CHAPTER 13

The epigenetics of aging

Athanasios Metaxakis[1], Ilias Gkikas[1,2] and Nektarios Tavernarakis[1,3]
[1]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Heraklion, Greece
[2]Department of Biology, University of Crete, Heraklion, Greece
[3]Department of Basic Sciences, Faculty of Medicine, University of Crete, Heraklion, Greece

13.1 Introduction

After three decades of extensive research on the molecular biology of aging, we can now specify several molecular and cellular processes that accelerate or delay aging in animal model systems. Molecular mechanisms that affect metabolism, caloric and dietary consumption, genomic stability, telomere attrition, autophagy and epigenetic alterations are the major anti-aging interventions shown so far to extend longevity, increase healthspan and delay the onset of age-related pathologies in animals.[1] Among them, the most complicated and least investigated are the epigenetic alterations that progress with aging. These are described as reversible alterations of chromatin that are heritable, but do not affect underlying DNA sequences and, consequently, permanent genetic information. Several causes, such as diet, genes, environmental and lifestyle factors influence epigenetic alterations, which, together with genetic information, preordain lifespan in animals. Consequently, differential epigenetic regulation of genetic information can sufficiently explain differences in longevity of identical twins and animals with the same genetic background. Several studies have shown that epigenetic information changes through aging and, most importantly, that these changes are associated with age-related progressive physiological deterioration and the development of age-related diseases, such as cancer, neurodegeneration and cardiovascular diseases.[2–4] Although the mechanisms underlying both the impact of aging on epigenetic alterations and of the latest age-related physiological decline are not fully understood, research on epigenetic phenomena that occur with age can provide novel anti-aging therapeutic approaches. This is due to the reversibility of epigenetic changes via the administration of drugs that correct these chemical modifications on proteins and nucleic acids.

Epigenetic alterations include various modifications of chromatin components, histones and DNA. These changes are described as the "epigenome" and can be even passed down to the offspring and impact their health in a transgenerational manner.[5] Histone modifications, chromatin remodeling, DNA methylation and altered expression of non-coding RNA molecules (ncRNAs) constitute epigenetic alterations.

Aging
DOI: https://doi.org/10.1016/B978-0-12-823761-8.00027-6

Through altering chromatin accessibility and genomic activity, the epigenome imposes various effects on cellular function. Chromatin activity, level of protein expression, the activity of transposable elements, integrity of telomeres and the stability of the genome have been suggested to mediate the effects of the epigenome on health and lifespan.

In this review, we will briefly describe the nature of the major epigenetic alterations and their relevance to longevity determination. Furthermore, we will present the primary findings that correlate epigenetic changes with the development of the major age-related diseases, cancer, neurodegenerative and cardiovascular diseases.

13.2 Epigenetic alterations and aging

Genetic activity is largely dependent on the accessibility of transcription factors to DNA. DNA is tightly bound by histone proteins, to compose chromatin. Depending on its flexibility, chromatin can be found in either two forms, euchromatin and heterochromatin. The former consists of a decondensed, highly transcriptable structure, while in the latter, the strong DNA-histone binding does not allow transcription factors to access DNA and ignite transcription. Consequently, genetic and environmental factors that alter chromatin tightness can affect transcription activity.[6] This is achievable through quantitative changes in the expression of histone proteins, expression of histone variants, histone post-translational modifications, such as acetylation and methylation, ATP-dependent remodeling, and DNA methylation. These modifications affect longevity via deregulation of genetic activity and genomic stability. Moreover, altered expression of ncRNAs has a regulatory role on protein translation.

13.2.1 Histone depletion

Several studies show that canonical histone levels are reduced through aging, while ectopic upregulation of histone biosynthesis increases lifespan.[7–11] Age-related changes in telomeres, histone chaperones, and lysosomal activity are suggested to cause histone depletion.[9,10] Strong evidence suggests that the effect of histone depletion on lifespan depends on the nature of the depleted histonic genetic area, and also on the degree of the depletion.[10,11] On the other hand, in mouse tissues and neural stem cells, the expression levels of H3 histone is not significantly changed with aging, but depending on the genomic location, the occupancy of H3 histone is differently affected.[12] As a result, chromatin at pro-inflammatory genes is more accessible and active; an observation that suggests differential nucleosome occupancy as a mechanism for reprogramming genetic expression through aging.

13.2.2 Non-canonical histone variants

With the exception of gradual histone depletion with age, non-canonical histone isoforms are increasingly expressed with age, such as the histone variants H3.3 and H2A.Z, accompanied by the downregulation of canonical histones.[13–17] For example, the H2A histone variant, H2A.J, accumulates with aging in mouse tissues and human skin.[18] H2A.J overexpression activates inflammatory genes, induces senescent-associated phenotypes and is suspected to contribute to the development of age-related chronic inflammation and diseases. Hence, not only quantitative, but also qualitative age-dependent alterations in histone expression affect healthspan.

13.2.3 Histone acetylation

A major chemical modification that alters the histone-DNA binding strength is acetylation of histonic lysine domains. The positively charged lysine domains significantly contribute to the attachment of histones on DNA. As a result, any chemical change that reduces the positive charge of lysine, weakens the interaction between histones and DNA. Such a chemical modification is the addition of acetyl moiety on the ε-amino groups of lysine, which neutralizes its positive charge and reduces histone-DNA interactions. Transfer of acetyl moiety is catalyzed by histone acetyltransferases (HATs), while deacetylation is catalyzed by histone deacetylases (HDACs). Activity of the HATs loosens histone-DNA interactions and increases transcription, while activity of HDACs has the opposite effect. Several reports indicate the importance of histone acetylation on longevity. Loss of HATs Gcn5, CREB-binding protein (CBP), and RTT109 in yeast, *Caenorhabditis elegans* and *Drosophila melanogaster* reduces longevity, while loss of members of the *sirtuin* genes, coding for evolutionary conserved NAD^+-dependent deacetylases, are associated with longevity extension in invertebrates and vertebrates.[19–25] CBP activity is reduced through aging and correlated with lifespan in several mice strains. In support, lifespan extension by dietary restriction (DR) in *C. elegans* is inhibited by the loss of the *cbp-1* gene, thus linking DR-induced longevity enhancement with increased acetylation. In addition, loss of acetyltransferase Gcn5 in yeast decreases lifespan through impeding interplay of metabolism and stress responses, chromatin-dependent gene regulation and genome stability. Contrarily, downregulation of the histone H4K12-specific acetyltransferase Chameau extends longevity in flies, through uncoupling age-related metabolic alterations from transcriptional regulation.[26]

Maybe the most remarkable examples that highlight the importance of histone acetylation on longevity determination come from studies on the activity of the Sirtuin deacetylases.[27] Sirtuins are involved in the regulation of cell metabolism, DNA repair, inflammation and apoptosis.[28] In yeast, deletion of the histone deacetylase gene *rpd3* and upregulation of the *Sir2* gene, which is activated by caloric restriction, extend lifespan.[27]

Similarly, downregulation of histone acetyltransferase Sas2 increases lifespan in yeast.[29] Similar effects have been described in worms, flies, mice and cells, thus showing that these findings are evolutionarily conserved.[19,20,22–24,30–32] Mechanistically, Sir2 maintains chromatin silencing through deacetylation of the residues H4K16 and H4K56 and recruitment of other silencing proteins. Sir2 protein levels decrease with aging, while H4K16 acetylation increases and histone abundance diminishes at subtelomeric regions. The above are suggestive for an abnormal upregulation of transcription at these loci, which is associated with the development of aging phenotypes.[29,33]

Some histone acetylation sites have been reported to be more important for lifespan determination, such as the H4K16. Sas2 targets H4K16 sites at the boundaries of euchromatin with telomeric regions and H4K16 hypoacetylation has been associated with defective DNA repair and premature senescence in mice.[34–36] Lifespan extension in flies via Chameau downregulation has been attributed to H4K12 hypoacetylation; deficiency of SIRT6 deacetylase promotes aging in mice via altered acetylation at H3K9 and H3K56, which cause telomeres dysfunction. H3K56 acetylation levels are critical for longevity in yeast, as in H4 N-terminal acetylation, which is regulated by caloric restriction.[7,23,26,29,37–39]

13.2.4 Histone methylation

Another type of histone modification that occurs with aging is histone methylation (HMT). Similar to histone acetylation, HMT is catalyzed by the addition of a methyl group by histone methyltransferases, while removal of methyl groups is catalyzed by histone demethylases. Depending on the histonic site, methylation can lead to enhanced or reduced transcription.[40] According to the heterochromatin loss model, transcriptionally inactive areas of chromatin become activated through aging, resulting in disparate profiles of gene activity and promoting aging.[41–44] Highly methylated histonic sites, such as H3K9, H4K20 and H3K64, are associated with transcriptional inactivity of heterochromatin.[45–47] Tight interconnection of histone hypomethylation and aging phenotypes is further supported by research in premature aging diseases. Patients with progeria syndromes have decreased expression of histone methyltransferases, reduced methylation at H3K9 and H3K27, loss of heterochromatin and changes in heterochromatin architecture.[44,48] Furthermore, mild mitochondria damage in *C. elegans* and mice induces activity of histone demethylases jmjd-1.2/PHF8 and jmjd-3.1/JMJD3, which delay aging through mitochondrial unfolded protein response (UPRmt).[49] On the other hand, in a mouse progeria model, inhibition of methyltransferase gene Suv39h1 improved DNA repair and increased longevity.[50]

Recent studies suggest a role for specific methylation patterns on longevity. In worms, trimethylation of H3K4 (H3K4me3) increases with aging. Reduction of the ASH-2 Trithorax complex proteins, which activate transcription by inducing

trimethylation of H3K4, decreases H3K4me3 and increases lifespan, while reduction of H3K4 demethylase RBR-2 decreases lifespan.[40,51] Similar results have been observed after downregulation of the ortholog of RBR-2 in flies, the demethylase Lid.[52] Inhibition of another demethylase in flies, the Dmel\Kdm4A H3K9me3 demethylase, reduces lifespan.[53] Trimethylation of H3K9 is abundant in heterochromatin, thus suggesting that alterations in the transcriptional activity of heterochromatin affect lifespan. In support, expression of H3K9me3 methyltransferase SUV39H1 is reduced through aging in mouse and human cells, which causes the reduction of H3K9me3 trimethylation, perturbs heterochromatin function and induces loss of B cell potential.[54] Trimethylation of H3K27 is increased with age and catalyzed by the transcription repressor Polycomb Repressive Complex-2 (PRC2).[55,56] Mutations in subunits of PRC2 in flies reduce H3K27me3, by increasing glycolysis and healthspan.[57,58] On the other hand, in human cells and *C. elegans*, trimethylation of H3K27 is reduced with age.[59–61] Reduction of the UTX-1 H3K27 demethylase in *C. elegans* extends lifespan by affecting the insulin pathway.[59] Accordingly, the link between H3K27me3 and aging is complex and cell type and/or animal model specific. Another methylation site, H3K36, is highly methylated proximally to the 3' end of actively transcribed genes, which is suggestive for a role in transcriptional termination and RNA processing.[62] Loss of H3K36 methyltransferase and mutations at the H3K36 site decrease lifespan in yeast, while loss of the Rph1 H3K36 demethylase increases H3K36me3 and enhances longevity.[63] In this study, the authors concluded that increased methylation at H3K36 suppresses cryptic transcript initiation and promotes longevity through recovering transcriptional fidelity in old yeast. The role of H3K36 methylation in the maintenance of transcriptional stability and longevity is presumably evolutionary conserved, since low levels of H3K36me3 are associated with altered length of 3′ untranslated regions (3′UTR) and shortened lifespan in worms and flies.[64,65]

Histone acetylation and methylation comprise the major and better described histone modifications. However, histones can be also modified through phosphorylation, ubiquitination and sumoylation. Although the biological importance of these modifications on cellular homeostasis and longevity are not yet elucidated, several reports suggest a modulatory role for histone phosphorylation on transcription regulation, DNA repair and chromatin compaction.[66] Ubiquitination is involved in transcription activity, inflammation signaling and HMT.[67] Sumoylation is involved in inflammation signaling and the epithelial–mesenchymal transition, which is related to cancer progression.[68]

13.2.5 ATP-dependent chromatin remodeling

The above described chemical histone modifications alter chromatin compactness and regulate transcriptional activity. Often these modifications function in concert with, or through activation of ATP-dependent chromatin remodeling factors to alter the

nucleosomes positions along DNA and modulate its accessibility to transcription factors and DNA replication machinery components.[69,70] For example, acetylation of the histone H3 N-terminal tail recruits and increases the affinity of the ATP-dependent chromatin remodelers SWI/SNF and RSC, which leads to nucleosome mobilization and chromatin remodeling.[71] The major groups of ATP-dependent chromatin-remodeling enzymes are the SWI/SNF, ISWI, Nurd/Mi/CHD, SWR1 and INO80, and recent studies prove their importance for lifespan determination.[72,73] In worms, chromatin remodeler SWI/SNF activates transcription at specific promoters in collaboration with the longevity promoting DAF-16/FOXO transcription factor. Inactivation of SWI/SNF decreases longevity and DAF-16/FOXO-mediated stress responses.[74] Moreover, loss of LET-418/Mi2, the catalytic subunit of the nucleosome remodeling and histone deacetylase complex (NuRD), increases longevity and environmental stress resistance in *C. elegans*, *Drosophila* and *Arabidopsis*.[75] In yeast, deletion of Isw2 increases response to genotoxic stress and extends yeast replicative lifespan, while deletion of components of the ortholog chromatin-remodeling complex in worms also extends lifespan.[76] PRC2, which is able to remodel chromatin and silence genes, has been implicated in the transcriptional dysregulation that the progeria primary fibroblasts exhibit.[77] These findings provide strong evidence for the evolutionarily-conserved role of ATP-dependent chromatin remodeling in facilitating stress responses and aging.

13.2.6 DNA methylation

Histone modifications are the primary targets of factors that affect epigenetics, such as diet, metabolism, environmental pollutants, drugs, etc. Their binding on DNA protects it from chemical modifications and restricts the accessibility of transcription factors. Nevertheless, epigenetic factors can directly chemically modify DNA, via methylation at cytosine residues which are mainly placed 5′ of guanine (CpG dinucleotide), located predominantly at intergenic, intronic and repetitive sequences. The latest are often generated by transposable elements and the increased methylation they exhibit might be related to the necessity of cells to inactivate such mobile DNA sequences and avoid genomic instability.[78] On the other hand, hypomethylated CpG dinucleotides are frequently located at promoters and first exons of the majority of genes (CpG islands). Transfer of a methyl group to cytosine is catalyzed by DNA methyltransferases (DNMTs), thus generating 5-methylcytosine (5mC). When DNA methylation occurs in promoters, it leads to transcriptional repression and causes gene silencing.[79] Although DNA methylation levels during the first years of life are similar between monozygotic twins, significant tissue-specific differences on DNA methylation appear with age, starting from childhood (epigenetic drift).[80–82] In animals and humans, a reduction of DNA methylation occurs with age both globally and tissue-specifically.[83–87] Methylation patterns of CD4 + T cells from newborn and centenarian individuals showed that DNA

methylation levels decrease with age. Likewise, CpGs dinucleotides are less methylated throughout the genome of centenarians, which is characterized by highly heterogeneous DNA methylation.[88] Age-dependent changes in DNA hypomethylation can lead to pathologies, through aberrant transcription. Progressive DNA hypomethylation at specific gene promoters has been implicated in the development of autoimmune responses.[89,90] On the other hand, age-related hypermethylation in promoters of genes that code for transcription and translation regulating factors can severely impact various cellular functions.[91–93] Contrarily, epigenomic analysis of pancreatic β cells revealed age-related differences in methylation patterns that were associated with the repression of proliferation and activation of metabolic regulators. B cell function was improved in old mice, suggesting that epigenetic alterations through aging do not necessarily lead to pathologies and physiological decline.[94]

Age-related changes in DNA methylation can be attributed to altered expressions of methyltransferases, demethylases and environmental factors. The importance of inadequate DNA methylation on health and lifespan has been clearly proven in animal models. In flies, functional dDnmt2, the gene expressing for DNA methyltransferase, is required for the maintenance of the normal lifespan of fruit flies, while its upregulation extends lifespan.[95] Enhanced DNMT2-induced longevity is achieved via retrotransposons silencing in *Drosophila* somatic cells and maintenance of telomeres' integrity.[96] In support of a beneficial role for DNA methyltransferases on health and longevity, mice with mutations in the gene coding for DNA methyltransferase 1 (Dnmt1) have decreased DNA methylation, decreased bone mineral density and body weight, impaired learning and memory functions in an age-dependent manner, but with canonical survival.[97] Additionally, mutations in the DNA methyltransferase 3 gene (Dnmt3a) cause premature neurodegeneration and death.[98] In honey bees, pharmacological demethylation enhances lifespan.[99] In mice and monkeys, age-related methylation drift was found to be associated with longevity, while caloric restriction diminished age-related methylation drift.[100] Furthermore, DNA methylation is implicated in transgenerational effects that regulate lifespan in offspring. In mice, old father offspring mice lived less and experienced stronger aging phenotypes compared to young father offspring mice. Genome-wide epigenetic analyses revealed differentially methylated promoters of genes expressing components of the lifespan regulator mTORC1 signaling pathway.[101] Interestingly, DNA methylation seems to cooperate with other epigenetic alterations, such as HMT, to regulate transcriptional activity at specific genomic areas, thus suggesting a strong interconnection between different epigenetic modifications.[102]

DNA methylation patterns at CpGs have been associated with aging and diseases such as cancer, obesity, and cardiovascular disease.[103–110] Clinical epigenetics aims to decipher such patterns and use them to predict the biological age of individuals, improving diagnostics and therapies.[111,112] The epigenome is formed by the co-action of genes, age, environmental factors and lifestyle. Hence, epigenetic profiles are very

informative regarding the depiction of the health status of an organism.[113] This has challenged the design of supervised machine learning approaches to analyze epigenetic profiles, with several studies having used machine learning to diagnose diseases.[114] There is a great deal of progress in the development of "epigenetic clocks," aging biomarkers made of DNA methylation profiles, which enable accurate age estimates.[115] However, only a few DNA methylation patterns of CpG sites can allow precise age prediction[116] and more studies are required to further advance this approach.

13.2.7 Non-coding RNA molecules

Non-coding RNA molecules are small or long RNAs that, despite not having a code for proteins, they regulate cellular function. They are classified into transfer RNAs, ribosomal RNAs, microRNAs (miRNAs), small interfering RNAs, piwi-interacting RNAs, small nucleolar RNAs (snoRNAs), small Cajal body-specific RNAs, extracellular RNAs and long non-coding RNAs (lncRNAs). Through their regulatory role on gene silencing, ncRNAs, especially miRNAs and lncRNAs, exert various effects on chromatin architecture, cell cycle, metabolism, etc., and their dysregulation is relevant to the progression of cellular senescence, cancer, cardiovascular, neuronal and immune pathologies.[117] In yeast, lifespan maintenance is regulated by the repression of rDNA non-coding transcription, which is achieved through Sir2. Mutations that reduce ncRNAs expression extend lifespan.[118] In human stem cells, ncRNAs expression from Alu sequences increases with age and causes senescence. Knockdown of ncRNAs expression reverses this effect.[119]

MiRNAs play a crucial role on cellular senescence and aging.[120] Through binding with the 3′UTR sequence of mRNA molecules, they inhibit translation and negatively modulate gene function. They cause heritable changes without directly altering the DNA sequence or chromatin structure, and their expression is differentially regulated through aging in mice and humans.[121–124] An essential pathway for health and lifespan determination, regulated by miRNAs, is the insulin pathway.[125] In *C. elegans*, several miRNAs regulate longevity and stress responses.[126,127] The miRNAs *lin-4* and *lin-14* have opposite roles on longevity, with *lin-14* serving as the target for *lin-4*. Reduction in *lin-14* activity is dependent on the DAF-16 and HSF-1 transcription factors, which are the mediators of the insulin pathway effects on healthspan.[128] In flies, a well-established longevity promoting intervention, caloric restriction (CR), is shown to alter expression of more than 100 lncRNAs, which serve as mediators of CR on healthspan.[129] In mice, the H19 lncRNA participates in a complex that interacts with histone lysine methyltransferases and facilitates repression of several genes, among which is the Igf2 (insulin-like growth factor 2).[130] Loss of this regulation occurs through aging in mice and the human prostate.[131] Hence, ncRNAs can regulate longevity via interfering with well-established metabolic pathways. Furthermore, ncRNAs are

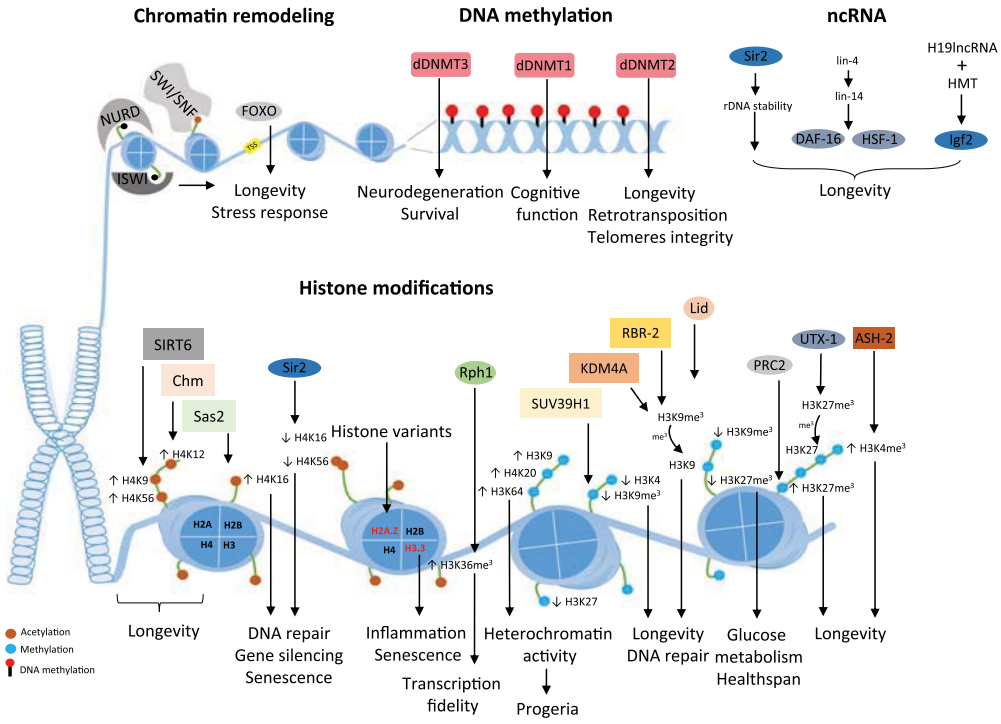

Figure 13.1 Epigenetic alterations that regulate health and lifespan.

also involved in forming the boundaries of heterochromatin.[132] The major epigenetic changes that affect health- and lifespan are summarized in Fig. 13.1.

13.3 Epigenetic alterations and age-related diseases

13.3.1 Cancer and epigenetics

Several studies show similarities between epigenetic changes that occur with aging and in cancer development.[68] Several histone modifications are prevalent in distinct cancer types. Hypoacetylation at H2BK12 is prevalent in osteosarcoma, hyperacetylated histone H3 is common in colorectal cancer, extensive hypoacetylation at H3K4 and H3K9 accompanies oral squamous cell carcinoma and ovarian tumors, and invasive colon cancer and glioma are characterized by upregulated H3K27ac, which has been shown to induce lncRNAs secretion in colon cancer cells.[133–137] Acetylation of lysine residues at histone 4 is also correlated with cancer. Acetylated H4K16, as also H4K20me3 are downregulated in breast, renal, colon and ovarian cancer, while acetylated H3K18 and H3K4me2 are upregulated in prostate, pancreatic, lung and kidney cancers.[138–145] Several other examples establish a causative relation between epigenetic alterations and

cancer. Demethylation of H3K9 has been associated with derepression of genes involved in breast and esophageal cancers.[146,147] P300 and CBP HATs suppress tumors and several cancers are characterized by their dysfunction.[148] Moreover, deacetylation of several non-histone proteins, including p53 and STAT3 transcriptional activator, is associated with cancer.[149] Expression of the polycomb group protein enhancer of zeste homolog 2 (EZH2) is higher in metastatic prostate cancer, while its downregulation inhibits cell proliferation in vitro. EZH2 regulates hypermethylation at H3K27 and represses gene activity in prostate cells, an effect that is mediated through histone deacetylase activity.[150] EZH2 is a marker for breast cancer and glioblastoma.[151,152] Several studies suggest a role for enhanced secretion of exosomes carrying lncRNAs in cancer development, through mediating intercellular communication in tumor microenvironments.[153] Interestingly, 25% of all cancers harbor mutations in genes encoding subunits of the SWI/SNF complexes. Novel findings support an anti-cancer role for SWI/SNF via repressing transcription and the facilitation of DNA damage repair.[154] Moreover, mutations in the genes that encode H1 isoforms B−E are causative to the development of B cell lymphomas, through inducing chromatin relaxation, upregulation of H3K36me2 and loss of repressive H3K27me3, which leads to derepression of developmentally-silenced genes.[155]

Recent findings suggest a role for miRNAs in cancer development. MiR-205 regulates differentiation and morphogenesis in epithelial cells and its aberrant expression is frequently detected in human cancers. Depending on the tumor type, it has been suggested to act as tumor-suppressor or as oncogene.[156] MiR-34a is shown to repress tumor progression through synergizing with p53 and transcription factors, via inhibition of the transition from epithelial cells to mesenchymal cells.[157] Also, a significant association between the expression of miR-181 and miR-200 family members and colorectal cancer has been observed.[158] Members of the miR-181 family have been suggested to perform their anti-cancerous function through downregulation of the hepatic transcriptional regulators, CDX2 and GATA6, and the Wnt signaling inhibitor NLK.[159] On the other hand, overexpression of miR-145 is shown to be carcinogenic, through altering methylation patterns and reducing activity of genes that regulate DNA damage response and apoptosis, consequently leading to overproliferation and enhanced epithelial to mesenchymal cells transition.[160]

Aging is a risk factor for cancer development and the retrotransposition is upregulated with aging, as in cancers, thus raising the possibility for a role of epigenetic drift on cancer development via age-related enhanced retrotransposition.[8,161,162] In yeast, age-related histone loss leads to increased retrotransposition, which causes genomic instability and disruption of cellular homeostasis, an age-related event which can be reverted via CR in mice.[8,163] Hypomethylation at repetitive regions such as Alu and long interspersed element-1 increases genomic instability and is associated with cancer.[164,165] On the other hand, cancer is induced by CpG dinucleotides hypermethylation at promoters of tumor suppressors and esophageal cells of individuals with a long smoking history and high methylation levels.[166−168] Moreover, carcinogenic factors

such as chronic inflammation, *Helicobacter pylori* and hepatitis B or C infections, as also with alcoholism, induce aberrant DNA methylation, which forms tissue- and carcinogenic factors-specific patterning and specificity.[137,169–174] Interestingly, the methylation degree can be indicative of exposure to carcinogens.[175]

13.3.2 Neuronal diseases and epigenetics

Epigenetic changes comprise a molecular link between aging and neurodegeneration, with etiology and symptomatology of neurodegeneration being, in many cases, linked to epigenetic effects.[176] Increased retrotransposition has been associated with neurodegeneration and reduced levels of DNA methyltransferases is a common feature in aging, Alzheimer's disease (AD) and Parkinson's diseases (PD).[177–179] In support, a deficiency in 5-hydroxymethylcytosine was found in a mouse model of Huntington's disease (HD).[180] On the other hand, several studies demonstrated increased DNA methylation in post-mortem tissues from cohorts of patients with AD.[181,182] Histone acetylation at the repetitive DNA sequences decreases with age in mice brains and altered histone acetylation has a causative role on age-dependent memory impairment.[183,184] Histone acetylation at certain residues is high in memory regulating brain areas, such as the hippocampus, with these residues being frequently affected in neurodegeneration.[185] Hypomethylation at neuronal enhancers in patients with AD is related to synapse degeneration.[186] Reduced PRC2 activity causes the upregulation of genes activated in HD and of genes that are known to induce neuronal cell death and neurodegeneration.[187]

MiRNAs also play various roles on neuroprotection and neurodegeneration, via non-elucidated mechanisms, with their concentration being dramatically decreased with age in the human brain.[188,189] In flies, expression of miR-34 is altered through aging and its loss causes brain degeneration and lifespan reduction. Its upregulation extends lifespan and inhibits human pathogenic polyglutamine disease protein-induced neurodegeneration. This is partially mediated via translation inhibition of Eip74EF.[190] On the other hand, samples from humans with AD and from mice with modeled AD, have different patterns of miRNAs expression compared to controls, as also with elevated levels of miR-34.[191,192] MiR-34 targets and decreases pro-survival factor Bcl2 and antiaging deacetylase SIRT1 and is suspected to play a causative role on neurodegeneration onset.[121,193] Levels of lncRNAs have been correlated with the expression of mutant alpha synuclein in presymptomatic PD.[194] Several lncRNA molecules are dysregulated in brains of patients with HD. Some of these have been suggested to target the neuroprotective transcriptional repressor, REST, a key mediator of transcriptional changes in neurodegenerative diseases.[195,196] Levels of another ncRNA, the miR-181c is decreased in the brains of AD patients, while its loss increases the levels of the amyloid precursor protein (Aβ).[197] The major epigenetic alterations that are involved in the development of cancer and neurodegeneration are depicted in Fig. 13.2.

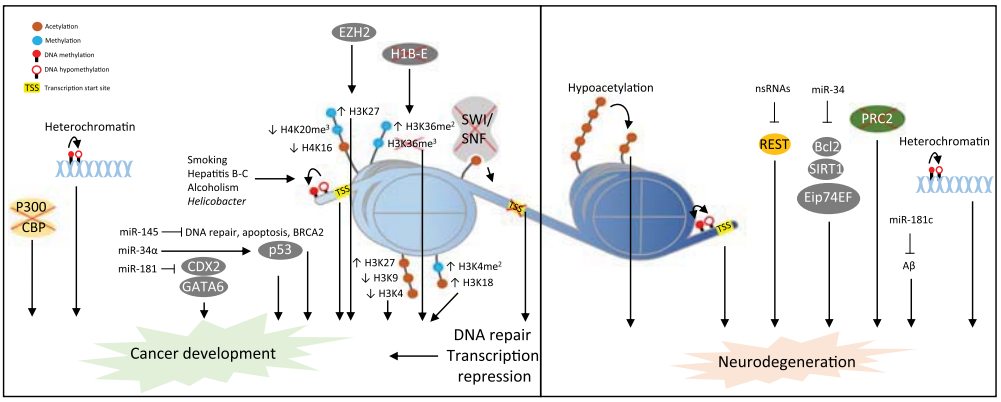

Figure 13.2 Epigenetic alterations implicated in cancer and neurodegeneration development.

13.3.3 Cardiovascular disease and epigenetics

One of the main risk factors of cardiovascular disease (CVD) is age. Several studies prove the major impact of epigenetic alterations in vascular function and arteriosclerosis, while histone deacetylase inhibitors are promising drugs to treat vascular diseases and arteriosclerosis.[198] When biological age is measured with the Horvath DNA methylation-based method, for each year of additional biological age, the risk for CVD occurrence increases by 4%.[199] Many studies reveal associations between epigenetic alterations through aging and CVD.[200] Hypermethylation of genes coding for superoxide dismutase-2 (SOD2), for histone 3 and for angiotensin I converting enzyme 2 promoter increases the risk of essential hypertension. Reduced global DNA methylation, hypomethylation of H3K79 and hyperacetylation at the promoter of the endothelial oxide synthetase gene (eNOS) are associated with hypertension.[201–203]

Epigenetic changes are also implicated in the development of hypercholesterolemia and atherosclerotic lesions. Patients with dyslipidemia have different methylation profiles in genes regulating mitochondrial function and lipid metabolism. Patients with hypercholesterolemia have hypermethylated promoters in genes that regulate transfer of cholesterol and formation of atherosclerotic lesions is associated with enhanced histone acetylation on H3K9 and H3K27 in the smooth muscle cells, as also altered methylation of several genes.[92,204–209] Methylation status of specific residues, such as H3K9, and the activity of the SWI/SNF chromatin remodeler have been causative to cardiomyocytes pathologies.[210,211] Finally, several ncRNAs are involved in age-related CVD.[212] Although there are not experimental proofs to establish a causative relationship between age-dependent epigenetic changes and CVD, these and several other findings suggest a strong correlation between epigenetic alterations and the development of CVD with age.[213]

With the exception of cancer, neurodegeneration and CVD, increasing evidence suggests that more age-related diseases, such as age-related renal, immune and metabolic diseases are correlated with age-related epigenetic changes.[214,215]

13.4 Conclusions

Age-dependent epigenetic changes constitute a longevity denominator that promotes age−related decline and pathologies. With age, several genetic, environmental and lifestyle agents alter epigenetic identity of individuals, leading to epigenetic drift, which can serve as a biomarker for "biological age" and functions as a regulator of physiology and lifespan, even of next generations. Epigenetic alterations mainly impact transcription regulation and proteins translation, which affect activity of genes involved in healthspan and lifespan determinations, such as genes participating in insulin signaling and responses to diet, genomic stability, telomeres attrition, cellular differentiation, senescence, stress responses and genes that are implicated in the onset and progress of age-dependent diseases. Although the same type of epigenetic alterations can impact cellular homeostasis and longevity in an opposite manner, dependent on the afflicted genomic areas, several studies have attributed epigenetic changes on specific genomic areas to distinct phenotypes and the onset of pathologies. However, research findings suggest that epigenetic alterations do not exclusively lead to pathologies and physiological decline, but they can even be beneficial for age-related physiological adaptations.[94]

Some difficulties impede the elucidation of the role of epigenetic mechanisms on aging and the development of age-related diseases. The same type of epigenetic alterations can have contradictory effects on health, depending on the specific histonic or genomic residue affected. Also, the same residual modifications can have opposite effects on cellular function in different animal model systems, thus making interpretation of research findings in humans' physiology puzzling. Moreover, the strong interconnection between different epigenetic alterations hinders causative relationships between such alterations and specific phenotypes. Nevertheless, in a simplistic, but solid, speculation, age-related epigenetic changes observed in humans possibly impacts aging phenotypes through the same mechanisms that laboratory-induced epigenetic alterations use to modulate cellular physiology in animal model systems.

The importance of clinical epigenetics for human medical treatment lies on the reversibility of epigenetic modifications. Adoption of a certain lifestyle, including increased physical activity, consumption of low-caloric foods and dietary polyphenols, changes in habits such as tobacco smoking and alcohol consumption can reduce the effects of epigenetic drift on physiological decline.[100,216−219] Moreover, a group of chemicals that enhance longevity through altering the epigenome has been described, which can potentially alleviate age-related deterioration and pathologies in humans (Table 13.1). Furthermore, large arsenals of drugs that target specific disease-related epigenetic modifications exist and can potentially confront the development and symptomatology of age-related human diseases[220] In the future, the usage of such drugs, combined with the analysis of the epigenome "fingerprint" of individuals, have the potential to revolutionize the contribution of clinical epigenetics in geriatrics, through improving both diagnostics and treatments of human diseases.

Table 13.1 Drugs and biomolecules related to both longevity regulation and epigenetics alterations.

Epigenetic mechanism	Interventions	Targets	Aging (Chronological or replicative)	Organism	References
DNA methyltransferases (DNMTs)	Decitabine	AID	Extension	*Mus musculus*	221
	Hydralazine	NRF2, PKA/SIRT1	Extension	*Caenorhabditis elegans*	222,223
	RG108	Vg	Extension	*Apis mellifera*	99
	EGCG	AMPK/SIRT1/ FOXO, glucose metabolism	Extension	*C. elegans, Drosophila melanogaster*	224,225
	Curcumin	REDOX signaling	Extension	*C. elegans, Saccharomyces cerevisiae, Rattus rattus*	226–228
	Genistein	SOD-3, HSP-16.2	Inconsistent extension	*C. elegans, M. musculus, D. melanogaster*	229–231
	Ursolic acid	Srl/PGC1α JNK	Extension	*D. melanogaster, C. elegans*	232,233
	Ascorbic acid	NA	Inconsistent extension	*M. musculus, D. melanogaster, C. elegans*	234–236
	Metformin	mTOR/AMPK KDM6A/UTX	Inconsistent extension	*D. melanogaster, C. elegans*	237–239
Histone modification (HDACs, HMTs, HDMs and HATs)	Resveratrol	SIRTs	Inconsistent extension	*C. elegans, D. melanogaster M. musculus*	240–243
	SB	FOXO/DAF-16, NRF2/SKN-1HSPs	Extension	*C. elegans, D. melanogaster, M. musculus*	26,36,244–246
	PBA	NA	Extension	*D. melanogaster,*	247
	TSA	HSP22	Extension	*D. melanogaster, C. elegans,*	248,249
	Quercetin	PaMTH1, MPF	Extension	*M. musculus, Podospora anserina, C. elegans*	250–252
	Spermidine	Autophagy	Extension	*M. musculus, S. cerevisiae, D. melanogaster C. elegans*	253,254
	Rapamycin	mTOR	Inconsistent extension	*M. musculus, D. melanogaster C. elegans*	255–259

EGCG, epigallocatechin-3-gallate; Vg, hemolymp vitellogenin; SB, sodium butyrate; AID, activation-induced cytidine deaminase; PBA, phenylbutyrate; TSA, trichostain A; NA, no information available.

Acknowledgment

This work was supported by grants from the European Research Council (ERC-GA695190-MANNA) and the General Secretariat for Research and Innovation of the Greek Ministry of Development and Investments.

Conflict of interest

The authors declare no conflict of interest.

References

1. Partridge L, Deelen J, Slagboom PE. Facing up to the global challenges of aging. *Nature* 2018;**561**:45–56.
2. Brunet A, Berger SL. Epigenetics of aging and aging-related disease. *J Gerontol A Biol Sci Med Sci* 2014;**69**(Suppl 1):S17–20.
3. Kennedy BK, Berger SL, Brunet A, Campisi J, Cuervo AM, Epel ES, et al. Geroscience: linking aging to chronic disease. *Cell* 2014;**159**:709–13.
4. Moskalev AA, Aliper AM, Smit-McBride Z, Buzdin A, Zhavoronkov A. Genetics and epigenetics of aging and longevity. *Cell Cycle* 2014;**13**:1063–77.
5. Jawaid A, Jehle KL, Mansuy IM. Impact of parental exposure on offspring health in humans. *Trends Genet* 2021;**37**:373–88.
6. Lee JH, Kim EW, Croteau DL, Bohr VA. Heterochromatin: an epigenetic point of view in aging. *Exp Mol Med* 2020;**52**:1466–74.
7. Feser J, Truong D, Das C, Carson JJ, Kieft J, Harkness T, et al. Elevated histone expression promotes life span extension. *Mol Cell* 2010;**39**:724–35.
8. Hu Z, Chen K, Xia Z, Chavez M, Pal S, Seol JH, et al. Nucleosome loss leads to global transcriptional up-regulation and genomic instability during yeast aging. *Genes Dev* 2014;**28**:396–408.
9. Ivanov A, Pawlikowski J, Manoharan I, van Tuyn J, Nelson DM, Rai TS, et al. Lysosome-mediated processing of chromatin in senescence. *J Cell Biol* 2013;**202**:129–43.
10. O'Sullivan RJ, Kubicek S, Schreiber SL, Karlseder J. Reduced histone biosynthesis and chromatin changes arising from a damage signal at telomeres. *Nat Struct Mol Biol* 2010;**17**:1218–25.
11. Liu L, Cheung TH, Charville GW, Hurgo BM, Leavitt T, Shih J, et al. Chromatin modifications as determinants of muscle stem cell quiescence and chronological aging. *Cell Rep* 2013;**4**:189–204.
12. Chen Y, Bravo JI, Son JM, Lee C, Benayoun BA. Remodeling of the H3 nucleosomal landscape during mouse aging. *Transl Med Aging* 2020;**4**:22–31.
13. Rogakou EP, Sekeri-Pataryas KE. Histone variants of H2A and H3 families are regulated during in vitro aging in the same manner as during differentiation. *Exp Gerontol* 1999;**34**:741–54.
14. Kreiling JA, Tamamori-Adachi M, Sexton AN, Jeyapalan JC, Munoz-Najar U, Peterson AL, et al. Age-associated increase in heterochromatic marks in murine and primate tissues. *Aging Cell* 2011;**10**:292–304.
15. Maze I, Wenderski W, Noh KM, Bagot RC, Tzavaras N, Purushothaman I, et al. Critical role of histone turnover in neuronal transcription and plasticity. *Neuron* 2015;**87**:77–94.
16. Tvardovskiy A, Schwammle V, Kempf SJ, Rogowska-Wrzesinska A, Jensen ON. Accumulation of histone variant H3.3 with age is associated with profound changes in the histone methylation landscape. *Nucleic Acids Res* 2017;**45**:9272–89.
17. Stefanelli G, Azam AB, Walters BJ, Brimble MA, Gettens CP, Bouchard-Cannon P, et al. Learning and age-related changes in genome-wide H2A.Z binding in the mouse hippocampus. *Cell Rep* 2018;**22**:1124–31.

18. Contrepois K, Coudereau C, Benayoun BA, Schuler N, Roux PF, Bischof O, et al. Histone variant H2A.J accumulates in senescent cells and promotes inflammatory gene expression. *Nat Commun* 2017;**8**:14995.
19. Rogina B, Helfand SL, Frankel S. Longevity regulation by Drosophila Rpd3 deacetylase and caloric restriction. *Science* 2002;**298**:1745.
20. Satoh A, Brace CS, Rensing N, Cliften P, Wozniak DF, Herzog ED, et al. Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. *Cell Metab* 2013;**18**:416–30.
21. Sasaki T, Maier B, Bartke A, Scrable H. Progressive loss of SIRT1 with cell cycle withdrawal. *Aging Cell* 2006;**5**:413–22.
22. Kawahara TL, Michishita E, Adler AS, Damian M, Berber E, Lin M, et al. SIRT6 links histone H3 lysine 9 deacetylation to NF-kappaB-dependent gene expression and organismal life span. *Cell* 2009;**136**:62–74.
23. Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gellon L, et al. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* 2006;**124**:315–29.
24. Kanfi Y, Naiman S, Amir G, Peshti V, Zinman G, Nahum L, et al. The sirtuin SIRT6 regulates lifespan in male mice. *Nature* 2012;**483**:218–21.
25. Chen J, Xie JJ, Jin MY, Gu YT, Wu CC, Guo WJ, et al. Sirt6 overexpression suppresses senescence and apoptosis of nucleus pulposus cells by inducing autophagy in a model of intervertebral disc degeneration. *Cell Death Dis* 2018;**9**:56.
26. Peleg S, Feller C, Forne I, Schiller E, Sevin DC, Schauer T, et al. Life span extension by targeting a link between metabolism and histone acetylation in Drosophila. *EMBO Rep* 2016;**17**:455–69.
27. Guarente L. Sir2 links chromatin silencing, metabolism, and aging. *Genes Dev* 2000;**14**:1021–6.
28. Watroba M, Dudek I, Skoda M, Stangret A, Rzodkiewicz P. Szukiewicz D. Sirtuins, epigenetics and longevity. *Aging Res Rev* 2017;**40**:11–19.
29. Dang W, Steffen KK, Perry R, Dorsey JA, Johnson FB, Shilatifard A, et al. Histone H4 lysine 16 acetylation regulates cellular lifespan. *Nature* 2009;**459**:802–7.
30. Kaeberlein M, McVey M, Guarente L. The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. *Genes Dev* 1999;**13**:2570–80.
31. Tissenbaum HA, Guarente L. Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. *Nature* 2001;**410**:227–30.
32. Rogina B, Helfand SL. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proc Natl Acad Sci USA* 2004;**101**:15998–6003.
33. Moazed D. Enzymatic activities of Sir2 and chromatin silencing. *Curr Opin Cell Biol* 2001;**13**:232–8.
34. Kimura A, Umehara T, Horikoshi M. Chromosomal gradient of histone acetylation established by Sas2p and Sir2p functions as a shield against gene silencing. *Nat Genet* 2002;**32**:370–7.
35. Suka N, Luo K. Grunstein M. Sir2p and Sas2p opposingly regulate acetylation of yeast histone H4 lysine16 and spreading of heterochromatin. *Nat Genet* 2002;**32**:378–83.
36. Krishnan V, Chow MZ, Wang Z, Zhang L, Liu B, Liu X, et al. Histone H4 lysine 16 hypoacetylation is associated with defective DNA repair and premature senescence in Zmpste24-deficient mice. *Proc Natl Acad Sci USA* 2011;**108**:12325–30.
37. Michishita E, McCord RA, Berber E, Kioi M, Padilla-Nash H, Damian M, et al. SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. *Nature* 2008;**452**:492–6.
38. Molina-Serrano D, Schiza V, Demosthenous C, Stavrou E, Oppelt J, Kyriakou D, et al. Loss of Nat4 and its associated histone H4 N-terminal acetylation mediates calorie restriction-induced longevity. *EMBO Rep* 2016;**17**:1829–43.
39. Yi SJ, Kim K. New insights into the role of histone changes in aging. *Int J Mol Sci* 2020;21.
40. Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. *Nat Rev Genet* 2012;**13**:343–57.
41. Villeponteau B. The heterochromatin loss model of aging. *Exp Gerontol* 1997;**32**:383–94.
42. Haithcock E, Dayani Y, Neufeld E, Zahand AJ, Feinstein N, Mattout A, et al. Age-related changes of nuclear architecture in Caenorhabditis elegans. *Proc Natl Acad Sci USA* 2005;**102**:16690–5.

43. Larson K, Yan SJ, Tsurumi A, Liu J, Zhou J, Gaur K, et al. Heterochromatin formation promotes longevity and represses ribosomal RNA synthesis. *PLoS Genet* 2012;**8**:e1002473.
44. Shumaker DK, Dechat T, Kohlmaier A, Adam SA, Bozovsky MR, Erdos MR, et al. Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging. *Proc Natl Acad Sci USA* 2006;**103**:8703–8.
45. Daujat S, Weiss T, Mohn F, Lange UC, Ziegler-Birling C, Zeissler U, et al. H3K64 trimethylation marks heterochromatin and is dynamically remodeled during developmental reprogramming. *Nat Struct Mol Biol* 2009;**16**:777–81.
46. Kourmouli N, Jeppesen P, Mahadevhaiah S, Burgoyne P, Wu R, Gilbert DM, et al. Heterochromatin and tri-methylated lysine 20 of histone H4 in animals. *J Cell Sci* 2004;**117**:2491–501.
47. Schotta G, Lachner M, Sarma K, Ebert A, Sengupta R, Reuter G, et al. A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. *Genes Dev* 2004;**18**:1251–62.
48. Zhang W, Li J, Suzuki K, Qu J, Wang P, Zhou J, et al. Aging stem cells. A Werner syndrome stem cell model unveils heterochromatin alterations as a driver of human aging. *Science* 2015;**348**:1160–3.
49. Merkwirth C, Jovaisaite V, Durieux J, Matilainen O, Jordan SD, Quiros PM, et al. Two conserved histone demethylases regulate mitochondrial stress-induced longevity. *Cell* 2016;**165**:1209–23.
50. Liu B, Wang Z, Zhang L, Ghosh S, Zheng H, Zhou Z. Depleting the methyltransferase Suv39h1 improves DNA repair and extends lifespan in a progeria mouse model. *Nat Commun* 2013;**4**:1868.
51. Greer EL, Maures TJ, Hauswirth AG, Green EM, Leeman DS, Maro GS, et al. Members of the H3K4 trimethylation complex regulate lifespan in a germline-dependent manner in C. elegans. *Nature* 2010;**466**:383–7.
52. Li L, Greer C, Eisenman RN, Secombe J. Essential functions of the histone demethylase lid. *PLoS Genet* 2010;**6**:e1001221.
53. Lorbeck MT, Singh N, Zervos A, Dhatta M, Lapchenko M, Yang C, et al. The histone demethylase Dmel\Kdm4A controls genes required for life span and male-specific sex determination in Drosophila. *Gene* 2010;**450**:8–17.
54. Djeghloul D, Kuranda K, Kuzniak I, Barbieri D, Naguibneva I, Choisy C, et al. Age-associated decrease of the histone methyltransferase SUV39H1 in HSC perturbs heterochromatin and B lymphoid differentiation. *Stem Cell Rep* 2016;**6**:970–84.
55. Baumgart M, Groth M, Priebe S, Savino A, Testa G, Dix A, et al. RNA-seq of the aging brain in the short-lived fish N. furzeri - conserved pathways and novel genes associated with neurogenesis. *Aging Cell* 2014;**13**:965–74.
56. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, et al. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science* 2002;**298**:1039–43.
57. Ma Z, Wang H, Cai Y, Wang H, Niu K, Wu X, et al. Epigenetic drift of H3K27me3 in aging links glycolysis to healthy longevity in Drosophila. *Elife* 2018;7.
58. Siebold AP, Banerjee R, Tie F, Kiss DL, Moskowitz J, Harte PJ. Polycomb repressive complex 2 and trithorax modulate drosophila longevity and stress resistance. *Proc Natl Acad Sci USA* 2010;**107**:169–74.
59. Jin C, Li J, Green CD, Yu X, Tang X, Han D, et al. Histone demethylase UTX-1 regulates C. elegans life span by targeting the insulin/IGF-1 signaling pathway. *Cell Metab* 2011;**14**:161–72.
60. Maures TJ, Greer EL, Hauswirth AG, Brunet A. The H3K27 demethylase UTX-1 regulates C. elegans lifespan in a germline-independent, insulin-dependent manner. *Aging Cell* 2011;**10**:980–90.
61. Ni Z, Ebata A, Alipanahiramandi E, Lee SS. Two SET domain containing genes link epigenetic changes and aging in Caenorhabditis elegans. *Aging Cell* 2012;**11**:315–25.
62. Bannister AJ, Schneider R, Myers FA, Thorne AW, Crane-Robinson C, Kouzarides T. Spatial distribution of di- and tri-methyl lysine 36 of histone H3 at active genes. *J Biol Chem* 2005;**280**:17732–6.
63. Sen P, Dang W, Donahue G, Dai J, Dorsey J, Cao X, et al. H3K36 methylation promotes longevity by enhancing transcriptional fidelity. *Genes Dev* 2015;**29**:1362–76.
64. Pu M, Ni Z, Wang M, Wang X, Wood JG, Helfand SL, et al. Trimethylation of Lys36 on H3 restricts gene expression change during aging and impacts life span. *Genes Dev* 2015;**29**:718–31.

65. Su L, Li H, Huang C, Zhao T, Zhang Y, Ba X, et al. Muscle-specific histone H3K36 dimethyltransferase SET-18 shortens lifespan of caenorhabditis elegans by repressing daf-16a expression. *Cell Rep* 2018;**22**:2716–29.
66. Rossetto D, Avvakumov N, Cote J. Histone phosphorylation: a chromatin modification involved in diverse nuclear events. *Epigenetics* 2012;**7**:1098–108.
67. Cao J, Yan Q. Histone ubiquitination and deubiquitination in transcription, DNA damage response, and cancer. *Front Oncol* 2012;**2**:26.
68. Roberti A, Valdes AF, Torrecillas R, Fraga MF, Fernandez AF. Epigenetics in cancer therapy and nanomedicine. *Clin Epigenetics* 2019;**11**:81.
69. Aalfs JD, Kingston RE. What does 'chromatin remodeling' mean? *Trends Biochem Sci* 2000;**25**:548–55.
70. Clapier CR, Cairns BR. The biology of chromatin remodeling complexes. *Annu Rev Biochem* 2009;**78**:273–304.
71. Chatterjee N, Sinha D, Lemma-Dechassa M, Tan S, Shogren-Knaak MA, Bartholomew B. Histone H3 tail acetylation modulates ATP-dependent remodeling through multiple mechanisms. *Nucleic Acids Res* 2011;**39**:8378–91.
72. Becker PB, Workman JL. Nucleosome remodeling and epigenetics. *Cold Spring Harb Perspect Biol* 2013;5.
73. Clapier CR, Iwasa J, Cairns BR, Peterson CL. Mechanisms of action and regulation of ATP-dependent chromatin-remodelling complexes. *Nat Rev Mol Cell Biol* 2017;**18**:407–22.
74. Riedel CG, Dowen RH, Lourenco GF, Kirienko NV, Heimbucher T, West JA, et al. DAF-16 employs the chromatin remodeller SWI/SNF to promote stress resistance and longevity. *Nat Cell Biol* 2013;**15**:491–501.
75. De Vaux V, Pfefferli C, Passannante M, Belhaj K, von Essen A, Sprecher SG, et al. The Caenorhabditis elegans LET-418/Mi2 plays a conserved role in lifespan regulation. *Aging Cell* 2013;**12**:1012–20.
76. Dang W, Sutphin GL, Dorsey JA, Otte GL, Cao K, Perry RM, et al. Inactivation of yeast Isw2 chromatin remodeling enzyme mimics longevity effect of calorie restriction via induction of genotoxic stress response. *Cell Metab* 2014;**19**:952–66.
77. Sebestyen E, Marullo F, Lucini F, Petrini C, Bianchi A, Valsoni S, et al. SAMMY-seq reveals early alteration of heterochromatin and deregulation of bivalent genes in Hutchinson-Gilford Progeria Syndrome. *Nat Commun* 2020;**11**:6274.
78. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet* 2012;**13**:484–92.
79. D'Aquila P, Rose G, Bellizzi D, Passarino G. Epigenetics and aging. *Maturitas* 2013;**74**:130–6.
80. Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, et al. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci USA* 2005;**102**:10604–9.
81. Kaminsky ZA, Tang T, Wang SC, Ptak C, Oh GH, Wong AH, et al. DNA methylation profiles in monozygotic and dizygotic twins. *Nat Genet* 2009;**41**:240–5.
82. Wong CC, Caspi A, Williams B, Craig IW, Houts R, Ambler A, et al. A longitudinal study of epigenetic variation in twins. *Epigenetics* 2010;**5**:516–26.
83. Berdyshev GD, Korotaev GK, Boiarskikh GV, Vaniushin BF. [Nucleotide composition of DNA and RNA from somatic tissues of humpback and its changes during spawning]. *Biokhimiia* 1967;**32**:988–93.
84. Romanov GA, Vanyushin BF. Methylation of reiterated sequences in mammalian DNAs. Effects of the tissue type, age, malignancy and hormonal induction. *Biochim Biophys Acta* 1981;**653**:204–18.
85. Busque L, Mio R, Mattioli J, Brais E, Blais N, Lalonde Y, et al. Nonrandom X-inactivation patterns in normal females: lyonization ratios vary with age. *Blood* 1996;**88**:59–65.
86. Richardson B. Impact of aging on DNA methylation. *Aging Res Rev* 2003;**2**:245–61.
87. Day K, Waite LL, Thalacker-Mercer A, West A, Bamman MM, Brooks JD, et al. Differential DNA methylation with age displays both common and dynamic features across human tissues that are influenced by CpG landscape. *Genome Biol* 2013;**14**:R102.
88. Heyn H, Li N, Ferreira HJ, Moran S, Pisano DG, Gomez A, et al. Distinct DNA methylomes of newborns and centenarians. *Proc Natl Acad Sci USA* 2012;**109**:10522–7.

89. Wei L, Liu B, Tuo J, Shen D, Chen P, Li Z, et al. Hypomethylation of the IL17RC promoter associates with age-related macular degeneration. *Cell Rep* 2012;**2**:1151–8.
90. Zhang Z, Deng C, Lu Q, Richardson B. Age-dependent DNA methylation changes in the ITGAL (CD11a) promoter. *Mech Aging Dev* 2002;**123**:1257–68.
91. Christensen BC, Houseman EA, Marsit CJ, Zheng S, Wrensch MR, Wiemels JL, et al. Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. *PLoS Genet* 2009;**5**:e1000602.
92. Post WS, Goldschmidt-Clermont PJ, Wilhide CC, Heldman AW, Sussman MS, Ouyang P, et al. Methylation of the estrogen receptor gene is associated with aging and atherosclerosis in the cardiovascular system. *Cardiovasc Res* 1999;**43**:985–91.
93. Oakes CC, Smiraglia DJ, Plass C, Trasler JM, Robaire B. Aging results in hypermethylation of ribosomal DNA in sperm and liver of male rats. *Proc Natl Acad Sci U S A* 2003;**100**:1775–80.
94. Avrahami D, Li C, Zhang J, Schug J, Avrahami R, Rao S, et al. Aging-dependent demethylation of regulatory elements correlates with chromatin state and improved beta cell function. *Cell Metab* 2015;**22**:619–32.
95. Lin MJ, Tang LY, Reddy MN, Shen CK. DNA methyltransferase gene dDnmt2 and longevity of Drosophila. *J Biol Chem* 2005;**280**:861–4.
96. Phalke S, Nickel O, Walluscheck D, Hortig F, Onorati MC, Reuter G. Retrotransposon silencing and telomere integrity in somatic cells of Drosophila depends on the cytosine-5 methyltransferase DNMT2. *Nat Genet* 2009;**41**:696–702.
97. Liu L, van Groen T, Kadish I, Li Y, Wang D, James SR, et al. Insufficient DNA methylation affects healthy aging and promotes age-related health problems. *Clin Epigenetics* 2011;**2**:349–60.
98. Nguyen S, Meletis K, Fu D, Jhaveri S, Jaenisch R. Ablation of de novo DNA methyltransferase Dnmt3a in the nervous system leads to neuromuscular defects and shortened lifespan. *Dev Dyn* 2007;**236**:1663–76.
99. Cardoso-Junior CAM, Guidugli-Lazzarini KR, Hartfelder K. DNA methylation affects the lifespan of honey bee (Apis mellifera L.) workers - evidence for a regulatory module that involves vitellogenin expression but is independent of juvenile hormone function. *Insect Biochem Mol Biol* 2018;**92**:21–9.
100. Maegawa S, Lu Y, Tahara T, Lee JT, Madzo J, Liang SD, et al. Caloric restriction delays age-related methylation drift. *Nat Commun* 2017;8.
101. Xie K, Ryan DP, Pearson BL, Henzel KS, Neff F, Vidal RO, et al. Epigenetic alterations in longevity regulators, reduced life span, and exacerbated aging-related pathology in old father offspring mice. *Proc Natl Acad Sci USA* 2018;**115**:E2348–57.
102. Pal S, Tyler JK. Epigenetics and aging. *Sci Adv* 2016;**2**:e1600584.
103. Heyn H, Esteller M. DNA methylation profiling in the clinic: applications and challenges. *Nat Rev Genet* 2012;**13**:679–92.
104. Aslibekyan S, Claas SA, Arnett DK. Clinical applications of epigenetics in cardiovascular disease: the long road ahead. *Transl Res* 2015;**165**:143–53.
105. Mill J, Heijmans BT. From promises to practical strategies in epigenetic epidemiology. *Nat Rev Genet* 2013;**14**:585–94.
106. Robertson KD. DNA methylation and human disease. *Nat Rev Genet* 2005;**6**:597–610.
107. Cui H, Cruz-Correa M, Giardiello FM, Hutcheon DF, Kafonek DR, Brandenburg S, et al. Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. *Science* 2003;**299**:1753–5.
108. Bhusari S, Yang B, Kueck J, Huang W, Jarrard DF. Insulin-like growth factor-2 (IGF2) loss of imprinting marks a field defect within human prostates containing cancer. *Prostate* 2011;**71**:1621–30.
109. Soubry A, Schildkraut JM, Murtha A, Wang F, Huang Z, Bernal A, et al. Paternal obesity is associated with IGF2 hypomethylation in newborns: results from a Newborn Epigenetics Study (NEST) cohort. *BMC Med* 2013;**11**:29.
110. Gluckman PD, Hanson MA, Buklijas T, Low FM, Beedle AS. Epigenetic mechanisms that underpin metabolic and cardiovascular diseases. *Nat Rev Endocrinol* 2009;**5**:401–8.
111. Jones PA, Issa JP, Baylin S. Targeting the cancer epigenome for therapy. *Nat Rev Genet* 2016;**17**:630–41.

112. How Kit A, Nielsen HM, Tost J. DNA methylation based biomarkers: practical considerations and applications. *Biochimie* 2012;**94**:2314–37.
113. Marioni RE, Shah S, McRae AF, Chen BH, Colicino E, Harris SE, et al. DNA methylation age of blood predicts all-cause mortality in later life. *Genome Biol* 2015;**16**:25.
114. Rauschert S, Raubenheimer K, Melton PE, Huang RC. Machine learning and clinical epigenetics: a review of challenges for diagnosis and classification. *Clin Epigenetics* 2020;**12**:51.
115. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of aging. *Nat Rev Genet* 2018;**19**:371–84.
116. Weidner CI, Lin Q, Koch CM, Eisele L, Beier F, Ziegler P, et al. Aging of blood can be tracked by DNA methylation changes at just three CpG sites. *Genome Biol* 2014;**15**:R24.
117. Statello L, Guo CJ, Chen LL, Huarte M. Gene regulation by long non-coding RNAs and its biological functions. *Nat Rev Mol Cell Biol* 2020.
118. Saka K, Ide S, Ganley AR, Kobayashi T. Cellular senescence in yeast is regulated by rDNA noncoding transcription. *Curr Biol* 2013;**23**:1794–8.
119. Wang J, Geesman GJ, Hostikka SL, Atallah M, Blackwell B, Lee E, et al. Inhibition of activated pericentromeric SINE/Alu repeat transcription in senescent human adult stem cells reinstates self-renewal. *Cell Cycle* 2011;**10**:3016–30.
120. Cho WC. OncomiRs: the discovery and progress of microRNAs in cancers. *Mol Cancer* 2007;**6**:60.
121. Jung HJ, Suh Y. MicroRNA in aging: from discovery to biology. *Curr Genomics* 2012;**13**:548–57.
122. Li N, Bates DJ, An J, Terry DA, Wang E. Up-regulation of key microRNAs, and inverse down-regulation of their predicted oxidative phosphorylation target genes, during aging in mouse brain. *Neurobiol Aging* 2011;**32**:944–55.
123. Noren Hooten N, Abdelmohsen K, Gorospe M, Ejiogu N, Zonderman AB, Evans MK. microRNA expression patterns reveal differential expression of target genes with age. *PLoS One* 2010;**5**:e10724.
124. Zhang J, Liu Q, Zhang W, Li J, Li Z, Tang Z, et al. Comparative profiling of genes and miRNAs expressed in the newborn, young adult, and aged human epididymides. *Acta Biochim Biophys Sin (Shanghai)* 2010;**42**:145–53.
125. Nigi L, Grieco GE, Ventriglia G, Brusco N, Mancarella F, Formichi C, et al. MicroRNAs as regulators of insulin signaling: research updates and potential therapeutic perspectives in type 2 diabetes. *Int J Mol Sci* 2018;19.
126. de Lencastre A, Pincus Z, Zhou K, Kato M, Lee SS, Slack FJ. MicroRNAs both promote and antagonize longevity in C. elegans. *Curr Biol* 2010;**20**:2159–68.
127. Pincus Z, Smith-Vikos T, Slack FJ. MicroRNA predictors of longevity in Caenorhabditis elegans. *PLoS Genet* 2011;**7**:e1002306.
128. Boehm M, Slack F. A developmental timing microRNA and its target regulate life span in C. elegans. *Science* 2005;**310**:1954–7.
129. Yang D, Lian T, Tu J, Gaur U, Mao X, Fan X, et al. LncRNA mediated regulation of aging pathways in Drosophila melanogaster during dietary restriction. *Aging (Albany NY)* 2016;**8**:2182–203.
130. Monnier P, Martinet C, Pontis J, Stancheva I, Ait-Si-Ali S, Dandolo L. H19 lncRNA controls gene expression of the Imprinted Gene Network by recruiting MBD1. *Proc Natl Acad Sci USA* 2013;**110**:20693–8.
131. Fu VX, Dobosy JR, Desotelle JA, Almassi N, Ewald JA, Srinivasan R, et al. Aging and cancer-related loss of insulin-like growth factor 2 imprinting in the mouse and human prostate. *Cancer Res* 2008;**68**:6797–802.
132. Cohen AL, Jia S. Noncoding RNAs and the borders of heterochromatin. *Wiley Interdiscip Rev RNA* 2014;**5**:835–47.
133. Hoang ML, Kinde I, Tomasetti C, McMahon KW, Rosenquist TA, Grollman AP, et al. Genome-wide quantification of rare somatic mutations in normal human tissues using massively parallel sequencing. *Proc Natl Acad Sci USA* 2016;**113**:9846–51.
134. Yamashita S, Iida N, Takeshima H, Hattori N, Maeda M, Kishino T, et al. A novel method to quantify base substitution mutations at the 10(-6) per bp level in DNA samples. *Cancer Lett* 2017;**403**:152–8.

135. Yamashita S, Kishino T, Takahashi T, Shimazu T, Charvat H, Kakugawa Y, et al. Genetic and epigenetic alterations in normal tissues have differential impacts on cancer risk among tissues. *Proc Natl Acad Sci USA* 2018;**115**:1328–33.
136. Issa JP, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, Baylin SB. Methylation of the oestrogen receptor CpG island links aging and neoplasia in human colon. *Nat Genet* 1994;**7**:536–40.
137. Issa JP, Ahuja N, Toyota M, Bronner MP, Brentnall TA. Accelerated age-related CpG island methylation in ulcerative colitis. *Cancer Res* 2001;**61**:3573–7.
138. Seligson DB, Horvath S, McBrian MA, Mah V, Yu H, Tze S, et al. Global levels of histone modifications predict prognosis in different cancers. *Am J Pathol* 2009;**174**:1619–28.
139. Juliano CN, Izetti P, Pereira MP, Dos Santos AP, Bravosi CP, Abujamra AL, et al. H4K12 and H3K18 acetylation associates with poor prognosis in pancreatic cancer. *Appl Immunohistochem Mol Morphol* 2016;**24**:337–-344.
140. Li Y, Li S, Chen J, Shao T, Jiang C, Wang Y, et al. Comparative epigenetic analyses reveal distinct patterns of oncogenic pathways activation in breast cancer subtypes. *Hum Mol Genet* 2014;**23**:5378–93.
141. Pfister S, Rea S, Taipale M, Mendrzyk F, Straub B, Ittrich C, et al. The histone acetyltransferase hMOF is frequently downregulated in primary breast carcinoma and medulloblastoma and constitutes a biomarker for clinical outcome in medulloblastoma. *Int J Cancer* 2008;**122**:1207–13.
142. Cao L, Zhu L, Yang J, Su J, Ni J, Du Y, et al. Correlation of low expression of hMOF with clinicopathological features of colorectal carcinoma, gastric cancer and renal cell carcinoma. *Int J Oncol* 2014;**44**:1207–14.
143. Wang Y, Zhang R, Wu D, Lu Z, Sun W, Cai Y, et al. Epigenetic change in kidney tumor: downregulation of histone acetyltransferase MYST1 in human renal cell carcinoma. *J Exp Clin Cancer Res* 2013;**32**:8.
144. Liu N, Zhang R, Zhao X, Su J, Bian X, Ni J, et al. A potential diagnostic marker for ovarian cancer: Involvement of the histone acetyltransferase, human males absent on the first. *Oncol Lett* 2013;**6**:393–400.
145. Cai M, Hu Z, Liu J, Gao J, Tan M, Zhang D, et al. Expression of hMOF in different ovarian tissues and its effects on ovarian cancer prognosis. *Oncol Rep* 2015;**33**:685–92.
146. Liu G, Bollig-Fischer A, Kreike B, van de Vijver MJ, Abrams J, Ethier SP, et al. Genomic amplification and oncogenic properties of the GASC1 histone demethylase gene in breast cancer. *Oncogene* 2009;**28**:4491–500.
147. Yang ZQ, Imoto I, Fukuda Y, Pimkhaokham A, Shimada Y, Imamura M, et al. Identification of a novel gene, GASC1, within an amplicon at 9p23–24 frequently detected in esophageal cancer cell lines. *Cancer Res* 2000;**60**:4735–9.
148. Tillinghast GW, Partee J, Albert P, Kelley JM, Burtow KH, Kelly K. Analysis of genetic stability at the EP300 and CREBBP loci in a panel of cancer cell lines. *Genes Chromosom Cancer* 2003;**37**:121–31.
149. Virani S, Colacino JA, Kim JH, Rozek LS. Cancer epigenetics: a brief review. *ILAR J* 2012;**53**:359–69.
150. Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, et al. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* 2002;**419**:624–9.
151. Kleer CG, Cao Q, Varambally S, Shen R, Ota I, Tomlins SA, et al. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc Natl Acad Sci USA* 2003;**100**:11606–11.
152. Suva ML, Riggi N, Janiszewska M, Radovanovic I, Provero P, Stehle JC, et al. EZH2 is essential for glioblastoma cancer stem cell maintenance. *Cancer Res* 2009;**69**:9211–18.
153. Sun Z, Yang S, Zhou Q, Wang G, Song J, Li Z, et al. Emerging role of exosome-derived long non-coding RNAs in tumor microenvironment. *Mol Cancer* 2018;**17**:82.
154. Mittal P, Roberts CWM. The SWI/SNF complex in cancer—biology, biomarkers and therapy. *Nat Rev Clin Oncol* 2020;**17**:435–48.
155. Yusufova N, Kloetgen A, Teater M, Osunsade A, Camarillo JM, Chin CR, et al. Histone H1 loss drives lymphoma by disrupting 3D chromatin architecture. *Nature* 2021;**589**:299–305.

156. Ferrari E, Gandellini P. Unveiling the ups and downs of miR-205 in physiology and cancer: transcriptional and post-transcriptional mechanisms. *Cell Death Dis* 2020;**11**:980.
157. Zhang L, Liao Y, Tang L. MicroRNA-34 family: a potential tumor suppressor and therapeutic candidate in cancer. *J Exp Clin Cancer Res* 2019;**38**:53.
158. Pop-Bica C, Pintea S, Cojocneanu-Petric R, Del Sal G, Piazza S, Wu ZH, et al. MiR-181 family-specific behavior in different cancers: a *meta*-analysis view. *Cancer Metastasis Rev* 2018;**37**:17–32.
159. Indrieri A, Carrella S, Carotenuto P, Banfi S, Franco B. The pervasive role of the miR-181 family in development, neurodegeneration, and cancer. *Int J Mol Sci* 2020;21.
160. Manvati S, Mangalhara KC, Kalaiarasan P, Chopra R, Agarwal G, Kumar R, et al. miR-145 supports cancer cell survival and shows association with DDR genes, methylation pattern, and epithelial to mesenchymal transition. *Cancer Cell Int* 2019;**19**:230.
161. Criscione SW, Zhang Y, Thompson W, Sedivy JM, Neretti N. Transcriptional landscape of repetitive elements in normal and cancer human cells. *BMC Genomics* 2014;**15**:583.
162. Lee E, Iskow R, Yang L, Gokcumen O, Haseley P, Luquette 3rd LJ, et al. Landscape of somatic retrotransposition in human cancers. *Science* 2012;**337**:967–71.
163. De Cecco M, Criscione SW, Peterson AL, Neretti N, Sedivy JM, Kreiling JA. Transposable elements become active and mobile in the genomes of aging mammalian somatic tissues. *Aging (Albany NY)* 2013;**5**:867–83.
164. Sartor MA, Dolinoy DC, Jones TR, Colacino JA, Prince ME, Carey TE, et al. Genome-wide methylation and expression differences in HPV(+) and HPV(-) squamous cell carcinoma cell lines are consistent with divergent mechanisms of carcinogenesis. *Epigenetics* 2011;**6**:777–87.
165. Zheng Y, Joyce BT, Liu L, Zhang Z, Kibbe WA, Zhang W, et al. Prediction of genome-wide DNA methylation in repetitive elements. *Nucleic Acids Res* 2017;**45**:8697–711.
166. Di Croce L, Raker VA, Corsaro M, Fazi F, Fanelli M, Faretta M, et al. Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. *Science* 2002;**295**:1079–82.
167. Finkel T, Serrano M, Blasco MA. The common biology of cancer and aging. *Nature* 2007;**448**:767–74.
168. Gautrey HE, van Otterdijk SD, Cordell HJ. Newcastle 85 + Study Core T, Mathers JC, Strathdee G. DNA methylation abnormalities at gene promoters are extensive and variable in the elderly and phenocopy cancer cells. *FASEB J* 2014;**28**:3261–72.
169. Maekita T, Nakazawa K, Mihara M, Nakajima T, Yanaoka K, Iguchi M, et al. High levels of aberrant DNA methylation in Helicobacter pylori-infected gastric mucosae and its possible association with gastric cancer risk. *Clin Cancer Res* 2006;**12**:989–95.
170. Niwa T, Tsukamoto T, Toyoda T, Mori A, Tanaka H, Maekita T, et al. Inflammatory processes triggered by Helicobacter pylori infection cause aberrant DNA methylation in gastric epithelial cells. *Cancer Res* 2010;**70**:1430–40.
171. Nishida N, Nagasaka T, Nishimura T, Ikai I, Boland CR, Goel A. Aberrant methylation of multiple tumor suppressor genes in aging liver, chronic hepatitis, and hepatocellular carcinoma. *Hepatology* 2008;**47**:908–18.
172. Hlady RA, Tiedemann RL, Puszyk W, Zendejas I, Roberts LR, Choi JH, et al. Epigenetic signatures of alcohol abuse and hepatitis infection during human hepatocarcinogenesis. *Oncotarget* 2014;**5**:9425–43.
173. Lambert MP, Paliwal A, Vaissiere T, Chemin I, Zoulim F, Tommasino M, et al. Aberrant DNA methylation distinguishes hepatocellular carcinoma associated with HBV and HCV infection and alcohol intake. *J Hepatol* 2011;**54**:705–15.
174. Takeshima H, Ushijima T. Accumulation of genetic and epigenetic alterations in normal cells and cancer risk. *NPJ Precis Oncol* 2019;**3**:7.
175. Takeshima H, Niwa T, Toyoda T, Wakabayashi M, Yamashita S, Ushijima T. Degree of methylation burden is determined by the exposure period to carcinogenic factors. *Cancer Sci* 2017;**108**:316–21.
176. Xylaki M, Atzler B, Outeiro TF. Epigenetics of the synapse in neurodegeneration. *Curr Neurol Neurosci Rep* 2019;**19**:72.

177. Chouliaras L, Mastroeni D, Delvaux E, Grover A, Kenis G, Hof PR, et al. Consistent decrease in global DNA methylation and hydroxymethylation in the hippocampus of Alzheimer's disease patients. *Neurobiol Aging* 2013;**34**:2091–9.
178. Reilly MT, Faulkner GJ, Dubnau J, Ponomarev I, Gage FH. The role of transposable elements in health and diseases of the central nervous system. *J Neurosci* 2013;**33**:17577–86.
179. Tan H, Qurashi A, Poidevin M, Nelson DL, Li H, Jin P. Retrotransposon activation contributes to fragile X premutation rCGG-mediated neurodegeneration. *Hum Mol Genet* 2012;**21**:57–65.
180. Wang F, Yang Y, Lin X, Wang JQ, Wu YS, Xie W, et al. Genome-wide loss of 5-hmC is a novel epigenetic feature of Huntington's disease. *Hum Mol Genet* 2013;**22**:3641–53.
181. De Jager PL, Srivastava G, Lunnon K, Burgess J, Schalkwyk LC, Yu L, et al. Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. *Nat Neurosci* 2014;**17**:1156–63.
182. Lunnon K, Smith R, Hannon E, De Jager PL, Srivastava G, Volta M, et al. Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease. *Nat Neurosci* 2014;**17**:1164–70.
183. Ryu SH, Kang K, Yoo T, Joe CO, Chung JH. Transcriptional repression of repeat-derived transcripts correlates with histone hypoacetylation at repetitive DNA elements in aged mice brain. *Exp Gerontol* 2011;**46**:811–18.
184. Peleg S, Sananbenesi F, Zovoilis A, Burkhardt S, Bahari-Javan S, Agis-Balboa RC, et al. Altered histone acetylation is associated with age-dependent memory impairment in mice. *Science* 2010;**328**:753–6.
185. Stilling RM, Ronicke R, Benito E, Urbanke H, Capece V, Burkhardt S, et al. K-Lysine acetyltransferase 2a regulates a hippocampal gene expression network linked to memory formation. *EMBO J* 2014;**33**:1912–27.
186. Li P, Marshall L, Oh G, Jakubowski JL, Groot D, He Y, et al. Epigenetic dysregulation of enhancers in neurons is associated with Alzheimer's disease pathology and cognitive symptoms. *Nat Commun* 2019;**10**:2246.
187. von Schimmelmann M, Feinberg PA, Sullivan JM, Ku SM, Badimon A, Duff MK, et al. Polycomb repressive complex 2 (PRC2) silences genes responsible for neurodegeneration. *Nat Neurosci* 2016;**19**:1321–30.
188. Szafranski K, Abraham KJ, Mekhail K. Non-coding RNA in neural function, disease, and aging. *Front Genet* 2015;**6**:87.
189. Ziats MN, Rennert OM. Identification of differentially expressed microRNAs across the developing human brain. *Mol Psychiatry* 2014;**19**:848–52.
190. Liu N, Landreh M, Cao K, Abe M, Hendriks GJ, Kennerdell JR, et al. The microRNA miR-34 modulates aging and neurodegeneration in Drosophila. *Nature* 2012;**482**:519–23.
191. Wang WX, Huang Q, Hu Y, Stromberg AJ, Nelson PT. Patterns of microRNA expression in normal and early Alzheimer's disease human temporal cortex: white matter vs gray matter. *Acta Neuropathol* 2011;**121**:193–205.
192. Wang X, Liu P, Zhu H, Xu Y, Ma C, Dai X, et al. miR-34a, a microRNA up-regulated in a double transgenic mouse model of Alzheimer's disease, inhibits bcl2 translation. *Brain Res Bull* 2009;**80**:268–73.
193. Meier I, Fellini L, Jakovcevski M, Schachner M, Morellini F. Expression of the snoRNA host gene gas5 in the hippocampus is upregulated by age and psychogenic stress and correlates with reduced novelty-induced behavior in C57BL/6 mice. *Hippocampus* 2010;**20**:1027–36.
194. Jiao F, Wang Q, Zhang P, Bu L, Yan J, Tian B. Expression signatures of long non-coding RNA in the substantia nigra of pre-symptomatic mouse model of Parkinson's disease. *Behav Brain Res* 2017;**331**:123–30.
195. Johnson R. Long non-coding RNAs in Huntington's disease neurodegeneration. *Neurobiol Dis* 2012;**46**:245–54.
196. Soldati C, Bithell A, Johnston C, Wong KY, Stanton LW, Buckley NJ. Dysregulation of REST-regulated coding and non-coding RNAs in a cellular model of Huntington's disease. *J Neurochem* 2013;**124**:418–30.

197. Geekiyanage H, Chan C. MicroRNA-137/181c regulates serine palmitoyltransferase and in turn amyloid beta, novel targets in sporadic Alzheimer's disease. *J Neurosci* 2011;**31**:14820–30.
198. Zhao TC, Wang Z, Zhao TY. The important role of histone deacetylases in modulating vascular physiology and arteriosclerosis. *Atherosclerosis* 2020;**303**:36–42.
199. Lind L, Ingelsson E, Sundstrom J, Siegbahn A, Lampa E. Methylation-based estimated biological age and cardiovascular disease. *Eur J Clin Invest* 2018;48.
200. Zhang W, Song M, Qu J, Liu GH. Epigenetic modifications in cardiovascular aging and diseases. *Circ Res* 2018;**123**:773–86.
201. Smolarek I, Wyszko E, Barciszewska AM, Nowak S, Gawronska I, Jablecka A, et al. Global DNA methylation changes in blood of patients with essential hypertension. *Med Sci Monit* 2010;**16**: CR149–55.
202. Fan R, Wang WJ, Zhong QL, Duan SW, Xu XT, Hao LM, et al. Aberrant methylation of the GCK gene body is associated with the risk of essential hypertension. *Mol Med Rep* 2015;**12**:2390–4.
203. Zhang D, Yu ZY, Cruz P, Kong Q, Li S, Kone BC. Epigenetics and the control of epithelial sodium channel expression in collecting duct. *Kidney Int* 2009;**75**:260–7.
204. Irvin MR, Zhi D, Joehanes R, Mendelson M, Aslibekyan S, Claas SA, et al. Epigenome-wide association study of fasting blood lipids in the Genetics of Lipid-lowering Drugs and Diet Network study. *Circulation* 2014;**130**:565–72.
205. Guay SP, Brisson D, Munger J, Lamarche B, Gaudet D, Bouchard L. ABCA1 gene promoter DNA methylation is associated with HDL particle profile and coronary artery disease in familial hypercholesterolemia. *Epigenetics* 2012;**7**:464–72.
206. Pagiatakis C, Sun D, Tobin SW, Miyake T, McDermott JC. TGFbeta-TAZ/SRF signalling regulates vascular smooth muscle cell differentiation. *FEBS J* 2017;**284**:1644–56.
207. Connelly JJ, Cherepanova OA, Doss JF, Karaoli T, Lillard TS, Markunas CA, et al. Epigenetic regulation of COL15A1 in smooth muscle cell replicative aging and atherosclerosis. *Hum Mol Genet* 2013;**22**:5107–20.
208. Liang H, Zhang C, Ban T, Liu Y, Mei L, Piao X, et al. A novel reciprocal loop between microRNA-21 and TGFbetaRIII is involved in cardiac fibrosis. *Int J Biochem Cell Biol* 2012;**44**:2152–60.
209. Greissel A, Culmes M, Burgkart R, Zimmermann A, Eckstein HH, Zernecke A, et al. Histone acetylation and methylation significantly change with severity of atherosclerosis in human carotid plaques. *Cardiovasc Pathol* 2016;**25**:79–86.
210. Thienpont B, Aronsen JM, Robinson EL, Okkenhaug H, Loche E, Ferrini A, et al. The H3K9 dimethyltransferases EHMT1/2 protect against pathological cardiac hypertrophy. *J Clin Invest* 2017;**127**:335–48.
211. Vieira JM, Howard S, Villa Del Campo C, Bollini S, Dube KN, Masters M, et al. BRG1-SWI/SNF-dependent regulation of the Wt1 transcriptional landscape mediates epicardial activity during heart development and disease. *Nat Commun* 2017;**8**:16034.
212. Pagiatakis C, Musolino E, Gornati R, Bernardini G, Papait R. Epigenetics of aging and disease: a brief overview. *Aging Clin Exp Res* 2019.
213. Soler-Botija C, Galvez-Monton C, Bayes-Genis A. Epigenetic biomarkers in cardiovascular diseases. *Front Genet* 2019;**10**:950.
214. Shiels PG, McGuinness D, Eriksson M, Kooman JP, Stenvinkel P. The role of epigenetics in renal aging. *Nat Rev Nephrol* 2017;**13**:471–82.
215. De Santis M, Selmi C. The therapeutic potential of epigenetics in autoimmune diseases. *Clin Rev Allergy Immunol* 2012;**42**:92–101.
216. Grazioli E, Dimauro I, Mercatelli N, Wang G, Pitsiladis Y, Di Luigi L, et al. Physical activity in the prevention of human diseases: role of epigenetic modifications. *BMC Genomics* 2017;**18**:802.
217. Arora I, Sharma M, Sun LY, Tollefsbol TO. The epigenetic link between polyphenols, aging and age-related diseases. *Genes (Basel)* 2020;11.
218. Hass BS, Hart RW, Lu MH, Lyn-Cook BD. Effects of caloric restriction in animals on cellular function, oncogene expression, and DNA methylation in vitro. *Mutat Res* 1993;**295**:281–9.

219. Chouliaras L, van den Hove DL, Kenis G, Dela Cruz J, Lemmens MA, van Os J, et al. Caloric restriction attenuates age-related changes of DNA methyltransferase 3a in mouse hippocampus. *Brain Behav Immun* 2011;**25**:616–23.
220. Pasyukova EG, Vaiserman AM. HDAC inhibitors: a new promising drug class in anti-aging research. *Mech Ageing Dev* 2017;**166**:6–15.
221. Tsai CT, Yang PM, Chern TR, Chuang SH, Lin JH, Klemm L, et al. AID downregulation is a novel function of the DNMT inhibitor 5-aza-deoxycytidine. *Oncotarget* 2014;**5**:211–23.
222. Dehghan E, Goodarzi M, Saremi B, Lin R, Mirzaei H. Hydralazine targets cAMP-dependent protein kinase leading to sirtuin1/5 activation and lifespan extension in C. elegans. *Nat Commun* 2019;**10**:4905.
223. Dehghan E, Zhang Y, Saremi B, Yadavali S, Hakimi A, Dehghani M, et al. Hydralazine induces stress resistance and extends C. elegans lifespan by activating the NRF2/SKN-1 signalling pathway. *Nat Commun* 2017;**8**:2223.
224. Wagner AE, Piegholdt S, Rabe D, Baenas N, Schloesser A, Eggersdorfer M, et al. Epigallocatechin gallate affects glucose metabolism and increases fitness and lifespan in Drosophila melanogaster. *Oncotarget* 2015;**6**:30568–78.
225. Xiong LG, Chen YJ, Tong JW, Gong YS, Huang JA, Liu ZH. Epigallocatechin-3-gallate promotes healthy lifespan through mitohormesis during early-to-mid adulthood in Caenorhabditis elegans. *Redox Biol* 2018;**14**:305–15.
226. Liao VH, Yu CW, Chu YJ, Li WH, Hsieh YC, Wang TT. Curcumin-mediated lifespan extension in Caenorhabditis elegans. *Mech Ageing Dev* 2011;**132**:480–7.
227. Stepien K, Wojdyla D, Nowak K, Molon M. Impact of curcumin on replicative and chronological aging in the Saccharomyces cerevisiae yeast. *Biogerontology* 2020;**21**:109–23.
228. Pirmoradi S, Fathi E, Farahzadi R, Pilehvar-Soltanahmadi Y, Zarghami N. Curcumin affects adipose tissue-derived mesenchymal stem cell aging through TERT gene expression. *Drug Res (Stuttg)* 2018;**68**:213–21.
229. Lee EB, Ahn D, Kim BJ, Lee SY, Seo HW, Cha YS, et al. Genistein from Vigna angularis extends lifespan in Caenorhabditis elegans. *Biomol Ther (Seoul)* 2015;**23**:77–83.
230. Li Y, Chen F, Wei A, Bi F, Zhu X, Yin S, et al. Klotho recovery by genistein via promoter histone acetylation and DNA demethylation mitigates renal fibrosis in mice. *J Mol Med (Berl)* 2019;**97**:541–52.
231. Altun D, Uysal H, Askin H, Ayar A. Determination of the effects of genistein on the longevity of Drosophila melanogaster meigen (Diptera; Drosophilidae). *Bull Environ Contam Toxicol* 2011;**86**:120–3.
232. Staats S, Wagner AE, Luersen K, Kunstner A, Meyer T, Kahns AK, et al. Dietary ursolic acid improves health span and life span in male Drosophila melanogaster. *Biofactors* 2019;**45**:169–86.
233. Negi H, Shukla A, Khan F, Pandey R. 3beta-Hydroxy-urs-12-en-28-oic acid prolongs lifespan in C. elegans by modulating JNK-1. *Biochem Biophys Res Commun* 2016;**480**:539–43.
234. DiTroia SP, Percharde M, Guerquin MJ, Wall E, Collignon E, Ebata KT, et al. Maternal vitamin C regulates reprogramming of DNA methylation and germline development. *Nature* 2019;**573**:271–5.
235. Selman C, McLaren JS, Meyer C, Duncan JS, Redman P, Collins AR, et al. Life-long vitamin C supplementation in combination with cold exposure does not affect oxidative damage or lifespan in mice, but decreases expression of antioxidant protection genes. *Mech Ageing Dev* 2006;**127**:897–904.
236. Pallauf K, Bendall JK, Scheiermann C, Watschinger K, Hoffmann J, Roeder T, et al. Vitamin C and lifespan in model organisms. *Food Chem Toxicol* 2013;**58**:255–63.
237. De Haes W, Frooninckx L, Van Assche R, Smolders A, Depuydt G, Billen J, et al. Metformin promotes lifespan through mitohormesis via the peroxiredoxin PRDX-2. *Proc Natl Acad Sci USA* 2014;**111**:E2501–9.
238. Chen J, Ou Y, Li Y, Hu S, Shao LW, Liu Y. Metformin extends C. elegans lifespan through lysosomal pathway. *Elife* 2017;6.
239. Cuyas E, Fernandez-Arroyo S, Verdura S, Garcia RA, Stursa J, Werner L, et al. Metformin regulates global DNA methylation via mitochondrial one-carbon metabolism. *Oncogene* 2018;**37**:963–70.

240. Bass TM, Weinkove D, Houthoofd K, Gems D, Partridge L. Effects of resveratrol on lifespan in Drosophila melanogaster and Caenorhabditis elegans. *Mech Aging Dev* 2007;**128**:546–52.
241. Park SJ, Ahmad F, Philp A, Baar K, Williams T, Luo H, et al. Resveratrol ameliorates aging-related metabolic phenotypes by inhibiting cAMP phosphodiesterases. *Cell* 2012;**148**:421–33.
242. Bhullar KS, Hubbard BP. Lifespan and healthspan extension by resveratrol. *Biochim Biophys Acta* 2015;**1852**:1209–18.
243. Staats S, Wagner AE, Kowalewski B, Rieck FT, Soukup ST, Kulling SE, et al. Dietary resveratrol does not affect life span, body composition, stress response, and longevity-related gene expression in Drosophila melanogaster. *Int J Mol Sci* 2018;19.
244. [Determination of geroprotective potential of sodium butyrate in Drosophila melanogaster: long-term effects]. *Adv Gerontol* 2013;**26**:111–16.
245. Edwards C, Canfield J, Copes N, Rehan M, Lipps D, Bradshaw PC. D-beta-hydroxybutyrate extends lifespan in C. elegans. *Aging (Albany NY)* 2014;**6**:621–44.
246. McDonald P, Maizi BM, Arking R. Chemical regulation of mid- and late-life longevities in Drosophila. *Exp Gerontol* 2013;**48**:240–9.
247. Kang HL, Benzer S, Min KT. Life extension in Drosophila by feeding a drug. *Proc Natl Acad Sci USA* 2002;**99**:838–43.
248. Zhang M, Poplawski M, Yen K, Cheng H, Bloss E, Zhu X, et al. Role of CBP and SATB-1 in aging, dietary restriction, and insulin-like signaling. *PLoS Biol* 2009;**7**:e1000245.
249. Tao D, Lu J, Sun H, Zhao YM, Yuan ZG, Li XX, et al. Trichostatin A extends the lifespan of Drosophila melanogaster by elevating hsp22 expression. *Acta Biochim Biophys Sin (Shanghai)* 2004;**36**:618–22.
250. Saul N, Pietsch K, Menzel R, Steinberg CE. Quercetin-mediated longevity in Caenorhabditis elegans: is DAF-16 involved? *Mech Aging Dev* 2008;**129**:611–13.
251. Warnsmann V, Hainbuch S, Osiewacz HD. Quercetin-induced lifespan extension in Podospora anserina requires methylation of the flavonoid by the O-methyltransferase PaMTH1. *Front Genet* 2018;**9**:160.
252. Wang H, Jo YJ, Oh JS, Kim NH. Quercetin delays postovulatory aging of mouse oocytes by regulating SIRT expression and MPF activity. *Oncotarget* 2017;**8**:38631–41.
253. Eisenberg T, Abdellatif M, Schroeder S, Primessnig U, Stekovic S, Pendl T, et al. Cardioprotection and lifespan extension by the natural polyamine spermidine. *Nat Med* 2016;**22**:1428–38.
254. Eisenberg T, Knauer H, Schauer A, Buttner S, Ruckenstuhl C, Carmona-Gutierrez D, et al. Induction of autophagy by spermidine promotes longevity. *Nat Cell Biol* 2009;**11**:1305–14.
255. Bjedov I, Toivonen JM, Kerr F, Slack C, Jacobson J, Foley A, et al. Mechanisms of life span extension by rapamycin in the fruit fly Drosophila melanogaster. *Cell Metab* 2010;**11**:35–46.
256. Villa-Cuesta E, Fan F, Rand DM. Rapamycin reduces Drosophila longevity under low nutrition. *IOSR J Pharm* 2014;**4**:43–51.
257. Robida-Stubbs S, Glover-Cutter K, Lamming DW, Mizunuma M, Narasimhan SD, Neumann-Haefelin E, et al. TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO. *Cell Metab* 2012;**15**:713–24.
258. Miller RA, Harrison DE, Astle CM, Fernandez E, Flurkey K, Han M, et al. Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction. *Aging Cell* 2014;**13**:468–77.
259. Gong H, Qian H, Ertl R, Astle CM, Wang GG, Harrison DE, et al. Histone modifications change with age, dietary restriction and rapamycin treatment in mouse brain. *Oncotarget* 2015;**6**:15882–90.