Epigenetic linkage of aging, cancer and nutrition

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ABSTRACT

epigenetic mechanisms play a pivotal role in the expression of genes and can be influenced by both the quality and quantity of diet. Dietary compounds such as sulforaphane (SFN) found in cruciferous vegetables and epigallocatechin-3-gallate (EGCG) in green tea exhibit the ability to affect various epigenetic modifications such as DNA methyltransferase (DNMT) inhibition, histone modifications via histone deacetylase (HDAC), histone acetyltransferase (HAT) inhibition, or noncoding RNA expression. Regulation of these epigenetic mechanisms has been shown to have notable influences on the formation and progression of various neoplasms. We have shown that an epigenetic diet can influence both cellular longevity and carcinogenesis through the modulation of certain key genes that encode telomerase and p16. Caloric restriction (CR) can also play a crucial role in aging and cancer. Reductions in caloric intake have been shown to increase both the life- and health-span in a variety of animal models. Moreover, restriction of glucose has been demonstrated to decrease the incidence of age-related diseases such as cancer and diabetes. A diet rich in compounds such as genistein, SFN and EGCG can positively modulate the epigenome and lead to many health benefits. Also, reducing the quantity of calories and glucose in the diet can confer an increased health-span, including reduced cancer incidence.

KEY WORDS: Epigenetics, Diet, Aging, Cancer, Nutrition

Introduction

Epigenetics is a term that was first introduced by developmental biologist Conrad H. Waddington in 1942 and usually refers to heritable changes in genetic expression without changing the DNA sequence itself (Waddington, 2012). Of the epigenetic mechanisms, there are three that are most important. These include DNA methylation, histone modification and RNA interference (RNAi) (Herceg, 2007). Methylation of DNA is generally regarded as one of the most important epigenetic modifications and occurs primarily at the cytosine residues of CpG dinucleotides (Razin and Riggs, 1980). This process is largely controlled by enzymes known as DNA methyltransferases (DNMTs) and often takes place within the regulatory regions of gene promoters and involves the addition of a methyl moiety to the cytosine in CpG dinucleotides (Li et al., 1993; Bestor, 2000). CpG dinucleotides are typically clustered in what is termed CpG islands, which are areas rich in CpG sites. These sites are found in roughly half of all human genes and can extend from 0.5 to 3 kb, occurring about every 100 kb within the genome (Antequera and Bird, 1993). Hypermethylation of these islands is usually associated with the silencing of gene expression. Inversely, hypomethylation in these regions often leads to gene reactivation (Li and Tollefsbol, 2010; Li et al., 1993).

Modification of histones involves changes within the basic structure of the chromatin unit known as the nucleosome. The nucleosome is composed of four core histones: H2A, H2B, H3 and H4. DNA 146 bp in length is wrapped around an octomer, which is made of two copies of each of the core histones and is held together by one linker histone, H1 (Luger et al., 1997; Li et al., 2011; Chervona and Costa, 2012). Remodeling of histones can often be a result of modifications via methylation, ubiquitylation, phosphorylation, biotinylation, sumoylation, ADP ribosylation and acetylation at the N-terminal group of lysine (K) residues. However, histone acetylation/deacetylation and methylation are among the most studied and considered to be the most prevalent of the modifications (Kouzarides, 2007; Li et al., 2011; Tollefsbol, 2014). Histone modifications and the resulting configuration of the chromatin are associated with both activation and silencing of genes. For example, acetylation of the histone lysine residue removes its positive charge, which reduces attraction to the negatively charged DNA strand and produces a loosened chromatin structure. This open state allows various transcription factors to access the DNA and promotes the transcriptional activation of genes (Clayton et al., 2006).

Epigenetic modifications via interference by RNA can also lead to varied gene expression. RNAi can prevent the accumulation of homologous transcripts by forming antisense transcripts. These transcripts can then lead to the formation of heterochromatin and consequent transcriptional silencing (Montgomery et al., 1998; Hardy and Tollefsbol, 2011). In addition, non-coding RNA such as microRNA (miRNA) can aid in the regulation of genes. miRNAs are single-stranded sequences ranging from 21 to 23 nucleotides in length and exhibit the potential to suppress gene expression by altering the stability of transcripts and targeting them for degradation (Tollefsbol, 2014). These miRNAs lead to post-transcriptional gene silencing by binding to the complementary sequences of target mRNA, causing increased degradation of the RNA (Ribarić, 2012). However, miRNAs have also been shown to increase transcriptional activity in some instances (Mathers et al., 2010; Tollefsbol, 2014). In additional pathways, miRNA has been shown to target gene expression via the promotion of DNA methylation and histone modification at the sites of certain promoters (Tan et al., 2009; Hawkins and Morris, 2008; Ribarić, 2012).

Nutrition and diet have been shown to be mediated through epigenetic mechanisms, and recent studies have elucidated that diet, in both its quantity and quality, is linked to aging and cancer incidence and prognosis (Li and Tollefsbol, 2011; Mercken et al., 2013; Meeran et al., 2010). Moreover, nutrition is thought to be the most influential of all the external environmental factors due to its ability to affect the transcriptional activity and expression of certain genes
administered a diet based on natural ingredients and their control diet was regulated in proportion. Whereas Colman et al. (Colman et al., 2009) fed their CR monkeys a purified diet and the controls were fed ad libitum.

Age is characterized by notable changes in the distribution of 5-methylcytosine, leading to an overall decrease in global DNA methylation patterns (Wilson et al., 1987; Singhal et al., 1987; Li et al., 2011). Caloric restriction is thought to increase the stability of the genome by reversing the loss of methylation that commonly occurs during aging, thus maintaining chromatin function (Li et al., 2011). For example, DNMT1 activity is greatly increased during CR in a potential response to the decreased levels of methylation during aging (Li et al., 2010a). Cancer cells are known to metabolize glucose much more rapidly compared with normal cells, a characteristic that makes them much more sensitive to reductions in glucose. Warburg (Warburg, 1956) first described the paradoxical preference of cancer cells to metabolize glucose via glycolysis even in the presence of ample amounts of oxygen where oxidative phosphorylation would be much more efficient in producing ATP. This apparent dependence on glucose by cancer cells has been successfully used in both cancer prognosis and monitoring (Rohren et al., 2004). Caloric restriction via glucose restriction has been shown to extend the lifespan of normal human lung fibroblasts in vitro compared with control cells receiving a normal amount of glucose (Li and Tollefsbol, 2011).

Cancer epigenetics
DNA methylation

The methylation status of DNA, especially within the promoter regions, can have a notable effect on both cancer incidence and progression. For example, hypermethylation of the promoters of certain cancer-related genes can lead to their inactivation and subsequent genetic instability and cancer development. These genes could include tumor suppressor genes such as p16RASFL/CDKN2A, p16INK4a (also known as p16 or CDKN2A), RUNX3 and TIG1 or regulatory genes, such as RAS association domain family 1A (RASSFlA) and retinoic acid receptor β (RARβ) (Baylin and Ohm, 2006; Li and Tollefsbol, 2010; Ribaric, 2012) (Fig. 1). Inversely, hypomethylation can lead to the activation of certain oncogenes, contributing to the development of cancer.

Fig. 1. Effects of caloric restriction on the reduction of age-related diseases and increased longevity. Caloric restriction (CR), at safe levels, has been shown to reduce the onset of various age-related diseases, as well as increase cellular longevity in both in vivo and in vitro models. CR can act as an activator of SIRT1, which inhibits FOXO proteins, leading to a reduction in apoptosis. Moreover, CR has been characterized as a modulator of aberrant DNA methylation patterns, which are often associated with the aging process. Studies have shown that hypermethylation of the p16 promoter can be facilitated by CR, causing a notable decrease in p16 expression and subsequent evasion of cellular senescence.
process of tumorigenesis (Gaudet et al., 2003). Aberrant methylation patterns are observed in almost all neoplasms, suggesting its importance as a molecular marker in cancer prevention, prognosis and therapeutic approaches.

In one of the most extensive methylation studies, Tsou et al. (Tsou et al., 2002) showed that in lung cancer, more than 40 genes displayed some type of alteration in DNA methylation patterns. Although hypomethylation is observed in cancers, hypermethylation of genes in cancer is much better characterized. Numerous genes have been shown to undergo hypermethylation in many cancers. For example, genes involved in cell cycle regulation such as \( p16 \), \( p15^{INK4b} \) (also known as \( p15 \) or CDKN2B), \( Rb \) and \( p14^{ARF} \), and those associated with DNA repair mechanisms like BRCA1 (breast cancer 1) and MGMT (methylguanine methyltransferase), exhibit a hypermethylated state in the majority of cancers (Das and Singal, 2004). Hypermethylation often leads to the loss of function of many of the gene products that are critical in the development of breast cancer, including those involved in steroid reception, cell adhesion and inhibition of matrix metalloproteinases (Yang et al., 2001).

Along with \( p16 \) and BRCA1, estrogen receptor (ER) alpha, progesterone receptor (PR) and E-cadherin are also hypermethylated in the majority of breast cancer genotypes. Although hypermethylation in cancers is more common and extensively characterized, hypomethylation can also play a critical role in the development of a variety of malignancies (Feinberg and Vogelstein, 1983; Liao et al., 2014). For example, a decrease in methylation is often seen in the solid tumors of cervical and ovarian cancers, prostate cancer, liver cancer and also in hematologic cancers such as B-cell chronic lymphocytic leukemia (Lin et al., 2001; Dueñas-González et al., 2005; Guerrero-Preston et al., 2007; Liao et al., 2014; Yang et al., 2013). In addition, Liao et al. (Liao et al., 2014) determined that global hypomethylation of tumor-initiating cells is an important signature in the poor prognosis of certain ovarian cancer patients.

**Histone modifications**

Modification of histones through mechanisms of acetylation, methylation, sumoylation, ADP-ribosylation, ubiquitylation and phosphorylation is also an important instrument in epigenetics and the development of neoplasms. This combination of histone modifications has a marked effect on gene expression via the recruitment of various transcriptional regulatory proteins that bind to specific, modified histone constructs (Choi and Lee, 2013). For example, promoters are typically activated when H3 is trimethylated at lysine 4 (H3K4me3) and inactivated upon methylation of H3 at Lys27 (H3K27me3) (Hon et al., 2009). Many cancers often exhibit aberrant histone modification patterns. A study performed by Fraga et al. (Fraga et al., 2005) found that cancer cells often exhibit losses in both histone acetylation and methylation, markedly in H4 at the acetylated Lys16 and trimethylated Lys20 residues. Aberrant targeting by modifying histone enzymes such as histone deacetylases (HDACs), histone acetyltransferases (HATs) and histone methyltransferases (HMTs) is often the underlying cause of many progressive cancer types. For instance, HDAC1 and HDAC2 expression has been shown to be upregulated in prostate and gastric cancers, respectively (Halkidou et al., 2004; Song et al., 2005). Moreover, a study by Simon et al. (Simon et al., 2012) reported a strong correlation between the increased frequency of spontaneous T-cell leukemia and the deletion of EZH2, a H3K27 methyltransferase, in mice. Aberrant histone modifications can not only lead to the development of cancers but also contribute to their poor prognosis. In breast cancer, global elevated levels of histone acetylation and methylation are often associated with a favorable prognosis. Conversely, moderate to low levels of certain histone acetylation (H3K18ac and H4K12ac) and histone methylation (H3K4me2, H4K20me3, H4R3me2) can correlate with poorer prognosis in certain carcinomas, especially basal carcinomas and HER-2-positive tumors (Elsheikh et al., 2009; Chervona and Costa, 2012). Chen et al. (Chen et al., 2013) were able to show a correlation between histone modification patterns and the cancer-specific survival rate in patients with oral squamous cell carcinoma. In this study, patients with low levels of H3K4ac and those with high levels of H3K27me3 exhibited an advanced tumor and nodal status, a progressed tumor stage and perineural invasion, further solidifying the critical role of aberrant histone modifications on both the progression and prognosis of cancers.

**microRNA**

Small noncoding RNAs known as miRNAs play a pivotal role in the alterations of post-transcriptional gene expression, and can act as important contributors in both the development and prevention of many different human diseases, including cancer. Various studies have shown that aberrant expression of miRNAs in cancer cells may contribute to both the onset of carcinogenesis as well as the prognosis of various neoplasms (Ferdin et al., 2010; Ross and Davis, 2011). For example, decreased expression of the let-7 family of miRNAs has been associated with increased tumorigenesis. However, increases in the expression of this family of miRNAs have been shown to inhibit the growth of various animal tumor cells, both in vivo and in vitro (Barh et al., 2010). In an investigation by Zhao et al. (Zhao et al., 2011), among the 25 differentially expressed miRNAs identified, certain members of the let-7 family were observed to be downregulated in breast cancer cells and showed an inverse correlation with ERα expression. This correlation was further confirmed by overexpressing let-7 miRNA in ER-positive MCF-7 breast cancer cells, and as a result of this overexpression, both inhibition of cell proliferation and the triggering of apoptosis were observed (Zhao et al., 2011). Furthermore, Saito et al. (Saito et al., 2006) was able to draw a connection between epigenetics and the regulation of miRNAs through an extensive expression profile of miRNAs in normal LD419 human fibroblasts and T24 human bladder cancer cells that had been treated with chromatin-modifying drugs. In this study, it was revealed that after treatment with the DNA-demethylating agent 5-Aza-2'-deoxycytidine, and histone deacetylase inhibitor, 4-phenylbutyric acid (PBA), 17 of the 313 examined human miRNAs were simultaneously upregulated. However, it should be noted that the miRNAs that were upregulated were notably different between the two cell lines. This variation in upregulated miRNAs between the two cell lines may indicate that differences in the methylation status and chromatin structure around miRNA genes in normal cells compared with cancer cells may play a role in miRNA expression; however, tissue specificity could also be responsible for the varied expression of these upregulated miRNAs (Saito et al., 2006; Yang et al., 2008). Epigenetic regulation of miRNAs was further confirmed in an investigation by Lujambio et al. (Lujambio et al., 2007) where a decrease in DNA methylation was shown to alter the expression of regulating miRNA in DNMT1- and DNMT3B-knockout HCT116 colorectal cancer cells. In this study, microarray analysis was used to show that 18 of the 320 human miRNAs profiled were upregulated more than threefold in the knockout cell line. One of the most notable targets in this study was mir-124a, which exhibits transcriptional inactivation via hypermethylation of its CpG island in various human tumor cell types (Lujambio et al., 2007; Yang et al., 2008).
p16 and p53

The cyclin-dependent kinase inhibitor p16 is commonly regarded to play a crucial role in tumor growth suppression and cellular senescence (Gil and Peters, 2006). p16 negatively regulates the cell cycle by inhibiting activation of cyclin D/CDK4/CDK6 and subsequently releasing the inhibitory retinoblastoma (Rb) gene, resulting in the failure of E2F release and cellular progression (Serrano et al., 1993; Li and Tollefsbol, 2011). p16 was shown to be deleted in ~20% of human breast tumors and epigenetically inactivated in an additional 20% of breast carcinomas (Tlsty et al., 2004; Sharpless, 2005). Using the p16-negative osteosarcoma cell line U2OS, Dai and Enders (Dai and Enders, 2000) were able to induce p16 and arrest cells in the G1 phase of the cell cycle. Moreover, they found that after 1 day of induction the cells underwent cellular arrest; however, after the inducer was removed, the level of p16 expression returned to baseline and cellular growth resumed within 3–5 days. Interestingly, after 6 days of continuous induction, cells exhibited a senescence-like morphology and DNA synthesis remained inhibited even after the removal of the inducer. This suggests that reactivation of p16 in certain cancers may exhibit a time dependence that eventually supersedes continued p16 expression levels. Studies have also shown that activation of p16 can play an important role in the formation of senescence-associated heterochromatin foci (SAHF), which contribute to cellular senescence by stabilizing the effects of the arrest (Narita et al., 2003). The SAHF are composed of reorganized DNA, enriched with proteins that are typically associated with heterochromatin formation (Narita et al., 2003; Bazarov et al., 2010). Our laboratory has previously reported the chromatin-remodeling patterns of the p16 promoter region have the potential to play important roles in the cellular fates of both normal and cancer cells, largely in response to glucose restriction (Li et al., 2010a).

p53 is a potent tumor suppressor and its elimination or mutant expression is one of the most important determinants in a majority of epithelia tissue transformations to malignancies (Fig. 2). In its wild-type form, p53 has been shown to either activate or inhibit cellular autophagy, successively leading to either cell death or survival, respectively (Tasdemir et al., 2008). Wild-type p53 binds to the promoter regions of target genes and modifies the acetylation of histones H3 and H4 via the recruitment of either co-activators or co-repressors. This epigenetic alteration results in increased gene expression via hyper-acetylated histones or a decrease in gene expression due to the deacetylation of histones H3 and H4 (Murphy et al., 1999; Barlev et al., 2001; Vrba et al., 2008). However, it has been reported and widely accepted that mutant forms of p53 acquire novel oncogenic functions compared with the wild-type form (Freed-Pastor and Prives, 2012; Rodriguez et al., 2012). Wild-type p53 is typically expressed at low levels because of its degradation via the proteasome, which is controlled mainly by the E3-ubiquitin ligase MDM2. However, mutant p53 forms, which are widely expressed in tumors, are thought to be able to evade proteolysis by blocking MDM2 transcription and causing subsequent aberrant interactions (Prives and White, 2008; Brosh and Rotter, 2009; Rodriguez et al., 2012). A recent study done by Cooks et al. (Cooks et al., 2013) reported the prolonged of TNF-α-induced NF-κB activation by mutant p53 in vivo in intestinal cells. In addition, a correlation was drawn between the accumulation of mutant p53, augmented NK-κB activation and the progression of inflamed cells to an invasive carcinoma (Cooks et al., 2013). Mutation of the p53 transcript (TP53), specifically TP53R172H, is reported to promote metastasis and occurs in 50–75% of human pancreatic ductal adenocarcinomas (PDACs) (Morton et al., 2010). TP53 mutations are also seen in breast cancers and its expression appears to be directly proportional to tumor stage. For example, tumors in stage T1 (<2 cm in diameter) exhibited little

Fig. 2. hTERT expression and telomere length in normal somatic cells and precancerous cells. Human telomerase reverse transcriptase (hTERT) activity is overexpressed in the majority of cancers. hTERT plays a pivotal role in the evasion of cellular senescence and subsequent immortalization of precancerous cells. In normal somatic cells, hTERT is only expressed at a basal level and telomeric ends are continually degraded with each cycle of cellular replication, eventually leading to senescence. However, in precancerous cells, telomeres are maintained via increased hTERT expression and cells are able to avoid mechanisms that would prevent immortalization.

![Diagram of hTERT expression and telomere length](image-url)
to no mutant TP53 expression, whereas T3 stage (>5 cm) tumors expressed a markedly higher mutant TP53 frequency (Olivier et al., 2006; Rivlin et al., 2011).

**Telomerase/TERT**

hTERT (human telomere reverse transcriptase) is the catalytic subunit of telomerase, the enzyme responsible for the maintenance of telomeric ends, and is widely expressed in over 90% of cancers. It is believed that this upregulation of TERT is a crucial mechanism for avoidance of cellular senescence (Fig. 2). Several studies have shown that TERT is regulated by various epigenetic modifications at promoter sites, including acetylation of histones and promoter methylation (Kyo et al., 2008; Meeran et al., 2010; Daniel et al., 2012). Contrary to the typical relationship between methylation and gene expression, increased TERT expression is associated with a hypermethylation of its 5′ regulatory region, whereas demethylation of this region inhibits transcription and leads to a decreased expression of the gene (Renaud et al., 2007; Meeran et al., 2010). Our laboratory has shown that the inhibition of HDACs and subsequent induction of histone acetylates allows for repressors to bind to the 5′ regulatory region of TERT. Moreover, downregulation of DNMTs and demethylation of CpG sites in exon 1 of TERT leads to the induction of RBP2, allowing repressor CTCF to bind to this region and contribute to the inhibition of TERT expression (Meeran et al., 2010). p16 has also been described to exhibit an inhibitory effect on the expression of TERT. Bazarov et al. (Bazarov et al., 2010) was able to show that transient exposure to p16 led to the suppression of telomerase in both MDA-MB-231 and MCF-7 breast cancer cells. Also, the induction of p16 caused an increase in the methylation of Lys27 on histone H3, leading to a transcriptional silencing of TERT (Bazarov et al., 2010). In addition, other studies have reported on the interconnectedness of p16 and TERT, and their roles in immortalization and carcinogenesis. For example, although induced expression of TERT is sufficient to drive the majority of primary human cells towards immortalization, additional inactivation of p16 is required for human keratinocyte and mammary epithelial cells to avoid senescence (Kiyono et al., 1998; Dickson et al., 2000). Shao et al. (Shao et al., 2008) found that activation of TERT alone was not sufficient to drive prostate epithelial cells to immortalization, and that methylation of the p16 promoter was necessary in this process.

**Caloric restriction, aging and cancer**

Quantity of diet has been characterized as a powerful mechanism in both the extension of lifespan and the development and prognosis of various cancers. In colorectal cancers, it has been proposed that dietary factors contribute to more than 70% of cases (Pericleous et al., 2013). The restriction of caloric intake displays effects at the cellular and organismal levels, altering various epigenetic mechanisms and signaling pathways (Li et al., 2011; Mercken et al., 2013). Rodriguez et al. (Rodriguez et al., 2012) were able to show a correlation between CR, in the form of glucose restriction, and a downregulation of mutant p53, which is widely expressed in a majority of tumors. It is known that cancer cells metabolize glucose at an elevated rate compared with normal cells, and it is this phenomenon that makes glucose restriction a novel therapeutic approach in the impairment of cancer growth and progression. Moreover, CR improves insulin sensitivity and causes its subsequent decrease in response to a reduction in glucose levels (Vera et al., 2013). A reduction of caloric consumption in humans has also been linked to reduced risks of diabetes and cardiovascular disease (Fontana and Klein, 2007; Cruzen and Colman, 2009). Increases in longevity and health-span have also been linked to CR. In fact, CR is reported to be one of the most effective and well-characterized mechanisms in extending the lifespan in a variety of animal models. CR has also been shown to both decrease the incidence and delay the onset of various age-related diseases, such as atherosclerosis and neurodegenerative diseases in humans and primates (Roth et al., 2001; Holloszy and Fontana, 2007; Colman et al., 2009). The exact mechanisms by which CR affects aging, age-related diseases and cancer are debated, although there is a strong suggestion that CR promotes protective mechanisms that allow evasion from DNA damage (Bordone and Guarente, 2005; Vera et al., 2013).

**Aging and CR**

Aging is often associated with oxidative stress and a decreased effectiveness of metabolic pathways. However, numerous studies have suggested that CR acts as a mechanism to offset these aberrations (Sohal and Weindruch, 1996; Li et al., 2011). Epigenetic mechanisms have also been determined to be major contributors in nutrition-related longevity and the decreased rates of aging (Li and Tollefsbol, 2011; Mercken et al., 2013). The downregulation of the insulin/IGF pathway is regarded as one of the most influential interventions that lead to the increased lifespan of lower organisms. However, Mercken et al. (Mercken et al., 2013) were able to show that CR resulted in a uniform and dramatic transcriptional reprogramming in human skeletal muscle, leading to a reversion of cellular function from metabolism to maintenance and repair activities. They found that a significant number of altered transcripts were involved in the IGF-1/insulin/FOXO pathway. Long-term CR leads to a downregulation of the IGF-1/insulin pathway, mainly through reduction of Akt phosphorylation and subsequent nuclear activation of FOXO transcription factors, which are modulators of cellular responses and longevity (Lanvin et al., 2007; Mercken et al., 2013). In the presence of insulin and/or growth factors, FOXO is directly phosphorylated and sequestered in the cytoplasm. However, in the absence of these factors, FOXO is translocated to the nucleus, where it can trigger a variety of cellular responses, especially resistance to oxidative stress (Greer and Brunet, 2008). SIRT1 has also been described to regulate the mediation of cellular responses by FOXO. FOXO3 and/or FOXO4 are directly deacetylated by SIRT1, causing a shift from the ability to induce apoptosis to the induction of cell cycle arrest and resistance to oxidative stress. This suggests that SIRT1 is able to modulate the responses of FOXO proteins under the stresses of CR from roles of cellular death to cellular survival (Brunet et al., 2004; Giannakou and Partridge, 2004; Fig. 1). In a study by Rebrin et al. (Rebrin et al., 2003), it was found that CR lowered the amounts of mitochondrial GSSG, the oxidized form of glutathione, in mice that had been given 40% fewer calories beginning at 4 months of age compared with mice fed ad libitum. These findings suggest that CR may be able to attenuate the pro-oxidizing shift of glutathione during the aging process of mice.

Epigenetics has also been reported to have notable effects on the aging process. For example, age causes dramatic changes in the genome-wide distribution of 5-methylcytosine, leading to an overall decrease in methylation (Knapskiwski et al., 2002). Conversely, this decrease of global methylation associated with aging does not occur in the promoter regions of many specific genes, which tend to switch to a state of methylation, resulting in gene silencing (Waki et al., 2003; Li et al., 2011). CR, however, is likely to recover these age-associated aberrant methylation patterns via specific loci control rather than globally (Muñoz-Najar and Sedivy, 2011).

Histone remodeling also plays an important role in the control of aging during CR. Our laboratory has shown that in vitro caloric
restriction leads to the enriched binding of HDAC1 to the promoter regions of p16 and TERT, leading to expression changes that promote human cellular longevity. In this study, CR via the reduction of glucose inhibited cellular senescence and significantly extended cellular lifespan in human lung fibroblasts. Glucose restriction induced the remodeling of chromatin, especially through the decreased binding of acetyl-H3 and dimethyl-H3K4 and the increased binding of trimethyl-H3K9 to the p16 promoter, leading to its transcriptional repression (Li and Tollefsbol, 2011). Moreover, glucose-restricted WI-38 fetal lung fibroblasts were shown to express increased levels of TERT and exhibited increased binding of active chromatin markers, acetyl-H3, acetyl-H4 and dimethyl-H3K4 to the TERT promoter (Li et al., 2010a). Moreover, SIRT1, an NAD-dependent histone deacetylase, is one of the most studied mediators in longevity resulting from CR. SIRT1 expression has been shown to increase with CR in mice, rats and humans (Baur et al., 2012). Our laboratory has also shown the induction of SIRT1 expression and enzymatic activity under conditions of restricted glucose (Li and Tollefsbol, 2011). However, there is still a question as to whether an increased expression of SIRT1 is necessary for the extension of lifespan under CR. Mercken et al. (Mercken et al., 2014) reported that calorie-restricted mice required SIRT1 for lifespan extension; however, high expressions of SIRT1 were not required. It should be noted that the life-extending properties of CR have been reported to be removed or minimalistic in some rodent strains (Turturro et al., 2004). A study done by Liao et al. (Liao et al., 2010) revealed that ILSXISS recombinant inbred mice exhibited a shortened lifespan under dietary restriction, supporting the concerns that life extension by CR may not be universal.

Cancer and CR
Carcinogenesis and its progression are often the result of epigenetic aberrations that lead to the activation or deactivation of specific genes that are typically regulated in normal cells. Several studies have shown that CR has the ability to prevent or reverse these divergent changes, leading to improved prognosis and lowered cancer incidence (Hass et al., 1993; Li et al., 2010a; Rodriguez et al., 2012). Epidemiological studies have provided additional support for the effects of CR on cancer risks. For example, inhabitants of Okinawa, Japan consume far fewer calories than those on the mainland and also exhibit continually lower cancer-related deaths when compared to residents on the Japanese mainland (Kagawa, 1978; Wilcox et al., 2007). CR has been previously reported to decrease levels of IGF-1, a critical component in the growth and development of many tissues (Mercken et al., 2013). This supports the finding that CR impacts the levels of cellular growth signals, although these reductions could in part be a result of the reduced glucose levels associated with CR (Gallagher et al., 2010).

Decreases in systemic leptin levels have also been linked to CR (Hursting et al., 2010). Several studies have shown that leptin levels, especially the leptin/adiponectin ratio, are associated with breast and endometrial cancer risk and prevention (Chen et al., 2006; Cleary et al., 2009; Ashizawa et al., 2010). A retrospective cohort study performed by Michels and Ekbohm (Michels and Ekbohm, 2004) reported that CR may elicit a protective mechanism from the development of invasive breast cancer. However, it should be noted that patients in this study underwent severe CR, with a large majority suffering from anorexia nervosa. Chronic inflammation is also believed to play a hallmark role in the development of cancers (Ono, 2008; Aggarwal and Gehlot, 2009). As an example of this concept, COX-2, an inflammatory mediator, is frequently upregulated in tumors. COX-2 is often associated with poor prognosis in many cancer types (Koki et al., 2002). It has been reported that CR lowers the incidence of inflammation and associated preneoplasia or neoplasia (Harvey et al., 2011; Harvey et al., 2013) and decreases the levels of circulating COX-2 in a variety of tissues and tumor types (Harvey et al., 2013; Hursting et al., 2013). The reduction in the levels of glucose and insulin secretion associated with CR also stimulates the release of glucagon by the pancreas, resulting in autophagy in liver cells, skeletal muscle and β-cells of the pancreas (Ezaki et al., 2011). It has been proposed that under the nutrient-limiting conditions of CR, cells are forced to shift energy investments from cell replication to growth and maintenance to ensure survival (Kapahi et al., 2010). This system of inducing autophagy and shifting energy away from cellular replication could serve as a key mechanism through which CR inhibits the formation of various neoplasms.

Epigenetic diet
In the same way that quantity of diet has been shown to play a pivotal role in the prolonging of life-span and the development of various diseases, quality of diet can also serve as an important mechanism in these processes. It has become increasingly evident that environmental factors can have marked effects on the epigenome and that these changes can be altered, in part, by diet. Because diet can influence epigenetic changes, utilizing dietary compounds to target, treat and even prevent certain diseases has become an area of great interest. The term ‘epigenetic diet’ was coined to refer to the consumption of certain foods, such as soy, grapes, cruciferous vegetables and green tea, which have been shown to induce epigenetic mechanisms that protect against cancer and aging (Hardy and Tollefsbol, 2011) (Table 1). Introduction of these food groups into a normal diet regime could serve as an effective therapeutic strategy for medicinal and chemopreventive purposes. Several studies have shown that practicing an epigenetic diet reduces the incidence of several diseases and even has the potential to mimic the effects of CR (Aggarwal and Shishodia, 2006; Ayissi et al., 2014). Sulforaphane (SFN) found in cruciferous vegetables, epigallocatechin-3-gallate (EGCG), found in green tea, genistein (soy) and resveratrol (grapes) are among the most characterized dietary compounds of the epigenetic diet (Fig. 3).

SFN is an isothiocyanate that elicits both proapoptotic and antiproliferative properties (Fimognari et al., 2008). SFN has also been shown to act as a HDAC inhibitor, increasing global and local histone acetylation and acting as a regulator of certain cancer-related genes (Dashwood and Ho, 2008; Telang et al., 2009). Other studies have shown that SFN possesses a dual role, as both a HDAC and DNMT inhibitor. Meeran et al. (Meeran et al., 2010) reported that SFN inhibits TERT expression in both a dose- and time-dependent manner in MCF-7 and MDA-MB-231 human breast cancer cells. Moreover, cells treated with SFN displayed a notable decrease in DNMTs, particularly DNMT1 and DNMT3A, which led to the demethylation of exon 1 of TERT and the subsequent binding of the TERT suppressor CCCTC-binding factor (CTCF). Lastly, SFN induced the hyperacetylation of the TERT promoter and allowed the binding of repressor proteins such as MAD1 (Meeran et al., 2010). Aging is often linked to oxidative stresses; however, SFN has been shown to activate the Nrf2 pathway and increase the expression of antioxidant genes, associating SFN not only with cancer prevention but also with aging (Greco et al., 2011).

Tea is second only to water as the most consumed beverage worldwide, and studies show that tea contains certain polyphenolic compounds that act as defense mechanisms, protecting plants from reactive oxygen species and photosynthetic stressors (Link et al.,...
miRNA expression. For example, miRNAs were found to be upregulated by EGCG in mice prostate tumors. Here, Siddiqui et al. (Siddiqui et al., 2011) reported a significant downregulation of miR-21 and an upregulation of tumor suppressor miR-330 in the tumors of mice treated with EGCG. Moreover, a broader investigation by Milenkovic et al. (Milenkovic et al., 2012) used miRNA microarrays to probe for 567 miRNAs in mice that had been supplemented with a variation of nine different polyphenols, including phenolic acids and flavonoids, for 2 weeks. It was shown that there was an average fold change of −2 for downregulated miRNAs and an average fold change of 2.24 for upregulated miRNAs. These findings suggest that supplementation with polyphenols at nutritionally achievable levels could regulate cellular functions via the modulation of miRNA expression (Milenkovic et al., 2012).

Genistein is a phytoestrogen found primarily in soybeans and is perhaps the most studied of the isoflavones, the largest class of polyphenolic compounds (Valls et al., 2009). Genistein, with its estrogen-like properties, exhibits chemopreventive qualities in several cancer types (Barnes, 1995). Fang et al. (Fang et al., 2005) showed that treatment of esophageal carcinoma cells with genistein can reactivate ERα encoded by ESR1 in MDA-MB-231 breast cancer cells via the decreased binding of the transcriptional repressor complex, Rb/p130–E2F4/5–HDAC1–SUV39H1–DNMT1 in the regulatory regions of the ERα gene promoter (Li et al., 2010b). Inhibition of telomerase has also been shown after EGCG treatment. In this study, EGCG downregulated hTERT expression in MCF-7 human breast cancer cells, largely as a result of TERT promoter demethylation and ablated histone H3 Lys9 acetylation (Berlesch et al., 2008).

Recent studies suggest that changes in miRNA expression may also be mediated by EGCG and various other polyphenols (Fig. 3). In an investigation by Tsang and Kwok (Tsang and Kwok, 2010), HepG2 human HCC cells treated with EGCG were analyzed by microarray and exhibited notable upregulation in the expression of 13 miRNAs and downregulation of 48 miRNAs. However, it should be mentioned that these modifications in miRNA expression were observed after treatment with 100 μmol l−1 EGCG, which is not considered to be physiologically achievable. Other studies have also drawn a correlation between EGCG treatment and alterations in miRNA expression. For example, miRNAs were found to be upregulated by EGCG in mice prostate tumors. Here, Siddiqui et al. (Siddiqui et al., 2011) reported a significant downregulation of miR-21 and an upregulation of tumor suppressor miR-330 in the tumors of mice treated with EGCG. Moreover, a broader investigation by Milenkovic et al. (Milenkovic et al., 2012) used miRNA microarrays to probe for 567 miRNAs in mice that had been supplemented with a variation of nine different polyphenols, including phenolic acids and flavonoids, for 2 weeks. It was shown that there was an average fold change of −2 for downregulated miRNAs and an average fold change of 2.24 for upregulated miRNAs. These findings suggest that supplementation with polyphenols at nutritionally achievable levels could regulate cellular functions via the modulation of miRNA expression (Milenkovic et al., 2012).

Table 1. Bioactive dietary compounds and their epigenetic functions

<table>
<thead>
<tr>
<th>Dietary compound</th>
<th>Structure</th>
<th>Food source</th>
<th>Epigenetic function(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epigallocatechin-3-gallate (EGCG)</td>
<td><img src="image" alt="Structure" /></td>
<td>Green tea</td>
<td>DNMT inhibitor, HAT inhibitor, miRNA regulator</td>
</tr>
<tr>
<td>Sulforaphane (SFN)</td>
<td><img src="image" alt="Structure" /></td>
<td>Cruciferous vegetables</td>
<td>HDAC inhibitor, DNMT inhibitor</td>
</tr>
<tr>
<td>Genistein</td>
<td><img src="image" alt="Structure" /></td>
<td>Soy and fava beans</td>
<td>DNMT and HDAC inhibitor, miRNA regulator</td>
</tr>
<tr>
<td>Curcumin</td>
<td><img src="image" alt="Structure" /></td>
<td>Turmeric (curry)</td>
<td>DNMT inhibitor</td>
</tr>
<tr>
<td>Resveratrol</td>
<td><img src="image" alt="Structure" /></td>
<td>Grapes, peanuts, mulberry,</td>
<td>DNMT and HDAC inhibitor, miRNA regulator</td>
</tr>
</tbody>
</table>

DNMT, DNA methyltransferase; HDAC, histone deacetylase; HAT, histone acetyltransferase.
cells and enhance their sensitivity to hormonal therapy (Li et al., 2013). Furthermore, genistein has also been reported to exhibit similar miRNA-regulating properties as EGCG. For example, genistein was shown to inhibit the expression of miR-27a in human uveal melanomal cells, which is believed to be associated with its growth inhibitory actions (Sun et al., 2009; Ross and Davis, 2011). Altering the expression of miRNA with genistein has also been linked to the regulation of the ARHI tumor suppressor gene in prostate cancer cells. In this study, miR-221 and miR-222 levels were downregulated ~30 and 55%, respectively, and the levels of ARHI (DIRAS3) mRNA were induced twofold after treatment (Chen et al., 2011).

Resveratrol is a stilbene polyphenol that exhibits antioxidant, anti-inflammatory and anti-cancer properties (Athar et al., 2009). The ability of resveratrol to inhibit proliferation has been shown in several cancer types, including cancers of the breast, liver, skin, prostate, lung and colon (Liu et al., 2010; Mao et al., 2010; Vanamala et al., 2010). Although resveratrol is not as potent as EGCG in DNMT inhibition, it can prevent the silencing of certain tumor suppressors such as BRCA1 (Stefanska et al., 2010). One of the most important properties of resveratrol, however, may be its strong ability to act as an activator of SIRT1, a known HDAC inhibitor (Howitz et al., 2003). It is hypothesized that resveratrol activates SIRT1 by mimicking the physiological pathways that stimulate SIRT1, leading to the negative regulation of survivin, an antiapoptotic protein (Borra et al., 2005; Wang et al., 2008; Hardy and Tollefsbol, 2011). Interestingly, resveratrol has also been described as a mimic of CR. For example, in a species of short-lived fish, Nothobranchius furzeri, resveratrol extended lifespan and delayed the onset of cognitive decline (Valenzano et al., 2006). Furthermore, long-term administration of resveratrol in mice led to CR-like gene expression profiles and delayed age-related deterioration; however, lifespan was not extended (Barger et al., 2008; Pearson et al., 2008).

Flavonoids, polyphenols, and various other compounds associated with the ‘epigenetic diet’ have also been shown to influence glycolysis and the energy metabolism of cancer cells. It is known that the loss of p53 function often associated with carcinogenesis has been linked to the upregulation of glucose transporters, facilitating the increased uptake of glucose characteristic of cancer cells. Moreover, recent studies have shown that mutations in the KRAS oncogene enhance the rate of glycolysis and subsequent uptake of glucose by upregulation of glucose transporter 1 (GLUT1) (Yun et al., 2009). Interestingly, Johnston et al. (Johnston et al., 2005) were able to show that flavonoids exist in the diet as conjugates with glucose and that they utilize glucose transporters, acting as competitive inhibitors to the uptake of glucose. EGCG or green tea polyphenols (GTPs) was shown to modulate the expression of key genes involved in the metabolism of glucose and fats in a chronic 4-week supplementation of obese New Zealand black mice. In this investigation, EGCG reduced the accumulation of body fat and significantly downregulated the expression of hepatic glucokinase, a key enzyme of liver glycolysis (Klaus et al., 2005).

Epidemiological studies have continually solidified the inverse relationship between the low-fat, soy-rich Asian diet and incidence of cancer (Messina et al., 2006). In a provocative investigation by...
Boros et al. (Boros et al., 2001), a radiolabeled glucose tracer was used to monitor the accumulation of glucose metabolites, and it was found that after treatment with genistein, pancreatic cancer cells exhibited a significant reduction in ribose synthesis via the non-oxidative branch of the pentose phosphate pathway. Oxidation of glucose to CO2 was also significantly inhibited; however, it should be noted that lipid synthesis was unaffected (Boros et al., 2001). In more recent studies, the modulation of mitochondrial functionality and oxidative stress by genistein was shown to be largely dependent on the ERα/ERβ ratio in breast cancer cells. Here, genistein treatment improved mitochondrial functionality in T47D cells (Nadal-Serrano et al., 2013; Pons et al., 2014). Collectively, these findings could be important in the use of dietary polyphenols as sufficient inhibitors of glucose and modulators of oxidative stress in carcinogenesis; however, further studies are needed to confirm both the efficacy and potency of this therapeutic approach.

Conclusion
With an ever growing population and an increasing percentage of individuals living into older age, the incidence of age-related diseases such as cancer becomes significantly more frequent. Nutrition and diet are some of the most influential lifestyle factors that contribute to health and the development and progression of chronic diseases. Thus, dietary therapies can be effective mechanisms of defense against the onset of these developing conditions. It has been widely shown that eating certain foods that contain bioactive dietary compounds, like SFN and EGCG, modulates epigenetic modifications such as DNA methylation, histone modification and noncoding RNA. These changes in the epigene aid in the prevention of neoplasms, resulting in cancer cell death, and potentiate the longevity effects of CR. In fact, CR is regarded as one of the most effective and established environmental manipulations that can extend life and health-span, delaying the development of age-associated disorders such as cancer, diabetes and neurodegenerative diseases. The focus on quality promoted by the ‘epigenetic diet’ and the importance of quantity under CR are crucial in healthy aging and also have a significant impact on cancer. There are still many questions that need to be answered in order to elucidate the exact role that epigenetics plays in aging and cancer. However, new discoveries are continually supporting the epigenetic reversal of aberrant mechanisms by dietary control and CR and linking them to a decreased incidence of cancer and an extended lifespan.

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Competing interests
The authors declare no competing financial interests.

Author contributions
M.D. was responsible for the written text and T.O.T. for editorial contributions.

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