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Epigenetic Response to Changes in Diets and in Response to Toxic Compounds

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Summary. The wellbeing is only partially encoded in DNA sequence. In addition to genetic factors, there exists a multi-level regulation of gene expression, including the one called epigenetic. Epigenetic mechanisms involve heritable but reversible changes in gene expression that occur without alteration in underlying DNA sequence. These mechanisms are highly sensitive to developmental and environmental cues. Diet is one of the very powerful environmental factors shaping the epigenetic response and contributing to the initiation and progression of diseases, such as cancer. The effect of certain bioactive food components such as folate, genistein, curcumin or toxins such as bisphenol A and heavy metal salts on epigenetic regulation of gene expression have been well studied. This review introduces the effects of aforementioned compounds on changes in DNA methylation and histone modifications pattern with great emphasis on disease development and cancer in particular.

Key words: epigenetic mechanisms, health, disease.

Introduction. The vast array of cellular processes in organisms is influenced not only by the genetic code, but by epigenetic regulations as well. To understand gene-environment interactions, the epigenome must be considered [1]. The term epigenetics was coined by developmental biologist Conrad Waddington, first in relation to early cellular determination[1]. The term now comprises much more – focusing on changes in gene expression that occur without changes to the nucleic acid sequence itself [1].

Manifestations of this phenomenon include, but are not limited to, DNA methylation, covalent modifications of histone amino (N) terminal tails (acetylation, methylation, phosphorylation and ubiquitination), chromatin folding and nuclear matrix attachment, and regulation by small non-coding RNAs [1, 2]. Via these mechanisms, chromatin architecture and possibly transcription factor access to promoters, are influenced – affecting gene expression [2]. These changes are heritably stable but are potentially reversible. They play pivotal in organism's development and are also influenced by aging and the environment [2]. Traditionally, gene-environment interactions focused on how organisms, with specific and different genotypes, were impacted by environmental exposures [1]. Within the past decade, awareness and support for the existence of epigenetic regulation of gene expression, has increased. The field of environmental epigenomics strives to uncover how environmental and nutritional factors affect gene expression in fetus and adult organism, with particular emphasis made on transgenerational nature and heritability of changes in epigenetic regulation linked to the phenotypic manifestations [1, 3].

Several lines of evidence have shown that epigenetic mechanisms have key roles in regulating gene expression for cellular differentiation, organogenesis, embryonic development, imprinting [1, 3]. However, just as epigenetic mechanisms are involved in influencing normal processes in cells, they can contribute to abnormalities as well. Global changes in chromatin structure occur in cancer cells during malignant transformation [2]. This dysregulation of the epigenome manifests itself by changes in the expression of numerous genes and upsetting metabolic pathways [2]. In this way, epigenetic mutations or epimutations may be as harmful as genetic mutations [4]. Increased risk of various human diseases, such as tumorigenesis [2], obesity [2], uremia [5], hyperhomocysteinemia [5] and number of other metabolic and neurodegenerative diseases may result from dysregulation of epigenetic mechanisms, causing abnormal gene expression [2].

The potential for disease characterization and prevention was substantial when epigenetic roles surfaced. For example, diseases with an important inflammatory component (chronic obstructive pulmonary disorder, cardiovascular disease, rheumatoid arthritis and Crohn's disease) were determined to lack a specific causal genetic factor [6]. Aberrant DNA methylation, histone modifications and microRNA patterns are characteristic hallmarks of inflammatory disease and cancer risk [6-8]. Sedentary lifestyle, obesity, metabolic syndrome and diet were also described as causative factors in cancer risk, especially when examining Asian immigrants (who have a low cancer risk in native areas) in Western countries [2]. As such, it was reasonable to predict diet composition as one of the leading modulators of cancer risk through epigenetic alterations in individuals.

Diet composition has the profound effect on different epigenetic components and this effect can be heritable. Maternal diet may influence her offspring's phenotype. For example, in the yellow agouti (A^{vy}) mouse model, methylation status of a promoter upstream of the agouti gene influenced the expression of that gene, changing the color of the coat. Dietary supplements of methyl donors changed the DNA methylation profile of the promoter and had impact on the offspring's phenotype [5]. Further details about the A^{vy} mice appear below and have been published (Dolinoy, 2008). In this model, the diet and epigenetic alterations of that particular epiallele were linked to adult-onset of obesity, diabetes and tumorigenesis [1]. In addition, the toxin, bisphenol A, also contributed to changes in methylation status. These results further demonstrated that changes in dietary and environmental components, leading to subsequent changes in the epigenome, were associated with abnormal gene expressions leading to disease risk [2]. DNA methylation is inherently connected with histone modifications and chromatin remodeling, and cancer tends to be the extreme endpoint of a variety of ailments. The causal link between diet, toxicology and epigenetics in human disease development is significant, but studies are still in their infancy [2]. The majority of analyses on epigenetic mediation of risk have associated DNA methylation and cancer through diet, revealing the main focuses of this review.

Bioactive Food Components

Although the examples of bioactive food components (BFCs) – those that are believed to have a positive health effects – are numerous, more detailed information on the effect of those compounds on modulating cancer risks only exists for folate, choline, zinc, epigallocatechin gallate (ECGC), diallyl disulfide, resveratrol, sulforaphane and genistein. These BFCs and micronutrients receive support from epidemiological and preclinical studies for their roles in modulating cancer risk [2, 5]. BFCs have been shown to influence epigenetic processes, with positive effects including control of proliferation, up-regulation of apoptosis, reduction of inflammation [2], and positive regulation of DNA repair efficiency, suppression of differentiation and angiogenesis [5]. Some BFCs, such as folate, genistein and EGCG were also shown to decrease incidences of human colon cancer and development of heart disease [2]. A comprehensive table of the numerous investigated food components, and the mechanisms they affect, has been published by Katarzyna Szarc vel Szic, et al. (2010) [6]. Among the most well described BFCs that have positive effects on decreasing risks of developing cancer and modulating DNA repair and genome stability are polyphenols (plant phytochemicals that include flavanoids). Table shows a short list of some of the BFCs and their source (tabl. 1).

Toxins

In vitro animal and human studies have delineated the some

Table 1. BFCs and their source

BFC compound	Source
Curcumin	Turmeric
Diallyl disulfide (DADS)	Garlic
Epigallocatechin gallate (ECGC)	Green tea
Folate/Folic acid/B_9 micronutrient	Green leaf vegetables, legumes, liver, egg yolks, among others
Genistein	Soy
Sulforaphane (SFN)	Cruciferous vegetables; broccoli sprouts

effects that toxins, another environmental factor, have on the epigenetic landscape. Environmental toxicants appear to have effects on DNA methylation, histone modifications and miRNA – all pertinent to the modulation of health and disease. Comprehensive tables of chemical factors, and their impacted mechanisms, were constructed by Baccarelli and Bollati (2009) [9]. Because environmental effects may be cumulative, it is difficult to establish cause-effect relationships between toxins and the epigenetic landscape. The majority of studies simply describe the epigenetic changes that were seen. This review will summarize some findings about common note-worthy chemicals. For instance, bisphenol A is used to manufacture polycarbonate plastic and is associated with increased body weight and increased risk for breast and prostate cancer, and changed reproductive function [5] (endocrine disruptor [1][1]). Metals (nickel, cadmium, lead and arsenic) are known to increase the production of reactive oxygen species (ROS) – which can cause DNA damage and interfere with methyltransferase interactions with DNA [9]. Other toxicants that have demonstrated epigenetic alterations, but are not mentioned here, are: trichloroethylene; dichloroacetic acid; air pollution (particulate matter), benzene, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX); and endocrine- and reproductive-disruptive toxicants (diethylstilbestrol, persistent organic pollutants, dioxin) [9].

Aberrant cellular function, thought to arise from dysregulation of specific genes [5], contributes to the development of the animal diseases discussed here. Incorrect gene expression may be the expression of a normally silenced gene (such as oncogenes), or the silencing of a normally expressed gene (i.e. tumor suppressors). Expression may be regulated by dietary or environmental compounds via influences on DNA methylation of the cytosine phosphate guanine dinucleotide (CpG) islands in the genome, particularly in promoters, chromatin remodeling through histone modifications, and gene silencing via RNA interference (RNAi) [5]. Below we present more details on the effect of BFCs and toxins on various epigenetic factors.

Effects on DNA Methylation

DNA cytosine methylation at CpG dinucleotides is the most studied [5] of the epigenetic mechanisms (widely in relation to cancers), and is most commonly analyzed to observe the effect on nutrition on epigenetic regulation. Chromatin structure is mainly comprised of DNA wrapped around histone- protein complexes [5]. Post replication, in mammals, DNA methyltransferases catalyze the transfer of the methyl group from the universal methyl donor S-adenosylmethionine (SAM) (dependent on other methyl donors and cofactors such as methionine, choline, vitamin B12 and folic acid [5]) to the 5'carbon on cytosine [2]. DNMT1 maintains the methylation patterns during replication, and DNMT3 (3a and 3b) control *de novo* methylation [2]. Methylation patterns are tissue and species specific [2]. Sequence-specific factors may also be involved in DMNT's abilities to target loci [5]. Removing methylation is considered to be a passive act through blocking the methylation of the newly synthesized DNA strands during replication. However, there is speculation that in mammals the methyl DNA binding protein (MBD2) and the DNMT3s have DNA demethylase activity [5]. These findings require further exploration. The addition of a methyl group to cytosine affects the major groove in the DNA helix. Since the methylation patterns on DNA act with histone tail modifications, this disruption alters how these proteins and how transcription factors attach, therefore affecting gene expression [2, 5]. Similarly, methylation at these sites inhibits transcription factor recognition and recruits binding proteins (e.g. MeCP2) and chromatin remodeling enzymes (such as histone deacetylases) [5]. Generally, methylation at specific sites, such as a promoter, will prevent or decrease the transcription of the affected gene [2]. This is critical, as aberrant hypomethylation of an oncogene, or aberrant hypermethylation of tumor suppressors (such as DNA repair genes) will contribute to cancer progression [2] and tumorigenesis [10]. In fact, in cancer cells, an early tumorigenic processes is the silencing of tumor suppressor genes at CpG island via hypermethylation [5]. As well, global DNA hypomethylation and increased DNA methyltransferase activity are commonly seen in tumor cells. Thus, many cancers are characterized by global genome hypomethylation and local gene-specific hypermethylations.

According to Ross (2010) there are four mechanism by which nutrients may influence DNA methylation levels/patterns [5]: 1) affecting the supply of methyl groups by altering the production or distribution of S-adenosylmethionine (SAM); 2) changing the activity of *de novo* or maintenance DNA methyltransferases; 3) influencing the activity of DNA demethylation processes; or 4) specific DNA methylation patterns, themselves, may alter nutrient metabolism and other cellular responses to the nutrient. Often more than one mechanism is in play in response to a particular nutrient and numerous alterations and deviations are found within each mechanism. The best known example of the effect on nutrients on DNA methylation comes from studies of Agouti (A^{vy} metastable epiallele) mice. Mice harboring this allele represent a unique model for the analysis of the effects of epigenetic reprogramming and the role of environment, including diet, on the phenotype [1, 2]. Metastable epialleles are genetically identical alleles that differ in the level of transcription due to developmentally early-occurring epigenetic modifications [1]. In the case of Agouti mice, the wild type allele encodes a paracrine signaling molecule that produced eumalanin (*a*), resulting in brown fur color or phaeomelanin (*A*) leading to yellow fur color. In the A^{vy} mice, there is an insertion of an intracisternal A particle (IAP) retrotransposon upstream of the transcription start site of the agouti gene. Since transposons are typically heavily methylated, methylation of 5' long terminal repeat (LTR) end of IAP affects the transposon expression as well as a*gouti* expression and results in the wild type phenotype (brown coat). Low methylation at the IAP leads to yellow coat. The yellow phenotype was also associated with an increased risk of obesity, susceptibility to cancer and to other chronic diseases [5].

In several round of experiments it was demonstrated that mother's diet of polyphenols, phytoestrogens and methyl group donors such as folic acid, vitamin B12, choline, etc. early in the fetus's development reversed the effect of hypomethylation at the IAP promoter, leading to higher frequency of occurrence of animals with brown color [1, 2, 5]. Diet supplementation had also a positive effect on reducing the risk of associated disease, such as cancer. Unfortunately this study is rather unique as no other animal model exists that would allow such a simple observation of the effect of diet on DNA methylation status. It can be hypothesized that methyl donor supplements in a mother's diet could alter an offspring's phenotype by changing methylation at the other loci as well [5]; however, it is not yet clear whether supplements differ in their capacity to cause changes in methylation and thus in phenotypic changes [5].

Similar changes in the coat color were observed upon consumption of genistein, another isoflavone. Supplementation of maternal diet with genistein caused partial reversal of methylation pattern and yellow color to brown color when concentrations of genistein were comparable to a human on a high soy diet (250 mg/kg) [5]. The progeny was followed to adult stage and it was found that DNA hypermethylation at the A^{vy} locus

caused by genistein protected animals again wait gain – providing additional proof that maternal diet could alter disease susceptibility in the progeny. It is suggested that genistein may mimick the effect of estrogen but may also function as a non-hormone, regulating various transcription factors [5].

In addition to the positive effect observed by consumption of BFCs, the agouti animals can be used for the analysis of the effect of various xenobiotic chemicals, thus functioning as biosensors [5]. After maternal feeding of 50 mg BPA/kg two weeks pre-mating and throughout gestation and lactation, the coat color of offspring was shifted to yellow, and methylation was decreased within the A^{vy} IAP. Curiously, when BPA was given together with genistein or methyl donors, the hypomethylation effect at IAP promoter was not observed [1]. Thus, these studies demonstrated that exposure to toxins can modify methylation pattern in parents and the offspring, leading to development of certain phenotype. In addition, it showed that such changes may be prevented or reversed by "proper" diet. Cumulative effect of proper diet or lack of proper diet was observed when animals with hypomethylation at IAP promoter (yellow fur coat) were propagated for three consecutive generations – changes in methylation, fur color and associated diseases, such as obesity amplified and became more pronounced as compared to first generation or animals that received folic acid/genistein diet [5].

Although agouti mice and IAP promoter are rather unique in their sensitivity to bioactive food and toxins, BFCs in diet have also been shown to reactivate "wrongly" silenced genes such as tumor suppressors [5, 11, 12]. It was demonstrated that application of EGCG (5-50 μM) or genistein (2-20 μmol/L) inhibited the activity of DNA methyltransferase, and resulting in hypomethylation of the promoter regions of tumor suppressor genes in neoplastic cultured cells[11,12]. The following genes were found to be "reactivated", increasing their expression in human colon, prostate, mammary and esophageal cell lines: p16INK4a, O^6-methylguanine methyltransferase, human mutL homolog1, retinoic acid receptor beta, among others [11, 12]. Although the mode of action of EGCG in this case is not quite clear, molecular modeling suggests that a gallate group on the D ring of EGCG interacted with the active site for cytosine on the DNMT [2]. This binding is stabilized by hydrogen bonds formed between the hydroxyl groups of two different residues on the protein.

Cadmium is among toxic substances that have negative effect on regulation of DNA methylation. It is a carcinogen [9], acting via ROS induction and DNA methylation. This particular metal inhibits DNMTs , possibly through interaction with the enzyme-DNA binding domain. This noncompetitive interference causes a reduction in genome methylation. It has been reported that cadmium has induced oncogene expression by inhibiting DNA methylation [9]. Cadmium is not the only environmental toxin altering DNA methylation. However, it does operate via a different mechanism. Inorganic arsenic is methylated for detoxification, which uses SAM – the methyl donor for DNA methylation via DNMTs. Malignant transformation was shown in rat liver, due to decreased SAM levels and DNMT activity. In addition, arsenic has been associated with hypermethylation of tumor suppressor promoters (such as p53 and p16). Interestingly, when folate was available, a global dose-dependent hypermethylation of blood DNA *in vivo* was seen, suggesting that arsenic-induced methylation is influenced by methyl availability [9].

Effects on Histone Modifications

The histone protein complexes around which DNA is wound are called nucleosomes [5]. They are comprised of an octamer of two histone H2A-H2B and two histone H3-H4 dimers. As mentioned, the N-terminal tails are posttranslationally modified, influencing DNA binding and chromatin remodeling. Deacetylation unmasks the histone's positive charge to increase binding to negative DNA and subsequent condensation of chromatin suppresses transcription [5]. Acetylation, in turn, has an opposite effect, decreasing positive charge and increasing histone affinity to DNA, thus increasing transcription rate. However, other modifications, such as methylation, influence chromatin relaxation or condensation based on a histone- and residue- specific basis. Modifications are also thought to enhance or suppress levels of gene expression, without complete activation or silencing. By influencing gene expression, histone modifications have been correlated to cancers. For example, the loss of monoacetylation and the trimethylation of histone H4 at specific genomic regions are biomarkers of cancer [5]. Acetylation modifications tend to be well understood in terms of aberrant gene expression. In cancer cells, there is an imbalance of histone acetyltransferase (HAT) and histone deacetylase (HDAC) activities [5]. Several active compounds, such as sulphoraphanes, diallyl disulfides (DADS), resveratrol and genistein are known to alter histone modifications. Sulphoraphanes (SFN), DADS and resveratrol (in wine) were shown to inhibit histone deacetylase activity resulting in an in increase in binding ability of transcription factors to DNA and thus upregulated gene expression [2]. SFN in the concentration of 3-15 μM inhibited HDAC activity and increased histone acetylation in various prostate epithelial cells (BPH-1, LnCaP, and PC-3), HCT116 human colorectal cancer cells as well as human embryonic kidney 293 cells [5]. Dose-dependent increase of histone H4 acetylation at the *p21* promoter, leading to increased p21 protein levels was observed upon SFN application. SFN was also shown to have an effect on live mice model. In the Apc^{min} mouse model, application of SFN in the dose of 443mg/kg increased histone acetylation in the *p21* promoter and promoters of several other genes in gastrointestinal cells, and was correlated with suppressed tumor development. Is there a parallel with human diet? Actually, there is! In healthy humans, consumption of one cup of broccoli sprouts (high in SFN) inhibited HDAC activity, and increased histone H3 and H4 acetylation, in blood mononuclear cells only 3-6 hours after eating [5]. Unfortunately a negative part of the study was an extremely small number of participants – just 3. In cancer cells, SFN has also been shown to induce apoptosis and inhibit growth and no such effect was observed in normal cells.

DADS administration into cancer cell lines was correlated with increased H4 and/or H3 acetylation in the *CDKN1A* promoter. Since CDKN1A positively regulates p21, activation of its promoter negatively affects cancer cells [13]. Indeed, scientist found increased levels of *CDKN1A* mRNA and p21 protein levels in response to DADS. These contributed to antiproliferation and G2/M phase cell cycle arrest in HT-29 and Caco-2 human cancer cell lines [5, 13]. DADS applied in the dose of 200 mg/ resulted in histone acetylation changes in rat liver (an *in vivo* application) and in non-tumorigenic isolated colonocytes as well as in Morris hepatoma 7777 cells. Since these concentrations of DADS are considered to be too large for human consumption, further research is required to correlate dose range and physiological effects for pharmacological use in animals and in human. Genistein appears to be also active in altering histone modifications, although its activity is different from SFN and DADS. Genistein application altered chromatin structure through the reduction of histone H3 lysine 9 (H3K9) methylation and deacetylation. In prostate cancer cells, genistein at concentrations of 10 and 15 μmol/L increased acetylation of histones H3 (H4K4) and H4 at *p21* and *p16* promoters, resulting in the increased gene expression and decreased cyclin levels. Higher concentration (50 μM) genistein reactivated aberrantly silenced tumor suppressor genes (PTEN, CYLD, p53, FOXO3a) [5, 14]. Although the mode of action of genistein is not entirely clear, it appears that genistein may have antagonistic effects to cancers that alter various epigenetic marks [5]. Negative influence of chemicals on histone modifications has been also documented.

Many studies showed that *in vitro* administration of nickel reduces histone acetylation and removes methyl groups from H3K9, among other altered histone modifications [9]. At non-toxic levels, nickel even decreased H4K12 acetylation in mammalian cells and decreased acetylation of all of the four H4 lysines in yeast cells. Nickels has also shown to increase H3K9 methy-

lation, which has been associated with DNA methylation and silencing [9]. The proposed mechanism of disruption involves a secondary structure that is promoted upon Ni^{2+} binding to histidine 18 in histone H4. This secondary structure affects the orientation of the side chains on N-terminal of H4.

Effects on microRNA

Noncoding RNAs have been shown to regulate posttranscriptional silencing. One type, microRNA (miRNA), are single-stranded (21-23 nucleotides) that are transcribed from DNA, but remain in the RNA stage [5, 9]. Mature miRNAs are partially complementary to particular messenger RNAs (mRNAs) and through a protein-complex mediator, can degrade the target mRNAs in plants or lead to translational inhibition in animals. It has been hypothesized that endogenous miRNAs, may even target gene promoters, and that miRNA dysregulation would aberrantly silence cancer-related genes [5]. Additionally, RNA-mediated transcriptional gene silencing was shown to correlate with changes in chromatin structure (regulating histone modifications and DNA methylation) – the impact of RNA-mediated silencing is being explored [5, 15].

Alterations in the level of small non-coding RNAs can be detrimental as single miRNA can target several hundred mRNAs, as well as several different miRNAs can target one mRNA, potentiating the inhibitory effect. Diets that alter the expression of miRNAs from their genomic locations or alter the steps of miRNA processing may contribute to various diseases. Diet defficienceis, rather than chemical carcinogens or viral mediators, can lead to tumor formation in the hepatocarcinogenesis (HCC) rodent model [5]. In this rat model, methyl deficiency and liver tumor formation are caused by the lack of methionine, choline, vitamin B12 and folic acid, causing genome-wide and gene-specific hypomethylation, and aberrant expression of epigenetic mediators (DNMTs, methyl CpG binding proteins, and HMTs). One of the effect of such massive changes is alterations of levels of miRNAs that regulate apoptosis, cell proliferation, and cell-to-cell connections. Expression of *miR-34a, miR-127, miR-200b* and *miR-16a* in animals with methyl source deficiency was inhibited. As a consequence, this resulted in the increased levels of several proteins that are the target of aforementioned miRNAs - E2F3, NOTCH1, BCL6, ZFHX1B, and BCL2 proteins associated with cancer development. When diet-induced changes in miRNA expression occurred early enough, the effects were persistent and did not revers when rats were fed a methyl-adequate diet.

Another BFC, curcumin was also shown to alter the miRNA profile in human BxPC-3 pancreatic cancer cells [5, 16]. Exposure of cancer cells for 72 hours to 10 μmol/L of curcumin upregulated 11 and downregulated 18 miRNAs. One of miRNAs, miR-22 was upregulated and its putative targets SP1 transcription factor and estrogen receptor 1 (ESR1) were downregulated. As a proof of principal, application of antisense miRNA-22 enhanced the expression of those targets, negating miR-22 effect. This study suggested that curcumin can mediate anticancer effects in pancreatic cells through epigenetic mechanisms [16]. Metals, such as arsenic, have also demonstrated impact on miRNA levels [9]. In human lymphoblastoid cells grown with sodium arsenite, miRNA profiles were changed[17]. In fact, the specific miRNAs that were altered, were the ones involved in one-carbon metabolism – connecting miRNA alterations to DNA methylation changes [17].

Discussion and Outlook

This review presented a very fascinating and rapidly developing field – nutritional epigenomics. Human epigenome and as a result - phenotype are constantly influenced by chemicals in the diet and in the environment. The compounds discussed here (folate, SFN, DADS, curcumin, genistein, cadmium, BPA, arsenic, nickel) barely touched the tip of the iceberg of the variety of compounds able to alter the epigenome. Effects of folates are the best known and there are hundreds of documented studies showing positive and negative effects of this compound. Folate itself, is a critical coenzyme for methylation and for nucleotide synthesis [18]. Its primary preventative mode of action was demonstrated for cancers of the lung, esophagus, brain, pancreas, bone marrow, cervix, and especially colorectum [18,19,20]. Although the studies are plenty, it is not always easy to correlate the results of various studies that involve animals to practical applications in relation to humans. Nevertheless, epigenetic modulators are currently being put into practice more often than ever. There has been recent success in using HDAC and DNMT inhibitors for therapeutic intervention of chronic inflammatory disease [6, 21]. Experiments involving agouti mice and the effect of diet on methylation pattern are useful because similar regulations may be found in human genome. Even though the transposon sequence in the agouti mouse model is not found in humans, the implications remain the same. There could be other metastable epialleles associated with other transposable elements that could be influenced by epigenetic mechanisms via maternal dietary supplements [5]. Similarly, the role of miRNAs in chromatin remodeling and the effect of diet are just beginning to be understood [8]. The mechanisms may involve repression of DNA and histone modification enzymes or of chromatin remodeling factors. However, evidence also suggests that chromatin around miRNA genes is epigenetically altered in cancer cells in a tumor- and tissue-specific manner [8]. The most important part remaining is the identification of all possible targets of those miRNAs, together with finding what diet supplements can regulate what miRNAs. The effects of curcumin only show that BFCs can regulate miRNA levels and influence disease outcomes. In addition, other non-coding RNAs may be affected in a similar or a different manner, increasing or reducing the progression of tumorigenesis [5, 22].

Foods contain many other known and unknown BFCs and their effect is yet to be explored. Some compounds consumed in the diet may also have negative epigenetic effects. For instance, soy intake is complicated. While genistein seems to have wondrous gene expression control implications through altering histone modifications, the constituent lunasin, also found in soy, may modify chromatin in an opposite manner [22]. At the same time, lunasin is also stated to have cancer prevention ability by inhibiting acetylation of histones and killing actively transforming cells [5, 22]. It acts by binding to deacetylated histones, which could upset the mode by which genistein works. This is one example where determining the timing of cellular vulnerability and the concentration of the compound used becomes important [5]. Compound concentration is indeed one of the main limiting factors as it is often difficult to evaluate the amount of BFC in a given food. Moreover, it is difficult to control the consumption of the particular food by an individual. In general, the concentrations of the bioactive food components are higher than what can be achieved nutritionally [2]. In consumed foods, there are low amounts of polyphenolic compounds and the effect of these on DNA methylation in humans is not clear[5]. In the agouti mice studies, the diets consisted of a many methyl donors and cofactors, implying that a combination of BFCs may be necessary. More so, excessive amounts of polyphenols may cause excessive and unwanted modifications as well [5]. For example, the EGCG levels used in aforementioned studies are so high that they are be up to 50-fold higher than blood and urine concentrations after drinking tea; such levels are perhaps reached after drinking 18 cups of green tea a day! Similarly, genistein concentrations used in agouti mice studies may be 3-10-fold higher than what can be achieved from eating soya products. Confounding factors also come into play. Polyphenols are rapidly metabolized in bodies through mechanisms of glucuronidation, sulfation and methylation [2]. These processes may contribute to the low internal availability of BFCs for *in vivo* effects.

As reported, most studies on environmental toxins and epigenetic changes were based on somatic cells of adults [9]. The effect of the alterations from these particular chemicals on the germ line is uncertain. For now, it is clear that environmental

chemicals cause epigenetic changes and that these changes are similar to those marks seen in patients with diseases or in diseased tissues[9]. Characterizing the link between the epigenetic change and the causative disease pathway is another direction in which to proceed. For each of these studied factors, the investigators seem to strive for similar future inquiries. There are still unresolved details surrounding the molecular mechanisms, quantities, frequency, duration, and timing of exposure, in order to bring about anticancer effects [5]. Considering the mechanisms of epigenetics, environment, aging and disease, uncovering whether these relationships are differentially modulated at dose- and life stage-dependent manners, would also be beneficial [2].

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Епігенетична реакція на зміни в дієті та реакція на токсичні сполуки

Резюме. Здоров'я тільки частково закодоване у ДНК. Окрім генетичних чинників, існує також багаторівнева регуляція експресії генів, включаючи епігенетичну. Епігенетичний механізм включає успадковані, проте зворотні зміни в експресії генів, які відбувається без перебудови в самій ДНК. Ці механізми є надзвичайно чутливими до стимулів розвитку й навколишнього середовища. Дієта є одним із найпотужніших чинників довколишнього середовища, формуючи епігенетичну реакцію й роблячи внесок у виникнення й перебіг таких хвороб як рак. Вплив деяких біоактивних харчових складників таких, як сіль фолієвої кислоти, дрік, куркума або ж таких токсинів як бісфенол А й солей важких металів на епігенетичну регуляцію експресії генів уже добре вивчена. В цій статті йдеться про вплив вищеназваних складників на зміни в метилуванні ДНК і гістонових модифікаціях з приділенням великої уваги розвитку хвороби, зокрема, такої як рак.

Ключові слова: епігенетичний механізм, здоров'я, хвороба.

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