Review Article

Epigenetics, Probiotic Metabolites and Colon Cancer Prevention: An Overview of Progress, Opportunities and Challenges

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Key Words

Probiotics · Probiotic metabolome · Colon cancer · Therapeutics

Abstract

Colorectal cancer (CRC) is a common epithelial neoplasia across the globe, with about 1.2 million newly diagnosed cases and over 600,000 mortalities annually. The underlying causes are numerous including dietary ingredients, environmental factors and gut metabolome that affect colon cellular gene regulation and post-translational modifications of the proteome. Collectively, these alterations initiate epigenetic modifications leading to the inception of various metabolic diseases and malignancies. Epigenetics is at the core of modern medicine since it deals with complex processes that turn the expression of certain genes on and off, and it elucidates the mechanisms and connections between genotypes and the environment during various stages of life. However, the majority of the vertical molecular mechanisms with reference to epigenomics, epigenetic and metabolic ailments are still unidentified and need further research. This article summarizes the burgeoning role of probiotic metabolites and some prominent therapeutic dietary ingredients in the management of CRC.

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Introduction

Health and diseases are largely driven by acquired genetic changes. Research insights have signified that every phenotype is a consequence of a complex interaction between genes, genotypes, epigenome and environmental factors.

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Cancer initiation and progression is controlled by both genetic and epigenetic mechanisms. Tens of millions of individuals identified with colon polyps are at increased risk for colon cancer. According to the American Cancer Society [1], colorectal cancer (CRC) is the second most lethal cancer; 50,830 people are predicted to die of colon cancer in 2013. Pieces of evidence have been obtained that diet and environmental factors directly or indirectly influence epigenetic mechanisms associated with colon cancer development. The inhibition of histone deacetylase (HDAC) activity and the disruption of the HDAC complex are some of the basic mechanisms involved [2, 3].

Epigenetic Overview of CRC

Epigenetics is generally referred to as the study of mechanisms that alter gene expression without altering the primary DNA sequence. Epigenetic mechanisms are heritable and reversible and involve changes in DNA methylation, histone modifications and small noncoding microRNAs (miRNAs) [4]. Different chemical modifications of DNA and histones have been found to have an intense impact on gene expression, and to be authentically copied through mitosis.

Modern science uses epigenetic alteration as a molecular tool for finding and treating diverse diseases including cancer. Over the past decades, epigenetic studies have mainly focused on embryonic development, aging and cancer. Presently, epigenetics is also highlighted in many other fields, such as inflammation, obesity, insulin resistance, type 2 diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, and immune diseases. As epigenetic modifications can be altered by external or internal environmental factors and have the ability to change gene expression, epigenetics is now considered an important mechanism in the unknown etiology of many diseases. Such induced epigenetic changes can be inherited during cell division, resulting in the permanent maintenance of the acquired phenotype.

Cancer is a multistep process derived from combinational crosstalk between genetic alterations and epigenetic influences through various environmental factors [5–7]. In the nutritional field, epigenetics is exceptionally important, because nutrients and bioactive food components can modify epigenetic phenomena and alter the expression of genes at the transcriptional level [2, 8, 9]. Several mechanisms are part of the epigenome which includes DNA methylation, histone acetylation, poly-ADP ribosylation and ATP-dependent chromatin remodeling. Epigenetic mechanisms controlling gene transcription are often involved in cell proliferation, differentiation and survival, and are casually linked with malignant development. Alterations in epigenetic processes including chromatin modifications, such as DNA methylation and histone acetylation, are common targets studied in cancer epigenomics [6, 10]. It has been shown that half of all tumor suppressor genes are inactivated in cancers, more often by epigenetic than by genetic mechanisms [8]. Increasing evidence suggests that bioactive dietary components impact epigenetic processes often involved in the reactivation of tumor suppressor genes, the activation of cell survival proteins and the induction of cellular apoptosis in many types of cancer [11]. In addition to the transcriptional silencing of tumor suppressor genes and protein expression, noncoding miRNAs also regulate the expression of a myriad of cellular proteins by affecting mRNA stability and translation by epigenetic processes in cancer progression [5, 6]. Interestingly, miRNAs can control the expression of various epigenetic modifying enzymes, such as DNA methyltransferases, histone methyltransferases, and HDACs, involved in carcinogenic processes [12, 13]. Recent evidence suggests that bioactive dietary components can target various oncogenic or tumor-suppressive miRNAs to alter the gene expression profile in cancer prevention [14, 15].

Bioactive Dietary Ingredients and CRC

Diet is a major factor that has multiple effects, including the alteration of both the transcriptome and the metabolome of the host, and thus it may reduce the incidence of CRC by as much as 80% [16–18]. Metabolites from dietary sources may affect the intestinal mucosa directly from the luminal side or indirectly by affecting the metabolism of the whole body. Some dietary components that are toxic when ingested in excess are also subject to microbial biotransformation in the gastrointestinal (GI) tract, and the metabolites [19] that are produced are assimilated into the circulatory stream, where they may shift the cellular balance towards undesirable situations, such as a susceptibility to or induction of genetic and epigenetic modifications in the cellular genome.

Mounting evidence advocates that some of the gut microbes and their metabolites may, either independently or in conjunction with each other, influence the risk of developing atopic disease [20]. The impact of nutrients on epigenetic alterations in the intestinal mucosa and colonocytes is of paramount interest, as aberrantly methylated nucleotides may serve as prognostic markers for CRC. Since epigenetic changes are potentially reversible, they provide promising targets for preventive as well as therapeutic interventions. Information is increasing on food habits, nutrients, and dietary components and their impact on cellular mechanisms and epigenetics in preventing CRC. Probiotics and prebiotics alter metabolic processes within the lumen of the gut and colonic epithelium, which may prevent carcinogenesis [21, 22]. Such processes include the biotransformation of certain organic compounds to carcinogens, the subsequent hepatic detoxification of these compounds, DNA damage and repair in the epithelium, and apoptosis of damaged cells. Evidence from experimental models shows that aberrant crypt foci are reduced and apoptosis of damaged cells is increased after the administration of probiotics [23–25].

Progression of CRC: Alterations in Cellular Genome Organization and Stability

Colon cancer risk is influenced by a balance between health-promoting microbial metabolites and potentially carcinogenic metabolites, such as secondary bile acids. Genomic instability is one of the first steps in the initiation of CRC. There are two major categories of genomic instability in CRC: chromosome instability (CIN) and micro- or minisatellite instability (MIN). Both conditions occur due to defects in the repair and replications of genomic DNA.

CIN refers to a persistent high rate of chromosome missegregation, which leads to alterations in the increase or loss of chromosome numbers in colon cells. CIN may originate from an event in mitosis leading to a chromosome error (like a kinetochore defect, alterations in the spindle apparatus, chromosomal adhesion defect or mitotic spindle checkpoint defect). CIN can also occur due to an error in premitotic phases, such as cellular replication stress that may lead to an aberration in the chromosome structure and dysregulation of the centrosome leading to multipolar mitosis [26]. All these events, plus chromosome pulverization resulting from errors in mitosis, ultimately lead to a damage to the genomic stability of cells and the incorporation of errors in cellular genome replications [27].

It is evident that p53, a transcription factor and tumor suppressor protein, is crucial in multicellular organisms, and is involved in the regulation and clustering of the centrosome, besides the elimination of aneuploid/polyploid cells. Erroneous cytokinesis and subsequent p53-dependent confiscation of the cell result in the development of polyploidy in a cell, which leads to aneuploid daughter cells via multipolar mitosis [28]. Conversely, the loss of p53 may result in a polyploidy-multipolar mitosis-aneuploid cycle, which is highly detrimental to the genomic stability of the cells.

MIN often refers to repetitive DNA expansions and contractions in the cell [26]. It is caused by imperfections in DNA repair and/or replication, and occurs primarily during G0/G1, S or G2 phases in the mitotic cell cycle. In addition, a MIN-type chromosomal modification or its underlying causes can also lead to mitotic errors and CIN [29]. Etiologically, CIN is more prevalent than MIN. According to a study by Dunican et al. [30], CIN was observed in approximately 85% of CRC cases and MIN in the remaining 15%. In addition, CRC contains a median of 76 nonsilent sequence mutations, of which 15 are ‘driver mutations’ and the rest are ‘passenger mutations’ [31], and around 55 aneuploidy events [32]. Epigenetic alterations in colon cancer affect the expression of hundreds of genes.

Probiotics as Versatile Therapeutic Agents

Probiotics, prebiotics and dietary phytochemicals (such as polyphenols, phytoestrogens, nonprotein amino acids and saponins, etc.) can alter the gut microbial population and influence the incidence of CRC [2, 19, 33, 34]. Among the beneficial effects of probiotics observed in humans are the stimulation of gut immunity through various mechanisms, prevention of diarrhea and enhanced tolerance to lactose [35]. In addition, lactobacilli have other prohealth attributes, such as the synthesis of vitamin B, the improvement of mineral and nutrient absorption, and the degradation or removal of certain antinutritional phytochemicals [36, 37]. However, the criteria for the selection of probiotics of human gut origin and traditionally fermented foods are somewhat empirical. The selection is based on parameters like enhancing the host endogenous defense mechanisms by ameliorating the humoral immunity, and it is thus promoting the gut immunological barriers [38]. In addition, probiotics have been found to stimulate nonspecific resistance to invading pathogens [39], thereby aiding in modulating host immunity to harmful antigens with a potential to downregulate hypersensitivity reactions [40].

Microbial Volatile and Short-Chain Fatty Acids

The expression and regulation of genes is highly dependent on and coordinated by nutrients, micronutrients and microbial metabolites [18, 41]. Fiber and some fermentable oligosaccharides are subjected to gut microbial breakdown, resulting primarily in the production of short-chain fatty acids (SCFAs), i.e. acetate, propionate and butyrate in the general ratio of 60:25:15 [42]. In addition, formate, valerate, caproate and branched-chain fatty acids, such as isobutyrate, 2-methylvalerate, and isovalerate, are also produced in low quantities from the catabolism of some branched-chain amino acids [42].

SCFAs are bioactive molecules and possess anti-inflammatory activities that play a vital role in the regulation of immune functions at the intestinal mucosal surface and are postulated to be important effector molecules with multiple roles. There is evidence for the ability of SCFAs to activate the apoptosis cascade and reduce the risk of cancer [43]. Studies have revealed that polyunsaturated fatty acids and volatile fatty acids usually interact and bestow protection against colon cancer [44, 45]. SCFAs have been found to induce apoptosis, presumably related to epigenetic modification, cell cycle arrest and activation of proapoptotic cellular genes. Although the incorporation of fatty acids into CRC chemotherapy regimens is still in its infancy, evidence is accumulating to allow the identification of the length of fatty acid chains capable of exerting most effectively antineoplastic activity. The data on the effect of butyrate on colon cancer is extensive, but it cannot be considered conclusive. Based on studies on the absorption and metabolism of SCFAs, the daily production of butyrate in the

human large bowel is estimated to be more than 200 mmol, which is readily absorbed across mucosa [46]. Based on observations in other species studied, it was inferred that human beings have a larger capacity for absorption and metabolism of SCFAs in the GI tract [46]. Three major pathways are involved in the uptake of butyrate in the GI tract: (1) diffusion of the undissociated form through lipid membranes of the distal colon, (2) countertransportation mediated by bicarbonate ions and (3) paracellular diffusion of the anionic form in the proximal colon [47]. It is believed that butyrate can minimize the incidence of CRC. Clustering of SCFAs within the colonic lumen aids in maintaining a favorable low pH, which is vital for the effectiveness of numerous enzymes and for inhibiting the metabolism of carcinogenic agents in the gut [48]. SCFAs exert several other important health-promoting actions, such as lowering intestinal pH, acting as energy sources for colonocytes, the stimulation of colonic blood flow, contraction of smooth muscle cells, transepithelial chloride secretion and proliferation of colonic epithelial cells through various proliferative stimuli [49]. Evidence shows that dietary fibers and SCFAs lower the incidence of inflammatory bowel disease by decreasing the expression of proinflammatory cytokines induced by nuclear factor kappa B and by stimulating the absorption of sodium and water [50, 51].

Therapeutic Attributes of Probiotic Metabolites

n-Butyrate is most widely studied as an energy source for colonocytes and as a chemopreventive agent [21, 52, 53]. In addition, n-butyrate is also known to influence cell-specific gene expression in intestinal cells, thereby influencing immune responses and oxidative and metabolic stress [54, 55]. Several lines of evidence indicate that SCFAs may serve as epigenetic drugs or HDAC inhibitors that play an important role as anticancer biomolecules with anti-proliferative effects against tumor cells [56, 57]. Sodium butyrate, used as an HDAC inhibitor, inhibits most HDAC enzymes except class III and class II HDAC6 and HDAC10 [58]. Fermented fecal supernatants were found to be rich in butyrate and propionate and to exhibit strong anti-HDAC activity in colon cancer cell lines [59].

The anti-HDAC activity was attributed to the disruption of processes involved in the generation of dendritic cells from bone marrow stem cells, primarily through associated sodium-coupled monocarboxylate transporter (S1c5a8)-dependent inhibition of HDACs [60]. S1c5a8 is responsible for the transportation of butyrate and propionate into cells, and it is likely that these acids block the development of dendritic cell precursor cells [60]. Cohort studies have been unable to detect significant effects, but most case-control investigations advocate a protective role of probiotics in fermented dairy foods against colon cancer [61]. The majority of the anticarcinogenic effects of butyrate have been observed in vitro using cancer cell lines. In these models, the addition of butyrate has been found to inhibit cellular proliferation and to induce apoptosis, necrosis and differentiation of carcinoma cell lines [62–65]. Butyrate has also been shown to stimulate a physiological pattern of cellular proliferation in the basal crypts in the colon as well as a reduction in the number and size of aberrant cryptic foci, which serve as earliest detectable neoplastic lesions in colon carcinoma [66]. Butyrate reduces the expression of the genes *cyclin D1* and *c-myc*, which are vital for the development of CRC [67]. An optimal anticancer drug would be one that destroys tumoral cells, but not healthy somatic cells. Butyrate is reported to induce cell cycle arrest, differentiation and/or apoptosis in certain colon cancer cell lines, thus providing further evidence of the potential of gut microbial butyrate to prevent colon cancer [68]. Another important mechanism by which butyrate interferes with colon carcinoma cells is the inhibition of HDAC, which leads to the hyperacetylation of histone residues. The aberrant histone acetylation leads to impaired transcription and silencing of genes involved in the control of cell cycle,

differentiation and apoptosis [69]. As an HDAC inhibitor, butyrate increases the expression of *p21* (WAF1) by selectively regulating the acetylation of gene-associated histones and by inducing cell cycle arrest at the G1 stage [70].

Probiotic Metabolome and Gut Epigenetic Targeting

Manipulation of the gut ecosystem through dietary probiotics and prebiotics to reduce the risk of colon cancers heralds niche areas of investigation in controlling gut disease [71, 72]. Epigenetic modification, which refers to the methylation of DNA through the covalent addition of a methyl moiety to the nucleotide cytosine, has an important role in the regulation of gene expression. Virtually every step of carcinogenesis or tumorigenesis is dependent on epigenetic modifications in the cellular genome. In the mammalian genome, the majority (90–98%) of CpG sites are methylated, except for certain CpG-enriched areas (CpG islands) that are not methylated. A few genes, called imprinted genes, are regulated by the methylation of CpG islands in their promoter. These genes are precisely replicated but are reversed during inheritance [73]. Hypomethylation of promoters is associated with an increased efficiency of gene transcription. Epigenetic modifications, including histone modification or acetylation as well as gene silencing mediated by noncoding RNAs, have also been noted. In human cancers, including CRC, epigenetic events are of significant concern [73]. The enhanced methylation of cytosine in CpG islands of tumor suppressor gene promoters impairs transcription and is used as a target to manipulate genes involved in the progression of carcinogenesis. Interestingly, epigenetic alterations are reversible, but they have the potential to alter the transcriptome profile. Numerous bioactive dietary components, namely curcumin (turmeric), genistein (some pulses and soybean), gallic acid, ellagic acid and epigallocatechin-3-gallate, polyphenols, and resveratrol (e.g. tea, some vegetables and fruits), and their gut metabolites interact with the cellular genome and metabolome of the host. They alter the methylation and histone acetylation required for the activation or silencing of genes in the cancer therapy or prevention [18, 19, 74, 75]. Probiotics and their metabolites can alter the population composition of gut bacterial species that can, in turn, alter the fermentation metabolites, particularly SCFAs. Miscellaneous biological activities attributed to probiotics could be the result of epigenetic alterations that may explain the wide range of anticarcinogenic effects attributed to probiotics. New findings about the effect of probiotics on the production of SCFAs and about the epigenetic effects of SCFAs will add to the current understanding of the associations between gut symbionts and the management of CRC through dietary interventions.

Future Directions

Researchers and clinicians alike are now realizing the significance of the applications of probiotics as well as bioactive dietary ingredients. Given the increasing commercial and clinical relevance of microbial food supplements, improving their stress tolerance profile and enabling them to overcome the physiological defenses of the host should be a prioritized concern of research.

Recent advances in epigenetics offer a better understanding of the underlying mechanisms of carcinogenesis, and provide insights into the discovery of potential cancer biomarkers for early detection, disease monitoring, prognosis and risk management.

Dietary interventions for preventing colon cancer have recently emerged as viable alternatives to manage colon cancers [2, 34]. It is envisaged that the supplementation of bacterial ingredients with the ability to synthesize anticancer metabolites (e.g. butyrate and anticancer

peptides) may confer health benefits in humans. In this sequel, it is desirable to investigate the mechanisms of action by which gut origin microorganisms promote cancer or tumor resistance. Besides, the role of active dietary phytochemicals should also be explored at the cellular as well as molecular level so as to utilize them as nutraceuticals for preventing CRC. The disruption of epigenetic processes can lead to altered gene expression and functions in malignant cells. Formulations of chemopreventive bioactive dietary metabolites as nanoparticulate systems could enhance their bioavailability for targeted colon cancer therapy.

Conclusion

The human population at large is exposed to inevitable critical factors (e.g. unhealthy food habits, chemicals and stresses) leading to the development of serious diseases like colon cancers. Dietary ingredients, gut microflora and their metabolic activities are being considered as one of the most significant environmental determinants that epigenetically influence the host metagenome and gene expression. Predictably, the exploration of epigenetic phenomena is certainly going to offer a novel model in the pursuit of etiological factors in various malignancies associated with environment and nutrition. Consequently, there is an increased need to further promote the interdisciplinary concerted teamwork among researchers and clinicians associated with gut microbiota, metabolomics, metagenomics, host microbial ecology, diet and nutrition, and disease pathogenesis in order to comprehend how these microbial molecules could interact with the (epi)genome and to invent effective therapeutic remedies for cancers, metabolic syndromes, diabetes, obesity, neurocognitive disorders and other malignancies.

Disclosure Statement

The authors declare no conflict of interest.

References

- 1 American Cancer Society: Statistics for colon cancer. 2013. <http://www.cancer.org/cancer/colonandrectum-cancer/detailedguide/colorectal-cancer-key-statistics>.
- 2 Kumar M, Nagpal R, Verma V, Kumar A, Kaur N, Hemalatha R, Gautam SK, Singh B: Probiotic metabolites as epigenetic targets in the prevention of colon cancer. *Nutr Rev* 2013;71:23–34.
- 3 Groh IA, Chen C, Lüske C, Cartus AT, Esselen M: Plant polyphenols and oxidative metabolites of the herbal alkenylbenzene methyleugenol suppress histone deacetylase activity in human colon carcinoma cells. *J Nutr Metab* 2013;2013:821082.
- 4 Kanwal R, Gupta S: Epigenetic modifications in cancer. *Clin Genet* 2012;81:303–311.
- 5 Ducasse M, Brown MA: Epigenetic aberrations and cancer (review). *Mol Cancer* 2006;5:60.
- 6 Esteller M: Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat Rev Genet* 2007;8:286–298.
- 7 Ellis L, Atadja PW, Johnstone RW: Epigenetics in cancer: targeting chromatin modifications. *Mol Cancer Ther* 2009;8:1409–1420.
- 8 Issa JP: Cancer prevention: epigenetics steps up to the plate. *Cancer Prev Res (Phila)* 2008;1:219–222.
- 9 Suter MA, Aagaard-Tillery KM: Environmental influences on epigenetic profiles. *Semin Reprod Med* 2009;27:380–390.
- 10 Herceg Z: Epigenetics and cancer: towards an evaluation of the impact of environmental and dietary factors. *Mutagenesis* 2007;22:91–103.
- 11 Landis-Piowar KR, Milacic V, Dou QP: Relationship between the methylation status of dietary flavonoids and their growth-inhibitory and apoptosis-inducing activities in human cancer cells. *J Cell Biochem* 2008;105:514–523.

- 12 Guil S, Esteller M: DNA methylomes, histone codes and miRNAs: tying it all together. *Int J Biochem Cell Biol* 2009;41:87–95.
- 13 Saito Y, Jones PA: Epigenetic activation of tumor suppressor microRNAs in human cancer cells (review). *Cell Cycle* 2006;5:2220–2222.
- 14 Sun Q, Cong R, Yan H, Gu H, Zeng Y, Liu N, Chen J, Wang B: Genistein inhibits growth of human uveal melanoma cells and affects microRNA-27α and target gene expression. *Oncol Rep* 2009;22:563–567.
- 15 Li Y, VandenBoom TG 2nd, Kong D, Wang Z, Ali S, Philip PA, Sarkar FH: Up-regulation of miR-200 and let-7 by natural agents leads to the reversal of epithelial-to-mesenchymal transition in gemcitabine-resistant pancreatic cancer cells. *Cancer Res* 2009;69:6704–6712.
- 16 Kumar M, Mohania D, Kumar A, Nagpal R, Behare PV, Aggarwal PK, Yadav M, Marotta F, Jain S, Yadav H: Metabolomics: an emerging tool for nutrition research. *Curr Topics Nutraceut Res* 2009;7:97–104.
- 17 Willett WC: Diet, nutrition, and avoidable cancer (review). *Environ Health Perspect* 1995;103(suppl 8):165–170.
- 18 Singh B, Mukesh M, Sodhi M, et al: Nutrigenomics, metabolomics and metabonomics: emerging faces of molecular genomics and nutrition; in Knapp JS, Cabrera WL (eds): *Metabolomics: Metabolites, Metabonomics and Analytical Technologies*. Hauppauge, Nova Science Publishers, Inc, 2011, pp 201–213.
- 19 Singh B, Bhat TK, Singh B: Potential therapeutic applications of some anti-nutritional plant secondary metabolites (review). *J Agric Food Chem* 2003;51:5579–5597.
- 20 O’Keefe SJ: Nutrition and colonic health: the critical role of the microbiota (review). *Curr Opin Gastroenterol* 2008;24:51–58.
- 21 Wollowski I, Rechkemmer G, Pool-Zobel BL: Protective role of probiotics and prebiotics in colon cancer (review). *Am J Clin Nutr* 2001;73(2 suppl):451S–455S.
- 22 de Moreno de LeBlanc A, Matar C, Perdígón G: The application of probiotics in cancer. *Br J Nutr* 2007;98(suppl 1):S105–S110.
- 23 Le Leu RK, Brown IL, Hu Y, Bird AR, Jackson M, Esterman A, Young GP: A synbiotic combination of resistant starch and *Bifidobacterium lactis* facilitates apoptotic deletion of carcinogen-damaged cells in rat colon. *J Nutr* 2005;135:996–1001.
- 24 Ohkawara S, Furuya H, Nagashima K, Asanuma N, Hino T: Oral administration of butyrylvibrio fibrisolvens, a butyrate-producing bacterium, decreases the formation of aberrant crypt foci in the colon and rectum of mice. *J Nutr* 2005;135:2878–2883, erratum in *J Nutr* 2009;139:194.
- 25 Geier MS, Butler RN, Howarth GS: Probiotics, prebiotics and synbiotics: a role in chemoprevention for colorectal cancer? *Cancer Biol Ther* 2006;5:1265–1269.
- 26 Rao CV, Yamada HY: Genomic instability and colon carcinogenesis: from the perspective of genes. *Front Oncol* 2013;3:130.
- 27 Crasta K, Ganem NJ, Dagher R, Lantermann AB, Ivanova EV, Pan Y, Nezi L, Protopopov A, Chowdhury D, Pellman D: DNA breaks and chromosome pulverization from errors in mitosis. *Nature* 2012;482:53–58.
- 28 Shi Q, King RW: Chromosome nondisjunction yields tetraploid rather than aneuploid cells in human cell lines. *Nature* 2005;437:1038–1042.
- 29 Burrell RA, McClelland SE, Endesfelder D, Groth P, Weller MC, Shaikh N, Domingo E, Kanu N, Dewhurst SM, Gronroos E, Chew SK, Rowan AJ, Schenk A, Sheffer M, Howell M, Kschischo M, Behrens A, Helleday T, Bartek J, Tomlinson IP, Swanton C: Replication stress links structural and numerical cancer chromosomal instability. *Nature* 2013;494:492–496.
- 30 Dunican DS, McWilliam P, Tighe O, Parle-McDermott A, Croke DT: Gene expression differences between the microsatellite instability (MIN) and chromosomal instability (CIN) phenotypes in colorectal cancer revealed by high-density cDNA array hybridization. *Oncogene* 2002;21:3253–3257, erratum in *Oncogene* 2002;21:7912.
- 31 Wood LD, Parsons DW, Jones S, Lin J, Sjöblom T, Leary RJ, Shen D, Boca SM, Barber T, Ptak J, Silliman N, Szabo S, Dezso Z, Ustyanksky V, Nikolskaya T, Nikolsky Y, Karchin R, Wilson PA, Kaminker JS, Zhang Z, Croshaw R, Willis J, Dawson D, Shipitsin M, Willson JK, Sukumar S, Polyak K, Park BH, Pethiyagoda CL, Pant PV, Ballinger DG, Sparks AB, Hartigan J, Smith DR, Suh E, Papadopoulos N, Buckhaults P, Markowitz SD, Parmigiani G, Kinzler KW, Velculescu VE, Vogelstein B: The genomic landscapes of human breast and colorectal cancers. *Science* 2007;318:1108–1113.
- 32 Cancer Genome Atlas Network: Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012;487:330–337.
- 33 Davis CD, Milner JA: Gastrointestinal microflora, food components and colon cancer prevention. *J Nutr Biochem* 2009;20:743–752.
- 34 Kumar M, Rakesh S, Nagpal R, Hemalatha R, Ramakrishna A, Sudarshan V, Ramagoni R, Shujaiddin M, Verma V, Kumar A, Tiwari A, Singh B, Kumar R: Probiotic *Lactobacillus rhamnosus* GG and *Aloe vera* gel improve lipid profiles in hypercholesterolemic rats. *Nutrition* 2013;29:574–579.
- 35 Wolvers D, Antoine JM, Myllyluoma E, Schrezenmeier J, Szajewska H, Rijkers GT: Guidance for substantiating the evidence for beneficial effects of probiotics: prevention and management of infections by probiotics. *J Nutr* 2010;140:698S–712S.
- 36 Osawa R, Fujisawa T, Pukall R: *Lactobacillus apodemi* sp nov, a tannase-producing species isolated from wild mouse faeces. *Int J Syst Evol Microbiol* 2006;56(Pt 7):1693–1696.
- 37 Rodríguez H, de las Rivas B, Gómez-Cordovés C, Muñoz R: Characterization of tannase activity in cell-free extracts of *Lactobacillus plantarum* CECT 748T. *Int J Food Microbiol* 2008;121:92–98.

- 38 Saggiaro A: Probiotics in the treatment of irritable bowel syndrome. *J Clin Gastroenterol* 2004;38(6 suppl):S104–S106, erratum in *J Clin Gastroenterol* 2005;39:261.
- 39 Resta-Lenert S, Barrett KE: Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). *Gut* 2003;52:988–997.
- 40 Snelling AM: Effects of probiotics on the gastrointestinal tract (review). *Curr Opin Infect Dis* 2005;18:420–426.
- 41 Inselman AL, Hansen DK, Lee HY, Nakamura N, Ning B, Monteiro JP, Varma V, Kaput J: Assessment of research models for testing gene-environment interactions. *Eur J Pharmacol* 2011;668(suppl 1):S108–S116.
- 42 Macfarlane S, Macfarlane GT: Regulation of short-chain fatty acid production (review). *Proc Nutr Soc* 2003;62:67–72.
- 43 Avivi-Green C, Polak-Charcon S, Madar Z, Schwartz B: Apoptosis cascade proteins are regulated in vivo by high intracolonic butyrate concentration: correlation with colon cancer inhibition. *Oncol Res* 2000;12:83–95.
- 44 Hofmanová J, Vaculová A, Koubková Z, Hýzd'álová M, Kozubík A: Human fetal colon cells and colon cancer cells respond differently to butyrate and PUFAs. *Mol Nutr Food Res* 2009;53(suppl 1):S102–S113.
- 45 Chapkin RS, Seo J, McMurray DN, Lupton JR: Mechanisms by which docosahexaenoic acid and related fatty acids reduce colon cancer risk and inflammatory disorders of the intestine. *Chem Phys Lipids* 2008;153:14–23.
- 46 Høverstad T: Studies of short-chain fatty acid absorption in man. *Scand J Gastroenterol* 1986;21:257–260.
- 47 Velázquez OC, Rombeau JL: Butyrate. Potential role in colon cancer prevention and treatment (review). *Adv Exp Med Biol* 1997;427:169–181.
- 48 Candela M, Maccaferri S, Turroni S, Carnevali P, Brigidi P: Functional intestinal microbiome, new frontiers in prebiotic design. *Int J Food Microbiol* 2010;140:93–101.
- 49 Cummings JH, Rombeau JL, Sakata T: *Physiological and Clinical Aspects of Short-Chain Fatty Acids*. Cambridge, Cambridge University Press, 1995.
- 50 Galvez J, Rodríguez-Cabezas ME, Zarzuelo A: Effects of dietary fiber on inflammatory bowel disease (review). *Mol Nutr Food Res* 2005;49:601–608.
- 51 Gibson GR, Roberfroid MB: Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics (review). *J Nutr* 1995;125:1401–1412.
- 52 Delzenne N, Cherbut C, Neyrinck A: Prebiotics: actual and potential effects in inflammatory and malignant colonic diseases (review). *Curr Opin Clin Nutr Metab Care* 2003;6:581–586.
- 53 Ma X, Fan PX, Li LS, Qiao SY, Zhang GL, Li DF: Butyrate promotes the recovering of intestinal wound healing through its positive effect on the tight junctions. *J Anim Sci* 2012;90(suppl 4):266–268.
- 54 Sauer J, Richter KK, Pool-Zobel BL: Physiological concentrations of butyrate favorably modulate genes of oxidative and metabolic stress in primary human colon cells. *J Nutr Biochem* 2007;18:736–745.
- 55 Walter J: Ecological role of lactobacilli in the gastrointestinal tract: implications for fundamental and biomedical research. *Appl Environ Microbiol* 2008;74:4985–4996.
- 56 Marks PA, Richon VM, Miller T, Kelly WK: Histone deacetylase inhibitors (review). *Adv Cancer Res* 2004;91:137–168.
- 57 Acharya MR, Sparreboom A, Venitz J, Figg WD: Rational development of histone deacetylase inhibitors as anti-cancer agents: a review. *Mol Pharmacol* 2005;68:917–932.
- 58 Davie JR: Inhibition of histone deacetylase activity by butyrate (review). *J Nutr* 2003;133(7 suppl):2485S–2493S.
- 59 Waldecker M, Kautenburger T, Daumann H, Busch C, Schrenk D: Inhibition of histone-deacetylase activity by short-chain fatty acids and some polyphenol metabolites formed in the colon. *J Nutr Biochem* 2008;19:587–593.
- 60 Singh N, Thangaraju M, Prasad PD, Martin PM, Lambert NA, Boettger T, Offermanns S, Ganapathy V: Blockade of dendritic cell development by bacterial fermentation products butyrate and propionate through a transporter (Slc5a8)-dependent inhibition of histone deacetylases. *J Biol Chem* 2010;285:27601–27608.
- 61 Saikali J, Picard C, Freitas M, Holt P: Fermented milks, probiotic cultures, and colon cancer (review). *Nutr Cancer* 2004;49:14–24.
- 62 Lan A, Lagadic-Gossman D, Lemaire C, Brenner C, Jan G: Acidic extracellular pH shifts colorectal cancer cell death from apoptosis to necrosis upon exposure to propionate and acetate, major end-products of the human probiotic propionibacteria. *Apoptosis* 2007;12:573–591.
- 63 Hinnebusch BF, Meng S, Wu JT, Archer SY, Hodin RA: The effects of short-chain fatty acids on human colon cancer cell phenotype are associated with histone hyperacetylation. *J Nutr* 2002;132:1012–1017.
- 64 Chirakkal H, Leech SH, Brookes KE, Prais AL, Waby JS, Corfe BM: Upregulation of BAK by butyrate in the colon is associated with increased Sp3 binding. *Oncogene* 2006;25:7192–7200.
- 65 Comalada M, Bailón E, de Haro O, Lara-Villoslada F, Xaus J, Zarzuelo A, Gálvez J: The effects of short-chain fatty acids on colon epithelial proliferation and survival depend on the cellular phenotype. *J Cancer Res Clin Oncol* 2006;132:487–497, erratum in *J Cancer Res Clin Oncol* 2007;133:211.
- 66 Alrawi SJ, Schiff M, Carroll RE, Dayton M, Gibbs JF, Kulavlat M, Tan D, Berman K, Stoler DL, Anderson GR: Aberrant crypt foci. *Anticancer Res* 2006;26:107–119.
- 67 Maier S, Daroqui MC, Scherer S, Roepcke S, Velcich A, Shenoy SM, Singer RH, Augenlicht LH: Butyrate and vitamin D₃ induce transcriptional attenuation at the cyclin D₁ locus in colonic carcinoma cells. *J Cell Physiol* 2009;218:638–642.

- 68 Daroqui MC, Augenlicht LH: Transcriptional attenuation in colon carcinoma cells in response to butyrate. *Cancer Prev Res (Phila)* 2010;3:1292–1302.
- 69 Scharlau D, Borowicki A, Habermann N, Hofmann T, Klenow S, Miene C, Munjal U, Stein K, Gleit M: Mechanisms of primary cancer prevention by butyrate and other products formed during gut flora-mediated fermentation of dietary fibre. *Mutat Res* 2009;682:39–53.
- 70 Chen YX, Fang JY, Lu J, Qiu DK: Regulation of histone acetylation on the expression of cell cycle-associated genes in human colon cancer cell lines. *Zhonghua Yi Xue Za Zhi* 2004;84:312–317.
- 71 Kumar M, Verma V, Nagpal R, Kumar A, Behare PV, Singh B, Aggarwal PK: Anticarcinogenic effect of probiotic-fermented milk and chlorophyllin on aflatoxin-B₁-induced liver carcinogenesis in rats. *Br J Nutr* 2012;107:1006–1016.
- 72 Nagpal R, Kaur A: Synbiotic effect of various prebiotics on in vitro activities of probiotic lactobacilli. *Ecol Food Nutr* 2011;50:63–68.
- 73 Migliore L, Migheli F, Spisni R, Coppedè F: Genetics, cytogenetics, and epigenetics of colorectal cancer. *J Biomed Biotechnol* 2011;2011:792362.
- 74 Singh B, Bhat TK, Sharma OP, Kanwar SS, Rahi P, Gulati A: Isolation of tannase-producing *Enterobacter ludwigii* GRT-1 from the rumen of migratory goats. *Small Rumin Res* 2012;102:172–176.
- 75 Santos IS, Ponte BM, Boonme P, Silva AM, Souto EB: Nanoencapsulation of polyphenols for protective effect against colon-rectal cancer. *Biotechnol Adv* 2013;31:514–523.