# 2 Cancer Biology and Nutrigenomics

*John A. Milner and Donato F. Romagnolo*

**Key Points**

1. Current cancer models comprise those that are inherited through the germline and represent only ~5% of total cases of human cancers. These tumors originate because of mutational events. The remaining ~95% originate as sporadic events and evolve as a result of exposure to the environment, which includes exposure to both environmental contaminants and dietary agents.
2. The multistage model of carcinogenesis identifies various phases, initiation, promotion, and progression, which determine the evolution of normal somatic cells to heterogeneous populations with cancer potential. This process appears to be influenced by tissue microenvironment and organization. Significant opportunities in nutrition and cancer prevention exist in the early stages of initiation and promotion prior to clonal expansion of heterogeneous populations. Targeting initiators, cocarcinogens, and promoters may provide the best opportunity in cancer prevention since the majority of advanced solid tumors are resistant to therapy.
3. Nutrigenomics represents a strategy that can be applied to the study and prevention of many diseases including cancer. It has been defined as a pyramidal approach that encompasses the study of molecular relationships between nutrients and genes (nutrigenetics), how these interactions influence changes in the profile of transcripts (transcriptomics), proteins (proteomics), and metabolites (metabolomics). DNA methylation and histone modifications are epigenetic events that mediate heritable changes in gene expression and chromatin organization in the absence of changes in the DNA sequence. The age-increased susceptibility to cancer may derive from accumulation of epigenetic changes and represents a potential target for therapies with bioactive compounds.
4. Factors that mediate the response to dietary factors include nuclear receptors and transcription factors, which function as sensors to dietary components and determine changes in the profile of transcripts.
5. Integration of high-throughput proteomic and metabolomic approaches with computational techniques is necessary to understand the complexity of the biological response to specific bioactive compounds or associations of nutrients and identify key molecular targets in cancer prevention and treatment.

**Key Words:** Nutrigenomics; cancer biology; multistage carcinogenesis; bioactive compounds; molecular targets; prevention

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## 1. INTRODUCTION

### *1.1. Multistage Carcinogenesis*

Cancer statistics indicate that only 5% of known cancers are linked to heredity, whereas the remaining 95% are sporadic, i.e., tumors originate in the absence of family history and are caused by a variety of factors. The classical model of multistage carcinogenesis identifies three phases: initiation, promotion, and progression. The first phase, initiation, is characterized by induction of unchecked replication, which may result from one or more events including exposure to DNA damaging agents, loss of DNA repair functions, fixation of mutations in tumor suppressor genes, or activation of protooncogenes *(1)*. A likely consequence of initiation is that selected cells may gain a resistant phenotype to certain carcinogens, DNA damaging agents, or escape programmed cell death. However, at this stage the presence of initiated cells is not necessarily associated with clonal growth. The second stage, promotion, is believed to be a quantitative phase during which initiated cells increase in number under the influence of selective, non-DNA reactive, pressures and in the context of a specific tissue microenvironment (e.g., stroma) *(2)*. Therefore, specific proliferative stimuli (e.g., estrogens) or chronic inflammation may have a central role as cocarcinogens and promote proliferation of existing focal lesions *(3)*. This process is essentially quantitative and facilitates growth of cancer foci under the influence of the tissue microenvironment (e.g., influence of paracrine factors) *(4)*. The net effect of events that occur during promotion may be the selective proliferation of initiated cells. The third phase, progression, has been characterized as a qualitative process during which cell heterogeneity arises and divergent cell populations grow inside focal lesions. The clonal evolution of specific cell subsets during progression contributes to the heterogeneity of tumors and is influenced by the tissue microenvironment or organization, which regulates the rate of cancer progression and metastasis *(1)*.

Key questions in cancer prevention pertain to identifying the timing in life of exposure to initiators; predicting the latency between time of exposure and cancer manifestation; and dissecting the molecular and biochemical mechanisms responsible for neoplastic growth. If these conditions were known, then they might be targeted with preventive naturally occurring dietary compounds or dietary patterns. For example, the importance of timing of nutrient exposure in cancer prevention is highlighted by experimental evidence showing that supplementation with the isoflavone genistein during the prepubertal or prepubertal plus adult periods protected against mammary carcinogenesis *(5)*. Similarly, epidemiological studies in Chinese women reported an inverse association between intake of soy during adolescence and the risk of breast cancer in adult life *(6)*. The risk of certain cancers (e.g., breast cancer) increases in association with Western diets compared to Mediterranean or native Mexican diets. Therefore, nutrition strategies need to be developed to prevent the effects of carcinogenic agents; target and eliminate premalignant lesions at early stages; and antagonize (i.e., induce apoptosis) the proliferation of clonal neoplastic populations *(7)*.

Based on the multistage model of carcinogenesis, multiple time points of intervention may exist. However, previous decades of experience in cancer therapeutics suggest that significant opportunities in nutrition and cancer prevention exist in the early stages of

initiation and promotion prior to clonal expansion of heterogeneous populations. As cancer cells diverge from progenitor cells, they may acquire differences in nutrient requirements, gain proliferative advantages, or become refractory to therapeutic bioactive food components and drugs. Targeting initiators, cocarcinogens, and promoters may provide the best opportunity in cancer prevention since the majority of advanced solid tumors is resistant to therapy *(8)*. Therefore, ideally bioactive components (e.g., *Symphytum officinale*) should not contribute to cancer risk per se *(9)* and halt or eliminate premalignant lesions. An interesting example is provided by polyphenols, which in combination with transition metals (e.g., copper) increase at higher levels in cancer cells the production of reactive oxygen species and cause DNA damage. Therefore, interactions between nutrients may be exploited in therapies specifically targeted to cancer cells *(10)*.

An important area of study is whether high doses of certain bioactive compounds or associations of bioactives could become co-carcinogens. An example of this potential risk is provided by folate, which may function as a cancer promoter of colonic lesions *(11)*. Similarly, exposure to indol-3-carbinol, an indole contained in cruciferous vegetables, increased in a rat model the incidence of uterine lesions including atypical hyperplasia and adenocarcinomas *(12)*. Therefore, studies are needed to determine the upper limits or thresholds of supplementation.

The main goal of nutrigenomics is to profile global changes induced by nutrients and develop dietary-intervention strategies to maintain homeostasis and prevent diseases including cancer *(13)*. The main challenge is that of integrating information pertaining to expression of more than 30,000 genes, for most of which the function is not known, and computing changes in expression for more than 100,000 proteins and several thousand metabolites *(14)*. A major drawback in developing prevention strategies comes from differences in approach between preclinical and clinical research. Most, if not all, preclinical studies with in vitro and animal models tend to focus on single bioactive food components without considerations of the complex interactions that occur among bioactive food components present in the human diet. This problem is addressed in part by epidemiological studies that focus on the average anticarcinogenic or procarcinogenic effects of specific groups of bioactive compounds (e.g., n-3 fatty acids) in the context of dietary exposure (e.g., Western vs. Asian diet). Nevertheless, results of population studies may not find statistical differences or be biased if the analysis comprises individuals with mutations in tumor suppressor genes or carrying specific polymorphisms. For example, individuals with the TT polymorpshism at nucleotide 677 for methylenetetrahydrofolate reductase (MTHFR) (~5–20% population worldwide) appear to be at decreased risk for colorectal adenomas in the presence of high plasma levels of folate *(15)*. Therefore, the interaction between levels of exposure to certain bioactive food components and genetics (nutrigenetics) may influence the risk of cancer in certain subpopulations and is an important component of nutrigenomic studies.

Whereas it is recognized that cancer requires multiple molecular changes, it is also known that certain genetic alterations play a hierarchical role in cancer development in certain tissues. For example, loss of BRCA-1 expression through epigenetic silencing may confer a high probability of breast cancer *(16)*. Loss of DNA repair functions controlled by BRCA-1 may lead to subsequent genetic alterations in genes that control proliferation and apoptosis. During the last two decades a tremendous amount

of information has been gathered concerning the role of signaling pathways in cancer development. Nutrigenomic strategies are an important tool to decode pyramidal effects and establish the minimum requirements for cancer development and prevention.

### *1.2. Gene × Diet Interactions*

Analysis of worldwide occurrence of cancer suggests that certain geographical areas have higher incidence for specific types of tumors. For example, historically Japan was a region with relatively low incidence of colon cancer, yet Japanese populations that relocated to the United States experience an increase in the incidence of colon cancers and a reduction in the incidence of stomach cancers. Similarly, increased incidence for breast and prostate cancer has been reported for migrant populations *(17–19)*. These observations suggest that heredity alone does not explain the susceptibility to these and other tumor types, and point to environmental and dietary factors as potential causative agents *(20)*. A second but very important factor that should be considered is that the overall occurrence of cancer increases with age. The age-increased susceptibility to cancer may derive from accumulation of epigenetics changes and represents a potential target for prevention with bioactive compounds.

If one excludes mutations in cancer susceptibility genes or occupational exposure to chemical carcinogens, diet alone likely represents the most significant risk factor in the etiology of sporadic tumors. In support of this notion, the 2007 AICR/WCRF Second Expert Report on Food, Nutrition, Physical Activity, and the Prevention of Cancer suggested that about one-third of all cancer deaths may be attributable to dietary factors *(21)*. The ability of dietary compounds to influence the risk of cancer may result from tissue-specific influences on cellular processes including inflammation, carcinogen metabolism, hormone regulation, cell differentiation, DNA repair, apoptosis, and cell growth cycle. Whereas about 30% of all cancer cases may relate to dietary habits, the impact of diet on these processes is influenced by the specific foods consumed, the specific type of cancer, and interactions among compounds, which may act additively or synergistically when combined in the human diet.

Modern approaches in cancer prevention should address the issue of tissue specificity and identify nutrients that in animal and cell culture models prevent tumors of the same type as those associated with human exposure *(8)*. An obvious challenge is the establishment of a cause–effect relationship between exposure to specific bioactive compounds and tissue-specific cancer prevention.

### *1.3. Nutrigenomics*

Conceptually, nutrigenomics represents a strategy that can be applied to the study and prevention of many diseases. It provides a pyramidal approach that encompasses the study of molecular relationships between nutrients and genes (nutrigenetics), how these interactions influence changes in the profile of transcripts (transcriptomics), proteins (proteomics), and metabolites (metabolomics) *(22)*. The underpinning concept is that thousands of bioactive compounds function as signals and influence the organism's response *(23)*. The opportunity of targeting nutrients–gene interactions to influence the cancer process is modulated by genetic variations in human populations, epigenetic

modifications that selectively and permanently alter gene expression, by complex interactions/associations among dietary components, and heterogeneity of cells within a certain tumor. Therefore, integration of information about gene polymorphisms, identification of gene targets that regulate cell and tissue-specific pathways, and development of diagnostic strategies to control for clinical heterogeneity are important to understand how nutrigenomics may be used in cancer prevention *(24)*. Other chapters in this volume will discuss specifically how nutrigenetics, epigenetics, transcriptomics, and metabolomics may help to assess the effects of specific nutrients on the cancer process. Here, we will highlight examples of how integration of nutrigenomic data may be useful to understand the correlation between consumption of specific bioactive compounds and protection toward specific tumor types.

A key point that should be made is that changes in gene or protein expression do not necessarily translate into changes in metabolic profiles. Conversely, changes in promoter activities and transcript profiles do not necessarily lead to changes in protein expression and functions. Therefore, integration and the parallel use of all components of nutrigenomics through high-throughput and computational techniques are necessary to understand the complexity of the biological response to specific bioactive compounds or associations of nutrients and identify key molecular targets *(25*, *26)*. The ultimate goal of nutrigenomics is that of developing genomics-based biomarkers that help in the early detection and prevention of diet-related diseases, including cancer. To reach this goal it is important to develop tissue-specific dietary responses that can be used as signatures or fingerprints to estimate risk *(27)*. The availability of nutritional biomarkers at early stages (e.g., initiation) may be used as prognostic tools. A complicating factor is that diet contains a large number of compounds and that each nutrient has different gene targets and affinities. An example is the cross talk of estrogens and isoflavones with estrogen receptors and how this interaction may affect the development and prevention of breast cancer *(28)*.

One of the mechanisms through which nutrients influence cellular responses is through interactions with members of the nuclear receptor superfamily of transcription factors, which integrate genomic and nongenomic effects. This family comprises many members that bind with various affinities to hormones, nutrients, and metabolites *(29)*. In essence, these receptors function as sensors and transmit signals to specific molecular targets to induce an adaptive response and changes in transcript levels. Receptors are binding proteins that translocate from the cytoplasm to the nucleus. Examples of these nuclear receptors are the glucocorticoid (GR), estrogen (ERα and β), retinoic acid (RARα, β, and γ), vitamin D (VDR), peroxisome proliferators activated receptor (PPARa, β/δ, and γ), and retinoid-X (RXRα, β, and γ) receptor. The RXR participates in the formation of heterodimers with many nuclear receptors including ER, VDR, and PPAR *(30)*. The formation of heterodimers and the presence of isoforms for receptors increase the number of potential complexes that can bind to specific DNA consensus sequence, thus amplifying the diversity of biological actions induced by receptor ligands. Numerous members of the nuclear receptor family are orphan since no apparent endogenous ligands have been found. Orphan receptors may function as activator or repressors. For example, the AhR activates the transcription of P450 genes, while repressing transcription of estrogen-inducible promoters *(31)*.

Consensus sequences for nuclear receptor complexes are harbored within or in close proximity to promoter regions and influence the chromatin organization and transcription of target genes. Nuclear receptors may act at as homodimers at inverted repeats (Class I), heterodimers at direct repeats (Class II), homodimers at direct repeats (Class III), and monomers at core sites (Class IV). The spatial arrangement and organization of these responsive elements is important to understand the responsiveness of certain genes to receptor ligands and cooperativity among multiple *cis*-regulatory elements. Receptors that are known to mediate responses to nutrients include members of the PPAR subfamily, which binds fatty acids and eicosanoid metabolites. In turn, the activated PPAR regulates the expression of genes involved in lipid metabolism *(32)*. A thorough overview of the application of transcriptomics to study PPAR-dependent gene regulation can be found in Bunger et al. *(33)*. The ER is known to bind to natural phytoestrogens (e.g., genistein) found in soy products *(34)* and the phytoalexin resveratrol. The AhR binds a vast number of compounds including tea cathechins and indoles (indol-3-carbinol (I3C)) and 3,3′diindolyilmethane (DIM) (more details in Chapter 32).

The molecular structure and concentration of ligands influences the ability to activate specific receptors and the direction and intensity of the response. Likely, the exposure to many dietary ligands occurs in the context of chronic exposure at concentrations (μM–mM) higher than those known for classical nuclear receptor ligands (nM) *(32)*. Therefore, the action of dietary ligands (e.g., isoflavones or resveratrol) for nuclear receptors (e.g., ER) may antagonize that of endogenous ligands (e.g., estrogen) and offer opportunities to prevent the growth of cancer lesions.

Transcription factors that have been shown to exert an important role in cancer include NFkB and the activator protein-1 (AP-1). Both factors play a key role in chronic inflammation, which is associated with cancer development. The transcription factor AP-1 is required for activation of NFkB and mediates the effects of tumor promoters including epidermal growth factor (EGF) and UV. Therefore, both AP-1 and NFkB have been identified as promising molecular targets for cancer prevention *(35–37)*. The activation of NFkB, e.g., by prostaglandins or bile acids *(38)* promotes cell proliferation and has been identified in solid tumors (breast, colon), leukemia, and lymphoma. NFkB is a homodimer and heterodimer of the Rel/NFkB family of proteins. In its inactive form, NFkB is sequestered to the cytosol bound to the cofactor IkB. The phosphorylation of IkB by the kinase Ikk leads to dissociation of IkB and translocation of NFkB to the nucleus where it targets promoter regions at specific binding sites. Dietary compounds found to inhibit the activity of NFkB include dimeric procyanidin B2 (B2), which prevents binding of NFkB to target DNA sequences. Procyanidins are oligomers formed from flavan-3-ols found in flavonoid-rich foods including cocoa, grapes, cranberries, red wine, and apples *(39)*. Other anti-NFkB bioactives include curcumin, a natural component of the rizhome of *Curcuma longa*, carnosol (rosemary and sage), benzylisothiocyanates and sulforaphane (cruciferous vegetables), and kaempferol *(40)*.

AP-1 comprises protein homodimers (jun-jun) and heterodimers (jun-fos, jun-ATF). It belongs to the class of basic leucine zipper (bZIP) transcription factors and binds promoters at sequence-specific sites, thus transactivating or repressing transcription. Bioactive compounds that inhibit AP-1 include rosmarinic acid *(41)*, isoliquiritigenin (a licorice flavonoid), luteolin *(42)*, conjugated linoleic acid *(43)*, eicosapentaenoic acid *(44)*, and sulforaphane.

A wealth of studies utilized DNA microarrays to understand cancer and identify transcriptional targets for bioactive food compounds. In general, investigations have attempted to classify clusters of genes based on their functional food categories. Epidemiological studies have suggested that high fish intake is associated with a decreased risk of colorectal cancer. This protective effect has been attributed to the high content of n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acids (EPA), and docosahexaenoic acid (DHA) in some fish. cDNA array studies suggested that the protective effects of n-3 PUFA were associated with upregulation of phase II enzymes (GSTT2) and Bax, and downregulation of pro-inflammatory genes (COX-2) *(45)*

Genome-wide analysis of gene expression in response to dietary fats has been used as a paradigm to study the nutrigenomics of PPARs. The PPAR receptor system is of interest primarily as a sensor to dietary lipids. However, most of the genome-wide analyses of PPAR functions have utilized pharmacological agonists *(33)*. Dietary fat-induced changes in gene expression have been examined before and during puberty in rodent models. Recent gene microarray analyses documented that expression of PPARγ was increased in the mammary gland of 50-day-old rats prepubertally fed low-fat n-3 PUFA. The reduction in mammary tumorigenesis was associated with increased expression of antioxidant genes and lower expression of genes that stimulate cell proliferation *(46)*. Combination of laser capture microdissection of mammary ductal epithelial cells and measurements of genes expression revealed fatty acid-enriched diets significantly stimulated proliferation and activation of cell cycle genes during puberty *(47)*. Oligonucleotide microarray analyses have been used to identify genes involved in enhanced growth of human prostate cancer. These studies revealed upregulation of IGF-1 receptor expression in prostate cancer xenografts under high-fat diets *(48)*. Increased expression of IGF-II and plasminogen activator inhibitor-1 (PAI-1) protein was detected in HepG2 cells following in vitro treatment with low (50 μM) levels of palmitate *(49)*. Both IGF-II and PAI-1 are involved in metabolic syndrome. The latter study combined mRNA expression profiling with in silico promoter analysis (Genomatix Gene2Promotor, Matinspector and Biobase Transfac programs) to identify common transcription factor binding sites in the promoter regions of palmitate-regulated genes. Genes were divided into groups of downregulated and upregulated genes. Recognition elements for the transcription factors AhR, EKLF, MAZF, MTF, NRSF, PAX5, and ZBPF were identified within the promoter regions of five downregulated metallothionein genes. The SREBP-1 binding element was found in the regulatory regions of the genes MT1E, MT1F, and MT2A. In the promoter regions of the upregulated genes, common recognition elements for the transcription factors EKLF, GREF, MZF, and ZBPF were identified. cDNA microarrays have also been used to detect changes in transcript expression of ~5,700 genes in radical prostatectomies in men after 6 weeks of low-fat/low-glycemic diets *(50)*.

Genomic-wide analyses based on ChIP and DNA microarrays illustrated that global features of estrogen-regulated genes included occupancy by ERα and histone acetylation *(51)*. The ER influences the expression of many genes by either binding as a homodimer to estrogen response elements (ERE), or by forming complexes with AP-1or specificity protein (Sp). These genes may be targeted by soy isoflavones because of their ability to act on the ER. Whole-genome microarray hybridization with 25,000 oligonucleotides has been used to investigate the impact of ER status (MCF-7=ER+ vs.

MDA-MB-231=ER–) and differences between normal (MCF-10A) and cancerous cells after lycopene exposure. These studies revealed that lycopene, which may have preventive roles in breast cancer, affected genes involved in apoptosis and cell cycle *(52)*. Differential expression studies in breast cancer cells documented that the phytoalexin resveratrol was more active in ER+ (MCF-7) than ER– (MDA-MB-231) breast cancer cells *(53)*. The specificity of resveratrol for ER+ breast cancer cells may provide the mechanistic basis for the development of prevention therapies against ER+ breast cancers.

Epigenetic events including DNA methylation and histone modifications can alter chromatin organization and modulate gene transcription in a site-specific and cooperative manner *(54)*. Octamers of core histones (two each of H2A, H2B, H3, and H4) are wrapped by ~146 bp of DNA to form the nucleosome, considered to be the minimum and basic structure of chromatin. Histone tails provide binding sites for regulatory proteins and are subject to dynamic posttranslational modifications including methylation, acetylation, phosphorylation, ADP-ribosylation, ubiquitination, and sumoylation *(55)*. DNA methylation of cytosines by DNA methyltransferases (DMNTs) results in chromatin reorganization governed by the methyl-binding proteins (MBPs). In addition, DNA methylation inhibits the binding of transcription factors and produces binding sites for chromatin modifying complexes such as the histone deacetylase complexes (HDACs). The removal of acetyl groups from amino-terminal lysine residues of histones by HDACs permits the subsequent methylation of histone H3 on lysine 9 (H3-K9) by the histone methyltransferases (HMTases), SUV39H1 and SUV39H2 *(56)*. The methylation of histone H3 lysine 9 allows for the recruitment of the HP-1 protein and also interferes with phosphorylation of histone H3 at serine-10 *(57)*. In contrast to histone deacetylation by HDACs, histone acetylation is generally associated with transcriptional activity *(55)*. Cofactors that contain histone acetyltransferase activity (HAT) include CBP/p300, p/CAF, and SRC-1 *(58)*.

Besides genetic changes, epigenetic alterations have been proposed to play a key role in the onset of a variety of tumors by inducing stable, heritable changes in gene expression that are propagated over cell generations *(59)*. Patterns and levels of DNA methylation and histone acetylation are reported to be profoundly altered in human pathologies including inflammation and cancer *(60)*. However, it is not clear how the cross talk between histone modifications and DNA methylation influences gene expression, and how the hierarchical order of these changes leads to silencing during tumor development.

It remains to be clearly established whether histone modifications need to occur before changes in DNA methylation or DNA methylation changes guide histone modifications. Answering this question has paramount implications in identifying the initiating events involved in cancer development and guide in the development of strategy for cancer prevention and treatment. Acetylations of histone H3Lys9 and Lys18 and deacetylation of Lys14 were associated with PI3K-dependent activation of PTEN through antioxidant-responsive element (ARE) *(61)*. Loss of histone H4K20 trimethylation was reported in early precursor lung cancer lesions *(62)*. Methylation of CpG islands in promoter regions has been reported for tumor suppressor genes, hormone receptors, and other genes involved in detoxification and DNA repair *(63)*. ERα and

BRCA-1 promoter methylation have been reported in subsets of sporadic breast cancers *(16)*. The CpG island methylator phenotype in colorectal cancer is frequently characterized by hypermethylation of promoter regions in tumor suppressor genes. This methylation event increases with age. Specific promoters found to be methylated in patients with CpG island methylator phenotype tumors include p16 and TIMP3. Methylation of these genes increased with age *(64)*.

Epigenetic changes can occur randomly or in response to the environment (diet). However, unlike genetic mutations, epigenetic events may be reversible *(65)* and represent viable targets for dietary intervention. For example, (–)-epigallocatechin-3-gallate (EGCG), the major polyphenol from green tea, and genistein, the major isoflavone present in soy, have been used to restore the expression of methylation-silenced genes including RARβ, p16, and $O^6$-methylguanine methyltransferase (MGMT) *(66, 67)*. The reversal effects of EGCG and genistein were attributed primarily to inhibition of DNA methyltransferase, although the effect of genistein was weaker than that of EGCG. The reactivation of p16 by genistein was associated with inhibition of cell growth and weak inhibition of HDAC activity. The combination of genistein plus sulforaphane, a HDAC inhibitor present in broccoli, augmented the reactivation of expression of RARβ, p16, and MGMT by genistein. In human cancer cells, sulforaphane, butyrate, and metabolites of garlic organosulfur compounds (allyl mercaptan, AM) inhibited HDAC activity *(68, 69)*. The depression in HDAC activity by AM was associated with increased histone acetylation, binding of Sp3 to the promoter region of p21, and cell cycle arrest. These results documented that combination of agents that target DNA methyltransferase and HDAC activity may be effective in epigenetic therapies targeted to reactivation of tumor suppressor and receptor genes and halt proliferation of cancer cells. In theory, one potential drawback of using bioactive compounds in cancer therapies is the lack of specificity. For example, a depression in DNA methyltransferase activity may lead to global hypomethylation leading to chromosomal instability and tumors *(70)*. Furthermore, HATs and HDACs and their inhibitors modify acetylation of nonhistone proteins, such as transcription factors. Ideally, these potential problems might be addressed using combinations of (weak) HDAC and DNMT inhibitors and concentrations sufficient to induce synergistic and tissue-specific effects.

The use of specific biomarker profiles that function as sentinel indicators may assist in the detection of changes in gene expression linked to nuclear receptors, transcription factors, signaling pathways, and metabolites *(71)*. Nevertheless, this approach is complicated by the multitude of compounds that affect multiple target genes, which in turn can exert polygenic effects and influence a large number of downstream targets. Moreover, more than 350 oncogenes and tumor suppressor genes have been implicated in the etiology of cancer *(1)*. Furthermore, intake of a particular bioactive food component or precursor does not equate to exposure at the tissue or cell levels *(72)*. The complexity of this system requires the development of genome-based tissue-specific signatures to correlate phenotypic responses to nutrition.

A specific target for nutrigenomic strategies is endocrine cancers, including breast, ovarian, endometrial, and prostate cancers. The vast majority of breast cancers is estrogen receptor (ER)-positive and occurs in postmenopausal women. Because breast tissues undergo complex programs of growth and development that are under the influence of

ovarian steroids, studies have considered nutrition factors that alter or interfere with estrogen and progesterone-dependent regulation.

The interest on isoflavones in breast cancer prevention derives from the fact breast cancer risk for women residing in geographical areas of high consumption of soy products during puberty is lower compared to that of women living in Western countries or Asian women who had a low soy intake *(73)*. However, clinical trials reported small *(74)* or no effect of supplementation with isoflavones on breast cancer risk *(75–78)*, and administration of isoflavones elicited in some cases an estrogen-like effect. Other studies indicated that the reduction in breast cancer risk due to soy intake was limited to Asian populations *(79)*. A case–control study conducted in Southeast China in 2004–2005 reported that premenopausal and postmenopausal women in the highest quartile of total isoflavone intake had a reduced risk for all receptor (ER/PR) status of breast cancer with a dose–response relationship. The protective effect was more pronounced for women with ER+/PR+ and ER–/PR– breast tumors *(80)*.

Several factors may be responsible for the inconsistent effects of soy-related diets on cancer outcome (Table 1). These include age, reproductive history, genetic background, dose and timing of exposure, and dietary patterns. For example, because of their binding affinity for the ER, isoflavones may function as agonists or antagonists depending on the concentration. The differential binding of isoflavones to the ER may interfere with or activate the genomic actions of the ER. Moreover, the agonist/competing effects of isoflavones for the ER may be modified by interactions with polymorphisms for the ER *(81)*. For example, polymorphisms in the ERβ have been shown to modify the association between isoflavone intake and breast cancer risk *(82)*. Given the role of cross talk between ER and isoflavones in breast cancer risk, genome-wide studies are required to examine the effects of isoflavones and exposure levels on promoter sequences that are targeted by the ER. DNA microarray technologies have been used to monitor genome-wide effects by isoflavones. For example, studies measured patterns of gene expression in the developing uterus and ovaries of Sprague-Dawley rats on GD 20, exposed to graded dosages of 17alpha-ethynyl estradiol (EE), genistein, or bisphenol A (BPA) from GD 11 to GD 20. Analysis of the transcript profile of these tissues was used to determine the estrogenicity of different compounds *(83)*. Studies that examined the impact of isoflavones on the epigenetic process reported that elevation of histone acetylation and coactivator activity of ER may reduce the risk of estrogen-related diseases *(84)*,

Nutrigenetics takes into account how the cellular response to specific nutrients is influenced by interindividual genetic variations including single nucleotide polymorphisms (SNPs). The cell response to isoflavones may also be influenced by the presence of mutations in specific tumor suppressor genes. For example, the growth of *BRCA1* mutant cells (SUM1315MO2) carrying the 185delAG *BRCA1* mutation was strongly inhibited by genistein, whereas this isoflavone only had a weak effect in cells expressing wild-type BRCA1 protein. The responsiveness of BRCA1 mutant cells was linked to higher expression of ERβ gene. These data suggested that genistein may be an efficient inhibitor of cancer development in *BRCA1* mutant breast cancer cells *(85)*. With respect to BRCA-1 status in ovarian cancers, genistein induced apoptosis in both wild-type and mutated BRCA-1 ovarian cancer (BG-1) cells. However, this effect was mediated by different pathways since genistein inhibited ERα in BRCA-1 deficient cells, whereas it activated ERβ when BRCA-1 was present *(86)*.

**Table 1**
**Isoflavones and Nutrigenomic Approaches in Female Cancers**

| *Experimental model* | *Dietary bioactive compounds* | *References* |
|---|---|---|
| *Transcriptomics* | | |
| Human MCF-7 breast cancer cells | Natural estrogens (17beta-estradiol, estriol, estrone, genistein) | Terasaka et al. *(91)* |
| Human MCF-7 breast cancer cells | Isoflavones (genistein, daidzein, glycitein, biochanin A and ipriflavone), flavones (chrysin, luteolin, and apigenin), flavonols (kaempferol and quercetin), and a coumestan, a flavanone and a chalcone (coumestrol, naringenin, and phloretin, respectively) | Ise et al. *(92)* |
| Uterus and ovaries of Sprague-Dawley rats | Genistein | Naciff et al. *(83)* |
| Human Ishikawa endometrial cancer cells | Genistein | Konstantakopoulos et al. *(93)* |
| Human MCF-7 breast cancer cells | Genistein | Shioda et al. *(94)* |
| Human MCF-7, T47D breast cancer cells | Genistein | Buterin et al. *(95)* |
| FVB female mice | Isoflavones, of which 66.5% was genistein, 32.3% daidzein, and 1.2% glycitein | Thomsen et al. *(96)* |
| *Epigenetics* | | |
| ER-mediated core histone acetylation, Chromatin preparations from *Drosophila* | Equol, genistein, daidzein | Hong et al. *(84)* |
| *Proteomics* | | |
| Rat mammary gland | Genistein | Rowell et al. *(97)* |
| *Metabolomics* | | |
| Premenopausal women | Soy or miso consumption | Solanky et al. *(98)* |
| Postmenopausal women | Soy milk | Pino et al. *(99)* |
| Postmenopausal female monkeys | Soy protein isolate | Wood et al. *(100)* |
| Premenopausal women | Soy (40 mg genistein/day) | Kumar et al. *(101)* |

Isoflavones may also alter gene expression by inducing chromatin modifications at target promoters. For example, genistein was shown to suppress DNA-cytosine methyltransferase-1 (DNMT) and reverse DNA hypermethylation in mammary cancer cells in vitro *(87)*. Therefore, epigenetic changes such as alterations in DNA methylation could account for the preventive effects of genestein and other soy isoflavones. A recent study reported that intake of soy isoflavones had an antiestrogenic effect and altered mammary promoter hypermethylation in healthy premenopausal women *(88)*. Low circulating levels of genistein were associated with decreased methylation of RARβ2 and CCND2, whereas promoter methylation of these genes increased with high circulating levels. Hypermethylation of both RARβ and CCND2 is correlated with breast carcinogenesis. The fact that the circulating levels of genestein may influence the direction and methylation levels represents important evidence of potential for epigenetic regulation by isoflavones in breast tissue.

The effects of isoflavones in mammary tissue have been related to either stimulation or repression of a number of processes. Pathways and processes that are stimulated by isoflavones include cell cycle arrest, apoptosis, cyclin-dependent inhibitors (p21 and p27), BRCA-1 and BRCA-2, PPARγ, MAPK signaling (p38 phosphorylation and JNK), and IGF-1 plasma levels. Conversely, isoflavones have been reported to downregulate cdc2 activity, Akt1, NFκB, AP-1, phosphorylation of ERK1/2, levels of VEGF and cell migration, xenobiotic metabolism, and enzymatic activities of estrogen sulfotransferases (SULT) *(73)*. The SULT enzymes regulate in endocrine tissue such as breast and endometrium the sulfonation of various substrates including estrogens and phenols *(89)*.

The chemical reactivity of isoflavones compared to that of estrogens may influence their preventative role in breast cancer. For example, genistein is metabolized to quinones with a short half-life, and it is subsequently hydrated to generate a catechol genistein which has estrogen-like properties, but low reactivity with DNA. Conversely, catechol estrogen quinones have a longer half-life and can damage DNA via depurination reactions *(90)*. Therefore, competition for quinone formation by genistein may reduce the formation of genotoxic quinone metabolites.

## 2. CONCLUSIONS

From the data discussed in this chapter it is apparent that clarifications of the mechanisms of action of bioactive compounds are complex (Table 2) and require simultaneous examination of changes in gene expression (transcriptomics), study of molecular relationships between nutrients and genes (nutrigenetics), how these interactions influence changes in the profile of proteins (proteomics), study of multiple signaling and metabolic pathways (metabolomics), and how associations of different compounds exert synergistic, additive, or opposing effects. DNA methylation and histone modifications are epigenetic events that mediate heritable changes in gene expression and chromatin organization in the absence of changes in the DNA sequence. Examples of large-scale analyses include microarray studies of estrogen-responsive genes in response to natural and industrial chemicals *(91)* and phytoestrogens *(92)*. Based on these studies, it emerges that nutritional strategies targeted to the prevention of endocrine tumors should consider multiple signaling pathways.

**Table 2**
**Factors Affecting Nutrigenomics Research**

| *Approach* | *Definition and factors* |
|---|---|
| Transcriptomics | *Identification of transcription factors that respond to nutrients and gene targets* |
| | RNA amplification procedure (quantity, quality, replicates, real-time PCR, high-density analysis) |
| | Quantity of starting tissue/cell material |
| | Fold change in expression |
| | Intraindividual and interindividual variations in healthy and diseased subjects |
| | Heterogeneity of cell populations and single-cell gene expression profiling |
| | Combination of gene variants (SNPs) |
| | Data processing and interpretation |
| Epigenetics | *Characterization of chromatin modifications that influence gene expression and impact of nutrients* |
| | Histone modifications |
| | DNA methylation |
| | Nucleosome organization |
| | Order, interdependence and intradependence, and reversibility of histone modifications |
| | Cross talk and mutual dependency between histone modifications, DNA methylation, and methyl-binding proteins |
| Proteomics | *Linking gene expression studies with protein functions* |
| | Protein structure |
| | Tissue and cellular localization |
| | Plasma levels |
| | Expression level |
| | Posttranslational modifications |
| | Protein–protein interactions |
| | Cellular function |
| | Bioinformatics and data processing |
| Metabolomics | *Linking exposure to biological effects induced by metabolites* |
| | Interindividual differences in metabolism and disposition |
| | Measurement of metabolites in specimens (urine, plasma) |
| | Recovery methods from tissue/plasma |
| | Metabolic origin and overlapping pathways |
| | Handling of metabolic data |

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