Nutritional Epigenomics: A Portal to Disease Prevention[1,2]

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ABSTRACT

Epigenetics can be defined as inheritable and reversible phenomena that affect gene expression without altering the underlying base pair sequence. Epigenomics is the study of genome-wide epigenetic modifications. Because gene expression changes are critical in both normal development and disease progression, epigenetics is widely applicable to many aspects of biological research. The influences of nutrients and bioactive food components on epigenetic phenomena such as DNA methylation and various types of histone modifications have been extensively investigated. Because an individual's epigenetic patterns are established during early gestation and are changed and personalized by environmental factors during our lifetime, epigenetic mechanisms are quite important in the development of transgenerational and adult obesity as well as in the development of diabetes mellitus. Aging and cancer demonstrate profound genome-wide DNA methylation changes, suggesting that nutrition may affect the aging process and cancer development through epigenetic mechanisms. *Adv. Nutr. 4: 530–532, 2013.*

Introduction

Nutrients can directly or indirectly interact with genes. Nutrient-induced changes in gene expression can have many downstream results, including alterations in metabolism and disease susceptibility. However, to optimize the use of nutrients for maintaining health and preventing certain diseases, more knowledge is needed regarding the role of nutrients at the molecular levels that affect critical gene function. Epigenetics is a biological phenomenon that involves the regulation of gene expression independent of the DNA base sequence. The field of nutritional epigenetics is further elucidating the nature of nutrient-gene and, more broadly, diet-gene interactions, thus providing support for the role of nutrition in acquiring new phenotypes. Aging is also associated with substantial changes in epigenetic profiles and recent work has implicated epigenetic mechanisms in the etiology of many age-associated diseases, including cancer. The "Nutritional Epigenomics: A Portal to Disease Prevention" symposium provided a forum to discuss how nutrients and diet modify individual genes as well as the whole genome, thereby affecting the most prevalent public health problems such as obesity, diabetes mellitus, aging, and cancer.

Dr. Kate Claycombe's Presentation Focused on Obesity and Adipose Tissue Epigenetics

Intrauterine under-nutrition such as a maternal low-protein (LP)[9] diet can epigenetically program the offspring for survival in a nutrient-poor postnatal environment. This compensatory response causes postnatal catch-up growth and development of obesity in later life. It is well established that obesity is associated with enlargement of existing fat cells as well as increased proliferation of adipocytes via differentiation from precursor cells. Several growth-stimulating auto-, para-, and endocrine hormones can induce adipose tissue expansion. Among them, insulin-like growth factor 2 (*Igf2*), which has one of the best known epigenetically imprinted genes, is associated with greater body weight

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[9] Abbreviations used: DMR, differentially methylated region; DNMT1, DNA methyltransferase; *GNMT*, glycine *N*-methyltransferase; HCC, hepatocellular carcinoma; HF, high fat; *Igf2*, insulin-like growth factor 2; LP, low protein; ZDF, Zucker diabetic fatty.

and obesity. Whether maternal LP diet-induced obesity is associated with *Igf2* expression and whether *Igf2* expression is differentially regulated in an adipose depot-specific manner has not yet been determined. In addition, whether maternal LP diet-induced obesity is associated with obesity-related comorbidities such as insulin resistance and the development of type 2 diabetes is unknown. Thus, Dr. Claycombe's group conducted animal model pre- and postnatal feeding studies using obese-prone Sprague-Dawley rats that were fed 8% (LP) or 20% (normal protein) protein diets for 3 wk prior to conception and throughout pregnancy and lactation. Using this animal model, they investigated whether prenatal LP and postnatal HF diets regulate the rate of adipose tissue catch-up growth, *Igf2* mRNA expression, and *Igf2* DNA methylation. To determine the postnatal diet effects, at weaning, the offspring were fed 10% (normal fat) or 45% [high fat (HF)] fat diets for 12 wk. The adipose tissue growth rate was increased up to 26-fold by LP prenatal and HF postnatal diets. In addition, adipose tissue *Igf2* mRNAs and DNA methylation were increased by LP prenatal and HF postnatal diets. The LP prenatal and HF postnatal diets also increased the number of small adipocytes in adipose tissue and decreased insulin sensitivity.

These findings suggest that prenatal LP and postnatal HF intakes result in adipose tissue catch-up growth through alterations in expression of *Igf2* gene and DNA methylation within adipocytes. The data also suggested that these alterations in adiposity are accompanied by an increased risk for development of type 2 diabetes.

Dr. J. Alfredo Martinez Presented on Dietary Factors, Epigenetic Modifications, and Obesity Outcomes

Epigenetics can provide some insights to understand genetic fetal programming, monozygotic twin differences, and chronic disease onset in adults that interact with dietary intake and nutritional processes. Actually, epigenetic research will contribute to an explanation for the way that cells/organisms carrying identical nucleotide sequences can generate different responses under the same nutrient exposure through mechanisms such as DNA methylation, small and non-coding RNAs, and chromatin architecture changes.

In recent years, different examples of dynamic changes in DNA methylation patterns due to the restriction or supplementation with different nutrients have been reported. Furthermore, in the adult state, some examples of diet-induced epigenetic changes have been also reported. Dr. Martinez and his colleagues have reported that high-fat/-sugar intake and situations of excessive body weight in rodents are associated with changes in DNA methylation patterns, affecting the promoter region of different genes involved in energy homeostasis and obesity such as leptin, pro-opiomelanocortin (*POMC*), fatty acid synthase (*FASN*), circadian locomotor output cycles kaput (*CLOCK*), and NADH dehydrogenase (ubiquinone) 1 β subcomplex subunit 6 (*NDUFB6*). On the other hand, epigenetic biomarkers are being identified in order to predict body weight maintenance after weight loss in humans, including TNFα, aquaporin 9 (*AQP9*), ATPase class V type 10A (*ATP10A*), and CD44 as well as some specific miRNAs.

It is becoming evident that inter-individual differences concerning the outcomes of nutritionally related chronic diseases such as diabetes and obesity depend not only on the dietary intake and the subject's DNA sequence, but also on the inherited epigenome and different nutritional influences (during the intrauterine or the adult periods) that modify the epigenetic marks and are able to affect gene expression, which includes DNA methylation, covalent histone modifications, chromatin folding, and, more recently described, the regulatory action of miRNAs.

Dr. Kevin L. Schalinske Presented about the Impact of Diabetes on DNA Methylation

Folate, homocysteine, and methyl group metabolism are interrelated pathways that are critically important for optimal health. Perturbation of these pathways by nutritional, genetic, or physiologic factors has been associated with a number of pathological conditions. Diabetes appears to be an aberrant physiological condition that is characterized by a disruption of folate, homocysteine, and methyl group metabolism.

In the initial type 1 diabetes studies utilizing streptozotocin-treated rats, Dr. Schalinske and colleagues found that hepatic expression of glycine *N*-methyltransferase (*GNMT*) was markedly elevated. GNMT is a key tissue-specific regulatory protein that functions to optimize both the supply of methyl groups from the folate-dependent 1-carbon pool and their utilization in *S*-adenosylmethionine–dependent transmethylation reactions, including the methylation of DNA by DNA methyltransferase (DNMT1). For type 1 diabetic rats, they found that hepatic DNA was hypomethylated in spite of increased expression of *DNMT1*. Increased hepatic *GNMT* expression and subsequent hypomethylation of DNA was diabetes specific, because it could be prevented in streptozotocin-mediated type 1 diabetes by insulin treatment. Although the kidney is also one of only a few tissues that expresses *GNMT*, induction by type 1 diabetes was transient and no change was exhibited with respect to DNA methylation.

They found that increased expression of hepatic *GNMT* was also evident in type 2 diabetes using Zucker diabetic fatty (ZDF) rats. Elevations in GNMT protein and activity were evident at both 12 and 21 wk of age in the liver of ZDF rats but only at 21 wk of age for the kidney. In contrast to his findings in type 1 diabetic rats, hepatic DNA in ZDF type 2 diabetic rats was hypermethylated. Moreover, the hepatic expression of *DNMT1* was also induced by a type 2 diabetic condition. No change in DNA methylation was observed in either renal or heart tissue.

Both type 1 and type 2 diabetes are characterized by marked changes in methyl group metabolism that may result in a methyl imbalance owing to the induction of *GNMT*. However, the impact on DNA methylation is not consistent; hepatic hypomethylation was observed in type 1 diabetes, whereas type 2 diabetes was characterized by

hypermethylation in the liver. Future studies are warranted to determine the biological importance of altered tissue-specific DNA methylation in both models of diabetes.

Dr. Sang-Woon Choi Presented about Diet, Epigenomics, and Aging

Nutrition has the potential to modulate the interactions between genes, aging, and disease susceptibility through epigenetic mechanisms. It is known that aging substantially alters epigenetic marks, but it is not yet known whether age-associated epigenetic changes are preprogrammed or random.

A Western-style rodent diet is high in fat and calories and low in vitamin D, calcium, fiber, methionine, and choline compared with a control AIN76A diet. Using a genome-wide DNA methylation array, Dr. Choi and colleagues identified 24 differentially methylated regions (DMRs) from the liver of mice fed a Western-style diet for 18 mo compared with mice fed the control diet. Through a network analysis, they found that the Western-style diet induced differential methylation localized to genes regulating cellular function and maintenance, lipid metabolism, and cardiovascular diseases.

Restricting caloric intake by 20–50% while still providing adequate micronutrients substantially extends mean and maximal lifespan, largely by retarding age-associated diseases such as cancer. They also conducted a genome-wide DNA methylation array from the liver of 24-mo-old calorie-restricted and control mice. Aging and calorie restriction generated distinct DNA methylation signatures. Interestingly, normal aging generates more DMRs than aging with a calorie-restricted diet, suggesting that the calorie restriction may attenuate age-related epigenetic changes. A network analysis found that calorie restriction changes DNA methylation in genes associated with obesity, cancer, epigenetic modification, and protein regulation.

In summary, the diet can yield distinct DNA methylation signatures in the liver during our lifetime. These studies suggest that the Western-style diet may increase disease susceptibility through epigenetic modifications and calorie restriction-induced longevity may be in part due to differential methylation at critical DMRs associated with disease susceptibility. Common loci of differential methylation can be further investigated as epigenetic biomarkers of diet and aging.

The Presentation of Dr. Simonetta Friso Focused on the Epigenomic Interface between Alcohol and Cancer

In cases of prolonged heavy alcohol drinking, the alcoholic liver may face a severe prolonged methyl deficiency, which is known to alter DNA methylation and is associated with the development of cancer. In fact, alcohol consumption in excess of 80 g/d is correlated with high rates (7.2–15%) of hepatocellular carcinoma (HCC).

The incidence of HCC in the United States has tripled while the 5-y survival rate has remained <12%. Even though HCC related to hepatitis C viral infection has become the fastest-rising cause of cancer-related death in the US, chronic alcohol consumption is still an important etiologic factor of HCC. To determine whether chronic alcohol consumption increases hepatic carcinogenesis by disturbing 1-carbon metabolism and subsequently altered DNA methylation, Dr. Friso and her group defined the DNA methylation profile in alcohol-related HCC. They enrolled 31 HCC patients associated with chronic alcohol use and examined their DNA methylation patterns. Genome-wide DNA methylation and gene expression array-based profiles show differential epigenomic patterns involving several known and novel metabolic pathways in alcohol-related HCC. Comparing with normal hepatic tissue, they found 2401 hypermethylated genes and 1244 hypomethylated genes, along with 1005 repressed genes and 670 induced genes from HCC. It is still unknown if this altered DNA methylation is the cause or the effect of alcohol-related HCC. However, because epigenetic phenomena are potentially reversible and influenced by nutrients, there is hope to find possible preventive/therapeutic strategies through DNA methylation.

This symposium revealed new insights into obesity and diabetes as well as highlighted recent advances in epigenomic techniques in the study of aging and cancer. From the 5 presentations, the audience gained a greater understanding of nutritional epigenetics, which is a field in its infancy. Much more research is to be done that has great potential to yield findings with important public health implications.

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