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REVIEW

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 mechanisms 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 (Ribarič, 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; Ribarič, 2012).

Nutrition and diet have been shown to be mediated through epigenetic mechanisms, and recent studies have eluded 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

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List of abbreviations

CR	caloric restriction
EGCG	epigallocatechin-3-gallate
ER	estrogen receptor
GTP	green tea polyphenol
hTERT	human telomerase reverse transcriptase
miRNA	micro RNA
PR	progesterone receptor
Rb	retinoblastoma protein
RNAi	RNA interference
SAHF	senescence-induced heterochromatin foci
SFN	sulforaphane
tRNA	transfer RNA

(Choi and Friso, 2010). Epigenetic modifications, potentiated by nutrition, have also been shown to delay the aging process, and certain changes within the epigenome have been adopted as potential biomarkers in many age-related diseases (Martin et al., 2013; Ross et al., 2008; Huffman, 2012). Nutrition in the form of caloric restriction (CR) is perhaps the best studied in its potential to delay the onset of aging. Caloric restriction generally involves a 30–40% reduction in the caloric intake while maintaining adequate nutrition. CR and its effect on longevity was first described by McCay et al. (McCay et al., 1989) who found that mice fed a calorie-restricted diet lived longer than mice that received a normal diet. Since then, increases in lifespan as a result of CR have been shown across a variety of species, including yeast, worms, flies, fish and even primates (Koubova and Guarente, 2003; Colman et al., 2009; Li et al., 2011). It should be noted, however, that there are discrepancies between two of the major CR studies conducted in Rhesus monkeys. Colman et al. (Colman et al., 2009) reported significant increases in longevity in CR primates when compared with those fed a control diet (*ad libitum*). By contrast, in a more recent study by Mattison et al. (Mattison et al., 2012), CR Rhesus monkeys did not exhibit significant survival outcomes compared with the controls, although some age- related diseases such as cancer were reduced in the CR primates. Differences in diet composition and administration could account for the opposing results of the two studies. For example, Mattison et al. (Mattison et al., 2012) 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 $p21^{WAF1/CIP1}$, $p16^{INK4a}$ (also known as *p16* or *CDKN2A*), *RUNX3* and *TIG1* or regulatory genes, such as RAS association domain family 1A (*RASSF1A*) and retinoic acid receptor β (*RARB*) (Baylin and Ohm, 2006; Li and Tollefsbol, 2010; Ribarič, 2012) (Fig. 1). Inversely, hypomethylation can lead to the activation of certain oncogenes, contributing to the

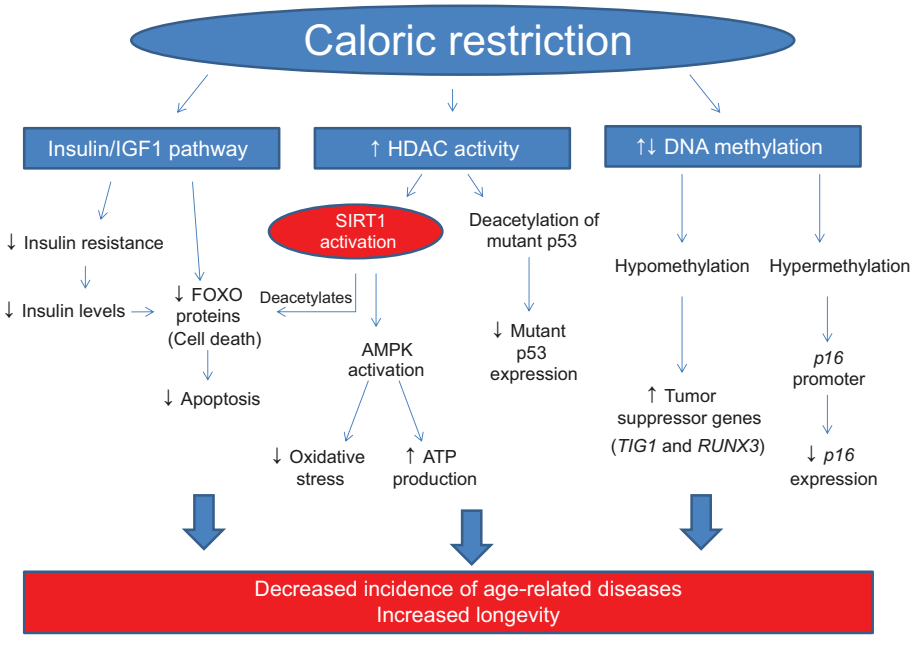


Fig. 1. Effects of caloric restriction on the reduction of age-related diseases and increased longevity. Caloric restriction (CR), at safe levels, has been shown to reduce the onset of various age-related diseases, as well as increase cellular longevity in both *in vivo* and *in vitro* models. CR can 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, sumolyation, 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 miRNA 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).

The Journal of Experimental Biology (2015) doi:10.1242/jeb.107110

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 that 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 prolongation 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 $TP53^{R172H}$, 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

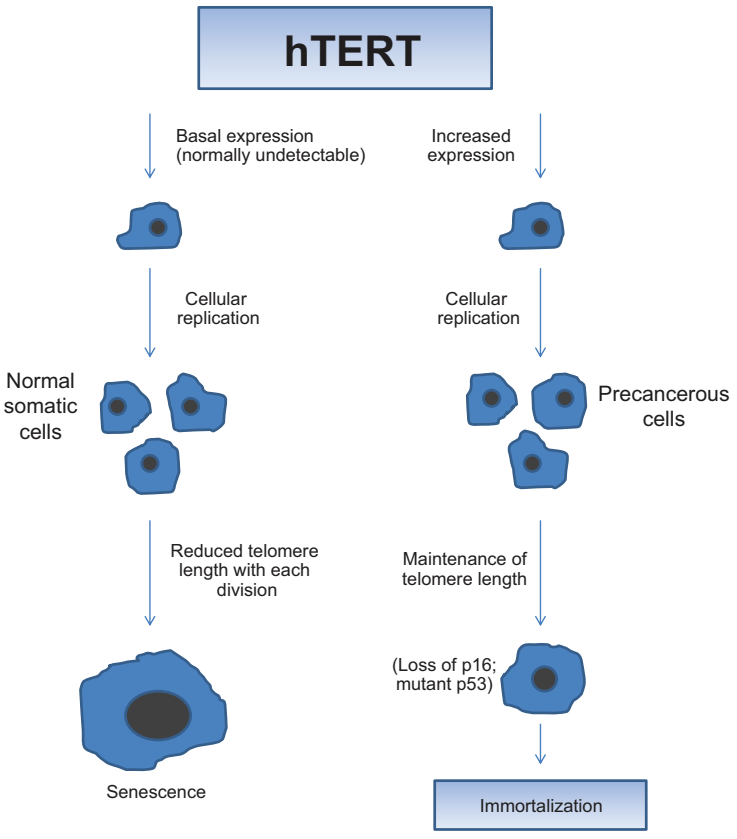


Fig. 2. hTERT expression and telomere length in normal somatic cells and precancerous cells. Human telomerase reverse transcriptase (hTERT) activity is overexpressed in the majority of cancers. hTERT plays a pivotal role in the evasion of cellular senescence and subsequent immortalization of precancerous 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.

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 acetylases 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 artherosclerosis 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 (Knapowski 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., 1999; Harper et al., 2006). 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; Willcox 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 systematic 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 Ekbom (Michels and Ekbom, 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

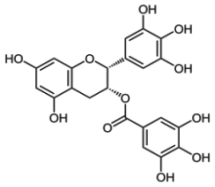
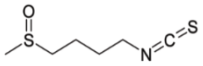
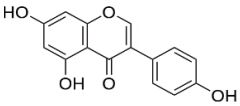
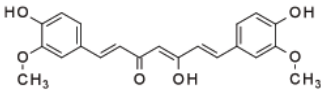
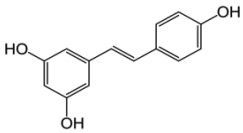
In the same way that quantity of diet has been shown to play a pivotal role in the prolonging of life-span and the development of various diseases, quality of diet can also serve as an important mechanism in these processes. It has become increasingly evident that environmental factors can have marked 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.,

The Journal of Experimental Biology (2015) doi:10.1242/jeb.107110

Table 1. Bioactive dietary compounds and their epigenetic functions

Dietary compound	Structure	Food source	Epigenetic function(s)
Epigallocatechin-3-gallate (EGCG)		Green tea	DNMT inhibitor, HAT inhibitor, miRNA regulator
Sulforaphane (SFN)		Cruciferous vegetables (broccoli, kale, cabbage, Brussels sprouts)	HDAC inhibitor, DNMT inhibitor
Genistein		Soy and fava beans	DNMT and HDAC inhibitor, miRNA regulator
Curcumin		Turmeric (curry)	DNMT inhibitor
Resveratrol		Grapes, peanuts, mulberry, cocoa	DNMT and HDAC inhibitor, miRNA regulator

DNMT, DNA methyltransferase; HDAC, histone deacetylase; HAT, histone acetyltransferase.

2010). These polyphenols, however, have also been shown to reduce disease and cancer incidence (Hardy and Tollefsbol, 2011). EGCG is the most abundant catechin in green tea and has been extensively studied for its anticarcinogenic properties (Lin et al., 1999). Moreover, there is growing evidence that links the consumption of EGCG to the inhibition of oral, breast, prostate, gastric, colorectal and a variety of other cancers (Shanmugam et al., 2011; Tu et al., 2011). EGCG has also been reported to exhibit DNMT inhibitory properties (Table 1). An earlier study conducted by Fang et al. (Fang et al., 2003) revealed that EGCG treatment lowered DNMT activity in esophageal cancer cells and resulted in the reversal of the hypermethylated state of tumor suppressor genes including *p16*, *RARB*, *MLH1* and *MGMT*. Findings in our laboratory have shown that green tea EGCG reactivates estrogen receptor-α (ERα encoded by *ESR1*) in MDA-MB-231 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 (Berletch 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).

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 resulted in a partial reversal of DNA hypermethylation and reactivated *p16*, *RARB* and *MGMT*. Importantly, a clinical study performed by Qin et al. (Qin et al., 2009) demonstrated that women who received daily doses of isoflavones, including genistein, exhibited an increased hypermethylation of cancer-related genes *RARB* and *CCND2*. Epidemiological studies have revealed that a higher intake of soy-rich diets is associated with a lower incidence of colorectal cancer in Asian countries (IARC, 2008). Moreover, studies show that genistein intake contributes to a reduced risk of brain, prostate and colorectal cancers and inhibits their growth via the suppression of telomerase activity (Ouchi et al., 2005; Perabo et al., 2008; Seibel et al., 2009; Khaw et al., 2012). As with EGCG treatment, our laboratory has also found that genistein can reactivate ERα in MDA-MB-231 breast cancer

The Journal of Experimental Biology (2015) doi:10.1242/jeb.107110

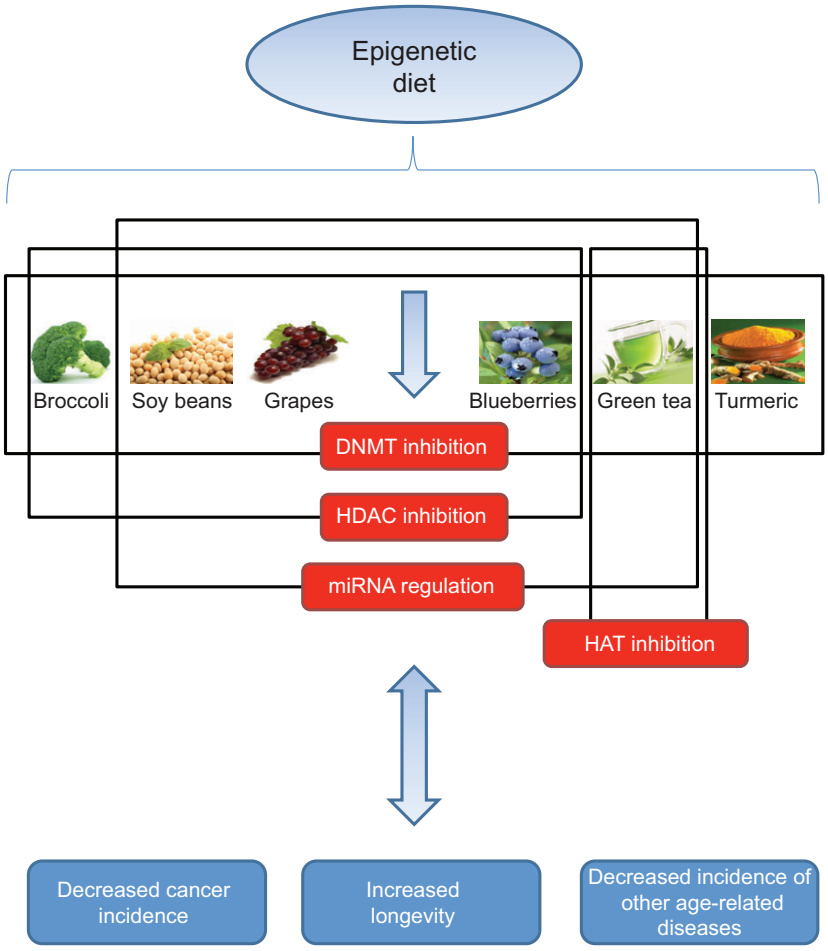


Fig. 3. Effects of an epigenetic diet. Consumption of foods that modulate epigenetic mechanisms has been shown to decrease the incidence of cancer and increase longevity, as well as prevent the onset of other age-related diseases. Cruciferous vegetables, such as broccoli, which are rich in sulforaphane, can act as HDAC inhibitors, regulating certain cancer-related genes. Genistein, which is found in soy beans, also exhibits chemopreventive properties and can result in both the partial reversal of aberrant DNA hypermethylation and the regulation of key miRNAs. Grapes contain resveratrol, a phenol that activates SIRT1 (a known HDAC inhibitor) and increases longevity, mimicking the effects of caloric restriction.

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 CO_2 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 that exhibit a low ERα/ERβ ratio; however, this trend was not seen in MCF-7 (high ERα/ERβ ratio) and MDA-MB-231 (ER-negative) breast cancer 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 epigenome 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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References

Aggarwal, B. B. and Gehlot, P. (2009). Inflammation and cancer: how friendly is the relationship for cancer patients? *Curr. Opin. Pharmacol.* **9**, 351-369.

Aggarwal, B. B. and Shishodia, S. (2006). Molecular targets of dietary agents for prevention and therapy of cancer. *Biochem. Pharmacol.* **71**, 1397-1421.

Antequera, F. and Bird, A. (1993). Number of CpG islands and genes in human and mouse. *Proc. Natl. Acad. Sci. USA* **90**, 11995-11999.

Ashizawa, N., Yahata, T., Quan, J., Adachi, S., Yoshihara, K. and Tanaka, K. (2010). Serum leptin-adiponectin ratio and endometrial cancer risk in postmenopausal female subjects. *Gynecol. Oncol.* **119**, 65-69.

Athar, M., Back, J. H., Kopelovich, L., Bickers, D. R. and Kim, A. L. (2009). Multiple molecular targets of resveratrol: Anti-carcinogenic mechanisms. *Arch. Biochem. Biophys.* **486**, 95-102.

Ayissi, V. B., Ebrahimi, A. and Schluesenner, H. (2014). Epigenetic effects of natural polyphenols: a focus on SIRT1-mediated mechanisms. *Mol. Nutr. Food Res.* **58**, 22-32.

Barger, J. L., Kayo, T., Vann, J. M., Arias, E. B., Wang, J., Hacker, T. A., Wang, Y., Raederstorff, D., Morrow, J. D., Leeuwenburgh, C. et al. (2008). A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice. *PLoS ONE* **3**, e2264.

Barh, D., Malhotra, R., Ravi, B. and Sindhurani, P. (2010). MicroRNA let-7: an emerging next-generation cancer therapeutic. *Curr. Oncol.* **17**, 70-80.

Barlev, N. A., Liu, L., Chehab, N. H., Mansfield, K., Harris, K. G., Halazonetis, T. D. and Berger, S. L. (2001). Acetylation of p53 activates transcription through recruitment of coactivators/histone acetyltransferases. *Mol. Cell* **8**, 1243-1254.

Barnes, S. (1995). Effect of genistein on in vitro and in vivo models of cancer. *J. Nutr.* **125 Suppl.**, 777S-783S.

Baur, J. A., Ungvari, Z., Minor, R. K., Le Couteur, D. G. and de Cabo, R. (2012). Are sirtuins viable targets for improving healthspan and lifespan? *Nat. Rev. Drug Discov.* **11**, 443-461.

Baylin, S. B. and Ohm, J. E. (2006). Epigenetic gene silencing in cancer – a mechanism for early oncogenic pathway addiction? *Nat. Rev. Cancer* **6**, 107-116.

Bazarov, A. V., Van Sluis, M., Hines, W. C., Bassett, E., Beliveau, A., Campeau, E., Mukhopadhyay, R., Lee, W. J., Melodyev, S., Zaslavsky, Y. et al. (2010). p16(INK4a)-mediated suppression of telomerase in normal and malignant human breast cells. *Aging Cell* **9**, 736-746.

Berletch, J. B., Liu, C., Love, W. K., Andrews, L. G., Katiyar, S. K. and Tollefsbol, T. O. (2008). Epigenetic and genetic mechanisms contribute to telomerase inhibition by EGCG. *J. Cell. Biochem.* **103**, 509-519.

Bestor, T. H. (2000). The DNA methyltransferases of mammals. *Hum. Mol. Genet.* **9**, 2395-2402.

Bordone, L. and Guarente, L. (2005). Calorie restriction, SIRT1 and metabolism: understanding longevity. *Nat. Rev. Mol. Cell Biol.* **6**, 298-305.

Boros, L. G., Bassilian, S., Lim, S. and Lee, W. N. (2001). Genistein inhibits nonoxidative ribose synthesis in MIA pancreatic adenocarcinoma cells: a new mechanism of controlling tumor growth. *Pancreas* **22**, 1-7.

Borra, M. T., Smith, B. C. and Denu, J. M. (2005). Mechanism of human SIRT1 activation by resveratrol. *J. Biol. Chem.* **280**, 17187-17195.

Brosh, R. and Rotter, V. (2009). When mutants gain new powers: news from the mutant p53 field. *Nat. Rev. Cancer* **9**, 701-713.

Brunet, A., Sweeney, L. B., Sturgill, J. F., Chua, K. F., Greer, P. L., Lin, Y., Tran, H., Ross, S. E., Mostoslavsky, R., Cohen, H. Y. et al. (2004). Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* **303**, 2011-2015.

Chen, D. C., Chung, Y. F., Yeh, Y. T., Chaung, H. C., Kuo, F. C., Fu, O. Y., Chen, H. Y., Hou, M. F. and Yuan, S. S. (2006). Serum adiponectin and leptin levels in Taiwanese breast cancer patients. *Cancer Lett.* **237**, 109-114.

Chen, Y., Zaman, M. S., Deng, G., Majid, S., Saini, S., Liu, J., Tanaka, Y. and Dahiya, R. (2011). MicroRNAs 221/222 and genistein-mediated regulation of ARHI tumor suppressor gene in prostate cancer. *Cancer Prev. Res. (Phila.)* **4**, 76-86.

Chen, Y. W., Kao, S. Y., Wang, H. J. and Yang, M. H. (2013). Histone modification patterns correlate with patient outcome in oral squamous cell carcinoma. *Cancer* **119**, 4259-4267.

Chervona, Y. and Costa, M. (2012). Histone modifications and cancer: biomarkers of prognosis? *Am J Cancer Res* **2**, 589-597.

Choi, S. W. and Friso, S. (2010). Epigenetics: a new bridge between nutrition and health. *Adv. Nutr.* **1**, 8-16.

Choi, J. D. and Lee, J. S. (2013). Interplay between epigenetics and genetics in cancer. *Genomics Inform* **11**, 164-173.

Clayton, A. L., Hazzalin, C. A. and Mahadevan, L. C. (2006). Enhanced histone acetylation and transcription: a dynamic perspective. *Mol. Cell* **23**, 289-296.

Cleary, M. P., Ray, A., Rogozina, O. P., Dogan, S. and Grossmann, M. E. (2009). Targeting the adiponectin:leptin ratio for postmenopausal breast cancer prevention. *Front. Biosci. (Schol. Ed.)* **1**, 329-357.

Colman, R. J., Anderson, R. M., Johnson, S. C., Kastman, E. K., Kosmatka, K. J., Beasley, T. M., Allison, D. B., Cruzen, C., Simmons, H. A., Kemnitz, J. W. et al. (2009). Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* **325**, 201-204.

Cooks, T., Pateras, I. S., Tarcic, O., Solomon, H., Schetter, A. J., Wilder, S., Lozano, G., Pikarsky, E., Forshew, T., Rosenfeld, N. et al. (2013). Mutant p53 prolongs NF-κB activation and promotes chronic inflammation and inflammation-associated colorectal cancer. *Cancer Cell* **23**, 634-646.

Cruzen, C. and Colman, R. J. (2009). Effects of caloric restriction on cardiovascular aging in non-human primates and humans. *Clin. Geriatr. Med.* **25**, 733-743, ix-x.

Dai, C. Y. and Enders, G. H. (2000). p16 INK4a can initiate an autonomous senescence program. *Oncogene* **19**, 1613-1622.

Daniel, M., Peek, G. W. and Tollefsbol, T. O. (2012). Regulation of the human catalytic subunit of telomerase (hTERT). *Gene* **498**, 135-146.

Das, P. M. and Singal, R. (2004). DNA methylation and cancer. *J. Clin. Oncol.* **22**, 4632-4642.
Dashwood, R. H. and Ho, E. (2008). Dietary agents as histone deacetylase inhibitors: sulforaphane and structurally related isothiocyanates. *Nutr. Rev.* **66 Suppl. 1**, S36-S38.
Dickson, M. A., Hahn, W. C., Ino, Y., Ronfard, V., Wu, J. Y., Weinberg, R. A., Louis, D. N., Li, F. P. and Rheinwald, J. G. (2000). Human keratinocytes that express hTERT and also bypass a p16(INK4a)-enforced mechanism that limits life span become immortal yet retain normal growth and differentiation characteristics. *Mol. Cell. Biol.* **20**, 1436-1447.
Dueñas-González, A., Lizano, M., Candelaria, M., Cetina, L., Arce, C. and Cervera, E. (2005). Epigenetics of cervical cancer. An overview and therapeutic perspectives. *Mol. Cancer* **4**, 38.
Elsheikh, S. E., Green, A. R., Rakha, E. A., Powe, D. G., Ahmed, R. A., Collins, H. M., Soria, D., Garibaldi, J. M., Paish, C. E., Ammar, A. A. et al. (2009). Global histone modifications in breast cancer correlate with tumor phenotypes, prognostic factors, and patient outcome. *Cancer Res.* **69**, 3802-3809.
Ezaki, J., Matsumoto, N., Takeda-Ezaki, M., Komatsu, M., Takahashi, K., Hiraoka, Y., Taka, H., Fujimura, T., Takehana, K., Yoshida, M. et al. (2011). Liver autophagy contributes to the maintenance of blood glucose and amino acid levels. *Autophagy* **7**, 727-736.
Fang, M. Z., Wang, Y., Ai, N., Hou, Z., Sun, Y., Lu, H., Welsh, W. and Yang, C. S. (2003). Tea polyphenol (-)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. *Cancer Res.* **63**, 7563-7570.
Fang, M. Z., Chen, D., Sun, Y., Jin, Z., Christman, J. K. and Yang, C. S. (2005). Reversal of hypermethylation and reactivation of p16INK4a, RARbeta, and MGMT genes by genistein and other isoflavones from soy. *Clin. Cancer Res.* **11**, 7033-7041.
Feinberg, A. P. and Vogelstein, B. (1983). Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature* **301**, 89-92.
Ferdin, J., Kunej, T. and Calin, G. A. (2010). Non-coding RNAs: identification of cancer-associated microRNAs by gene profiling. *Technol. Cancer Res. Treat.* **9**, 123-138.
Fimognari, C., Lenzi, M. and Hrelia, P. (2008). Chemoprevention of cancer by isothiocyanates and anthocyanins: mechanisms of action and structure-activity relationship. *Curr. Med. Chem.* **15**, 440-447.
Fontana, L. and Klein, S. (2007). Aging, adiposity, and calorie restriction. *JAMA* **297**, 986-994.
Fraga, M. F., Ballestar, E., Villar-Garea, A., Boix-Chornet, M., Espada, J., Schotta, G., Bonaldi, T., Haydon, C., Ropero, S., Petrie, K. et al. (2005). Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nat. Genet.* **37**, 391-400.
Freed-Pastor, W. A. and Prives, C. (2012). Mutant p53: one name, many proteins. *Genes Dev.* **26**, 1268-1286.
Gallagher, E. J., Fierz, Y., Ferguson, R. D. and LeRoith, D. (2010). The pathway from diabetes and obesity to cancer, on the route to targeted therapy. *Endocr. Pract.* **16**, 864-873.
Gaudet, F., Hodgson, J. G., Eden, A., Jackson-Grusby, L., Dausman, J., Gray, J. W., Leonhardt, H. and Jaenisch, R. (2003). Induction of tumors in mice by genomic hypomethylation. *Science* **300**, 489-492.
Giannakou, M. E. and Partridge, L. (2004). The interaction between FOXO and SIRT1: tipping the balance towards survival. *Trends Cell Biol.* **14**, 408-412.
Gil, J. and Peters, G. (2006). Regulation of the INK4b-ARF-INK4a tumour suppressor locus: all for one or one for all. *Nat. Rev. Mol. Cell Biol.* **7**, 667-677.
Greco, T., Shafer, J. and Fiskum, G. (2011). Sulforaphane inhibits mitochondrial permeability transition and oxidative stress. *Free Radic. Biol. Med.* **51**, 2164-2171.
Greer, E. L. and Brunet, A. (2008). FOXO transcription factors in ageing and cancer. *Acta Physiol. (Oxf.)* **192**, 19-28.
Guerrero-Preston, R., Santella, R. M., Blanco, A., Desai, M., Berdasco, M. and Fraga, M. (2007). Global DNA hypomethylation in liver cancer cases and controls: a phase I preclinical biomarker development study. *Epigenetics* **2**, 223-226.
Halkidou, K., Gaughan, L., Cook, S., Leung, H. Y., Neal, D. E. and Robson, C. N. (2004). Upregulation and nuclear recruitment of HDAC1 in hormone refractory prostate cancer. *Prostate* **59**, 177-189.
Hardy, T. M. and Tollefsbol, T. O. (2011). Epigenetic diet: impact on the epigenome and cancer. *Epigenomics* **3**, 503-518.
Harper, J. M., Leathers, C. W. and Austad, S. N. (2006). Does caloric restriction extend life in wild mice? *Aging Cell* **5**, 441-449.
Harvey, A. E., Lashinger, L. M. and Hursting, S. D. (2011). The growing challenge of obesity and cancer: an inflammatory issue. *Ann. N. Y. Acad. Sci.* **1229**, 45-52.
Harvey, A. E., Lashinger, L. M., Otto, G., Nunez, N. P. and Hursting, S. D. (2013). Decreased systemic IGF-1 in response to calorie restriction modulates murine tumor cell growth, nuclear factor-κB activation, and inflammation-related gene expression. *Mol. Carcinog.* **52**, 997-1006.
Hass, B. S., Hart, R. W., Lu, M. H. and Lyn-Cook, B. D. (1993). Effects of caloric restriction in animals on cellular function, oncogene expression, and DNA methylation in vitro. *Mutat. Res.* **295**, 281-289.
Hawkins, P. G. and Morris, K. V. (2008). RNA and transcriptional modulation of gene expression. *Cell Cycle* **7**, 602-607.
Herceg, Z. (2007). Epigenetics and cancer: towards an evaluation of the impact of environmental and dietary factors. *Mutagenesis* **22**, 91-103.
Holloszy, J. O. and Fontana, L. (2007). Caloric restriction in humans. *Exp. Gerontol.* **42**, 709-712.
Hon, G. C., Hawkins, R. D. and Ren, B. (2009). Predictive chromatin signatures in the mammalian genome. *Hum. Mol. Genet.* **18**, R195-R201.
Howitz, K. T., Bitterman, K. J., Cohen, H. Y., Lamming, D. W., Lavu, S., Wood, J. G., Zipkin, R. E., Chung, P., Kisielewski, A., Zhang, L. L. et al. (2003). Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. *Nature* **425**, 191-196.
Huffman, K. (2012). The developing, aging neocortex: how genetics and epigenetics influence early developmental patterning and age-related change. *Front. Genet.* **3**, 212.
Hursting, S. D., Smith, S. M., Lashinger, L. M., Harvey, A. E. and Perkins, S. N. (2010). Calories and carcinogenesis: lessons learned from 30 years of calorie restriction research. *Carcinogenesis* **31**, 83-89.
Hursting, S. D., Dunlap, S. M., Ford, N. A., Hursting, M. J. and Lashinger, L. M. (2013). Calorie restriction and cancer prevention: a mechanistic perspective. *Cancer Metab* **1**, 10.
IARC (2008). Cancer incidence in five continents. *IARC Sci. Publ.* **IX**, 1-837.
Johnston, K., Sharp, P., Clifford, M. and Morgan, L. (2005). Dietary polyphenols decrease glucose uptake by human intestinal Caco-2 cells. *FEBS Lett.* **7**, 1653-1657.
Kagawa, Y. (1978). Impact of Westernization on the nutrition of Japanese: changes in physique, cancer, longevity and centenarians. *Prev. Med.* **7**, 205-217.
Kapahi, P., Chen, D., Rogers, A. N., Katewa, S. D., Li, P. W., Thomas, E. L. and Kockel, L. (2010). With TOR, less is more: a key role for the conserved nutrient-sensing TOR pathway in aging. *Cell Metab.* **11**, 453-465.
Khaw, A. K., Yong, J. W., Kalthur, G. and Hande, M. P. (2012). Genistein induces growth arrest and suppresses telomerase activity in brain tumor cells. *Genes Chromosomes Cancer* **51**, 961-974.
Kiyono, T., Foster, S. A., Koop, J. I., McDougall, J. K., Galloway, D. A. and Klingelhutz, A. J. (1998). Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. *Nature* **396**, 84-88.
Klaus, S., Pültz, S., Thöne-Reineke, C. and Wolfram, S. (2005). Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. *Int. J. Obes.* **29**, 615-623.
Knapowski, J., Wieczorowska-Tobis, K. and Witowski, J. (2002). Pathophysiology of ageing. *J. Physiol. Pharmacol.* **53**, 135-146.
Koki, A., Khan, N. K., Woerner, B. M., Dannenberg, A. J., Olson, L., Seibert, K., Edwards, D., Hardy, M., Isakson, P. and Masferrer, J. L. (2002). Cyclooxygenase-2 in human pathological disease. *Adv. Exp. Med. Biol.* **507**, 177-184.
Koubova, J. and Guarente, L. (2003). How does calorie restriction work? *Genes Dev.* **17**, 313-321.
Kouzarides, T. (2007). Chromatin modifications and their function. *Cell* **128**, 693-705.
Kyo, S., Takakura, M., Fujiwara, T. and Inoue, M. (2008). Understanding and exploiting hTERT promoter regulation for diagnosis and treatment of human cancers. *Cancer Sci.* **99**, 1528-1538.
Lanvin, O., Bianco, S., Kersual, N., Chalbos, D. and Vanacker, J. M. (2007). Potentiation of ICI182,780 (Fulvestrant)-induced estrogen receptor-alpha degradation by the estrogen receptor-related receptor-alpha inverse agonist XCT790. *J. Biol. Chem.* **282**, 28328-28334.
Li, Y. and Tollefsbol, T. O. (2010). Impact on DNA methylation in cancer prevention and therapy by bioactive dietary components. *Curr. Med. Chem.* **17**, 2141-2151.
Li, Y. and Tollefsbol, T. O. (2011). p16(INK4a) suppression by glucose restriction contributes to human cellular lifespan extension through SIRT1-mediated epigenetic and genetic mechanisms. *PLoS ONE* **6**, e17421.
Li, E., Beard, C. and Jaenisch, R. (1993). Role for DNA methylation in genomic imprinting. *Nature* **366**, 362-365.
Li, Y., Liu, L. and Tollefsbol, T. O. (2010a). Glucose restriction can extend normal cell lifespan and impair precancerous cell growth through epigenetic control of hTERT and p16 expression. *FASEB J.* **24**, 1442-1453.
Li, Y., Yuan, Y. Y., Meeran, S. M. and Tollefsbol, T. O. (2010b). Synergistic epigenetic reactivation of estrogen receptor-α (ERα) by combined green tea polyphenol and histone deacetylase inhibitor in ERα-negative breast cancer cells. *Mol. Cancer* **9**, 274.
Li, Y., Daniel, M. and Tollefsbol, T. O. (2011). Epigenetic regulation of caloric restriction in aging. *BMC Med.* **9**, 98.
Li, Y., Meeran, S. M., Patel, S. N., Chen, H., Hardy, T. M. and Tollefsbol, T. O. (2013). Epigenetic reactivation of estrogen receptor-α (ERα) by genistein enhances hormonal therapy sensitivity in ERα-negative breast cancer. *Mol. Cancer* **12**, 9.
Liao, C. Y., Rikke, B. A., Johnson, T. E., Diaz, V. and Nelson, J. F. (2010). Genetic variation in the murine lifespan response to dietary restriction: from life extension to life shortening. *Aging Cell* **9**, 92-95.
Liao, Y. P., Chen, L. Y., Huang, R. L., Su, P. H., Chan, M. W., Chang, C. C., Yu, M. H., Wang, P. H., Yen, M. S., Nephew, K. P. et al. (2014). Hypomethylation signature of tumor-initiating cells predicts poor prognosis of ovarian cancer patients. *Hum. Mol. Genet.* **23**, 1894-1906.
Lin, J. K., Liang, Y. C. and Lin-Shiau, S. Y. (1999). Cancer chemoprevention by tea polyphenols through mitotic signal transduction blockade. *Biochem. Pharmacol.* **58**, 911-915.
Lin, C. H., Hsieh, S. Y., Sheen, I. S., Lee, W. C., Chen, T. C., Shyu, W. C. and Liaw, Y. F. (2001). Genome-wide hypomethylation in hepatocellular carcinogenesis. *Cancer Res.* **61**, 4238-4243.
Link, A., Balaguer, F. and Goel, A. (2010). Cancer chemoprevention by dietary polyphenols: promising role for epigenetics. *Biochem. Pharmacol.* **80**, 1771-1792.
Liu, P. L., Tsai, J. R., Charles, A. L., Hwang, J. J., Chou, S. H., Ping, Y. H., Lin, F. Y., Chen, Y. L., Hung, C. Y., Chen, W. C. et al. (2010). Resveratrol inhibits human lung adenocarcinoma cell metastasis by suppressing heme oxygenase 1-mediated nuclear factor-kappaB pathway and subsequently downregulating expression of matrix metalloproteinases. *Mol. Nutr. Food Res.* **54 Suppl. 2**, S196-S204.

Luger, K., Mäder, A. W., Richmond, R. K., Sargent, D. F. and Richmond, T. J. (1997). Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **389**, 251-260.
Lujambio, A., Ropero, S., Ballestar, E., Fraga, M. F., Cerrato, C., Setién, F., Casado, S., Suarez-Gauthier, A., Sanchez-Cespedes, M., Git, A. et al. (2007). Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. *Cancer Res.* **67**, 1424-1429.
Mao, Q. Q., Bai, Y., Lin, Y. W., Zheng, X. Y., Qin, J., Yang, K. and Xie, L. P. (2010). Resveratrol confers resistance against taxol via induction of cell cycle arrest in human cancer cell lines. *Mol. Nutr. Food Res.* **54**, 1574-1584.
Martin, S. L., Hardy, T. M. and Tollefsbol, T. O. (2013). Medicinal chemistry of the epigenetic diet and caloric restriction. *Curr. Med. Chem.* **20**, 4050-4059.
Mathers, J. C., Strathdee, G. and Relton, C. L. (2010). Induction of epigenetic alterations by dietary and other environmental factors. *Adv. Genet.* **71**, 3-39.
Mattison, J. A., Roth, G. S., Beasley, T. M., Tilmont, E. M., Handy, A. M., Herbert, R. L., Longo, D. L., Allison, D. B., Young, J. E., Bryant, M. et al. (2012). Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. *Nature* **489**, 318-321.
McCay, C. M., Crowell, M. F. and Maynard, L. A. (1989). The effect of retarded growth upon the length of life span and upon the ultimate body size. 1935. *Nutrition* **5**, 155-171, discussion 172.
Meeran, S. M., Patel, S. N. and Tollefsbol, T. O. (2010). Sulforaphane causes epigenetic repression of hTERT expression in human breast cancer cell lines. *PLoS ONE* **5**, e11457.
Mercken, E. M., Crosby, S. D., Lamming, D. W., JeBailey, L., Krzysik-Walker, S., Villareal, D. T., Capri, M., Franceschi, C., Zhang, Y., Becker, K. et al. (2013). Calorie restriction in humans inhibits the PI3K/AKT pathway and induces a younger transcription profile. *Aging Cell* **12**, 645-651.
Mercken, E. M., Hu, J., Krzysik-Walker, S., Wei, M., Li, Y., McBurney, M. W., de Cabo, R. and Longo, V. D. (2014). SIRT1 but not its increased expression is essential for lifespan extension in caloric-restricted mice. *Aging Cell* **13**, 193-196.
Messina, M., McCaskill-Stevens, W. and Lampe, J. W. (2006). Addressing the soy and breast cancer relationship: review, commentary, and workshop proceedings. *J. Natl. Cancer Inst.* **98**, 1275-1284.
Michels, K. B. and Ekbom, A. (2004). Caloric restriction and incidence of breast cancer. *JAMA* **291**, 1226-1230.
Milenkovic, D., Deval, C., Gouranton, E., Landrier, J. F., Scalbert, A., Morand, C. and Mazur, A. (2012). Modulation of miRNA expression by dietary polyphenols in apoE deficient mice: a new mechanism of the action of polyphenols. *PLoS ONE* **7**, e29837.
Montgomery, M. K., Xu, S. and Fire, A. (1998). RNA as a target of double-stranded RNA-mediated genetic interference in Caenorhabditis elegans. *Proc. Natl. Acad. Sci. USA* **95**, 15502-15507.
Morton, J. P., Timpson, P., Karim, S. A., Ridgway, R. A., Athineos, D., Doyle, B., Jamieson, N. B., Oien, K. A., Lowy, A. M., Brunton, V. G. et al. (2010). Mutant p53 drives metastasis and overcomes growth arrest/senescence in pancreatic cancer. *Proc. Natl. Acad. Sci. USA* **107**, 246-251.
Muñoz-Najar, U. and Sedivy, J. M. (2011). Epigenetic control of aging. *Antioxid. Redox Signal.* **14**, 241-259.
Murphy, M., Ahn, J., Walker, K. K., Hoffman, W. H., Evans, R. M., Levine, A. J. and George, D. L. (1999). Transcriptional repression by wild-type p53 utilizes histone deacetylases, mediated by interaction with mSin3a. *Genes Dev.* **13**, 2490-2501.
Nadal-Serrano, M., Pons, D. G., Sastre-Serra, J., Blanquer-Rosselló, M. M., Roca, P. and Oliver, J. (2013). Genistein modulates oxidative stress in breast cancer cell lines according to ERα/ERβ ratio: effects on mitochondrial functionality, sirtuins, uncoupling protein 2 and antioxidant enzymes. *Int. J. Biochem. Cell Biol.* **45**, 2045-2051.
Narita, M., Nũnez, S., Heard, E., Narita, M., Lin, A. W., Hearn, S. A., Spector, D. L., Hannon, G. J. and Lowe, S. W. (2003). Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell* **113**, 703-716.
Olivier, M., Langerød, A., Carrieri, P., Bergh, J., Klaar, S., Eyfjord, J., Theillet, C., Rodriguez, C., Lidereau, R., Bièche, I. et al. (2006). The clinical value of somatic TP53 gene mutations in 1,794 patients with breast cancer. *Clin. Cancer Res.* **12**, 1157-1167.
Ono, M. (2008). Molecular links between tumor angiogenesis and inflammation: inflammatory stimuli of macrophages and cancer cells as targets for therapeutic strategy. *Cancer Sci.* **99**, 1501-1506.
Ouchi, H., Ishiguro, H., Ikeda, N., Hori, M., Kubota, Y. and Uemura, H. (2005). Genistein induces cell growth inhibition in prostate cancer through the suppression of telomerase activity. *Int. J. Urol.* **12**, 73-80.
Pearson, K. J., Baur, J. A., Lewis, K. N., Peshkin, L., Price, N. L., Labinskyy, N., Swindell, W. R., Kamara, D., Minor, R. K., Perez, E. et al. (2008). Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. *Cell Metab.* **8**, 157-168.
Perabo, F. G., Von Löw, E. C., Ellinger, J., von Rücker, A., Müller, S. C. and Bastian, P. J. (2008). Soy isoflavone genistein in prevention and treatment of prostate cancer. *Prostate Cancer Prostatic Dis.* **11**, 6-12.
Pericleous, M., Mandair, D. and Caplin, M. E. (2013). Diet and supplements and their impact on colorectal cancer. *J. Gastrointest. Oncol.* **4**, 409-423.
Pons, D. G., Nadal-Serrano, M., Blanquer-Rossello, M. M., Sastre-Serra, J., Oliver, J. and Roca, P. (2014). Genistein modulates proliferation and mitochondrial functionality in breast cancer cells depending on ERalpha/ERbeta ratio. *J. Cell. Biochem.* **115**, 949-958.
Prives, C. and White, E. (2008). Does control of mutant p53 by Mdm2 complicate cancer therapy? *Genes Dev.* **22**, 1259-1264.
Qin, W., Zhu, W., Shi, H., Hewett, J. E., Ruhlen, R. L., MacDonald, R. S., Rottinghaus, G. E., Chen, Y. C. and Sauter, E. R. (2009). Soy isoflavones have an antiestrogenic effect and alter mammary promoter hypermethylation in healthy premenopausal women. *Nutr. Cancer* **61**, 238-244.
Razin, A. and Riggs, A. D. (1980). DNA methylation and gene function. *Science* **210**, 604-610.
Rebrin, I., Kamzalov, S. and Sohal, R. S. (2003). Effects of age and caloric restriction on glutathione redox state in mice. *Free Radic. Biol. Med.* **35**, 626-635.
Renaud, S., Loukinov, D., Abdullaev, Z., Guilleret, I., Bosman, F. T., Lobanenkov, V. and Benhattar, J. (2007). Dual role of DNA methylation inside and outside of CTCF-binding regions in the transcriptional regulation of the telomerase hTERT gene. *Nucleic Acids Res.* **35**, 1245-1256.
Ribarič, S. (2012). Diet and aging. *Oxid. Med. Cell. Longev.* **2012**, 741468.
Rivlin, N., Brosh, R., Oren, M. and Rotter, V. (2011). Mutations in the p53 tumor suppressor gene: important milestones at the various steps of tumorigenesis. *Genes Cancer* **2**, 466-474.
Rodriguez, O. C., Choudhury, S., Kolukula, V., Vietsch, E. E., Catania, J., Preet, A., Reynoso, K., Bargonetti, J., Wellstein, A., Albanese, C. et al. (2012). Dietary downregulation of mutant p53 levels via glucose restriction: mechanisms and implications for tumor therapy. *Cell Cycle* **11**, 4436-4446.
Rohren, E. M., Turkington, T. G. and Coleman, R. E. (2004). Clinical applications of PET in oncology. *Radiology* **231**, 305-332.
Ross, S. A. and Davis, C. D. (2011). MicroRNA, nutrition, and cancer prevention. *Adv Nutr* **2**, 472-485.
Ross, S. A., Dwyer, J., Umar, A., Kagan, J., Verma, M., Van Bemmel, D. M. and Dunn, B. K. (2008). Introduction: diet, epigenetic events and cancer prevention. *Nutr. Rev.* **66 Suppl. 1**, S1-S6.
Roth, G. S., Ingram, D. K. and Lane, M. A. (2001). Caloric restriction in primates and relevance to humans. *Ann. N. Y. Acad. Sci.* **928**, 305-315.
Saito, Y., Liang, G., Egger, G., Friedman, J. M., Chuang, J. C., Coetzee, G. A. and Jones, P. A. (2006). Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. *Cancer Cell* **9**, 435-443.
Seibel, J., Molzberger, A. F., Hertrampf, T., Laudenbach-Leschowski, U. and Diel, P. (2009). Oral treatment with genistein reduces the expression of molecular and biochemical markers of inflammation in a rat model of chronic TNBS-induced colitis. *Eur. J. Nutr.* **48**, 213-220.
Serrano, M., Hannon, G. J. and Beach, D. (1993). A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature* **366**, 704-707.
Shanmugam, M. K., Kannaiyan, R. and Sethi, G. (2011). Targeting cell signaling and apoptotic pathways by dietary agents: role in the prevention and treatment of cancer. *Nutr. Cancer* **63**, 161-173.
Shao, G., Balajee, A. S., Hei, T. K. and Zhao, Y. (2008). p16INK4a downregulation is involved in immortalization of primary human prostate epithelial cells induced by telomerase. *Mol. Carcinog.* **47**, 775-783.
Sharpless, N. E. (2005). INK4a/ARF: a multifunctional tumor suppressor locus. *Mutat. Res.* **576**, 22-38.
Siddiqui, I. A., Asim, M., Hafeez, B. B., Adhami, V. M., Tarapore, R. S. and Mukhtar, H. (2011). Green tea polyphenol EGCG blunts androgen receptor function in prostate cancer. *FASEB J.* **25**, 1198-1207.
Simon, C., Chagraoui, J., Krosl, J., Gendron, P., Wilhelm, B., Lemieux, S., Boucher, G., Chagnon, P., Drouin, S., Lambert, R. et al. (2012). A key role for EZH2 and associated genes in mouse and human adult T-cell acute leukemia. *Genes Dev.* **26**, 651-656.
Singhal, R. P., Mays-Hoopes, L. L. and Eichhorn, G. L. (1987). DNA methylation in aging of mice. *Mech. Ageing Dev.* **41**, 199-210.
Sohal, R. S. and Weindruch, R. (1996). Oxidative stress, caloric restriction, and aging. *Science* **273**, 59-63.
Song, J., Noh, J. H., Lee, J. H., Eun, J. W., Ahn, Y. M., Kim, S. Y., Lee, S. H., Park, W. S., Yoo, N. J., Lee, J. Y. et al. (2005). Increased expression of histone deacetylase 2 is found in human gastric cancer. *APMIS* **113**, 264-268.
Stefanska, B., Rudnicka, K., Bednarek, A. and Fabianowska-Majewska, K. (2010). Hypomethylation and induction of retinoic acid receptor beta 2 by concurrent action of adenosine analogues and natural compounds in breast cancer cells. *Eur. J. Pharmacol.* **638**, 47-53.
Sun, Q., Cong, R., Yan, H., Gu, H., Zeng, Y., Liu, N., Chen, J. and Wang, B. (2009). Genistein inhibits growth of human uveal melanoma cells and affects microRNA-27a and target gene expression. *Oncol. Rep.* **22**, 563-567.
Tan, Y., Zhang, B., Wu, T., Skogerbø, G., Zhu, X., Guo, X., He, S. and Chen, R. (2009). Transcriptional inhibiton of Hoxd4 expression by miRNA-10a in human breast cancer cells. *BMC Mol. Biol.* **10**, 12.
Tasdemir, E., Chiara Maiuri, M., Morselli, E., Criollo, A., D'Amelio, M., Djavaheri-Mergny, M., Cecconi, F., Tavernarakis, N. and Kroemer, G. (2008). A dual role of p53 in the control of autophagy. *Autophagy* **4**, 810-814.
Telang, U., Brazeau, D. A. and Morris, M. E. (2009). Comparison of the effects of phenethyl isothiocyanate and sulforaphane on gene expression in breast cancer and normal mammary epithelial cells. *Exp. Biol. Med. (Maywood)* **234**, 287-295.
Tlsty, T. D., Crawford, Y. G., Holst, C. R., Fordyce, C. A., Zhang, J., McDermott, K., Kozakiewicz, K. and Gauthier, M. L. (2004). Genetic and epigenetic changes in mammary epithelial cells may mimic early events in carcinogenesis. *J. Mammary Gland Biol. Neoplasia* **9**, 263-274.
Tollefsbol, T. O. (2014). Dietary epigenetics in cancer and aging. *Cancer Treat. Res.* **159**, 257-267.
Tsang, W. P. and Kwok, T. T. (2010). Epigallocatechin gallate up-regulation of miR-16 and induction of apoptosis in human cancer cells. *J. Nutr. Biochem.* **21**, 140-146.

Tsou, J. A., Hagen, J. A., Carpenter, C. L. and Laird-Offringa, I. A. (2002). DNA methylation analysis: a powerful new tool for lung cancer diagnosis. *Oncogene* **21**, 5450-5461.

Tu, S. H., Ku, C. Y., Ho, C. T., Chen, C. S., Huang, C. S., Lee, C. H., Chen, L. C., Pan, M. H., Chang, H. W., Chang, C. H. et al. (2011). Tea polyphenol (-)-epigallocatechin-3-gallate inhibits nicotine- and estrogen-induced α9-nicotinic acetylcholine receptor upregulation in human breast cancer cells. *Mol. Nutr. Food Res.* **55**, 455-466.

Turturro, A., Witt, W. W., Lewis, S., Hass, B. S., Lipman, R. D. and Hart, R. W. (1999). Growth curves and survival characteristics of the animals used in the biomarkers of aging program. *J. Gerontol. A Biol. Sci. Med. Sci.* **54**, B492-B501.

Valenzano, D. R., Terzibasi, E., Genade, T., Cattaneo, A., Domenici, L. and Cellerino, A. (2006). Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. *Curr. Biol.* **16**, 296-300.

Valls, J., Millán, S., Martí, M. P., Borràs, E. and Arola, L. (2009). Advanced separation methods of food anthocyanins, isoflavones and flavanols. *J. Chromatogr. A* **1216**, 7143-7172.

Vanamala, J., Reddivari, L., Radhakrishnan, S. and Tarver, C. (2010). Resveratrol suppresses IGF-1 induced human colon cancer cell proliferation and elevates apoptosis via suppression of IGF-1R/Wnt and activation of p53 signaling pathways. *BMC Cancer* **10**, 238.

Vera, E., Bernardes de Jesus, B., Foronda, M., Flores, J. M. and Blasco, M. A. (2013). Telomerase reverse transcriptase synergizes with calorie restriction to increase health span and extend mouse longevity. *PLoS ONE* **8**, e53760.

Vrba, L., Junk, D. J., Novak, P. and Futscher, B. W. (2008). p53 induces distinct epigenetic states at its direct target promoters. *BMC Genomics* **9**, 486.

Waddington, C. H. (2012). The epigenotype. *Int. J. Epidemiol.* **41**, 10-13.

Waki, T., Tamura, G., Sato, M. and Motoyama, T. (2003). Age-related methylation of tumor suppressor and tumor-related genes: an analysis of autopsy samples. *Oncogene* **22**, 4128-4133.

Wang, R. H., Zheng, Y., Kim, H. S., Xu, X., Cao, L., Luhasen, T., Lee, M. H., Xiao, C., Vassilopoulos, A., Chen, W. et al. (2008). Interplay among BRCA1, SIRT1, and Survivin during BRCA1-associated tumorigenesis. *Mol. Cell* **32**, 11-20.

Warburg, O. (1956). On the origin of cancer cells. *Science* **123**, 309-314.

Willcox, B. J., Willcox, D. C., Todoriki, H., Fujiyoshi, A., Yano, K., He, Q., Curb, J. D. and Suzuki, M. (2007). Caloric restriction, the traditional Okinawan diet, and healthy aging: the diet of the world's longest-lived people and its potential impact on morbidity and life span. *Ann. N. Y. Acad. Sci.* **1114**, 434-455.

Wilson, V. L., Smith, R. A., Ma, S. and Cutler, R. G. (1987). Genomic 5-methyldeoxycytidine decreases with age. *J. Biol. Chem.* **262**, 9948-9951.

Yang, X., Yan, L. and Davidson, N. E. (2001). DNA methylation in breast cancer. *Endocr. Relat. Cancer* **8**, 115-127.

Yang, N., Coukos, G. and Zhang, L. (2008). MicroRNA epigenetic alterations in human cancer: one step forward in diagnosis and treatment. *Int. J. Cancer* **122**, 963-968.

Yang, B., Sun, H., Lin, W., Hou, W., Li, H., Zhang, L., Li, F., Gu, Y., Song, Y., Li, Q. et al. (2013). Evaluation of global DNA hypomethylation in human prostate cancer and prostatic intraepithelial neoplasm tissues by immunohistochemistry. *Urol. Oncol.* **31**, 628-634.

Yun, J., Rago, C., Cheong, I., Pagliarini, R., Angenendt, P., Rajagopalan, H., Schmidt, K., Willson, J. K., Markowitz, S., Zhou, S. et al. (2009). Glucose deprivation contributes to the development of KRAS pathway mutations in tumor cells. *Science* **325**, 1555-1559.

Zhao, Y., Deng, C., Wang, J., Xiao, J., Gatalica, Z., Recker, R. R. and Xiao, G. G. (2011). Let-7 family miRNAs regulate estrogen receptor alpha signaling in estrogen receptor positive breast cancer. *Breast Cancer Res. Treat.* **127**, 69-80.