

REVIEW ARTICLE

The Aging Epigenome and The Rejuvenation Strategies

Anna Meiliana^{1,2,3,*}, Nurrani Mustika Dewi^{2,3}, Andi Wijaya^{2,3}¹Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km 21, Jatinangor 45363, Indonesia²Prodia Clinical Laboratory, Jl. Supratman No 43, Bandung 40114, Indonesia³Prodia Education and Research Institute, Jl. Kramat Raya No. 150, Jakarta, 10430, Indonesia

*Corresponding author. E-mail: anna.meiliana@prodia.co.id

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Abstract

BACKGROUND: Aging is an unavoidable part of life, defined by a gradual loss in tissue and organ function and an increasing chance of death. Current studies of aging connected the genetic and epigenetic changes to cause this process.

CONTENT: When the aging-related epigenetic alterations is accumulated, it may result in irregular gene expression, metabolic instability, stem cell senescence and exhaustion, and imbalance of tissue homeostasis, which all accelerate the aging process. Altered epigenetic gene regulatory mechanisms such as DNA methylation, histone

modification and chromatin remodeling, and non-coding RNAs can induce aging process, thus manipulating these processes give a chance for the success of age-delaying interventions.

SUMMARY: Given updated tools and technologies to investigate the epigenetic regulation affecting aging processes, new therapeutic strategies to delay this process can be developed to increase longevity and improve quality of life.

KEYWORDS: aging, epigenetic, senescence, autophagy, mitochondria, metabolism, rejuvenation

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Introduction

Aging has been linked with a loss of function at the cellular, tissue, and organismal levels, as well as with a broad variety of illnesses, including cardiovascular and neurological diseases, metabolic disorders, and several types of cancer. Health span is a term that refers to the period of disease-free physiological health (for example, normal cognition and mobility) and significantly indicates human aging. Understanding the factors regulating aging process, lifespan, and health span can open opportunities to reverse the hallmarks of aging and promote a longer youthful period.(1)

The aging hallmarks can be influenced by genetic and also epigenetic.(2) By controlling the transcriptional

machinery's access to DNA, gene expression dynamically or chronically by chromatin and epigenetic factors can impact, even through cell division. These factors include enzymes that alter DNA or histones (H2A, H2B, H3, and H4), and variant histones (H3.3, macroH2A, and H2A.Z) directly. Numerous epigenetic studies in all aging models showed that in local and global chromatin remodeling, histones become loss and altered. There is also global and local changes in DNA methylation, histone post-translational modification, and chromatin structure and remodeling, and substantial nuclear rearrangement, particularly in mammalian systems. (3) Non-coding RNAs, microRNAs, and circular RNAs, also provide layers of epigenetic control and affect the aging process.(4)

Recent investigations have discovered that significant chromatin alterations occur throughout aging, generally

accompanied by gradual loss of constitutive heterochromatin (5,6), and that processes influence transgenerational inheritance, most crucially, the environmental and epigenetic variables (such as sirtuins) which may directly regulate aging kinetics. In yeast normal aging, declining histone was found, while restoration of histone levels promotes lifetime extension.(7) These findings reveal that aging may be slowed without changing genomic sequences, but due to the epigenetic forms. Metabolic modification, partial reprogramming, heterochronic parabiosis, pharmacological delivery, and senescent cell ablation (8), stem cell function restoration, mitochondrial activity rescue, suppression of active transposons, and repression of age-related chronic inflammation, 'resetting' the epigenome might lead to rejuvenation of cells, tissues, and species.(2)

Epigenetic Regulation of Aging

Epigenetic pathways have been widely recognized to play essential roles in both health and illness, including aging process (9), and now even recognized as indicators of aging. Epigenetic drift due to long-term environmental stress

exposure result in disrupted phenotype, gene expression, and even immune system (10). This links how the environmental can alter the epigenome and affect our healthspan.

The exact causation between these epigenetic modifications and the aging process, on the other hand, is still on investigation based on two evidence. First, epigenetic drift or mutations that accumulate with age can lead to genomic instability and changes in gene expression patterns defined by an increase in gene expression noise, both of which have been linked to aging. Second, epigenetic changes that accumulate in one generation may be transmitted down to the next generation, influencing the offspring aging acceleration.(2) Environmental perturbations can cause heritable epigenetic (11), and the children of elderly paternal mice have shorter lives and show aging characteristics earlier than the offspring of young paternal mice (12). The epigenetic bases of aging and rejuvenation can be found in Figure 1.

The epigenome is the cross-point of the genome and environment. Epigenome and transcriptome can be affected by lifestyle and behavioral factors such as prenatal exposures, childhood adversity, diet, physical activity, exercise, stress, exposure to pollutants and toxins, smoking,

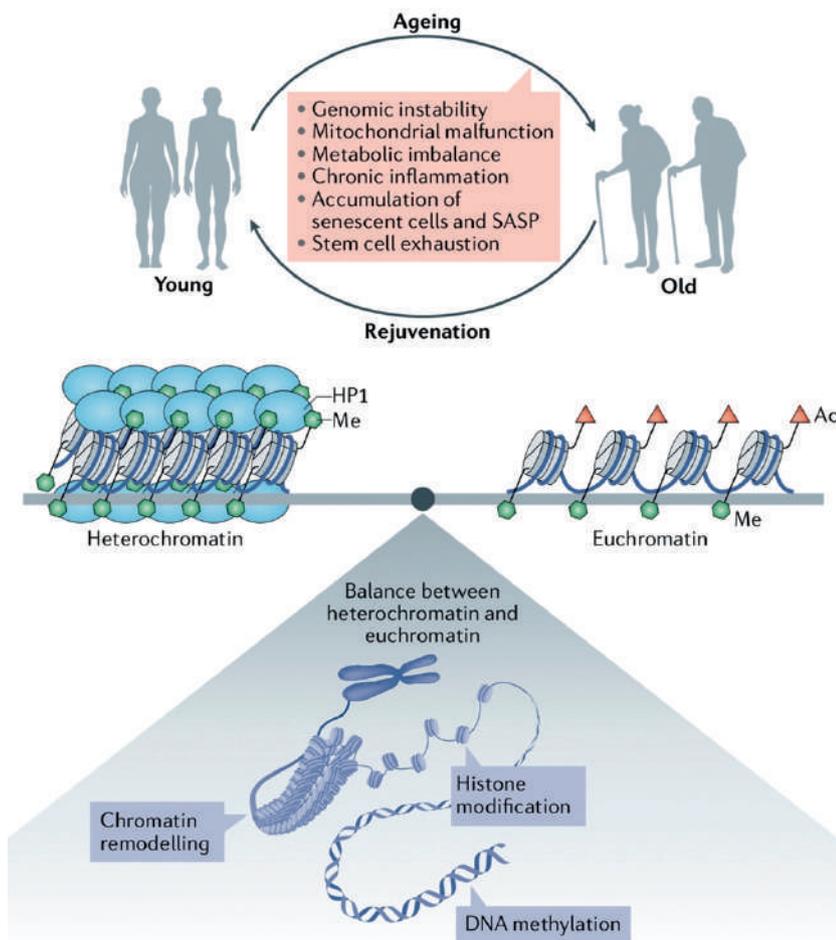


Figure 1. The epigenetic bases of aging and rejuvenation.(2) (Adapted with permission from Springer Nature).

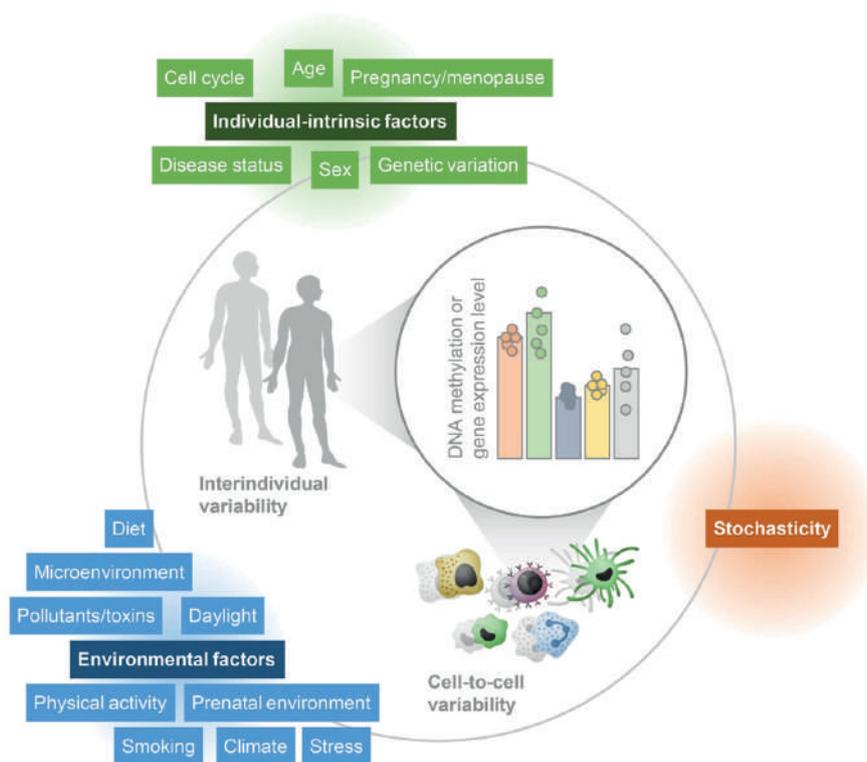


Figure 2. Overview of the different types and sources of epigenetic and transcriptional variability.(19) (Adapted with permission from Wiley Periodicals).

climate, season, daylight, culture, education, socio-economic variables, result in phenotype (Figure 2). Some changes even can have a long-term influence, and be handed on to future generations.(13-19)

Post-synthesis chemical alteration of three kinds of essential macromolecules: DNA, RNA, and proteins, is required for the regulation and adaptation of practically all biological activities. Methylation, one of the most common modifications, comprises an alkylation process in which a methyl group replaces a hydrogen atom. Methyltransferases act as the “writers” in methylation process assisted by methyl “readers”, while demethylases is the “erasers”, utilizing S-adenosylmethionine (SAM) as the methyl donor. The fact that their dysregulation is connected to a variety of diseases emphasizes the relevance of the multiple methylation mechanisms.(20-24) DNA methyltransferase 1 (DNMT1), DNMT3A, and DNMT3B are three enzymes that methylate DNA and keep genomic methylation patterns under check. The methylation by adding a methyl group to the 5' position of the cytosine pyrimidine ring mostly in palindromic CpG dinucleotides (5mC). While the first step of active DNA demethylation started by the oxidation of 5-methyl group of 5mC by the ten–eleven translocation (TET) dioxygenases create 5-hydroxymethylcytosine (5hmC), 5-formylcytosine, or 5-carboxylcytosine.(25)

SAM is transformed to S-adenosylhomocysteine (SAH) during the methylation step, which limits methyltransferase

function.(26) As a result, alterations in the cellular SAM-to-SAH ratio can affect methyltransferases. CpG methylation of proto-oncogene promoters can increase cellular levels of SAM and inhibit proto-oncogenes.(27) Different enzymes can methylate different classes of macromolecule.(28) According to the 'heterochromatin loss hypothesis of aging' theory, aging causes cellular malfunction due to reduced heterochromatin and/or incorrect redistribution of heterochromatin-silencing proteins. While the processes causing heterochromatin remodeling are unknown, the interactions between the chromatin machinery and nuclear peripheral proteins such nuclear lamins may be involved (Figure 3).(1) Loss of heterochromatin with age is frequently accompanied with modification of histone methylations associated with 'active' chromatin.(29-31).

The methylation landscapes of DNA and histones alter as a cell matures. Early research claimed that global DNA hypomethylation is a sign of aging; however, more recent research revealed that DNA methylation loss occurs especially in constitutive-heterochromatin repeat regions, whereas promoter CpGs are hypermethylated. (32,33) The genomic transposition of retrotransposable elements is associated with the formation of DNA double-strand breaks.(34-36) This can induce genome instability and cause disease.(37) A stable epigenome, on the other hand, has been linked to lifespan and cancer resistance.(38)

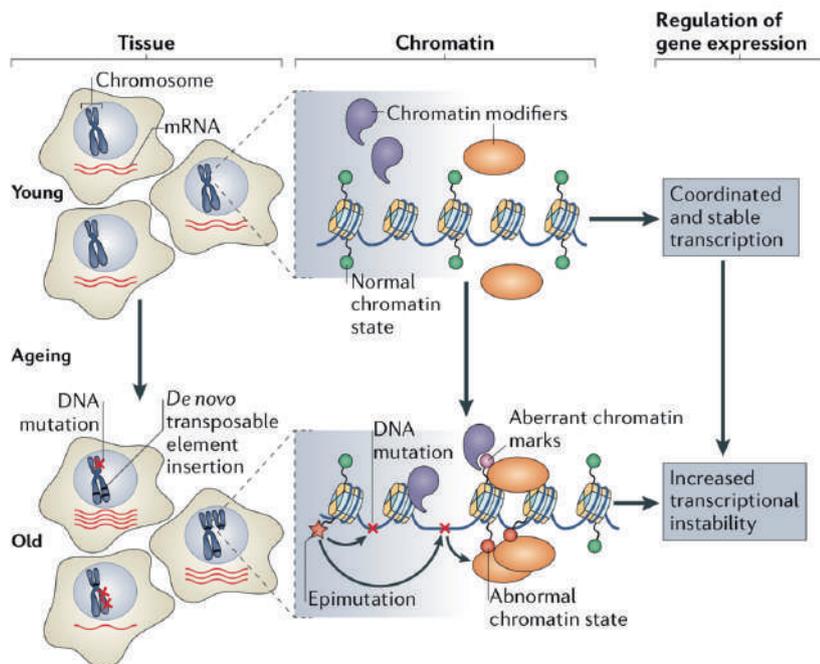


Figure 3. A model for the possible crosstalk between chromatin changes and transcription and genomic instability during aging.(1) (Adapted with permission from Springer Nature).

In aging blood and tissues, there has been an increase in locus-specific DNA hypermethylation (39,40), produces a transcriptional pattern that is age-related (40). In model organisms, a variety of DNA methylation 'clocks' have been identified (41), may be used to estimate age (42), predict mortality (43) and lifespan (44,45).

Two common chromatin alteration themes in aging influenced by environmental cues, nutritional signaling, and metabolic status include global upregulation of activating marks and downregulation of repressive marks, and gene-specific changes in chromatin which influence essential longevity genes expression. Significant epigenetic alterations that may be linked to aging identification bring up big challenges and opportunity to improve our aging-related metabolism, such as which chromatin modification or enzymes can induce which pathway, especially related to the mitochondrial aging.(46)

The Metabolic Regulation of Aging

A variety of metabolic changes seen in aging, including regulation of mitochondrial activity, a decrease in insulin sensitivity, and changes in substrate use.(47) Dissecting how metabolic pathways are influenced by environmental or genetic alterations to regulate longevity may provide greater information. In mice and rats, lifelong caloric restriction (CR) can lengthen lifespan by up to 50% compared to ad libitum-fed control animals in some circumstances (48), led more studies to figure out how reducing food intake leads to

longer life, and how the genetic and physiological processes mediate this effect (49). The mechanistic target of rapamycin (mTOR) is one clear connection. mTOR is a serine-threonine kinase that acts as an intracellular energy sensor and is found in two different protein complexes in mammalian cells: mTORC1 and mTORC2. mTOR is activated in response to growth factor stimulation or an increase in intracellular amino acid levels, and it then governs a slew of downstream events that control development and metabolism.(50)

Another set of possible mediators relating nutrition availability to lifespan is the sirtuin family of proteins. Both histone and nonhistone protein deacetylation of nicotinamide adenine dinucleotide (NAD), and the acyl moieties removal from longer carbon lengths such as malonyl and succinyl groups, from several enzymes, all performed by sirtuin family members.(51) The relationship suggests that this protein family may be at the intersection of metabolism and lifespan, as answering how CR can increase the lifespan in yeast (52) and flies (53), although some studies questioned it (55).

Another critical linkage between nutrition and lifespan are the metabolic sensor adenosine monophosphate (AMP)-activated protein kinase (AMPK), and autophagy process, which is the important lysosomal-dependent recycling process. When intracellular energy supplies (ATP) are exhausted, AMPK is activated and lower-energy adenine nucleotides (AMP and ADP) rise to compensate and regulates a number of metabolic pathways that, in general, enhance energy supply while decreasing demand.(54) The AMPK capacity to regulate autophagy later recognized as a

key modulator of organismal aging.(55) Intriguingly, these effects can act in a non-cell-autonomous manner in the same way as mTOR signaling does.

A mutation in a receptor of *C. Elegans* receptor that is similar to the mammalian insulin/Insulin-like Growth Factor 1 (IGF-1) receptor double its lifespan compare to the wildtype, and this suggested a strong link between insulin/IGF-1 signaling and lifespan.(56,57) Surprisingly, genetic studies of long-lived humans have frequently linked mutations in the IGF-1 receptor or FOXO transcription factors to a longer lifespan and better health.(58,59) Finally, the importance of the somatotrophic axis in coordinating growth, metabolism, and lifespan is highlighted by secreted substances such as insulin/IGF-1, or, as noted in the case of dwarf mice, secretion of factors such as growth hormone. (60) Thus, neuroendocrine modulation is vital for energy homeostasis and aging.(61,62)

Bulk histone acetylation altered patterns are frequently seen in aging tissues and have been linked to age-related diseases including cancer and dementia (1,20,63), suggesting a large-scale chromatin modifications (64). Class III HDACs (sirtuins) have previously been demonstrated to mediate the connection of histone acetylation to general metabolism (65), and that CR-induced improvements in healthy longevity via overexpression of the Sir2 deacetylase (66). Resveratrol from wine known to boost the activity of AMPK, SIRT1, and peroxisome proliferator-activated receptor gamma coactivator 1- α (PGC-1 α), demonstrated to have a favorable metabolic influence on obese adults.(67) Somehow, different results were observed on *C. elegans* and *D. melanogaster* about how sirtuins involved in aging process due to strict control of the genetic background of the animals.(68,69)

Acetyl-CoA, a major molecule produced predominantly in mitochondria, is used by KATs.(70) Acetyl-CoA that has not been metabolized by the tricarboxylic acid (TCA) cycle can be transferred to the cytosol by being converted to citrate, which is then cleaved back into oxaloacetate and acetyl-CoA by ATP citrate lyase (ACLY/ATPCL) in the cytosol.(71) Because acetyl-CoA may readily transport into the nucleus, reduction of ACLY/ATPCL causes reduced histone acetylation levels in animals (71), tying metabolism and histone modification together (72-74).

Flies with decreased ATPCL activity had longer lifespan, indicating that increased histone acetylation is linked to the aging process.(75) Another study found that increasing pyruvate carboxylase activity is connected to age-related memory impairment in *Drosophila*, links acetyl-CoA levels to organismal aging.(64) The discovery of major

metabolic pathways connected to longevity has initiated interest to pharmacologically targeting these parameters with hopes of slowing aging and alleviating age-related disorders. The natural plant polyphenol resveratrol was the first chemical screen found as one such sirtuin activator. (76) The question of whether resveratrol works as a direct or indirect sirtuin activator however is still controversial. (77) Despite the lack of understanding of the mechanism, resveratrol has been shown to have positive cardiovascular and metabolic benefits in animal models (78,79), non-human primates (80,81) and human subjects (67).

Low-carbohydrate diets (LCDs) shift the metabolic away from carbohydrate oxidation toward fatty acid oxidation. The ketogenic diet (KD), the most extreme LCD, has been proven to create an anti-inflammatory metabolic state and boost ketone body levels in rats, similar to key aspects of CR.(82) KD significantly increased median lifespan and survival compared to controls. Only those mice with KD showed signs of physiological function preservation as they grew older. In a tissue-dependent way, the KD raised protein acetylation levels and controlled mTORC1 signaling. In this study, mice with a KD have a longer lifespan and are healthier.(83)

Another developing strategy is to increase intracellular NAD⁺ metabolite levels to combat aging and age-related disease. In both model species and humans, biochemical levels of tissue NAD⁺ appear to decrease with age.(84) Although the exact cause of this age-related loss is unknown, a decrease in NAD⁺ metabolite levels appear to diminish the sirtuin function, as these proteins are mostly NAD⁺-dependent enzymes. NAD supplementation stimulates the UPR system, improves metabolic indices, and, most importantly, increases longevity.(84-87) Other research shows that restoring 'youthful' NAD⁺ levels may help to delay the onset of age-related illnesses.(88)

Mitochondria: Masters of Epigenetics

Aging has been linked to a general decline of mitochondrial function.(46,89-91) A genetic mutation in the mitochondrial DNA polymerase induce accelerated aging in mice.(92) The altered serum metabolome of mice with an accelerated aging phenotype, including lower citrate levels, a sign of mitochondrial activity, supports the functional relevance of metabolic decline throughout aging.(93) Muscle fibers taken from aged adult individuals had lower oxygen consumption rates, implying that this tissue has lower mitochondrial activity.(94) Somehow, lowering metabolic activity by

calorie restriction or inhibiting the activities of important metabolic regulators like IGF-1, mTOR, and AMPK has been linked to a longer lifespan.(95) Contradictively, enhanced mitochondrial activity at earlier stages of aging (midlife) associated with a shorter lifespan.(75,96) This might explain why, in midlife, lowering calorie intake and hence decreasing metabolic activity can delay age-related metabolic gain.(64)

As mentioned before, nuclear-encoded mitochondrial proteins have been implicated in the organisms' control of longevity.(97,98) Most of these genetic manipulations affected the expression of electron transport chain components, impairing mitochondrial function while also extending longevity. Human aging is often associated with mitochondrial function loss (99). Studies have recently discovered that mitochondrial stress exposure to *C. elegans* larvae induce a particular and long-lasting epigenetic response which protects the worm as they adult and increases longevity.(100-102)

Mitochondrial function depends on the coordination of nuclear and mitochondrial genomes, the transcription and translation of both genomes are coregulated. For example, the oxidative phosphorylation (OXPHOS) complexes were made up of protein components encoded in both genomes and hence must be built in a stoichiometric way.(103,104) The nucleus regulates mitochondrial function through a process known as "anterograde regulation" (signaling

from the nucleus to the mitochondria), which stimulates biogenesis and regulates mitochondrial activity to fulfill cellular demands (Figure 4). Mitochondria, on the other hand, can affect nuclear gene expression via a "retrograde reaction" (signaling from mitochondria to the nucleus), changing cellular function via reprogramming metabolism. Mitonuclear communication, or bidirectional regulation, is a strong network that maintains cellular homeostasis while also controlling response to a range of stimuli.(105) In addition, environmental changes and cellular metabolism can alter the epigenetic regulation of mitochondrial function and the mechanisms that affect both nuclear and mitochondrial epigenome.(106)

Environmental variation including nutritional and stress, are translated into gene expression modifications through epigenome alteration. Epigenetic modifications control the expression of certain genes without affecting the DNA sequence, but only modification in the chromatin structure either in open or condensed state.(107) Post-translational histone modifications and DNA methylation are the key epigenetic processes implicated in this shift. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are enzymes to modulate chromatin packing to control gene expression. HATs transfer an acetyl group from acetyl-CoA to the ϵ -amino group of lysine residues, relaxing the chromatin and boosting accessibility to transcriptional activators by hiding the positive charge of the lysine.

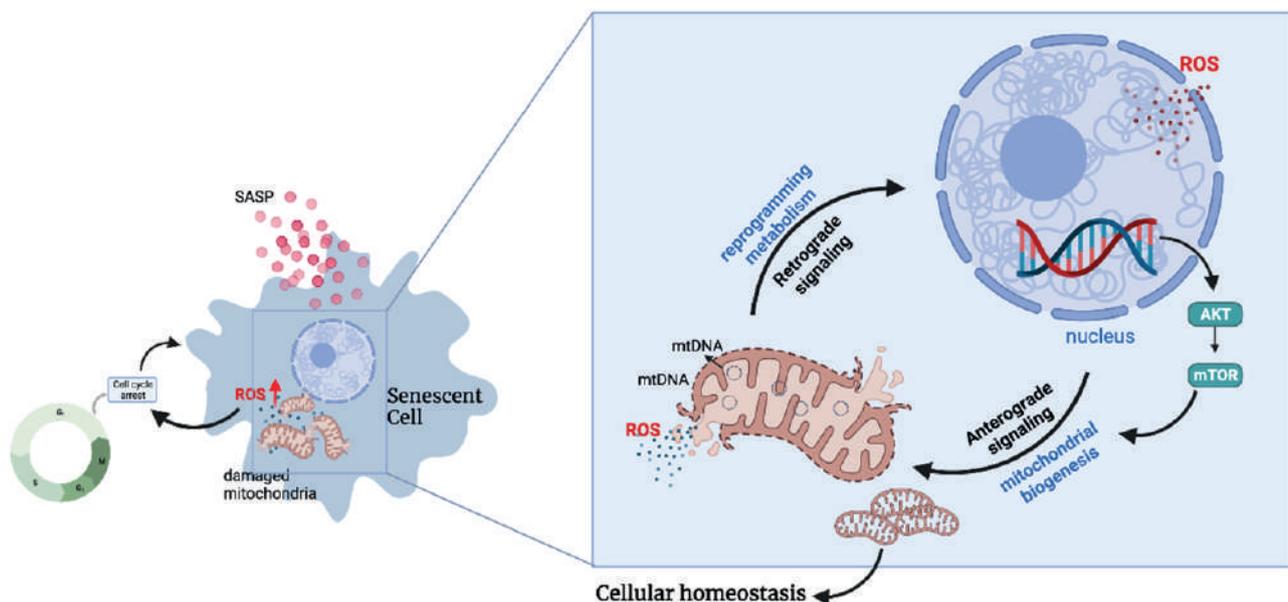


Figure 4. Throughout life, cells were exposed to stress, such as senescence. Senescent cells release a lot of reactive oxygen species (ROS) and pro-inflammatory senescence associated secretory phenotype (SASP). ROS can damage mitochondria and nuclear DNA which enforce cell cycle arrest, and further induce mitochondrial biogenesis. (Created by author in biorender.com).

The function of HATs is dependent on acetyl-CoA levels. Glycolysis and pyruvate, fatty acid oxidation, acetate, ketone bodies, and amino acids are all examples of metabolic processes and intermediates that can produce acetyl-CoA. (108,109) Citrate synthase in mitochondria converts acetyl-CoA and oxaloacetate to citrate in the TCA cycle. Citrate is then exported to the cytosol, where it is transformed to acetyl-CoA and oxaloacetate in an inverse process by ATP-citrate lyase (ACLY).(71) Acetyl-CoA levels in cells are highly correlated with energy generation: The acetyl-CoA levels rise as the energy production is high, to increase histone acetylation and gene expression. When cellular metabolism is sluggish, decreasing acetyl-CoA levels reduce histone acetylation and inhibiting gene expression through chromatin condensation.(108) Mitochondrial activity, namely the TCA cycle, is a key regulator of histone acetylation and, as a result, global gene expression.

The most common methylations are found on histone 3 (H3) and 4 (H4) lysine residues. Histone methylation, unlike acetylation, works by changing a single lysine (K) or arginine (R) amino acid residue on a histone protein to activate or inhibit transcription. Lysine can be mono-, di-, or trimethylated during the methylation process, and arginine usually be mono- or dimethylated.(110,111)

Histone methyltransferases (HMTs) are enzymes that methylate histones, while histone demethylases (HDMs) remove methyl marks. SET domain lysine methyltransferases, non-SET domain lysine methyltransferases, and arginine methyltransferases are the three types of HMTs. SAM is used as a precursor in methyl group transfer in all of the aforementioned classes.(118,120) As a result, mitochondrial activity can affect SAM production and hence modulate histone methylation. Although SAM is produced in the cytosol through the methionine-homocysteine cycle, this cycle is dependent on the folate cycle and ATP, both of which are dependent on mitochondrial metabolism.(118,121)

Stress in mitochondria can alter the epigenome by causing substantial chromatin remodeling, as evidenced by alterations in H3K9me2 methylation patterns mediated by HMT MET-2 and nuclear cofactor LIN-65. H3K9me2 methylation increases chromatin compaction, so the transcriptional apparatus is unable to initiate transcription. During mitochondrial stress, ATFS-1 and DVE-1, the transcriptional regulators open and bind loci carrying UPRmt genes and downregulation of global transcription. These variables cause the UPRmt to be activated in order to maintain mitochondrial proteostasis and improve mitochondrial lifespan. Importantly, LIN-65 and DVE-1 nuclear translocation are interdependent, indicating that

mitochondrial stress-induced chromatin reconfiguration and UPRmt transcriptional activation are highly controlled and interrelated processes.(101) Overall, mitochondrial function affects longevity by regulating the epigenome, linking two important characteristics of aging, mitochondrial failure and epigenetic changes (112), to lifetime determination.

Regulation of Chromatin and Gene Expression by Metabolic Enzymes and Metabolites

Environmental changes can be sensed by cells and modulate particular epigenome conversion through a range of signaling components such as histone- and DNA-modifying enzymatic activity.(113) Cell homeostasis, growth, proliferation, migration, differentiation, and death, need cell metabolism regulation to support all critical cellular functions. Cell metabolism started at the gene expression level to adjust the cells' extracellular and intracellular signals due to environmental change. In turn, cell metabolism can alter chromatin changes and hence chromatin functions, either directly or indirectly, through the activity of metabolic enzymes and metabolites. Hence, DNA-based process and cell metabolism are interlinked as a respond to the environment, and any disruption to this process can induce disease development.(114)

Enzymes that alter DNA or histones via cofactors are involved in chromatin control. These enzymes either add small chemical units (PTMs) or modify the location or content of the nucleosome (*i.e.*, of histone variants). This regulation is thought to be partially dependent on the changing amounts of cellular metabolites that function as cofactors for enzymes like acetyl-CoA, or S-adenosyl methionine utilized by methyltransferases, flavin was utilized by demethylases, kinases utilize ATP as acetyl, methyl, or phospho groups donors, while deacetylases utilize nicotinamide adenine dinucleotide (NAD).(113)

Although it is plausible that a number of metabolites are involved in various elements of epigenetic regulation on a conceptual level.(74,115) The hypothesis is that metabolite levels may fluctuate in response to diverse physiological stressors, therefore affecting enzymes involved in chromatin remodeling. The subcellular concentration of certain metabolites may influence the activation or inhibition of enzyme activity at the local level. Thus, the presence of "niches" of chromatin-associated metabolites has been suggested, which might explain why otherwise indistinguishable histones and DNA are modified

locus-specifically.(74) The metabolites may be related to enzyme activity involved in chromatin remodeling and DNA methylation on a functional level.

A metabolite is an intermediate or end product of metabolism. It serves as cofactors and regulators for enzymes, including those in chromatin remodeling, thus metabolites have important roles in methylation, hydroxylation, acetylation, crotonylation, 2-hydroxyisobutyrylation, O-GlcNAcylation, and poly (ADP) ribosylation (PARylation). Suggested that a diverse array of metabolites can regulate methyltransferase and demethylase activities to dynamically control histone and DNA methylation. Cell signaling regulates the production of these metabolites, and the enzymes responsible for their production can be mutated, thus determining biological outcomes.(114)

It was recently discovered that several metabolic enzymes, which are normally located in the cytoplasm or mitochondria, can also be present in the nucleus. These enzymes include glycolytic enzymes pyruvate kinase M2 isoform (PKM2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH); TCA (also known as MAT2A), 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 (PFKFB4), and fructose-1,6-bisphosphatase 1 (FBP1). These enzymes can provide metabolites to chromatin-modifying enzymes or transcription regulators in the nucleus, therefore

regulating chromatin shape and gene expression. They may also play other roles in controlling chromatin and gene expression that are unrelated to metabolite synthesis, such as direct alteration of histones and transcription regulators (Figure 5).(114)

Numerous types of enzymes can have their activity regulated by variations in metabolite availability or by the usage of particular metabolites as coenzymes.(113) The class III HDACs are a group of mammalian proteins that were initially discovered due to their resemblance to the yeast gene Sir2. There are seven members of sirtuin family in mammals in mitochondria (Sirt3, Sirt4, and Sirt5), nucleus (Sirt1, Sirt6, and Sirt7), and in cellular compartments (Sirt1, Sirt6, and Sirt7) (116). Sir2 was first discovered as a NAD⁺-dependent deacetylase and associated with longevity regulation (117–119), although the effects in various organisms is still debated (68).

A key concept rooting chromatin's involvement in regulating cell physiology and homeostasis is that nuclear activity needs coordination and reaction to the overall metabolic state of the cell. However, nuclear activities involving DNA-associated chromatin transactions distinct by location and regulation from those involving cytoplasm-based intermediate metabolic enzymes. A novel hypothesis to explain this dichotomy is that changes in metabolism may

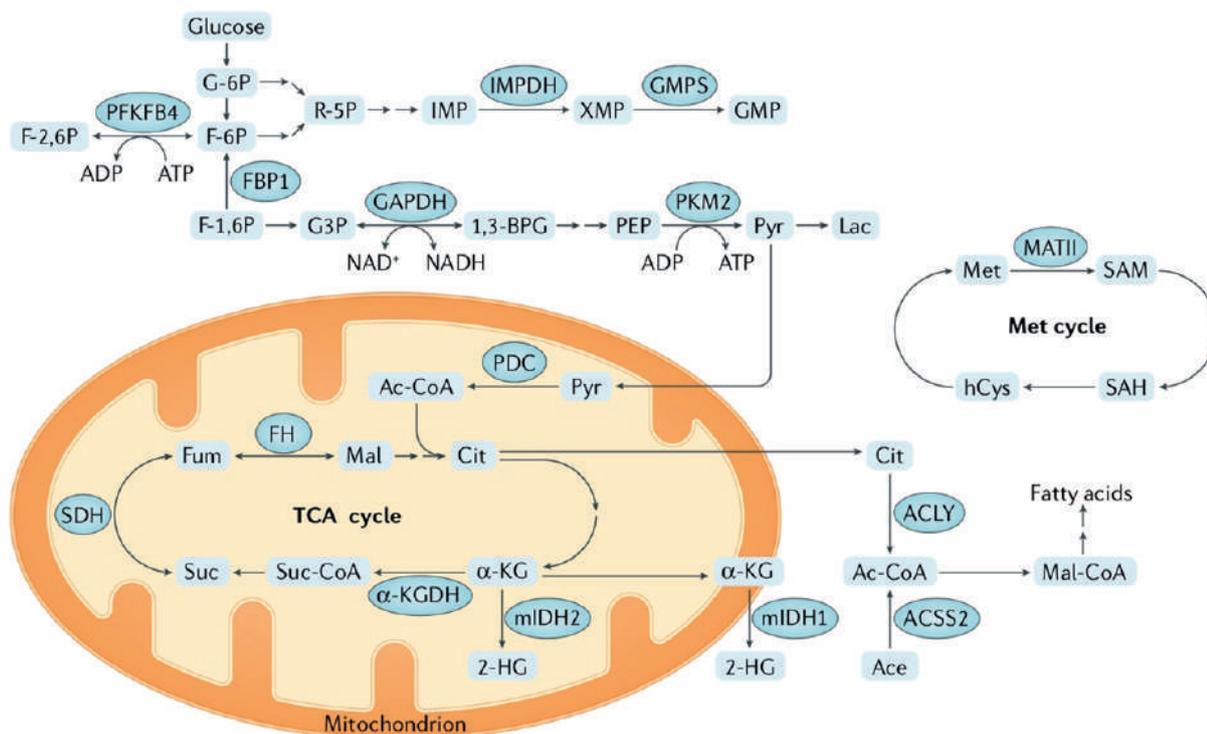


Figure 5. Core metabolic functions of metabolic enzymes that also function in epigenetic modifications.(114) (Adapted with permission from Springer Nature).

directly affect the amounts of acetyl-CoA, SAM, NAD, and FAD in the nucleus, which may subsequently affect DNA and histone modifications, and also transcription.(74)

Cell metabolism and gene expression should be tightly coupled and mutually regulated to perform essential biological function. Many factors can change the enzymatic activity and location at once such as nutrients, growth factors, and cytokines, and also stressors, both physiological or pathological. These alterations not only respond directly to cellular metabolic demands, but also govern cellular homeostasis, growth, proliferation, survival, and a variety of other activities via gene reprogramming. Enzymes' metabolites including acyl-CoA, SAM, fumarate, succinate, 2-HG, NAD⁺, NAM, and ketone bodies can function as direct substrates for DNA, histone modification, or regulate the DNA activity. The enzymes can function for non-metabolic activities such as protein kinases for direct phosphorylate histones or being the transcription factors and co-activators for gene transcription machinery. When metabolic enzymes located in the nucleus, they can merge with transcription factors, histone writers, readers, and also erasers.(114) It is critical whether epigenetic modifications contribute to cellular aging, given the long-established links between life duration, chromatin enzymes, and metabolic changes. The finding that yeast Sir2, a critical protein involved in heterochromatic silencing and lifespan promotion, is a NAD⁺-dependent HDAC has raised the possibility that chromatin plays a role in aging. (52,117-120)

Autophagy as Promoter of Longevity

Autophagy is the body's way of cleaning out damaged cells by catabolic process, in order to regenerate newer, healthier cells, and maintain cellular homeostasis. Autophagy facilitates lysosomes to do the break down and recycling of intracellular macromolecules and organelles. It was initially identified as a survival strategy in yeasts subjected to nutrient deprivation, a circumstance that accelerates the activity much above basal levels.(121) Numerous lines of evidence suggest that aging has an effect on the autophagy process, where autophagy-reporter analyses and gene expression studies in various species showed that autophagy has declined over time, and genetic experiments in short-lived animals showed that autophagy modulation can promote lifespan.(121)

Autophagy has four primary implications on age-related processes. First, by increasing the cytoplasmic

turnover, autophagy can neutralize the damaged organelles and macromolecular complexes. Second, when the homeostasis enhanced, genomic and epigenomic were stabilized, and the sensitivity of nutrition sensing increased. Third, autophagy significantly improve anti-inflammatory and immunological properties, therefore promoting system homeostasis. And fourth, and maybe most importantly, its actions may be connected to circadian clocks. The evidence for a bidirectional relationship between circadian clocks and autophagy-flux is growing.(122)

While the genetic data indicates that bulk autophagy is involved in aging processes, there is also evidence that impairments in the turnover of particular cargoes via selective autophagy may play a significant role in age-related diseases and aging. The accumulation of defective mitochondria is a common feature of aging and a variety of age-related illnesses.(89,91,112,123) Although the fundamental mechanisms causing age-related loss of mitochondrial function remain unknown and may include a variety of processes, a decrease in mitophagy has been hypothesized to play a critical role.(91,123,124) Mitophagy's molecular processes have been explored in depth in mammalian cell culture and genetic studies in model species (125-127), and found to be linked to the pathogenesis of age-related illnesses such as heart disease (128), retinopathy (129), fatty liver disease (130), pulmonary hypertension (131), kidney disease (132), and neurodegenerative disorders.

Numerous studies in varied species indicate that various pro-longevity treatments are related with certain alteration in lipid metabolism.(133) Autophagy's role in intracellular lipid droplet destruction, dubbed lipophagy, has been found in recent years.(134) The first conclusive evidence that lipid droplets can be recycled by autophagy came from experiments in cultured hepatocytes with decreased ATG5 levels.(135,136) The ability of autophagy to control lipid metabolism increases its physiological importance in the cellular energy balance modulation. Alterations in lipophagy may contribute to the aging metabolic syndrome (137), which is characterized by obesity, dysregulated lipoprotein metabolism, inappropriate glucose management, and hypertension. Additionally, lipophagy has been associated with cancer (138) and atherosclerosis (139).

Aggrephagy is the selective type of autophagy process which degrade protein aggregates, or oligomeric versions of proteins. Some protein aggregates in degenerated neurons such as tau, α -synuclein, and mutant HTT can induce toxicity and neurodegenerative disorders such as Alzheimer disease, Parkinson disease, and Huntington disease.(140-142), so upregulating autophagy for these aggregates by

chemical (143), genetic (144) or by hormetic heat shock (145) can improve neurodegeneration symptoms in a variety of animal models, including *C. elegans*, *D. melanogaster*, zebrafish, and mice (146). Indeed, aging is a significant risk factor for the majority of neurodegenerative disorders.

The molecular sensors that initiate autophagy in response to CR also regulate circadian clocks.(122) As a result, autophagy is regularly increased in a circadian clock-dependent way, and autophagy induction has a circadian clock-regulating effect.(147) Autophagy, in a variety of forms, has lately emerged as a critical process of stress adaption throughout the eukaryotic kingdom, posing a potential (but complicated) target for the treatment of a variety of human diseases.(148,149) Macroautophagy, in particular (hereinafter referred to as autophagy), destroys potentially hazardous or disposable cytoplasmic components to maintain cellular homeostasis under physiological and stress circumstances.(150) Over the last decade, many studies has suggesting that autophagy and aging are inextricably connected. Numerous ATG genes have been demonstrated to be needed for the extended lifespan generated by conserved longevity paradigms in model organisms ranging from yeast to mice.(121)

Epigenetic Regulation of Senescence

Aging-related cellular damage activates mechanisms that regulate cell proliferation and differentiation, as well as stem cell-mediated regeneration, in order to prevent the irreversible breakdown of cellular and tissue homeostasis. Cellular senescence and regeneration mechanisms are particularly relevant to aging and AADs.(151) Human diploid cells have a finite lifetime after they terminally exit the cell cycle.(152) This process is referred as cellular senescence. Although cellular senescence is considered to contribute to aging, this theory is still disputed. Numerous studies have demonstrated the accumulation of senescent cells in aged tissues *in vivo* (153-155), such as in progeria patients' fibroblasts which had shorter replicative lifespans (156).

Senescence is a cell cycle halt that occurs in reaction to stress and is connected with age-related tissue deterioration *in vivo*.(157) More than 30% chromatin rearrangement were altered in senescent cells and develop senescence-associated heterochromatin foci (SAHF), a highly condensed chromatin regions.(29) It was associated with heterochromatic histone modifications, heterochromatic proteins, histone variant macroH2A, high-mobility group A (HMGA) proteins, and

late replicating regions in the genome.(158,159) These alterations are associated with the downregulation of lamin B1 transcription in senescence.(29,160,161) Autophagic degradation of lamin B1 was linked with destabilization of heterochromatic lamin-associated domains (LADs), and repressive histones.(162)

Senescent cells also known to have a significant abundance of H4K16ac (acetylation of lysine 16 on H4) near active gene promoter elements where it overlaps with HIRA and newly synthesized H3.3 peaks.(163) HIRA, a non-replicating histone chaperone that deposits variant histone H3.3 and H4, is essential for H4K16ac steady-state stability. These changes imply that the senescent state is associated with a dynamic and unbalanced chromatin environment that is qualitatively distinct from the proliferative state.(3)

Numerous processes promoting senescence have been found with the majority of which involve the buildup of damage to DNA and other cell macromolecules. (164-170) The chromatin shape is important for cell and organism life span control.(171-173) Aging is associated with heterochromatin reduction, as shown in senescent cells that expression of histone-encoding genes and the number of repressive heterochromatin marks, such as DNA methylation and histone methylation, including H3K9me3, H3K27me3, and H4K20me3 marks were reduced.(9)

Senescence is irreversible, caused by prolonged activation of the tumor suppressor RB-p16 and p53-p21 pathways.(174,175) This pathway activation can initiate the senescence-associated secretory phenotype (SASP) mediated by transforming growth factor β (TGF β) and nuclear factor- κ B (NF- κ B). Long term SASP activation can be harmful since it impairs intercellular communication between immune-responsive to senescent cells and, therefore preventing immune system-mediated to eliminate those senescent cells.

Failure to eliminate senescent cells during aging might exacerbate physiological deterioration by promoting cellular senescence in adjacent cells via gap junction, mediated cell-cell interactions.(112) Artificially induced senescence clearance in aged mice with mitotic checkpoint protein BubR1 deficiency increase its lifespan, as well as inhibits atherogenesis in low-density lipoprotein receptor-deficient mice, and preserves renal and cardiac function in wild-type mice.(179-181) The SASP may contribute to local and systemic malfunction and illness, and because senescent cells increase with age, targeting senescent cells serve as potential drug targets for promoting a prolonged, healthy lifespan.(176)

Emerging Rejuvenation Strategies

Aging was first considered as irreversible, but recent studies showed that it was not. Indeed, suppression of high-nutrient-sensing pathways (*e.g.*, the IGF and mTOR pathways) and activation of low-nutrient-sensing proteins (*e.g.*, AMPK and sirtuins) both increase longevity in many model animals.(177,178) Even when administered late in life, nutritional treatments such as dietary restriction and pharmaceutical interventions such as the mTOR inhibitor rapamycin can improve aging.(83,179-183) The question is whether the aging of cells, organs, and organisms can be reversed or 'rejuvenated' rather than just delayed. Numerous rejuvenation methods targeting these characteristics have recently developed, classified as systemic (blood) factors, metabolic alterations, senescent cell ablation, and cellular reprogramming. While various treatments appear to target disparate aspects of aging (95,184-187), then more questions raised, whether they share common mechanisms of action? Is there any cost associated with the rejuvenating effect? Can rejuvenation techniques ultimately be utilized

to increase human health and lifespan, as well as to combat age-related diseases?

Figure 6 shows the mechanisms and target cells of the rejuvenation strategies. Restoring a young epigenome by maintaining the stem cell pool may be critical for tissue function and lifespan extension in aged animals. (8,188) Some studies investigate how epigenetic process involved in recovering the youthful function in old stem cells subjected to rejuvenating treatments. The epigenetic rejuvenation extends longevity by correcting other aging-related characteristics, including as mitochondrial failure, genomic instability induced by retrotransposon activation, and chronic inflammation.

Dysfunctional mitochondria accumulated in aging cells.(91) After CR, partial reprogramming, heterochronic parabiosis, and pharmacological treatment, these mitochondria were restored.(88,189-191) In muscle and white fat tissues, CR promotes mitochondrial biogenesis. (192) Resveratrol supplementation increases the amount of mitochondria in muscle, increases physical activity, and increases the average longevity of mice.(78) Reprogramming mitochondria restores them to a condition similar in

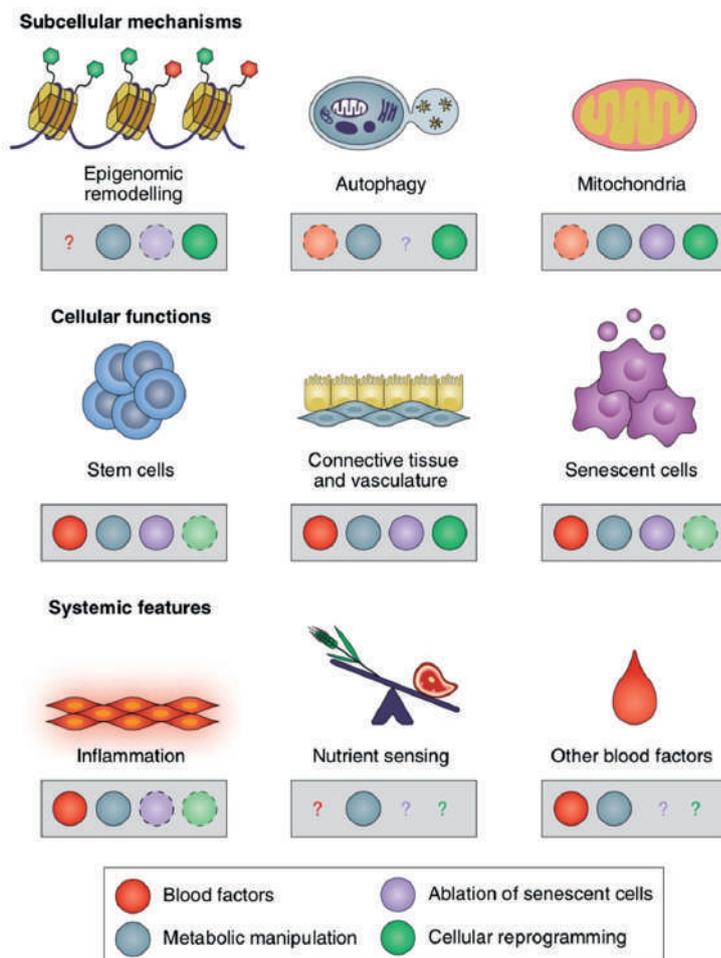


Figure 6. Potential common mechanisms and target cells of the rejuvenation strategies.(8) (Adapted with permission from Springer Nature).

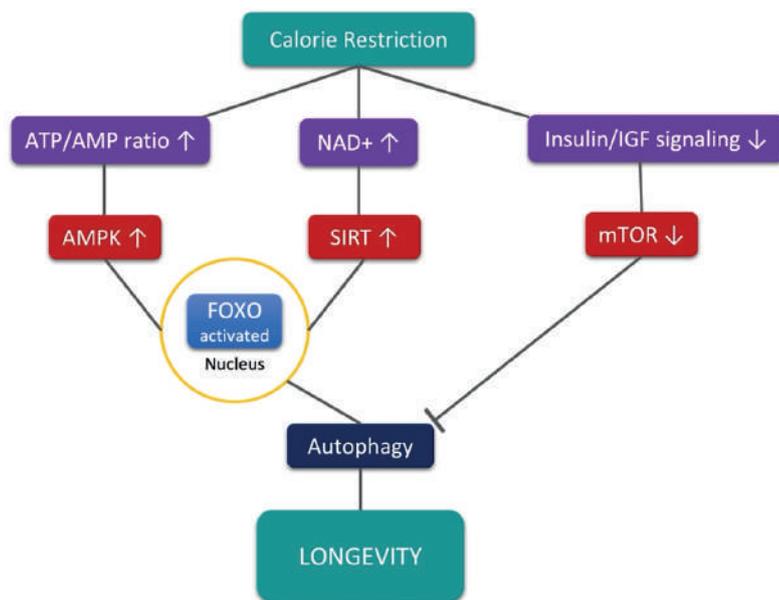


Figure 7. The regulation of autophagy by calorie restriction. In low energy intake condition, ATP/AMP ratio and NAD⁺ level are higher while Insulin/IGF signaling was lower. This activates the energy sensors AMPK and SIRT, but inhibit mTOR activity. AMPK and SIRT upregulate the expression of forkhead box transcription factors (FOXOs) in the nucleus. SIRT1 deacetylates the core autophagy machinery and thereby increases autophagy.

embryonic stem cells.(193) Additionally, heterochronic parabiosis decreases the swelling and vacuolization of mitochondria in skeletal muscle of elderly mice.(194)

Attenuation of oxidative phosphorylation (91), and regulation of mitochondrial unfolded protein response are two mitochondria-dependent strategies for lifespan extension. (189) By providing substrates for epigenetic changes, mitochondria govern lifespan mechanistically, acting as essential platforms for metabolism, epigenetic regulation, and aging.(114) TCA cycle's metabolic intermediates and byproducts function as cofactors and substrates for a variety of epigenetic enzymes.(64,88,91,192,195)

NAD⁺, the cofactor for sirtuins, is another critical molecule that connects epigenetic control to mitochondria. (189) Increased NAD⁺ levels increase mitochondrial function, replenish stem cell pools, and prolong the lives of mice.(196,197) Nicotinamide mononucleotide (NMN) plasma levels decline with aging. NAD⁺ precursors include nicotinamide riboside and NMN.(198) Supplementing aged mice with NAD⁺ precursors slows mitochondrial function decreases, ameliorates muscular, neuronal, and melanocyte stem cell senescence, ameliorates age-related physiological losses, and prolongs longevity.(88,198-204)

The rejuvenation techniques we discussed here have the potential to promote epigenetic reprogramming, implying that the epigenetic program can be experimentally reprogrammed to a younger state throughout later stages of life. Not only does a cell's epigenomic landscape represent its identity, but also its health and biological age.(1,40,42) Senescent cells have a distinct chromatin state (158,205), and their secreted cytokines (for example, IL-6) can cause

epigenomic alterations.(206,207) It has been hypothesized that epigenomic modification in cell results in the rejuvenating effect.(208) Dietary treatments such as calorie restriction and dietary restriction-mimicking drugs such as resveratrol combining with NAD⁺ (Figure 7) or Acetyl-CoA supplementation have been proved in many studies to increase the autophagy activities and rejuvenate our cells, hence increase the longevity.(1,181,209)

Nutrigenomic and nutrigenetic assessments, together with gut microbiomes and metabolites profiling are what we currently have to construct a personalize lifestyle and determine what kind of diet and supplementation as well as exercise we need (210) since genetic and environmental treatments have been demonstrated to extend life expectancy, by acting primarily through epigenome modification.(211) Aging process is flexible and that old cells, tissues, and organs may be rejuvenated. Additionally, they offer the intriguing prospect of translation for the treatment of human aging and age-related illnesses. Undoubtedly, the future years will bring exciting advances in our continuous efforts to better understand, postpone, and maybe reverse aging.(8)

Conclusion

Aging process is characterized by tissues and organs functional decline, and the increased risk of aging-associated disorders. Our increasing knowledge about how epigenetic changes along our aging process guide future research on treatments to rejuvenate the epigenome and delay the aging processes. Many treatments were proposed to delay the

onset of aging, prevent the aging-associated diseases, and extend the healthspan as well as lifespan. These treatments including metabolic manipulation, partial reprogramming, heterochronic parabiosis, pharmaceutical administration and senescent cell ablation.

Authors Contribution

AM drafted and wrote the manuscript, NMD edited the manuscript, AW proposed the manuscript topic, supervised, and edited the manuscript.

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