperplastic polyps, adenomas, as well as in human colon cancers that precedes defects in the APC pathway [75]. Somatostatin receptor 2 (Sst2), which mediates anti-proliferative responses to the hormone somatostatin [77, 78], was also more abundant in bean-fed animals and levels tended to decrease following AOM treatment. These results are consistent with decreased expression of sst2 in human colon tumors [79] and with findings from Xiao et al. [80] who demonstrated enhanced colonic mRNA and serum protein levels of somatostatin in rats fed either whey or soy protein isolate. Bean-fed rats also exhibited a lower expression of the mitotic genes Ccnb1, Cdc2, and Top2a. Top2a is involved in a variety of processes including DNA replication, chromosome segregation, and maintenance of chromosome structure [81]. Protein expression has been detected primarily in the actively proliferating cells at the base of the crypt and levels increase during tumorigenesis [82]. Binding of cyclin B1 to Cdc2 is required for cells to enter mitosis at the G2 checkpoint [83, 84]. Although these genes can be regulated at the transcriptional level in a p53-dependent manner [84], the fold change differences between diets would more likely suggest an increase in the proportion of cells undergoing terminal differentiation. This is further supported by the anatomical distribution of Topoisomerase II alpha and CEACAM1 along the crypt-lumen axis and implies a general effect of bean-feeding on maintaining normal crypt cell homeostasis.

Other clusters of genes similarly affected by bean-feeding highlight differences in fiber sources on colon cell physiology. For example, the most highly induced class of genes affected by bean diets included those involved in water channel and ion transport (Aqp8, Scnn1g, Slc12a7, Tfec, Slc5a1, Slc16a1) and energy metabolism (Hmgcs2, Aldob, Pck1, Hadhβ, Ech1). These changes are consistent with the physiological effects of fermentable fibers on intestinal function. Bacterial fermentation of dietary fibers and resistant starch produces the short-chain fatty acids (SCFA), acetate, propionate, and butyrate [85, 86]. SCFAs are trophic to the normal colonic epithelium, enhance water and sodium absorption, increase mucosal blood flow, and modulate enterohormone release [87, 88]. Butyrate in particular is an important energy source for colonocytes and can induce growth arrest, differentiation, and/or apoptosis of colon epithelial cells in vitro [89, 90]. Augenlicht et al. [90, 91] demonstrated that butyrate metabolism, through mitochondrial β-oxidation, is important for induction of apoptosis both in vitro and in vivo. Dysregulation of genes involved in energy metabolism, such as Hmgcs2, have been observed in inflammatory conditions of the colon [92] and during tumorigenesis [42, 93], suggesting a potential link between enhanced colonic expression and reduced cancer risk.

Despite the large number of genes influenced by carcinogen or diet alone, only 5 transcripts were significantly affected by both treatments. It was originally hypothesized that gene changes within this group would be the most important to understanding dietary modulation of tumorigenesis, with particular interest in those that paralleled tumor incidence data. We identified that transcripts for antimicrobial genes (Pla2g2a, RatNP-3) and extracellular matrix components (Colla1, Ftn1) were induced by AOM treatment in all diets, but to a much lesser extent in bean-fed animals, whereas somatostatin receptor 2 (Sstr2) showed the opposite trend. sPLA2 (Pla2g2a) and NP defensin 3 (RatNP-3) exhibit antimicrobial activity and together with other proteins play an important role in mucosal epithelial defense [94–100]. sPLA2 is a multifunctional protein induced in a variety of inflammatory and neoplastic conditions [101–107]. Enhanced expression has been reported in colon tumors of patients with familial adenomatous polyposis [108], in areas adjacent to sporadic colon tumors [109], in inflammatory bowel disease [101, 104, 105, 110], as well as in carcinogen-induced tumors in rodents [41, 111], suggesting a promoting role in colonic neoplasia. Aside from antimicrobial activity [95], sPLA2 may coordinate immune defenses by enhancing neutrophil function [112] and contributing to eicosanoid synthesis [103, 113, 114]. Similar to sPLA2, NP defensin 3 exhibits antimicrobial activity as well as other immune modulatory roles [99, 115, 116]. Normal colonic expression of alpha defensins is low [96, 97], but expression is induced during inflammation [96, 97, 117, 118], potentially by the proinflammatory cytokines IL-1β, TNF-α, and IL-6 [119]. Recently, increased levels of human neutrophil (HNP) defensins 1–3 were identified in individuals with colorectal tumors [120–122] as well as in the serum and colon from patients with active IBD [117], indicating that alpha defensins may be a plausible and early biomarker for gastrointestinal disease. Dietary modulation of these genes in addition to a lower expression of other innate immune genes, including toll-like receptor 4 (Tlr4) and Dmbt1, further suggest bean feeding inhibits tumor promotion by limiting microbially induced inflammation.
Alterations in extracellular matrix (ECM) components are commonly observed during inflammation and carcinogenesis [123–126] and contribute to number of processes including cell adhesion and migration, wound healing, angiogenesis, and immune cell migration and activation [123, 127]. Additionally, changes in the distribution and expression of different ECM proteins have also been reported along the crypt axis [128–130] suggesting a role in normal epithelial migration and differentiation [130–132]. Coll1a1 expression is upregulated in colon cancer and in other hyperproliferative disorders [133], but normal colonic expression has not been previously reported. Fibronectin has been identified as a downstream target of the Wnt/β-catenin pathway, which is frequently altered in colorectal carcinogenesis [134, 135]. Additionally, Kolachala et al. [136] reported expression is localized to surface epithelial cells and protein levels increase during the active and restitution phase of dextran sodium-induced colitis in mice. This was associated with induction of the α5-integrin receptor, increased cell attachment, and activation of the NFκB signaling pathway. Although important in restitution to injury, several bacteria also contain binding sites for fibronectin, and increased apical secretion may influence adherence to mucosal surfaces, thereby potentiating inflammation [137–140]. The lower expression of these genes in bean-fed animals treated with carcinogen may represent fewer preneoplastic lesions and/or a more general effect of diet on maintaining mucosal integrity.

Our findings provide a strong basis for future studies on legumes and colon cancer prevention, however, there are a few limitations to the current study. First, although microarrays are a powerful tool for biomedical research, one limitation to this technology is decreased sensitivity for the detection of low-abundance genes [141]. Additionally, differences in gene expression may not directly reflect changes in protein levels nor account for other posttranslational modifications such as protein phosphorylation that may also be involved in dietary modulation of colon cancer [141]. Additional studies should be undertaken to address the functional involvement of gene changes and corresponding proteins from this study to further confirm postulated roles in chemoprevention. Another potential limitation was the use of colonic mucosal scrapings rather than whole colon tissue. Mucosal scrapings yield a heterogeneous cell population consisting primarily of colonocytes, with lesser amounts of intraepithelial lymphocytes, macrophages, and endothelial cells. Although cancer evolves from genetic and epigenetic alterations arising in epithelial cells, there is increasing recognition for the importance of the microenvironment in the carcinogenic process [142]. For example, infiltrating immune cells in the lamina propria may contribute to tumor development through generation of reactive oxygen species [143, 144] as well as local production of cytokines, chemokines, and other lipid mediators which can influence carcinogenesis by promoting angiogenesis and disrupting cell cycle regulation [142]. Although our primary interests were in epithelial gene changes, gene expression patterns in other cell types were likely underrepresented and should be considered in future studies to more fully understand the impact of legumes on colon cancer development.

5. Conclusions

In summary, dietary habits are strongly associated with colon cancer risk, and this research lends further support to epidemiological and experimental data that consumption of bean-based diets inhibits colon cancer development. The finding that beans reduce markers of colonic inflammation is consistent with the inverse association of long-term non-steroidal anti-inflammatory drug (NSAID) use on CRC risk [145, 146]. Further, it is well recognized that individuals previously treated for colon cancer are at a higher risk of recurrence of the disease [147]. This observation is proposed to be related to molecular abnormalities in areas surrounding cancer tissue [148, 149]. Results from this study suggest that some abnormalities may be related to changes in cytokineti, the innate immune system, and extracellular matrix components. We speculate dietary modulation of these genes is associated with reduced inflammation, possibly through enhancing mucosal barrier function. Confirming some of these gene changes in humans, further identifying what causes these changes to occur and determining if bean consumption can reverse these changes would strengthen the relationship of bean consumption on colon cancer inhibition and may provide useful adjunct therapy for those at risk.

Conflict of Interests

The authors declare no conflict of interests.

Acknowledgments

Financial support was provided by the United States Agency for International Development through the Dry Grain Pulse Collaborative Research Support Program and the Michigan State University AgBioResearch Unit. The authors would like to sincerely thank Annette Thelen and the Genomics Technology Support Facility at Michigan State University for technical assistance and support with microarray hybridization and processing.

References

[1] J. Ferlay, H. Shin, F. Bray et al., “GLOBOCAN 2008, Cancer Incidence and Mortality Worldwide. IARC CancerBase No. 10 [Internet],” Lyon, France, 2010.
[2] B. Armstrong and R. Doll, “Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices,” International Journal of Cancer, vol. 15, no. 4, pp. 617–631, 1975.
[3] R. Doll and R. Peto, “The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today,” Journal of the National Cancer Institute, vol. 66, no. 6, pp. 1191–1308, 1981.
[4] WCRF and AICR, Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective, World Cancer Research Fund & American Institute for Cancer Research, Washington, DC, USA, 2007.
[5] J. D. Potter, “Colorectal cancer: molecules and populations,” Journal of the National Cancer Institute, vol. 91, no. 11, pp. 916–932, 1999.
[6] K. A. Steinmetz and J. D. Potter, “Food-group consumption and colon cancer in the Adelaide case-control study. I. Vegetables and fruit,” International Journal of Cancer, vol. 53, no. 5, pp. 711–719, 1993.

[7] G. E. Fraser, “Associations between diet and cancer, ischemic heart disease, and all-cause mortality in non-Hispanic white California Seventh-day Adventists,” American Journal of Clinical Nutrition, vol. 70, no. 3, 1999.

[8] P. N. Singh and G. E. Fraser, “Dietary risk factors for colon cancer in a low-risk population,” American Journal of Epidemiology, vol. 148, no. 8, pp. 761–774, 1998.

[9] L. L. Marchand, J. H. Hankin, L. R. Wilkens, L. N. Kolonel, H. N. Englyst, and L. C. Lyu, “Dietary fiber and colorectal cancer risk,” Epidemiology, vol. 8, no. 6, pp. 658–665, 1997.

[10] H. Deneo-Pellegrini, P. Boffetta, E. De Stefani, A. Ronco, P. Brennan, and M. Mendilaharsu, “Plant foods and differences between colon and rectal cancers,” European Journal of Cancer Prevention, vol. 11, no. 4, pp. 369–375, 2002.

[11] E. Lanza, T. J. Hartman, P. S. Albert et al., “High dry bean intake and reduced risk of advanced colorectal adenoma recurrence among participants in the polyp prevention trial,” Journal of Nutrition, vol. 136, no. 7, pp. 1896–1903, 2006.

[12] S. E. Pories, N. Ramchurren, I. Summerhayes, and G. Steele, “Relation of vegetable, fruit, and grain consumption to colorectal adenomatous polyps,” American Journal of Epidemiology, vol. 144, no. 11, pp. 1015–1025, 1996.

[13] P. Correa, “Epidemiological correlations between diet and cancer frequency,” Cancer Research, vol. 41, no. 9, pp. 3685–3690, 1981.

[14] R. F. Jacoby, X. Llor, B. B. Teng, N. O. Davidson, and T. A. Brasitus, “Mutations in the K-ras oncogene induced by 1,2-dimethylhydrazine in preneoplastic and neoplastic rat colonic mucosa,” Journal of Clinical Investigation, vol. 87, no. 2, pp. 624–630, 1991.

[15] A. A. Vivona, B. Shpitz, A. Medline et al., “K-ras mutations in aberrant crypt foci, adenomas and adenocarcinomas during azoxymethane-induced colon carcinogenesis,” Carcinogenesis, vol. 14, no. 9, pp. 1777–1781, 1993.

[16] S. E. Forries, N. Ramchurren, I. Summerhayes, and G. Steele, “Animal models for colon carcinogenesis,” Archives of Surgery, vol. 128, no. 6, pp. 647–653, 1993.

[17] M. Takahashi, K. Fukuda, T. Sugimura, and K. Wakabayashi, “β-catenin is frequently mutated and demonstrates altered cellular location in azoxymethane-induced rat colon tumors,” Cancer Research, vol. 58, no. 1, pp. 42–46, 1998.

[18] M. Persé and A. Cerar, “Morphological and molecular alterations in 1,2 dimethylhydrazine and azoxymethane induced colon carcinogenesis in rats,” Journal of Biomedicine and Biotechnology, vol. 2011, Article ID 473964, 2011.

[19] J. Chen and X. F. Huang, “The signal pathways in azoxymethane-induced colon cancer and preventive implications,” Cancer Biology & Therapy, vol. 8, no. 14, pp. 1313–1317, 2009.

[20] C. De Filippo, G. Caderni, M. Bazzicalupo et al., “Mutations of the ApC gene in experimental colorectal cancerogenesis induced by azoxymethane in F344 rats,” British Journal of Cancer, vol. 77, no. 12, pp. 2148–2151, 1998.

[21] M. Takahashi, M. Mutoh, T. Kawamori, T. Sugimura, and K. Wakabayashi, “Altered expression of β-catenin, inducible nitric oxide synthase and cyclooxygenase-2 in azoxymethane-induced rat colon carcinogenesis,” Carcinogenesis, vol. 21, no. 7, pp. 1319–1327, 2000.

[22] Y. Yamada, N. Yoshimi, Y. Hirose et al., “Frequent β-catenin gene mutations and accumulations of the protein in the putative peneplastic lesions lacking macroscopic aberrant crypt foci appearance, in rat colon carcinogenesis,” Cancer Research, vol. 60, no. 13, pp. 3323–3327, 2000.

[23] M. Takahashi, S. Nakatsugi, T. Sugimura, and K. Wakabayashi, “Frequent mutations of the β-catenin gene in mouse colon tumors induced by azoxymethane,” Carcinogenesis, vol. 21, no. 6, pp. 1117–1120, 2000.

[24] R. N. DuBois, A. Radhika, B. S. Reddy, and A. J. Entingh, “Increased cyclooxygenase-2 levels in carcinogen-induced rat colonic tumors,” Gastroenterology, vol. 110, no. 4, pp. 1259–1262, 1996.

[25] T. Ohta, M. Takahashi, and A. Ochiai, “Increased protein expression of both inducible nitric oxide synthase and cyclooxygenase-2 in human colon cancers,” Cancer Letters, vol. 239, no. 2, pp. 246–253, 2006.

[26] K. Watanabe, T. Kawamori, S. Nakatsugi, and K. Wakabayashi, “Cox-2 and inos, good targets for chemoprevention of colon cancer,” BioFactors, vol. 12, no. 1–4, pp. 129–133, 2000.

[27] K. Guda, C. Giardina, P. Nambiar, H. Cui, and D. W. Rosenberg, “Aberrant transforming growth factor-β signaling in azoxymethane-induced mouse colon tumors,” Molecular Carcinogenesis, vol. 31, no. 4, pp. 204–213, 2001.

[28] J. Shao, H. Sheng, R. Aramandla et al., “Coordinate regulation of cyclooxygenase-2 and TGF-β1 in replication error-positive colon cancer and azoxymethane-induced rat colonic tumors,” Carcinogenesis, vol. 20, no. 2, pp. 185–191, 1999.

[29] N. Bellam and B. Pasche, “Tgf-beta signaling alterations and colon cancer,” Cancer Treatment and Research, vol. 155, pp. 85–103, 2010.

[30] M. R. Bennink and A. S. Om, “Inhibition of Colon Cancer (CC) by soy phytochemicals but not by soy protein,” The FASEB Journal, vol. 12, no. 5, p. A655, 1998.

[31] M. R. Bennink, A. S. Om, and Y. Miyagi, “Inhibition of colon cancer (CC) by soy flour but not by genistin or a mixture of isoflavones,” The FASEB Journal, vol. 13, p. A50, 1999.

[32] L. Hanenberg and M. R. Bennink, “Consumption of black beans and navy beans (Phaseolus vulgaris) reduced azoxymethane-induced colon cancer in rats,” Nutrition and Cancer, vol. 44, no. 1, pp. 60–65, 2002.

[33] E. A. Rondini and M. R. Bennink, “Defatted soy flour, but not soy concentrate, reduces azoxymethane-induced colon carcinogenesis,” Journal of Nutrition, vol. 132, p. 5898S, 2002.

[34] R. Hakak, S. Korourian, M. J. J. Ronis, J. M. Johnston, and T. M. Badger, “Soy protein isolate consumption protects against azoxymethane-induced colon tumors in male rats,” Cancer Letters, vol. 166, no. 1, pp. 27–32, 2001.

[35] J. S. Hughes, C. Ganthavorn, and S. Wilson-Sanders, “Dry beans inhibit azoxymethane-induced colon carcinogenesis in F344 rats,” Journal of Nutrition, vol. 127, no. 12, pp. 2328–2333, 1997.

[36] G. Bobe, K. G. Barrett, R. A. Mentor-Marcel et al., “Dietary cooked navy beans and their fractions attenuate colon carciogenesis in azoxymethane-induced Ob/Ob mice,” Nutrition and Cancer, vol. 60, no. 3, pp. 373–381, 2008.

[37] T. Liu, A. O. Mokuolu, C. V. Rao, B. S. Reddy, and P. R. Holt, “Regional chemoprevention of carcinogen-induced tumors in rat colon,” Gastroenterology, vol. 109, no. 4, pp. 1167–1172, 1995.

[38] P. G. Reeves, F. H. Nielsen, and G. C. Fahey, “AIN-93 purified diets for laboratory rodents: final report of the American Journal of Nutrition and Metabolism 13
Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet,” *Journal of Nutrition*, vol. 123, no. 11, pp. 1939–1951, 1993.

[39] D. W. Huang, B. T. Sherman, and R. A. Lempicki, “Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources,” *Nature Protocols*, vol. 4, no. 1, pp. 44–57, 2009.

[40] D. W. Huang, B. T. Sherman, and R. A. Lempicki, “Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists,” *Nucleic Acids Research*, vol. 37, no. 1, pp. 1–13, 2009.

[41] K. Fujiwara, M. Ochiai, T. Ohta et al., “Global gene expression analysis of rat colon cancers induced by a food-borne carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine,” *Carcinogenesis*, vol. 25, no. 8, pp. 1495–1505, 2004.

[42] I. C. Lawrance, C. Fiocchi, and S. Chakravarti, “Ulcerative colitis and Crohn’s disease: distinctive gene expression profiles and novel susceptibility candidate genes,” *Human Molecular Genetics*, vol. 10, no. 5, pp. 445–456, 2001.

[43] M. R. Bennink, “Dietary soy reduces colon carcinogenesis in human and rats: soy and colon cancer,” *Advances in Experimental Medicine and Biology*, vol. 492, pp. 11–17, 2001.

[44] R. Nagashima, K. Maeda, Y. Imai, and T. Takahashi, “Lamina propria macrophages in the human gastrointestinal mucosa: their distribution, immunohistological phenotype, and function,” *Journal of Histochemistry and Cytochemistry*, vol. 44, no. 7, pp. 721–731, 1996.

[45] I. Maric, P. G. Holt, M. H. Perdue, and J. Bienenstock, “Class II MHC antigen (Ia)-bearing dendritic cells in the epithelium of the rat intestine,” *Journal of Immunology*, vol. 156, no. 4, pp. 1408–1414, 1996.

[46] D. C. Baumgart and S. R. Carding, “Inflammatory bowel disease: cause and immunobiology,” *The Lancet*, vol. 369, no. 9573, pp. 1627–1640, 2007.

[47] A. M. Mowat and J. L. Viney, “The anatomical basis of intestinal immunity,” *Immunological Reviews*, vol. 156, pp. 145–166, 1997.

[48] H. Tlaskalová-Hogenová, M. A. Farré-Castany, R. Štefánková et al., “The gut as a lymphopoietic organ: the role of intestinal epithelial cells in mucosal immunity,” *Folia Microbiologica*, vol. 40, no. 4, pp. 385–391, 1995.

[49] H. Lue, M. Thiele, J. Franz et al., “Macrophage migration inhibitory factor (MIF) promotes cell survival by activation of the Akt pathway and role for CSN5/JAB1 in the control of autocrine MIF activity,” *Oncogene*, vol. 26, no. 35, pp. 5046–5059, 2007.

[50] I. Leng, C. N. Metz, Y. Fang et al., “MIF signal transduction initiated by binding to CD74,” *Journal of Experimental Medicine*, vol. 197, no. 11, pp. 1467–1476, 2003.

[51] E. E. Deschner and M. Lipkin, “Study of human rectal epithelial cells in vitro. III. RNA, protein, and DNA synthesis in polyps and adjacent mucosa,” *Journal of the National Cancer Institute*, vol. 44, no. 1, pp. 175–185, 1970.

[52] K. Fujiwara, M. Ochiai, T. Ubagai et al., “Differential gene expression profiles in colon epithelium of two rat strains with distinct susceptibility to colon carcinogenesis after exposure to PHP in combination with dietary high fat,” *Cancer Science*, vol. 94, no. 8, pp. 672–678, 2003.

[53] H. M. Lee, G. H. Greeley, and E. W. Engleander, “Age-associated changes in gene expression patterns in the duodenum and colon of rats,” *Mechanisms of Ageing and Development*, vol. 122, no. 4, pp. 355–371, 2001.

[54] N. Khansari, Y. Shakiha, and M. Mahmoudi, “Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer,” *Recent Patents on Inflammation and Allergy Drug Discovery*, vol. 3, no. 1, pp. 73–80, 2009.

[55] A. Federico, F. Morgillo, C. Tuccillo, F. Giardello, and C. Loguercio, “Chronic inflammation and oxidative stress in human carcinogenesis,” *International Journal of Cancer*, vol. 121, no. 11, pp. 2381–2386, 2007.

[56] M. Macarthur, G. L. Hold, and E. M. El-Omar, “Inflammation and Cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal malignancy,” *American Journal of Physiology*, vol. 286, no. 4, pp. G515–G520, 2004.

[57] C. J. Van Der Woude, J. H. Kleibeuker, P. L. M. Jansen, and H. Moshage, “Chronic inflammation, apoptosis and (pre)malignant lesions in the gastro-intestinal tract,” *Apotosis*, vol. 9, no. 2, pp. 123–130, 2004.

[58] E. Shacter and S. A. Weitzman, “Chronic inflammation and cancer,” *Oncology*, vol. 16, no. 2, pp. 217–230, 2002.

[59] H. Bartsch and J. Nair, “Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer: role of lipid peroxidation, DNA damage, and repair,” *Langenbeck’s Archives of Surgery*, vol. 391, no. 5, pp. 499–510, 2006.

[60] M. B. Grisham, D. Jourd’heuil, and D. A. Wink, “Chronic inflammation and reactive oxygen and nitrogen metabolism—implications in DNA damage and mutagenesis,” *Alimentary Pharmacology and Therapeutics*, vol. 14, supplement 1, pp. 3–9, 2000.

[61] D. K. Podolsky, “Mucosal immunity and inflammation V. Innate mechanisms of mucosal defense and repair: the best offense is a good defense,” *American Journal of Physiology*, vol. 277, no. 3, pp. G495–G499, 1999.

[62] M. A. McGuckin, S. K. Lindén, P. Sutton, and T. H. Florin, “Mucin dynamics and enteric pathogens,” *Nature Reviews Microbiology*, vol. 9, no. 4, pp. 265–278, 2011.

[63] J. R. Turner, “Molecular basis of epithelial barrier regulation: from basic mechanisms to clinical application,” *American Journal of Pathology*, vol. 169, no. 6, pp. 1901–1909, 2006.

[64] J. R. Turner, “Intestinal mucosal barrier function in health and disease,” *Nature Reviews Immunology*, vol. 9, no. 11, pp. 799–809, 2009.

[65] C. A. E. Clayburgh, N. Mittal et al., “Epithelial NF-xB enhances transmucosal fluid movement by altering tight junction protein composition after T cell activation,” *American Journal of Physiology*, vol. 176, no. 1, pp. 158–167, 2010.

[66] J. Matrician, N. Barnich, and D. Ardid, “Immunopathogenesis of inflammatory bowel disease,” *Self/Nonself Immune Recognition and Signaling*, vol. 1, no. 4, pp. 299–309, 2010.

[67] S. Y. Salim and J. D. Söderholm, “Importance of disrupted intestinal barrier in inflammatory bowel diseases,” *Inflammatory Bowel Diseases*, vol. 17, no. 1, pp. 362–381, 2011.

[68] M. A. A. Schepens, A. J. Schoneville, C. Vink et al., “Supplemental calcium attenuates the colitis-related increase in diarrhea, intestinal permeability, and extracellular matrix breakdown in HLA-B27 transgenic rats,” *Journal of Nutrition*, vol. 139, no. 8, pp. 1525–1533, 2009.

[69] G. An, B. Wei, B. Xia et al., “Increased susceptibility to colitis and colorectal tumors in mice lacking core 3-derived O-glycans,” *Journal of Experimental Medicine*, vol. 204, no. 6, pp. 1417–1429, 2007.

[70] A. P. Soler, R. D. Miller, K. V. Laughlin, N. Z. Carp, D. M. Klurfeld, and J. M. Mullin, “Increased tight junctional permeability is associated with the development of colon cancer,” *Carcinogenesis*, vol. 20, no. 8, pp. 1425–1431, 1999.
[104] M. M. Haapamäki, J. M. Grönroos, H. Nurmi, K. Irjala, K. A. Alanen, and T. J. Nevalainen, "Phospholipase A2 in serum and colonic mucosa in ulcerative colitis," *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 59, no. 4, pp. 279–288, 1999.

[105] M. M. Haapamäki, J. M. Grönroos, H. Nurmi, K. Alanen, M. Kallajoki, and T. J. Nevalainen, "Gene expression of group II phospholipase A2 in intestine in ulcerative colitis," *Gut*, vol. 40, no. 1, pp. 95–101, 1997.

[106] T. J. Nevalainen, M. M. Haapamäki, and J. M. Grönroos, "Roles of secretory phospholipases A2 in inflammatory diseases and trauma," *Biochimica et Biophysica Acta*, vol. 1488, no. 1-2, pp. 83–90, 2000.

[107] T. J. Nevalainen, G. G. Graham, and K. F. Scott, "Antibacterial actions of secreted phospholipases A2. Review," *Biochimica et Biophysica Acta*, vol. 1781, no. 1-2, pp. 1–9, 2008.

[108] B. P. Kennedy, C. Soravia, J. Mo et al., "Overexpression of the nonpancreatic secretory group II PLA2 messenger RNA and protein in colorectal adenomas from familial adenomatous polyposis patients," *Cancer Research*, vol. 58, no. 3, pp. 500–503, 1998.

[109] L. Trible, L. T. Jensen, K. Jørgensen et al., "Increased expression and activity of group IIA and X secretory phospholipase A2 in peritumoral versus central colon carcinoma tissue," *Anticancer Research*, vol. 27, no. 5, pp. 3179–3185, 2007.

[110] T. Minami, Y. Shinomura, J. I. Miyagawa, H. Tojo, M. Okamoto, and Y. Matsuzawa, "Immuno-histochemical localization of group II phospholipase A2 in colonic mucosa of patients with inflammatory bowel disease," *American Journal of Gastroenterology*, vol. 92, no. 2, pp. 289–292, 1997.

[111] A. P. Femia, C. Luceri, S. Toti, A. Giannini, P. Dolaro, and G. Caderni, "Gene expression profile and genomic alterations in colonic tumours induced by 1,2-dimethylhydrazine (DMH) in rats," *BMC Cancer*, vol. 10, article 194, 2010.

[112] G. Zallen, E. E. Moore, J. L. Johnson et al., "New mechanisms by which secretory phospholipase A2 stimulates neutrophils to provoke the release of cytotoxic agents," *Archives of Surgery*, vol. 133, no. 11, pp. 1229–1233, 1998.

[113] C. O. Bingham and K. Frank Austen, "Phospholipase A2 enzymes in eicosanoid generation," *Proceedings of the Association of American Physicians*, vol. 111, no. 6, pp. 516–524, 1999.

[114] L. A. Marshall and D. W. Morgan, "Modulation of arachidonic acid metabolism: focus on phospholipase A2(s)," *Drug News and Perspectives*, vol. 11, no. 2, pp. 82–91, 1998.

[115] N. Droin, J. B. Hendra, P. Ducoroy, and E. Solary, "Human defensins as cancer biomarkers and antitumour molecules," *Journal of Proteomics*, vol. 72, no. 6, pp. 918–927, 2009.

[116] R. N. Cunliffe and Y. R. Mahida, "Antimicrobial peptides in innate intestinal host defence," *Gut*, vol. 47, no. 1, pp. 16–17, 2000.

[117] S. Kanamura, H. Uto, M. Numata et al., "Human neutrophil peptides 1-3 are useful biomarkers in patients with active ulcerative colitis," *Inflammatory Bowel Diseases*, vol. 15, no. 6, pp. 909–917, 2009.

[118] R. N. Cunliffe, M. Kamal, F. R. A. J. Rose, P. D. James, and Y. R. Mahida, "Expression of antimicrobial neutrophil defensins in epithelial cells of active inflammatory bowel disease mucosa," *Journal of Clinical Pathology*, vol. 55, no. 4, pp. 298–304, 2002.

[119] M. Rodríguez-García, H. Oliva, N. Climent, F. García, J. M. Gatell, and T. Gallart, "Human immature monocyte-derived dendritic cells produce and secrete α-defensins 1-3," *Journal of Leukocyte Biology*, vol. 82, no. 5, pp. 1143–1146, 2007.

[120] H. Mothes, C. Melle, G. Ernst, R. Kaufmann, F. Von Eggeling, and U. Settmacher, "Human Neutrophil Peptides 1-3—early markers in development of colorectal adenomas and carcinomas," *Disease Markers*, vol. 25, no. 2, pp. 123–129, 2008.

[121] J. Albrethsen, R. Bogebo, S. Gammeltoft, J. Olsen, B. Winther, and H. Raskov, "Upregulated expression of human neutrophil peptides 1,2 and 3 (HNP 1-3) in colon cancer serum and tumours: a biomarker study," *BMC Cancer*, vol. 5, article 8, 2005.

[122] C. Melle, G. Ernst, B. Schimmels et al., "Discovery and identification of α-defensins as low abundant, tumour-derived serum markers in colorectal cancer," *Gastroenterology*, vol. 129, no. 1, pp. 66–73, 2005.

[123] L. Sorokin, "The impact of the extracellular matrix on inflammation," *Nature Reviews Immunology*, vol. 10, no. 10, pp. 712–723, 2010.

[124] H. Obtani, "Stromal reaction in cancer tissue: pathophysiological significance of the expression of matrix-degrading enzymes in relation to matrix turnover and immune/inflammatory reactions," *Pathology International*, vol. 48, no. 1, pp. 1–9, 1998.

[125] D. L. Worthley, A. S. Giraud, and T. C. Wang, "The extracellular matrix in digestive cancer," *Cancer Microenvironment*, vol. 3, no. 1, pp. 177–185, 2010.

[126] G. I. Abdev and N. L. Lazarevich, "Control of differentiation in progression of epithelial tumors," *Advances in Cancer Research*, vol. 95, pp. 61–113, 2006.

[127] S. H. Kim, J. Turnbull, and S. Guimond, "Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor," *Journal of Endocrinology*, vol. 209, no. 2, pp. 139–151, 2011.

[128] S. Groos, G. Hünefeld, and L. Luciano, "Epithelial cell turn-over–extracellular matrix relationship in the small intestine of human adults," *Italian Journal of Anatomy and Embryology*, vol. 106, no. 2, pp. 353–361, 2001.

[129] P. Simon-Assmann, M. Keding, and K. Haffen, "Immunocytochemical localization of extracellular-matrix proteins in relation to rat intestinal morphogenesis," *Differentiation*, vol. 32, no. 1, pp. 59–66, 1986.

[130] D. Gagné, J. F. Groulx, Y. D. Benoît et al., "Integrin-linked kinase regulates migration and proliferation of human intestinal cells under a fibronectin-dependent mechanism," *Journal of Cellular Physiology*, vol. 222, no. 2, pp. 387–400, 2010.

[131] S. Westcarr, P. Farshori, J. Wyche, and W. A. Anderson, "Aptosis and differentiation in the crypt-villus unit of the rat small intestine," *Journal of Submicroscopic Cytology and Pathology*, vol. 31, no. 1, pp. 15–30, 1999.

[132] P. H. Vachon, A. Simonneau, F. E. Herring-Gillam, and J. F. Beaulieu, "Cellular fibronectin expression is down-regulated at the mRNA level in differentiating human intestinal epithelial cells," *Experimental Cell Research*, vol. 216, no. 1, pp. 30–34, 1995.

[133] A. M. DeGiorgio-Miller, L. J. Treharne, R. J. McAnulty, P. D. Coleridge Smith, G. J. Laurent, and S. E. Herrick, "Procollagen type I gene expression and cell proliferation are increased in lipodermatosclerosis," *British Journal of Dermatology*, vol. 152, no. 2, pp. 242–249, 2005.

[134] D. Gradl, M. Kühl, and D. Wedlich, "The Wnt/Wg signal transducer β-catenin controls fibronectin expression," *Molecular and Cellular Biology*, vol. 19, no. 8, pp. 5576–5587, 1999.

[135] H. Takayasu, H. Horie, E. Hiyama et al., "Frequent deletions and mutations of the β-catenin gene are associated with..."
overexpression of cyclin D1 and fibronectin and poorly differentiated histology in childhood hepatoblastoma," *Clinical Cancer Research*, vol. 7, no. 4, pp. 901–908, 2001.

[136] V. L. Kolachala, R. Bajaj, L. Wang et al., "Epithelial-derived fibronectin expression, signaling, and function in intestinal inflammation," *Journal of Biological Chemistry*, vol. 282, no. 45, pp. 32965–32973, 2007.

[137] B. Walla, F. E. Castaneda, L. Wang et al., "Polarized fibronectin secretion induced by adenosine regulates bacterial-epithelial interaction in human intestinal epithelial cells," *Biochemical Journal*, vol. 382, no. 2, pp. 589–596, 2004.

[138] L. L. Graham, T. Friel, and R. L. Woodman, "Fibronectin enhances Campylobacter fetus interaction with extracellular matrix components and INT 407 cells," *Canadian Journal of Microbiology*, vol. 54, no. 1, pp. 37–47, 2008.

[139] R. Isenmann, M. Schwarz, E. Rozdzinski et al., "Interaction of fibronectin and aggregation substance promotes adherence of Enterococcus faecalis to human colon," *Digestive Diseases and Sciences*, vol. 47, no. 2, pp. 462–468, 2002.

[140] M. E. Konkel, J. E. Christensen, A. M. Keetch, M. R. Monteville, J. D. Klena, and S. G. Garvis, "Identification of a fibronectin-binding domain within the Campylobacter jejuni CadF protein," *Molecular Microbiology*, vol. 57, no. 4, pp. 1022–1035, 2005.

[141] P. Jaluria, K. Konstantopoulos, M. Betenbaugh, and J. Shioloach, "A perspective on microarrays: current applications, pitfalls, and potential uses," *Microbial Cell Factories*, vol. 6, article 4, 2007.

[142] J. Terzić, S. Grivennikov, E. Karin, and M. Karin, "Inflammation and colon cancer," *Gastroenterology*, vol. 138, no. 6, pp. 2101–2114.e5, 2010.

[143] J. E. Klaunig, Z. Wang, X. Pu, and S. Zhou, "Oxidative stress and oxidative damage in chemical carcinogenesis," *Toxicology and Applied Pharmacology*, vol. 254, no. 2, pp. 86–99, 2011.

[144] L. B. Meira, J. M. Bugni, S. L. Green et al., "DNA damage induced by chronic inflammation contributes to colon carcinogenesis in mice," *Journal of Clinical Investigation*, vol. 118, no. 7, pp. 2516–2525, 2008.

[145] F. V. N. Din, E. Theodoratou, S. M. Farrington et al., "Effect of aspirin and NSAIDs on risk and survival from colorectal cancer," *Gut*, vol. 59, no. 12, pp. 1670–1679, 2010.

[146] R. E. Harris, "Cyclooxygenase-2 (cox-2) blockade in the chemoprevention of cancers of the colon, breast, prostate, and lung," *Inflammopharmacology*, vol. 17, no. 2, pp. 55–67, 2009.

[147] A. M. Abulafi and N. S. Williams, "Local recurrence of colorectal cancer: the problem, mechanisms, management and adjuvant therapy," *British Journal of Surgery*, vol. 81, no. 1, pp. 7–19, 1994.

[148] T. Ushijima, "Epigenetic field for cancerization," *Journal of Biochemistry and Molecular Biology*, vol. 40, no. 2, pp. 142–150, 2007.

[149] B. J. M. Braakhuis, M. P. Tabor, J. A. Kummer, C. R. Leemans, and R. H. Brakenhoff, "A genetic explanation of slaughter’s concept of field cancerization: evidence and clinical implications," *Cancer Research*, vol. 63, no. 8, pp. 1727–1730, 2003.