Nutrition, Epigenetic Mechanisms, and Human Disease

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Edited by
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This book is dedicated to our parents
whose unfailing support, encouragement, and affection
tided us over many difficulties.

Nilanjana Maulik, PhD, FAHA, FACN
Gautam Maulik, PhD

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Preface

A uniquely tailored diet that corresponds to the demands of our genetic signature is becoming an emerging indispensable need, as nutrition research is shifting its focus from epidemiology and physiology to effects of nutrients at the molecular level. Nutrigenomics relates to the application of high-throughput genomic tools in nutrition research to unravel the influence of micro- and macronutrients as potent dietary signals regulating metabolic pathways (dietary signature) and unmask how susceptible genotypes predispose to diet-related diseases. Since the last decade, extensive research on nutrigenomics has unveiled numerous epigenetic mechanisms that are influenced by our dietary signature and are capable of modifying an individual's susceptibility to diet-related disorders. The primary objective of this volume is to illustrate how nutrition can influence epigenetic inheritance and the mechanisms that underlie the modification of metabolic imprint of an individual, so that our enriched understanding of nutrigenomics can be applied to master a tailored diet that can alleviate imprinted metabolic syndromes. Specifically, the focus of the book will be on three key areas: discussion of the basics of nutrigenomics and epigenetic regulation, types of nutrition influencing the genetic imprinting, and the role of nutrition in modulating an individual's predisposition to cancer.

Nutrigenomics aims at devising dietary-intervention strategies to alleviate diet-related diseases and to restore normal metabolic homeostasis of the body. Epigenetic mechanisms like DNA methylation and transposon insertion have been shown to play at the nexus between nutrition and the genetic signature of an individual. Chromatin remodeling across the genome mediated via epigenetic mechanisms and transient nutritional stimuli can wield persistent changes on the genomic profile that are likely to be passed on to the subsequent generations. Genomic imprinting refers to a unique type of epigenetic regulation whereby differential modification of the parental alleles at certain genetic loci in the parental germlines (imprinting control regions) takes place depending on whether the allele is passed on to the offspring through the male or female gamete. Genomic imprinting mechanisms have been shown to be influenced by maternal modifier genes (after fertilization) resulting in the removal of paternal imprints on sperm DNA as well as by the dietary signature. Human epidemiologic studies reveal that metabolic imprinting is affected by poor perinatal and neonatal nutrition as well as maternal nutritional imbalance, which might result in predispositions to adult obesity, cardiovascular disease, atherosclerosis, hypertension, cancer, and type 2 diabetes.

This book addresses a very complex scenario related to nutrition—epigenetic changes related to human health and diseases. The contents are highly relevant, focused, and very timely. Recently, the National Institutes of Health (NIH) has added "epigenetic" to its roadmap; therefore a book on nutrition and epigenetics is certainly in demand. It is written by world-recognized experts in the field of nutrition, epigenetic regulations and gene expression related to aging, various cancers, vascular function, lung inflammation, diabetes, metabolic syndrome, and neurodegenerative

diseases. Selected topics from this field have been covered in some books, but no comprehensive text on epigenetics, nutrition, and human health and disease is available. This handbook includes 14 contributions from leading scientists. After Chapter 1, "Nutritional Epigenetics and Disease Prevention: Are We There Yet?," the book deals with various ongoing researches on nutrition-mediated regulation of epigenetic mechanisms and various disease scenarios. We are very sure this invaluable reference book is of interest to all health care–related professionals as well as nutritionists, biochemists, cancer biologists, pharmacologists, and mutagenesists.

This book is intended for biochemists, molecular biologists, cell biologists, biomedical researchers, and clinical researchers.

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Gautam Maulik, PhD

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The Editors

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Gautam Maulik, PhD, is an instructor at the Department of Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts. Dr. Maulik earned his master's degree in biochemistry in 1983 from Calcutta University, Calcutta, India. Immediately after earning his degree, Dr. Maulik enrolled in the doctoral program in the Department of Biochemistry at Jadavpur University, Calcutta, India. After completing his PhD, Dr. Maulik came to the United States in 1994 to continue his research on free radical-mediated oxidative stress. Dr. Maulik has since joined the Dana Farber Cancer Institute/Harvard Medical School where he has continued to produce outstanding research. His most profound accomplishment since joining Harvard Medical School has been the development of a sophisticated cancer detection method and anticancer drugs. Based on his recent work, new anticancer drugs have been developed and are currently being tested for lung cancer and other

malignancies. Dr. Maulik has already identified some of the factors and molecular pathways that are involved in the pathogenesis of lung cancer. He has published more than 50 articles in esteemed scientific journals, including *Cancer Research, Oncogene, Clinical Cancer Research, Nucleic Acids Research,* and *PNAS,* and has presented his research at important scientific conferences throughout the world, including in the United States, Japan, and India. Dr. Maulik has also published three book chapters.

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1 Nutritional Epigenetics and Disease Prevention
Are We There Yet?

Mukesh Verma

CONTENTS

1.1 EPIGENETICS MECHANISM AND GENE REGULATION

Our genome contains information related to gene structure and function but when and how long that information is utilized is determined by our epigenome. Epigenetics includes alterations in gene expression that do not include a change in DNA sequence during growth, development, and disease states (Ballestar and Esteller 2008). For normal function of a cell or organ, epigenetic regulation is needed; however, this regulation is disturbed during disease initiation and progression. Thus our genome is the "hardware" and our epigenome is the "software" of the body. Genetic information is static whereas epigenetic information is dynamic and transient. In the body, all the cells have the same genome but each cell has a different epigenome. The phenotype of a cell is determined by its epigenome (Murrell et al. 2005). Environmental factors (e.g., exposure to radiation, infectious agents), nutrients, toxins, and disease states affect the epigenome resulting in altered gene expression (Verma 2003; Kumar and Verma 2009) (Figure 1.1). Epigenetic changes can be measured quantitatively and followed during the progression/regression and recurrence of a disease (Ganesan et al. 2009; Feinberg 2010; Verma et al., 2004; 2006). This chapter compiles existing knowledge regarding the application of epigenetics toward understanding the dynamic interrelationship between bioactive food components (and/or a combination thereof) and cancer prevention.

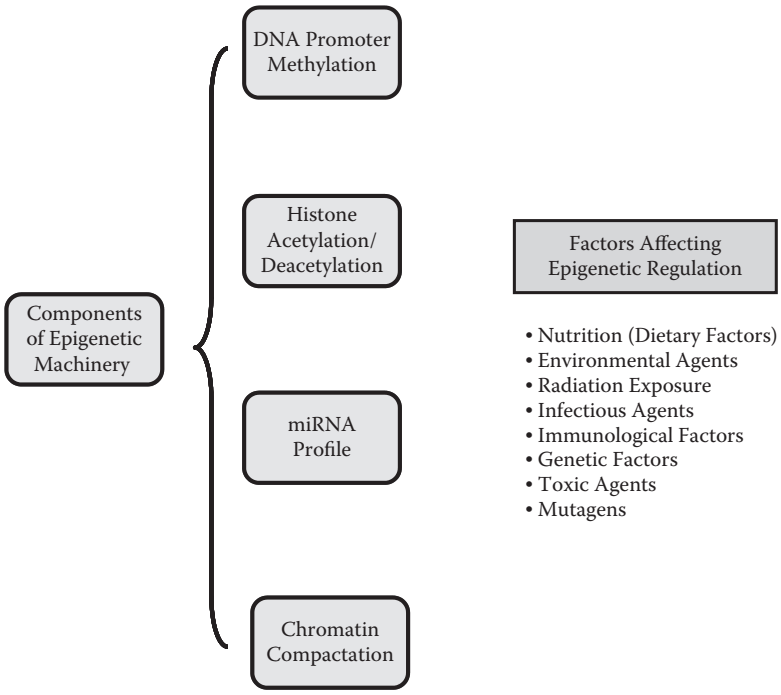

FIGURE 1.1 Components of epigenetics machinery and factors that influence gene regulation epigenetically. Factors mentioned here may work independently or in combination. A few factors affect DNA, whereas others affect proteins and nucleic acids simultaneously.

1.2 COMPONENTS OF EPIGENETIC MACHINERY

The major components of epigenetics are DNA methylation (methylation code), histone modification (histone code), chromatin compactation and relaxation, gene imprinting, and microRNA (miRNA) profile (Figure 1.1). Chromatin, which is composed of nucleosomes, is the key component of epigenetics. Nucleosomes are comprised of histone proteins arranged as octamers associated with 146 bp of DNA via its negatively charged phosphate backbone (Lustberg and Ramaswamy 2009; Mitsiades and Anderson 2009). The amino terminal part of histones protrudes out and becomes susceptible to enzymatic modifications, specifically at lysine residues, but also at other amino acids. More than 100 histone modifications of amino acids have been reported (Ganesan et al. 2009; Verma and Kumar 2009). The dimeric H3 and H4 form a tetramer, whereas H2A and H2B remain as dimmer.

The DNA in promoter regions of several genes contain CpG islands (regions rich in GC content), which are covalently modified due to methylation of cytosines at the 5′ position (Hitchins and Ward 2009; Laird 2010). This process is called hypermethylation. A number of tumor suppressor genes get inactivated due to hypermethylation of their promoters (Verma et al. 2006). On the other hand, a few genes, such as oncogenes, are methylated in their normal states and become hypomethylated in disease states, resulting in their activation (Ballestar and Esteller 2008; Laird 2010).

Throughout life, equilibrium is needed in the methylation state of the whole epigenome. Enzymes that are involved in methylation are called methyl transferases. These enzymes either initiate or maintain methylation. Proteins that bind to methylated DNA have also been identified and characterized and are referred to as methylated binding proteins (MBPs). The roles of other proteins, such as the polycomb group of proteins, have also been defined (Gieni and Hendzel 2009).

Repetitive regions, such as LINE and Alu, are hypermethylated in the normal state and are hypomethylated during growth and development. This process prevents chromosomal instability, translocation, and gene disruption caused by activation of transposons (Ballestar and Esteller 2008; Esteller 2008).

Quantitative measurements of methylation levels help in disease detection, progression, and follow-up to treatment. For example, hypermethylation of the glutathione gene (GSTP1) occurs only in prostate cancer and not in benign states (Bryzgunova et al. 2008). Thus human populations can be screened based on the methylation status of the GSTP1 gene to distinguish high-risk individuals. Furthermore, the methylation of cytosine can be reversed by chemicals, such as azacytidine and deoxycytidine, and inactive genes can be activated by chemical agents, both of which provide therapeutic potential (Ganesan et al. 2009). There are a few drugs that have been approved by the Food and Drug Administration (FDA) that have demonstrated promising results in clinical trials (Verma 2010).

MicroRNAs (miRNAs) are small noncoding RNAs with a length of 21–25 bp that possess the ability to suppress translation of a gene by binding to partially complementary messenger RNA (mRNA) (Ku and McManus 2008; Chen et al. 2009). For example, Let-7 and miR-15/miR-16 inactivate oncogenes RAS and BCL2, respectively (Esteller 2008; Garzon et al. 2009). Recent research indicates that selected miRNAs are tissue and disease specific. Cell development, differentiation, and death is affected by miRNAs (Sekine et al. 2009). miRNAs can also be used for disease detection and treatment follow-up (Chen et al. 2009). Technologies exist to perform epigenome-wide miRNA profiling to identify differentially expressed miRNAs in disease states.

The orientation and modulation of histones contribute to the heterochromatin and euchromatin states. Histone acetylation/deacetylation may result in turning off of the cell cycle regulatory genes, inactivation of tumor suppressor genes, and activation of oncogenes (Lane and Chabner 2009). Enzymes that mediate acetylation (acetyltransferases) and deacetylation (deacetylases) are well characterized in different cell types (Lane and Chabner 2009; Villagra et al. 2009). Histone modifications also include biotinylation, methylation, phosphorylation, sumoylation, and ubiquitination (Verma and Kumar 2009). Acetylation of H3-lysine has been observed at locations 9, 14, 18, and 23, whereas the lysine locations of that on H4 is at 5, 8, 12, and 16. The interaction of acetyl groups occurs at the epsilon amino group of lysine, resulting in histone neutralization. Other amino acids of histones that generally undergo alteration include arginine and serine (Ma et al. 2009; Marson 2009; Verma and Kumar 2009).

One additional type of gene regulation is gene imprinting, which is paternal or maternal allele-specific expression of a limited number of genes (50–80) (Jelinic and Shaw 2007; Vu et al. 2010). Without proper imprinting control, abnormal growth occurs. Examples of diseases regulated epigenetically via imprinting are

TABLE 1.1
Clinical Samples for Epigenetic Analysis

Sample	Suitable Analysis
Bronchoalveolar lavage	DNA isolation and methylation profiling
Buccal cells	DNA isolation and methylation profiling
Ductal lavage fluid	Proteomic analysis (histone and nonhistones) and methylation profiling
Cervical swab	Proteomic analysis (histone and nonhistones) and methylation profiling; methylation profile of infectious agents (HPV)
Duodenal fluid	Proteomic analysis (histone and nonhistones) and methylation profiling
Ejaculate	DNA isolation and methylation profiling (for example, GSTP1 methylation in prostate cancer)
Exfoliated cells	Proteomic analysis (histone and nonhistones) and methylation profiling
Nipple aspirate	Proteomic analysis (histone and nonhistones) and methylation profiling
Pleural lavage	DNA isolation and methylation profiling
Saliva	DNA isolation and methylation profiling, proteomic analysis, and miRNA profiling
Sputum	DNA isolation and methylation profiling, proteomic analysis, and miRNA profiling
Stool	DNA isolation and methylation profiling
Tissue	DNA isolation and methylation profiling, proteomic analysis, and miRNA profiling
Urine	DNA isolation and methylation profiling (for example, bladder cancer)

Beckwith-Wiedemann syndrome (BWS), Silver-Russell syndrome (SRS), and X-chromosome inactivation (Zhao et al., 2009). Methylation of DNA occurs in the imprinting loci called imprinting control regions (ICRs). Loss of imprinting (LOI) of IGF2 has been proposed in stem cell proliferation and cancer (Dammann et al. 2010; Timp et al. 2009). A variety of biospecimens can be utilized for epigenetic analysis (Table 1.1).

1.3 NIH EPIGENOMICS ROADMAP

The National Institutes of Health (NIH) has initiated the Epigenomics Roadmap Program (www.roadmapepigenomics.org), which is comprised of five major initiatives: Reference Epigenome Mapping Centers, Epigenomic Data Analysis and Coordinating Centers, Technology Development in Epigenetics, Discovery of Novel Epigenetic Marks, and Epigenomics of Human Health and Diseases. This program proposes that the origins of health and susceptibility to disease are the result of epigenetic regulation of the genetic information. Specifically, epigenetic mechanisms that control stem cell differentiation and organogenesis contribute to the biological response to environmental and other factors in the form of stimuli that contribute in disease development. To accomplish this, the Roadmap Epigenomics Program plans to develop standardized platforms, procedures, and reagents for epigenomics research; conduct demonstration projects to evaluate how epigenomes change;

develop new technologies for single-cell epigenomic analysis and in vivo imaging of epigenetic activity; and create a public data resource to accelerate the application of epigenomics approaches. This program will transform biomedical research by developing comprehensive reference epigenome maps, developing new technologies for comprehensive epigenomic analyses, and providing novel strategies for disease detection, diagnosis, treatment, and prognosis. Since several institutes are participating in this initiative, a number of diseases are covered in the roadmap. A few examples of epigenetic approaches, already funded by this program, in different diseases are described next.

In vascular epigenomics, Gary Gibbons's group (Morehouse School of Medicine) has proposed that the high prevalence of hypertensive vascular disease among African Americans is due to gene and environment interaction mediated via vascular epigenome. Hypertensive vasculature complications involve long-term changes in vessel function and structure and may contribute to diseases such as stroke. The underlying mechanisms are not completely understood. This group proposes that a group of genes are "vasculopathic" and the other group is "vasculoprotective." The methylation profile and histone modifications will be studied for whole genome and disease-specific methylation profile, and histone modifications will be identified. The participants in this proposal are age- and sex-matched controls and cases of African American origin. As a follow-up of this study, profiles will be developed from samples of cases undergoing treatment with different food habits and lifestyles. The data obtained will be available for public and will be an excellent resource to develop new prevention, intervention, and treatment strategies in vascular diseases.

Jessica Connelly of the University of Virginia is conducting research to test whether methylation plays a major role in endothelial cells and smooth muscle cells undergoing phenotypic switching during atherosclerosis initiation and progression. Normal and disease-affected tissue samples will be utilized in this case-control study to identify differentially expressed methylation profile. Contribution of genetic factors in epigenetic regulation of disease-related genes will also be accomplished. Another group, led by Yongmei Liu (Wake Forest University Health Sciences), has focused on investigating association of global methylation profile in circulating monocytes in relation to atherosclerosis and monocyte gene expression in the Multi-Ethnic Study of Atherosclerosis (MESA). More than 1500 samples of Caucasian, African American, and Hispanic origin will be analyzed by these investigators. After identifying disease-associated methylation marks, validation of these marks will be accomplished in more samples. Contribution of environmental, lifestyle, and dietary factors will also be evaluated.

A number of chronic conditions, such as cognitive decline and dementia, are developed during old age that impair older persons' ability to interact optimally in the community. Neuropathology of cognitive decline in old-age diseases, such as Alzheimer's disease, cerebrovascular disease, Lewy Body disease, accounts for only 20% of the cognitive behavior. David Benett (Rush University Medical Center) has proposed that there are other factors that may contribute to the remaining cognitive decline in old age. Life experiences (socioeconomic status, psychological distress, chronic non-neuronal diseases) are not related to known neuropathological process

but contribute in disease development. Preliminary evidence of altered epigenetic marks in these diseases exists, but a systematic study has not been completed. David Benett is conducting epigenome-wide methylation analysis of brain tissues from participants in the Rush Memory and Aging Project and the Religious Orders Study. Furthermore, data from genomewide association studies (GWASs) of brain tissues will be used to establish correlation of epigenetics and genetics in cognitive diseases and brain disorders. Another group, led by Paul Coleman of Sun Health Research Institute, plans to utilize more than 500 samples of Alzheimer's disease from the Brain Bank at this institute to perform methylation profile and compare with genotyping data. It is our hope that the data will help in early diagnosis of the disease and in identifying new targets for treatment. Jonathan Mill of Kings College, London, is exploring epigenetic regulation in Alzheimer's disease using well-characterized postmortem Alzheimer's-disorder brains, and he will cover different regions of the brain in his analysis. Detailed clinical data on these patients is available before their death. Further functional analysis of potential genes will also be accomplished. Roel Ophoff of University of California at Los Angeles plans to investigate schizophrenia-associated epigenetic changes because mutations are rare in this disease and it seems logical to evaluate alternative mechanisms.

Type 2 diabetes mellitus (T2DM) is developed mostly in adults, but a few cases in younger ages have also been reported. Francine Einstein of the Albert Einstein College of Medicine is evaluating the epigenetic regulation in utero and its contribution to T2DM development during the lifetime. Stem cells will be utilized in this project. It is expected that understanding the contribution of intrauterine conditions to chronic adult disease may lead to novel epigenetic markers that may help in identifying high-risk individuals and populations. Evan Rosen of Beth Israel Deaconess Medical Center plans to study adipocyte methylation patterns to identify insulin resistance–associated epigenetic marks.

Autism is a neurological disorder with features like impaired social interaction and restricted and repetitive behavior, and it starts quite early in life. Margaret Fallin of Johns Hopkins University thinks that autism and related disorders have an epigenetic basis. Experiments are being conducted to test whether environment plays a major role in disease development and whether epigenetic regulation is predominant in that genetic regulation in autism. Samples from the Johns Hopkins National Children's study will be utilized in this study. The research will help us understand: are there regions of the epigenome susceptible to environment before and during pregnancy; and are there epigenomic regions that correlate with newborn and infant development phenotypes related to diabetes?

Glaucoma and age-related muscular degeneration and their regulation by epigenetic mechanisms will be studied by Shannath Merbs of Johns Hopkins University. About 4 million people are affected by these diseases in the United States. Samples from age- and sex-matched control cases will be analyzed for global methylation profiles. These samples were taken by laser capture microdissection so that the methylation profile can be obtained in retinal ganglia cells and in photoreceptor and retinal pigment epithelium cells.

Epigenetic regulation of bipolar disorders (BPDs)—such as the discordance of identical twins, significant fluctuations in disease initiation, progression, and

development, and sex and paternal origin effects—will be studied by Art Petronis (Center for Addiction and Mental Health, Canada). The prefrontal cortex of individuals affected with BPD and schizophrenia will be utilized to discover disease-associated methylation profiles in various genes.

Asthma epigenetics will be studied by David Schwartz of the National Jewish Health Center by analyzing methylation profiles in T cells, airways epithelium, and mononuclear cells during disease development. Such studies will help in designing novel prevention and treatment strategies in asthma.

1.4 NUTRIENTS AND THEIR CONTRIBUTION IN EPIGENETIC REGULATION OF DIFFERENT DISEASES

Multiple factors interact with genes and contribute to phenotypes of disease development. Along with environmental factors, dietary components have a major role in both disease prevention and development (Coppedè 2009; Ross et al. 2008). The folate pathway has been studied as a candidate biochemical and metabolic pathway for colon cancer (Carr et al. 2008). This pathway has been conserved among species, indicating its significance (Johnson and Belshaw 2008). Genetic variants in relevant genes have shown associations with diseases such as cancer, heart disease, and neural tube defects. In colon cancer adenomas, dietary folic acid supplementation has a protective effect, whereas either no effects or adverse effects have been observed in relation to colon cancer recurrence. Genetic explanations alone cannot explain these observations; therefore attempts are being made to understand these associations by alternative mechanisms, such as epigenetics (Carr et al. 2008; Ulrich 2008).

Although reports indicate that nutrition plays a role in disease prevention, especially cancer, interactions among dietary bioactive food compounds and food combinations remain understudied. Colon cancer is one of the few areas of nutritional epigenetics that has been well studied (Johnson and Belshaw 2008). Folic acid is a well-known methyl donor, and several foods are fortified with folic acid. Folic acid one-carbon metabolism (FOCM) is an excellent example of a complex pathway with interconnected subpathways for folic acid and methionine metabolism, which in turn have their own feedback loops (Kim et al. 2009). Furthermore, folate biochemistry is well defined, and enzymes involved in the metabolism of folate, whether they exist in the cytoplasm or mitochondria, are well characterized (Ulrich 2008). Since methyl groups are the key component in CpG methylation, their levels influence gene expression. Alterations in homocysteine levels, DNA methylation, purine and thymidylate synthesis, and incorporation of uracil into DNA (misincorporation) occur simultaneously in the cells and contribute to DNA damage and repair pathway.

In metabolic syndromes, Plagemann et al. (2009) has proposed that overfeeding, by way of epigenetic factors, contributes to obesity, and subsequently to diabetes and cardiovascular diseases. Their conclusions are based on methylation profiling of the proopiomelanocortin gene, which encodes a polypeptide hormone precursor that undergoes extensive tissue-specific posttranslational modifications by an enzyme, prohormone convertase. The phenotypes included in the study were obesity, hyperleptinemia, hyperglycemia, hyperinsulinemia, and an increased insulin/glucose

ratio. Histone demethylase has also been proposed to influence metabolic syndromes (Inagaki et al. 2009).

It is important to know the bioactive food components, their quality, and mode of interaction with the body in order to apply the effects of nutrients and their components to a healthy lifestyle. Genes controlling the synthesis and metabolism of bioactive food components are regulated genetically and epigenetically. A comprehensive understanding of genotype (genetics) and its relation with phenotype (epigenetics) is needed if nutrition is to be applied for disease intervention and prevention purposes (Milner 2008).

1.5 CHALLENGES AND OPPORTUNITIES IN THE FIELD, FUTURE DIRECTIONS, AND CONCLUDING REMARKS

Major challenges in the field of nutritional epigenetics are the large number of input variables, relatively few intermediate markers and measurements, dynamic nature of nutrients, and limited outcome measurements. One approach to address these problems could be the application of a systems biology approach where *in silico* models are developed based on biological information to test these models first in animals and then in human populations. Taking colon cancer as a prototype, existing databases, such as the Colon Cancer Family Registry Folate Study, should be utilized to develop models to understand dietary influences on epigenetics and disease development. As the next step, single-pathway approaches can be expanded to include a genomewide approach because technologies exist for measuring genomewide epigenetic changes (Feinberg 2010; Laird 2010). Combining observational studies with experimental studies may result in risk-prediction models with implications for identifying populations at high risk of developing diseases. Incorporating genomic information in epigenetic databases may also be useful in understanding the biology of the underlying disease, developing intervention targets, and ultimately improving health. There are a few bioactive food components that have activity with deacetylate histones, but this effect is general and not gene specific. Gene-specific inhibitors are needed to treat specific diseases.

Research questions for the future include the following:

- How do bioactive food components regulate epigenetic events in different diseases?
- How do bioactive food components alter epigenetic patterns and restore gene function?
- How do these components circumvent and compensate for pathways that are altered during the disease development?
- How can we make gene-specific epigenetic inhibitors?
- How can we measure the temporality in epigenetic profile caused by bioactive food components?

Epigenetics in general and nutrition epigenetics in particular have the potential to make a tremendous impact in disease prevention, control, and management. However,

validation studies have not been completed to evaluate this potential in different diseases; therefore it would be premature to declare that nutrients can prevent or treat diseases. Once the human epigenome is completed and additional nutritional epigenetic studies have been conducted, it may be possible to achieve this goal.

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