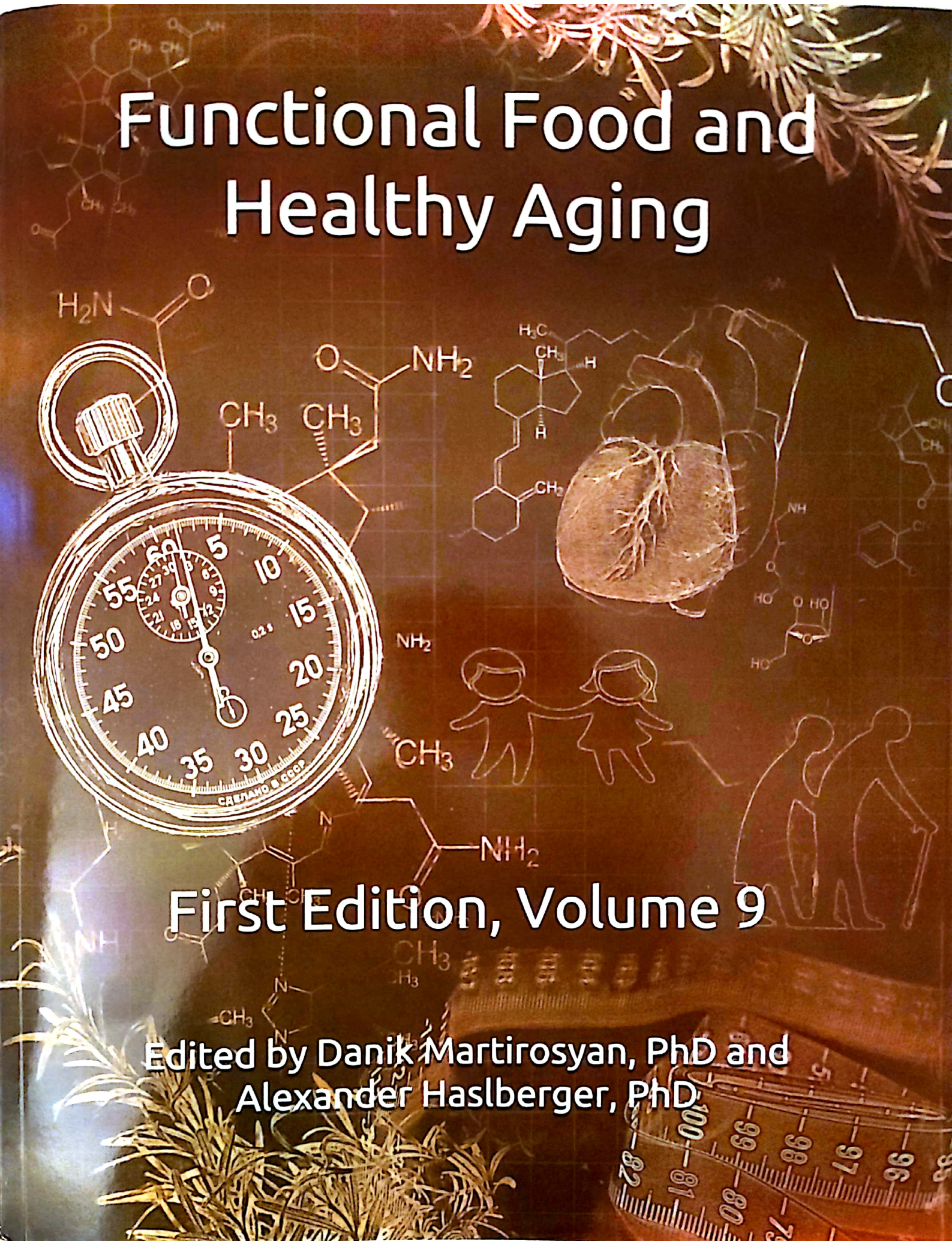
Functional Food and Healthy Aging



First Edition, Volume 9

Edited by Danik Martirosyan, PhD and Alexander Haslberger, PhD

Functional Food and Healthy Aging

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Chapter 7

Epigenetically Active Nutraceuticals to Address Personal Molecular Mechanisms of Aging

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INTRODUCTION

Health preservation and disease prevention are central objectives for establishing a healthy lifestyle and nutrition. Nowadays, the idea of healthy aging and attempts to increase longevity have resulted in science-based innovations such as precision medicine and precision nutrition. Molecular mechanisms in the development of complex diseases and premature aging are analyzed at every step by scientific disciplines addressing the hallmarks of aging.

Following an enormously broadened understanding of the personal genetic background, the fast development of epigenetics displays the personal regulation of gene expression and DNA integrity by environmental factors such as stress or nutrition. Additionally, the detailed characterization of personal aspects of the human microbiome shows the need for systemic OMIC approaches for the understanding of pathologies and coherent markers for them. The fields of nutrigenetics and nutri-epigenetics are analyzing mechanisms and markers in this area.

The use of molecular markers enables the detection of ongoing pathological mechanisms and interventions before the onset of symptoms. Medical and nutritional or dietary prevention and intervention need then to be personalized. These developments will result in preventive and personalized health care. Markers from the areas of genetics, epigenetics, microbiota, gene expression, and metabolomics are going to be integrated for the assessment of optimized personal pre- and post- intervention. Medical drugs, functional foods, and nutrition are more and more used for personalized treatment of identified molecular mechanisms of concern.

A strongly increased, worldwide awareness of health preservation has boosted the fast development of functional foods and nutritional concepts. This includes the health-supporting effects of caloric restriction and fasting. Selected bacteria, algae, cells, or plants and their metabolites or extracts are screened for health-promoting effects and developed into functional foods. Scientific literature shows an often

overwhelming flood of information on their activities in different health areas. Key areas of interest are cancerogenesis, metabolic and nervous diseases, as well as immune functions.

However, nutrient availability and diet are to date the most thoroughly studied environmental factor that affects longevity.

The Understanding of Aging

Aristotle was one of the first philosophers to show a serious interest in health and aging. He considered it a natural process, and so within the purview of his philosophy of nature, aging is a process that living things undergo and life is essentially tied up with the soul. Aristotle recognizes many different psychic powers, but he sees the power of nutrition as, in some sense, the most fundamental, since it alone can be separated from the others, while the other powers are always held in conjunction with nutrition [1].

In recent times, healthy aging was defined as a major objective, and WHO defines Healthy Aging as "the process of developing and maintaining the **functional ability** that enables **wellbeing** in older age" https://www.who.int/ageing/healthy-ageing/en/

Additionally, "successful" aging has been connected to the concept of resilience, which is "the process of adapting well in the face of adversity, trauma, tragedy, threats, or significant sources of stress" or "bouncing back" from difficult experiences [2]. This includes the idea of **Hormesis**, where the induction of a reduced amount of stress leads to an increment in health and viability [3].

Following the findings of the human genome project, genetic aspects of aging, such as the role of Daf-2 and insulin signalling in longevity [11,12], have attracted attention. The increasing interest in epigenetics and the central role of gene-environment interactions resulted in both improved molecular understanding as well as translational concepts towards healthy aging and longevity.

Aging is a complex multifactorial biological process shared by all living organisms. It is manifested by a gradual decline of normal physiological functions in a time-dependent manner. Organismal aging holds significant importance for human health because it increases susceptibility to many diseases, including cancer, cardiovascular disorders, neurodegenerative diseases, and metabolic disorders, such as diabetes. Cellular aging is linked to cellular senescence, a specialized process of growth arrest considered to be a potential endogenous anticancer mechanism, during which there is irreversible growth arrest in response to potentially oncogenic stimuli. Cellular senescence bears many similarities to the aging process. Although the interactions of mechanisms are still poorly understood, there are continued efforts to delineate longevity pathways conserved among all eukaryotes to promote human longevity [4]. The understanding of aging per se as a disease has been discussed critically [5].

Hallmarks of Aging

Modern molecular biology has summarized major molecular mechanisms, hallmarks of aging, which determine biological aging (**Figure 1**) [6]. These hallmarks are: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular

senescence, stem cell exhaustion, and altered intercellular communication [6]. Most of these hallmarks of aging are affected by epigenetic processes.

Figure 1. Hallmarks of aging

Aging and Epigenetics

Among the hallmarks of aging, epigenetic alterations represent one crucial mechanism behind the deteriorated cellular functions observed during aging and in age-related disorders. By definition, epigenetics represents the reversible heritable mechanisms that occur without any alteration of the underlying DNA sequence [7].

Although the chromosomes in our genome carry the genetic information, the epigenome is responsible for the functional use and stability of that valuable information; it connects the genotype with the phenotype. These epigenetic changes can either be previously accepted spontaneously or driven more likely by external or internal influences. Epigenetics potentially serves as the missing link to explain why the pattern of aging is different between two genetically identical individuals, such as identical twins [8]–[10]. Although longevity studies on the human population have shown that genetic factors could explain a fraction (20 to 30%) of the differences observed in the life spans of monozygotic twins, the majority of the remainder of variation is thought to have arisen through epigenetic drift during their lifetime [11]–[13].

Similarly, different environmental stimuli, including diet, cause differential alterations of stored epigenetic information to create a striking contrast in physical appearance, reproductive behavior, and life span. In turn, the resulting variability in the pattern of epigenetic information within individual cells in the population during aging leads to transcriptional drift and genomic instability. Being established by

enzymes, epigenetic information is reversible. Hence, epigenetics holds great prospects for targeting by therapeutic interventions, as opposed to genetic changes, which are currently technically irreversible in humans. Accordingly, delineating and understanding the epigenetic changes that happen during aging is a major ongoing area of study, which may potentially lead the way to the development of novel therapeutic approaches to delay aging and age-related diseases [14].

There are different types of epigenetic information encoded within our epigenome, DNA methylation, chromatin remodeling, posttranslational modifications of the histone proteins, structural and functional variants of histones, and transcription of noncoding RNAs (ncRNAs) [15]–[19].

DNA methylations are one of the most extensively studied and best characterized epigenetic modifications during aging [6], [20]–[24]. In young cells, the majority of CpGs within the genome have cytosine methylation. CpG methylation within promoters leads to transcriptional repression through the formation of compact chromatin structures, such as heterochromatin. Conversely, promoters of genes that are highly expressed are often devoid of DNA methylation. As identical twins age, the pattern of DNA methylation becomes more and more divergent because of epigenetic drift caused by environmental factors or spontaneous stochastic changes in the process of transmission of DNA methylation. Epigenetic drift leads to unpredictable differences in the methylome among aging individuals. Caloric restriction delays age-related methylation drift [25]–[27].

This fact indicates that at least parts of the DNA methylation changes are associated with lifestyle mechanisms. With a few exceptions, mammalian aging is more commonly associated with CpG hypomethylation, especially at repetitive DNA sequences. This is likely to be at least partly responsible for the loss of heterochromatin during aging. The global decrease in DNA methylation upon aging may be attributed to the progressive decline in levels of the DNA methyltransferase DNMT1 [28]. In addition to the general DNA hypomethylation that occurs during aging, progressive loss of DNA methylation occurs at specific gene promoters [29], [30] and at specific CpG sites to repress expression of specific genes [28], [31] – [41]. The methylome of CD4+ T cells of centenarians compared to CD4+ T cells from newborns shows a global decrease as well as heterogeneous DNA patterns. Through analyzing these specific CpG sites, predictions of age can be done due to age related methylation patterns [42], [43].

While age measures chronological time, **the epigenetic clock** (**Figure 2**) [44] uses well-established, age-related methylation patterns as biomarkers for individual, physiological age.

Referred to as ‘DNA methylation age’ (DNAmAge), it provides an accurate estimate of age across a range of tissues and some of the most promising biomarkers of aging. DNAmAge has also permitted the identification of individuals who show substantial deviations from their actual chronological age, and this ‘accelerated biological aging’ has been associated with unhealthy behaviors, frailty, cancer, diabetes, cardiovascular diseases (CVD), dementia, and mortality risk [1].

In the last few years, meta-analyses have been undertaken to investigate the extent to which DNAmAge in blood predicts mortality risk. They reported a significant association between DNAmAge and mortality risk [1].

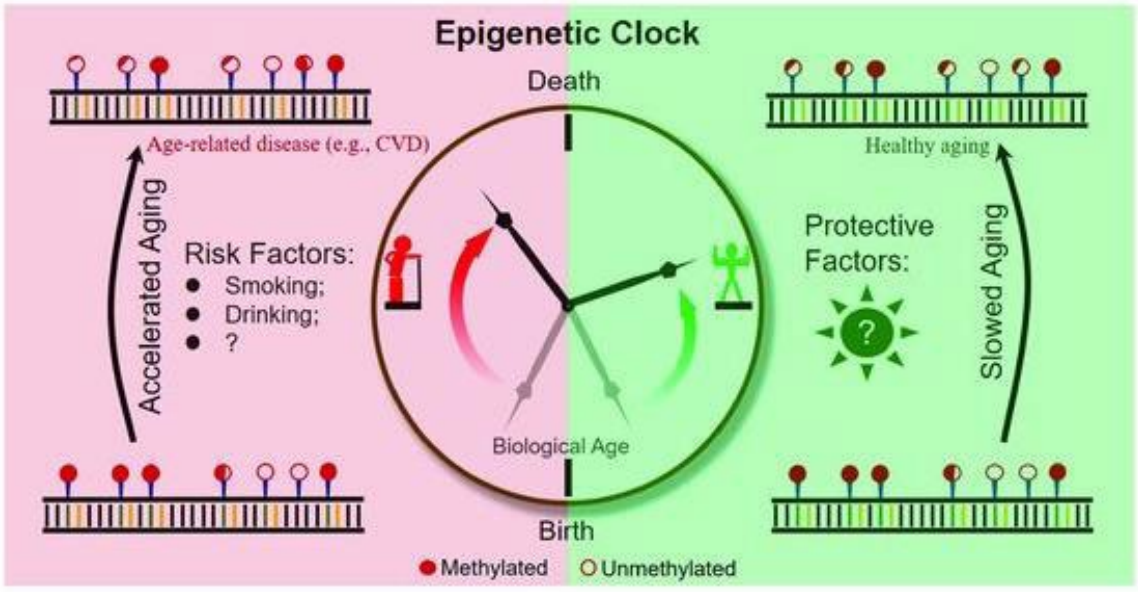

Figure 2. Epigenetic clock. Dynamic changes of DNA methylation throughout the human lifetime exhibits a strong correlation with age and age-related outcomes. Age prediction models with high accuracy are based on age-dependent methylation changes in certain CpG loci. Epigenetic clocks, namely epigenetic or DNA methylation age, serve as a new standard to track chronological age and predict biological age. Measures of age acceleration have been developed to assess the health status of a person. There is evidence that an accelerated epigenetic age exists in patients with certain age-related diseases (e.g., Alzheimer's disease, cardiovascular disease)

Similar to the changes in the methylation pattern during aging, the total methylation and methylation at specific sites change during replicative senescence. Notably, cell passage numbers and the population doublings can be accurately predicted from the methylation pattern at specific CpG sites [45], [46]. This altered DNA methylation pattern during replicative senescence correlates with the expression level of SIRT1 [47]–[49]. One of the remaining challenges in the analyses of DNA methylation during aging is to identify the causal pathways that contributes to the functional decline of the DNA methylome [50].

Environmental factors, such as exercise or circadian rhythms [51], are shown to influence gene expression and longevity in different organisms. However, nutrient availability and diet is to date the most thoroughly studied environmental factor to affect longevity.

Histone modifications are key epigenetic regulators that control chromatin structure and gene transcription, thereby impacting various important cellular phenotypes. Over the past decade, a growing number of studies have indicated that changes in various histone modifications have a significant influence on the aging process. Furthermore, it has been revealed that the abundance and localization of histone modifications are responsive to various environmental stimuli, such as diet. It is well accepted that a healthy diet and lifestyle exert a positive effect on aging [52], while exposure to multiple stressors can have the opposite

outcome. Dietary choices during every stage of life, from embryo to old age, can significantly impact our growth and health [53].

The basic unit of chromatin is the nucleosome, a dynamic consisting of 147 base pairs of DNA wrapped around an octamer of histone proteins. This octamer comprises two copies of each of the core histones: H2A, H2B, H3 and H4. Histones can be decorated with various post-translational modifications (PTMs), including acetylation, methylation, phosphorylation, and ubiquitination. These PTMs are deposited and removed by specialized histone modifying enzymes [54]. Due to these characteristics, histone modifications are reversible and thus able to dynamically modulate chromatin structure to activate or silence gene expression.

Different dietary interventions like a high-fat (HF) diet, low protein (LP) diet, and caloric restriction showed that extreme dietary conditions affect multiple nutrient-sensing pathways and can cause global histone modification changes [55] that have impacts on lifespan. Sirtuins are probably the best studied family of enzymes implicated in changing the epigenome as a response to environmental signals. Sirtuins are histone deacetylases (HDACs) that mediate lysine deacetylation through NAD hydrolysis, yielding O-acetyl-ADP-ribose nicotinamide. As NAD-dependent deacetylases, sirtuins are a perfect candidate to mediate lifespan responses to nutrients, having a dual role as NAD-sensors and transcriptional regulators through the deposition of PTMs in histones and other target proteins [56]. Sir2/SIRT1 is the most studied sirtuin in aging. It has been extensively reported that raising its activity by altering NAD+/NADH levels, genetic manipulation, or chemical stimulation robustly extends lifespan. Sirtuins have been implicated in lifespan regulation [57], and NAD+ levels increase during fasting or exercising.

Several natural compounds can act as regulators of the epigenome. For example, sulforaphane, an isothiocyanate from broccoli and cabbage, and allyl compounds from garlic can have HDAC inhibitory effects. Phenethyl isothiocyanate (PEITC), present in cruciferous vegetables, and epigallocatechin-3-gallate (EGCG) from green tea are found to have HDAC inhibitory activity as well. Sodium butyrate, found in cheese and butter, can regulate histone acetylation. Luteolin, found in parsley, thyme, peppermint, basil herb, celery, artichoke, and curcumin from turmeric can block HDAC activity. Diindolylmethane, a digestion by-product of indole-3-carbinol from different vegetables (including broccoli, cabbage, cauliflower, mustard and radish) generated in the stomach, causes proteasomal degradation of the histone deacetylases HDAC1, HDAC2 and HDAC3 [58]. Dietary polyphenols such as resveratrol, quercetin, and catechins like EGCG also have an effect activating the HDAC Sir2/SIRT1 [59].

One of the earlier proposed models of aging was the "**heterochromatin loss model of aging**" [60]–[62]. This model suggests that the loss of heterochromatin that accompanies aging leads to changes in global nuclear architecture and the expression of genes residing in those regions, directly or indirectly causing aging and cellular senescence. Loss of transcriptional silencing due to decay of the heterochromatin occurs during aging in all eukaryotes examined from yeast to humans, and there is evidence that accelerating or reversing this process can either shorten or lengthen life span. Gene silencing requires the absence of histone acetylation within heterochromatin regions. Accordingly, treatment with histone deacetylase (HDAC)

inhibitors or deletion of genes encoding HDACs, such as SIR2, shortens life span, whereas chemical activation or overexpression of SIR2 or sirtuins extends life span [63]–[65], [65]–[71]. However, DNA methylation and histone modifications are intertwined to exert the changes observed during aging.

Noncoding RNAs It is now widely accepted that approximately 60 to 90% of the human genome is transcribed, giving rise to an enormous array of ncRNAs. Until recently, most of the studies focused on the short ncRNAs, such as miRNAs, but the functional importance of long ncRNAs (lncRNAs) is now of special interest. miRNAS are involved in post-transcriptional gene silencing by inducing mRNA degradation or translational repression by binding to a target messenger RNA. Recent studies have also shown that miRNAs regulate age-associated processes and pathologies in a diverse array of mammalian tissues, including brain, heart, bone, and muscle [72].

Disruption of ncRNA function has been implicated in numerous disease conditions, such as cancer, neurodegenerative disorders, cardiovascular disorders, and aging [73]–[76] [77]. A majority of miRNAs are expressed differently with age. ncRNAs can be controlled by environmental and dietary factors, particularly by nutrients, diets, or bioactive compounds (**Figure 3**) [78]-[81]. Changes in the expression of specific sets of miRNA responses and effects in numerous disease conditions have been shown for SFA, PUFA, FF such as Resveratrol, DFA, PUFA, curcumin, quercetin, Catechins, and minerals or vitamins [79].

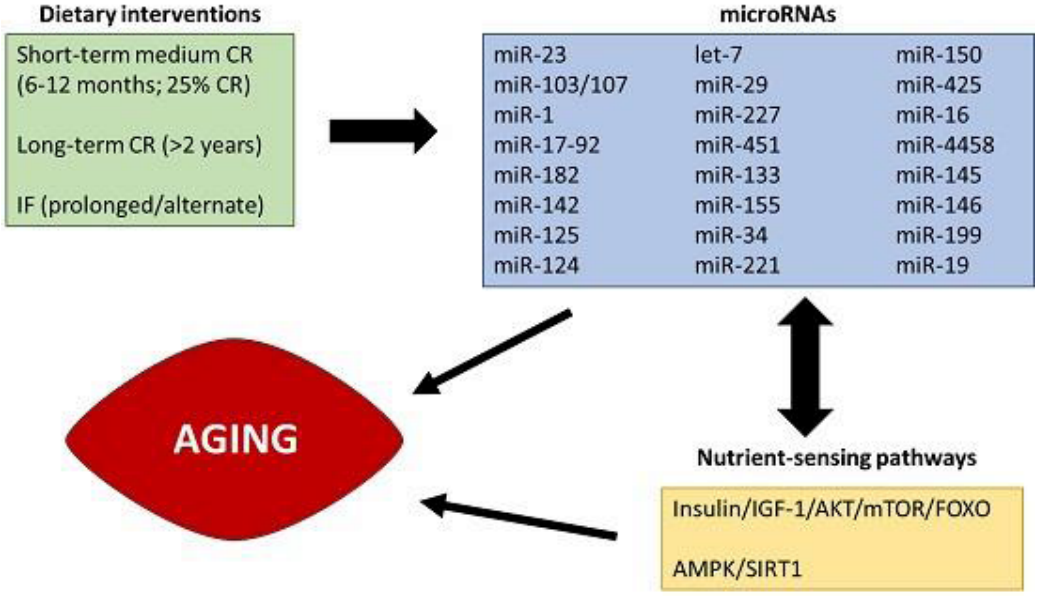

Figure 3. Aging and miRNAs nutrient-sensing pathways become deregulated with age. These pathways are a link between diet and aging. MicroRNAs have emerged as important regulators of cellular functions and can be modified by diet. Some microRNAs target genes encoding proteins and enzymes belonging to the nutrient sensing-pathways and, therefore, may play key roles in the modulation of the aging process [81]. Polyphenols targeting age-associated microRNAs could be a novel strategy for aging.

In recent years, evidence has gathered that microRNAs can be found in food and can be absorbed into the circulatory system and organs of humans and other animals where they regulate gene expression and

biological processes. These food-derived dietary microRNAs may serve as a novel functional component of food. The absorption, stability, and physiological effects of dietary microRNA in recipients, especially in mammals, are currently under heavy debate [80].

Mechanisms of Epigenetic Active FF

It is well established that a balanced diet enhances life expectancy and helps to prevent or treat certain diseases, such as obesity, diabetes, cancer, and mental disorders. The term **'nutriepigenomics'** describes interactions of nutrients and their effects on human health through epigenetic modifications. Epigenetic alterations at critical time points during development, especially within the first 1000 days of life, can result in stable changes and predispose individuals to disease later in life. Numerous nonmendelian features of metabolic syndrome, cancer, or central nervous system disorders, and clinical differences between men and women or monozygotic twins, are associated with epigenetic effects of fetal and/or lifelong nutrition.

All aspects of nutrition affect the epigenetic regulations of DNA methylation, histone modification, and ncRNAs (**Figure 4**) [82].

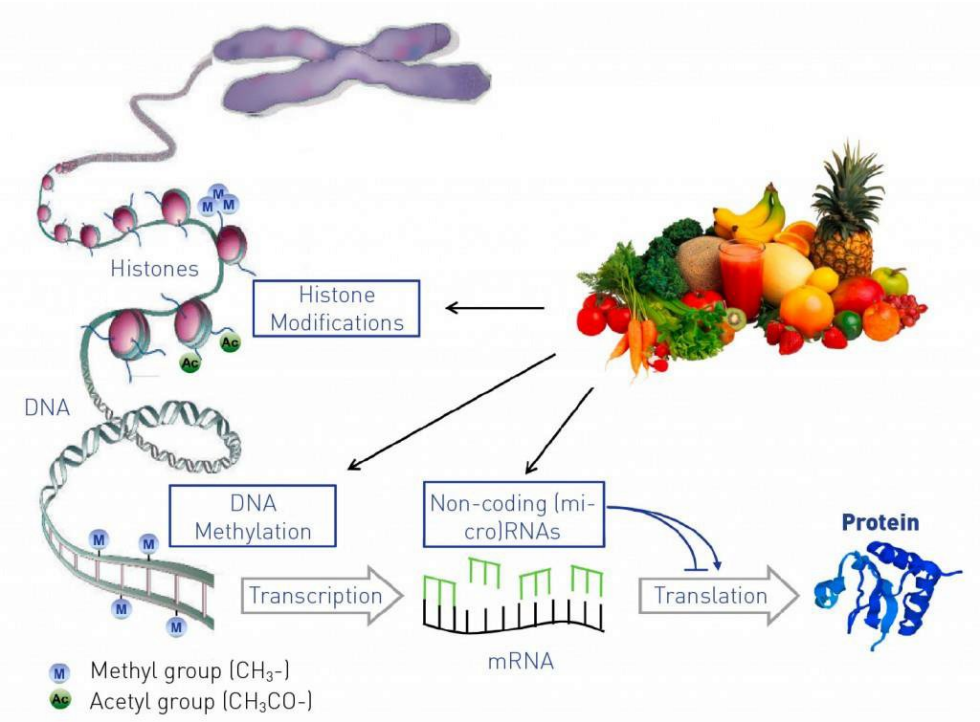

Figure 4. Polyphenols address all epigenetic mechanisms.

DNA methylation Polyphenols (bioflavonoids and catechins) such as quercetin, fisetin, myricetin, catechin, epicatechin, and epigallochatechin-3-gallate (EGCG) have been shown to inhibit DNA methylation by effecting DNMT activity (**Figure 5**) [83]. Polyphenol structures all contain a catechin group, which is the main component allowing the inhibition of DNMT activity. Dietary catechols can be methylated by the enzyme catechol-O-methyltransferase (COMT) using the same methyl donator SAM as in DNA

methylation. This specific methylation mechanism is called O-methylation. During O-methylation of various polyphenols, SAM is turned into SAH, which is a feedback inhibitor of DNMTs. It is thought that higher SAH concentrations through O-methylation may lead to the inhibition of DNMTs and thus indirectly inhibit DNA methylation. The second mechanism is the direct inhibition of DNMTs through EGCG, which is independent of the O-methylation pathway. EGCG is a noncompetitive inhibitor of DNMTs and its effect is enhanced by the presence of Mg2+ [84]. EGCG has been shown to to reactivate silenced tumor suppressor genes and reduce global DNA methylation by downregulating DNMT activity. Moreover, EGCG has been linked to antioxidant, anti-inflammatory, and DNA repairing effects, which also may play an important role in cancer prevention. Furthermore, there have been no negative side effects reported in adequate green tea consumption, while too high doses of EGCG caused cell apoptosis [85].

The key methyl donor for DNA and protein methyltransferases, SAM, is synthesized in the methionine cycle while accompanied by various nutrients present in the diet, including methionine, folate, choline, betaine, vitamins B2, B6, and B12. These nutrients act as precursors and contribute to the production of SAM at different sites of the cycle. Deficiencies in these nutrients result in changes in the SAM pool, which can influence DNMTS' reaction kinetics and DNA methylation as well [86]. Nutrients affecting one of the two metabolites of the **1-carbon metabolism:** S-Adenosylmethionin, a ubiquitous methyl donor, or S-adenosylhomocysteine, an inhibitor of methyltransferases, can alter the methylation of DNA and histones. Methylated promoter and other regulatory regions of a gene are usually associated with gene repression, whereas DNA demethylation within these regions leads to gene activation.

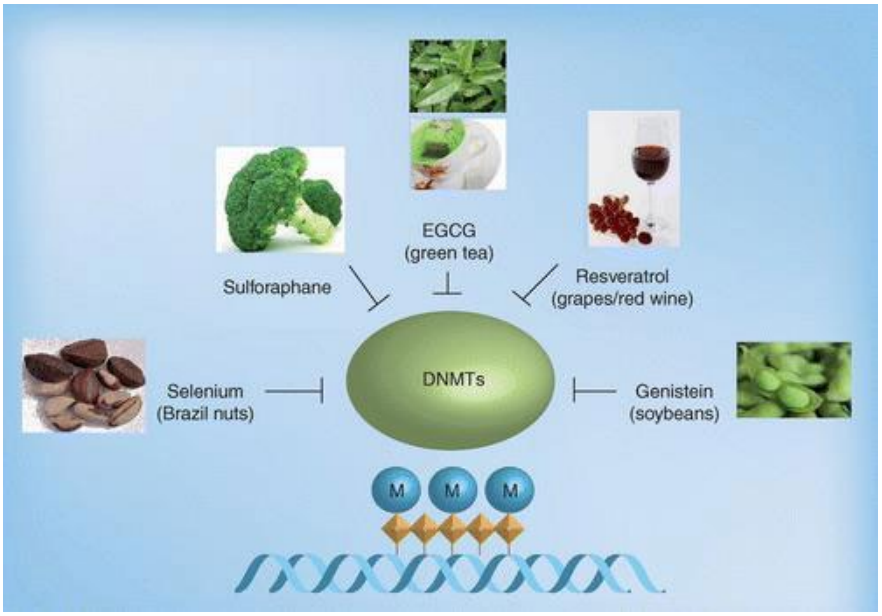

Figure 5. Effects of epigenetic active plant ingredients on cytosine methylation

Histone modification Polyphenols, including curcumin, genistein, epigallocatechin gallate (EGCG), and resveratrol, are also well known for their beneficial effects via modulation of nuclear factor kappa B (NFkB) expression and chromatin remodeling through regulation of histone deacetylases (HDACs) (**Figure 6**) [83]. Components such as gut microbial derived butyrate, sulforaphane, and curcumin affect histone acetyltransferase (HATs) and/or HDACs activities, leading to changes in chromatin structure. Vitamins such as biotin, niacin, and pantothenic acid influence histone modifications. For example, biotin influences histone biotinylation AQ5 and niacin histone ADP-ribosylation. Resveratrol, butyrate, sulforaphane, and diallyl sulfide inhibit HDACs, whereas curcumin inhibits histone acetyl transferases HATs [87]. Although the action of many bioactive substances is specific to enzymes and proteins involved in the regulation of different components of the epigenome, interaction with other nutrients and lifestyle factors in physiological and pathological conditions must also be taken into account. In addition, epigenetic components exert effects over each other. This adds an additional layer of complexity to the action of epigenetically active nutrients. Studies demonstrate that DNA methylation and histone modifications that act together to establish chromatin structure are involved in miRNA regulation and vice versa [10]. Thus, deeper knowledge of bioactive nutrients/diets for characterization of their effects on the epigenome modifying enzymatic activities (acetylation, methylation, phosphorylation, ribosylation, oxidation, ubiquitination, and sumoylation) is needed [88], and often the molecular mechanisms are not yet well understood.

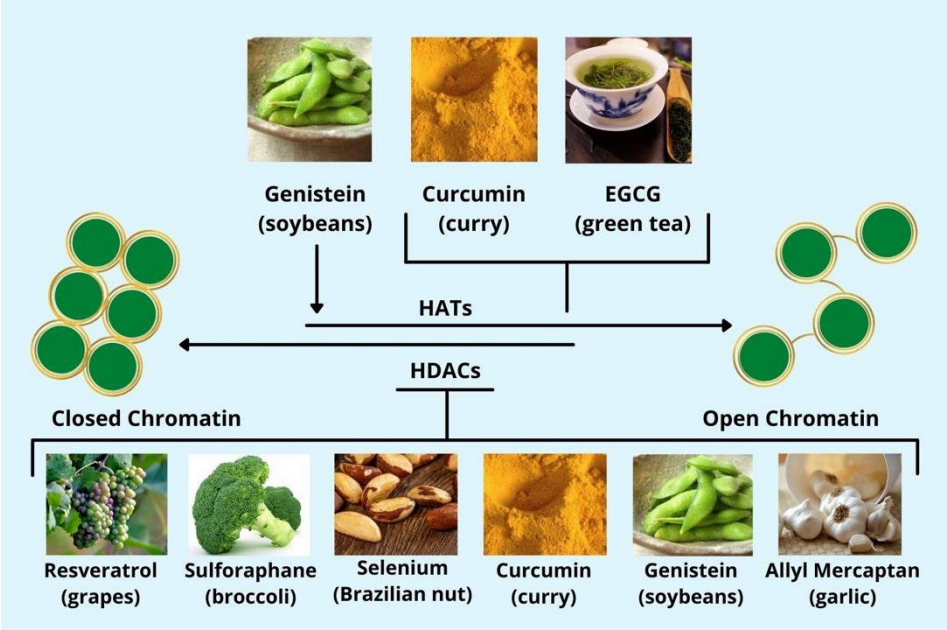

Figure 6. Effects of epigenetic active plant ingredients on histones, chromatin, and gene expression

Sirtuins One of the most important modifiers of histones are Sirt enzymes, histone deacetylases. Sirtuins, commonly referred to as silent mating type information regulation 2 homologous (SIRT), are a

class of proteins which can be found in all living organisms. In an effort to find yeast mutants with longer life durations, sirtuins were first discovered in the 1990s [89].

Humans possess a total of seven sirtuins (SIRT1-SIRT7) (**Figure 7**, modified) [90]. SIRT1 is mainly located in the cell nucleus, but it can also be found in the cytosol. SIRT2 is also located in the cytosol, where it has its main site. SIRT3, SIRT4 and SIRT5 are mitochondrial proteins, but SIRT3 can also be located in the cell nucleus and cytosol under different cellular conditions. SIRT6 and SIRT7 are located in the cell nucleus and nucleolus, respectively [91].They act as energy sensors in our cells and are activated when there is a lack of energy. Sirtuins are therefore multifunctional and regulate many metabolic processes as well as the aging process. In fact, an increased activity of a yeast's sirtuin, silent information regulator two (Sir2), can extend its life. It ensures the silencing of certain chromatin regions by deacetylating histones. This attenuation of chromatin activities, such as during replication, recombination, and transcription, seems to be essential for prolonging the life of Sirt2.

Sirtfood	Major Sirtuin-Activating Nutrients
Bird's-eye chilli	Luteolin, Myricetin
Buckwheat	Rutin
Capers	Kaempferol, Quercetin
Celery, including its leaves	Apigenin, Luteolin
Cocoa	Epicatechin
Coffee	Caffeic acid, Chlorogenic acid
Extra virgin olive oil	Oleuropein, Hydroxytyrosol
Green tea (especially matcha green tea)	Epicgallocatechin gallate (EGCG)
Kale	Kaempferol, Quercetin
Lovage	Quercetin
Medjool dates	Gallic acid, Caffeic acid
Parsley	Apigenin, Myricetin
Red chicory	Luteolin
Red onion	Quercetin
Red wine	Resveratrol, Piceatannol
Rocket	Quercetin, Kaempferol
Soy	Daidzein, Formononetin
Strawberries	Fisetin
Turmeric	Curcumin
Walnuts	Gallic acid

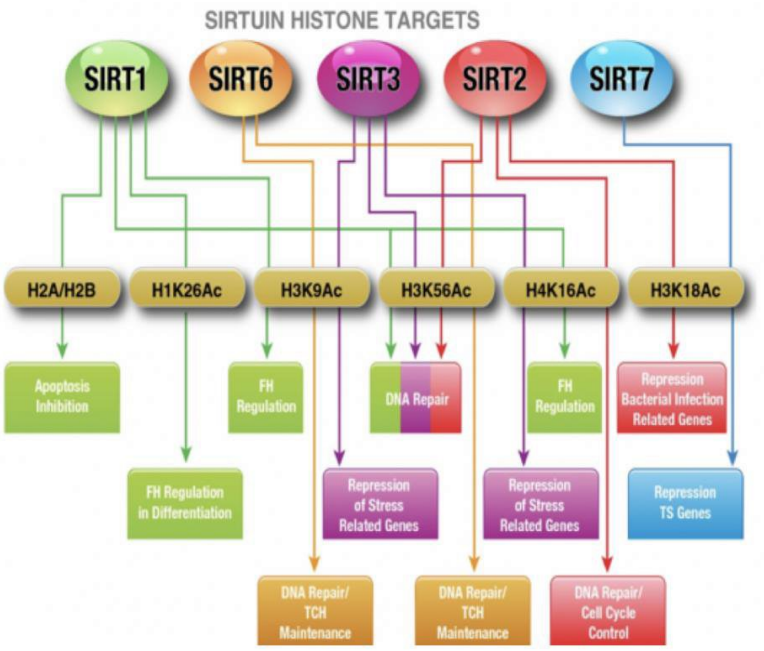

Figure 7. Sirtuins, activating nutrients, histone targets and functions

SIRT1 mediates **oxidative stress response** by directly deacetylating several transcription factors that regulate antioxidant genes. Notably, SIRT1 activates several members of the FOXO family of transcription factors which promote the expression of stress response genes, including SOD2. For example, SIRT1 functions in an autoregulatory loop along with the early growth response protein ERG1 to regulate SOD2 to protect contracting muscle cells from oxidative stress. SIRT1 also promotes mitochondrial biogenesis by activating peroxisome proliferator-activated receptor co-activator 1-α (PGC-1α). PGC-1α increases mitochondrial mass and upregulates the expression of oxidative stress genes, including glutathione peroxidase

(GPx1), catalase, and manganese SOD (MnSOD). Finally, SIRT1 inactivates the p65 subunit of NF-κB through direct deacetylation. NF-κB inhibition suppresses the inducible nitric oxide synthase (iNOS) and nitrous oxide production, and thus may lower the cellular ROS load [89].

Sirtuins possess broad enzymatic activities and deacylases, including deacetylase, desuccinylase, demaloynylase, deglutarylase, long-chain deacylase, lipoamidase, and ADP-ribosyltransferase. All these enzymatic activities specifically require NAD+. The coupling of nicotinamide adenine dinucleotide (NAD+) breakdown and protein deacylation is a unique feature of sirtuins (**Figure 8**) [92].

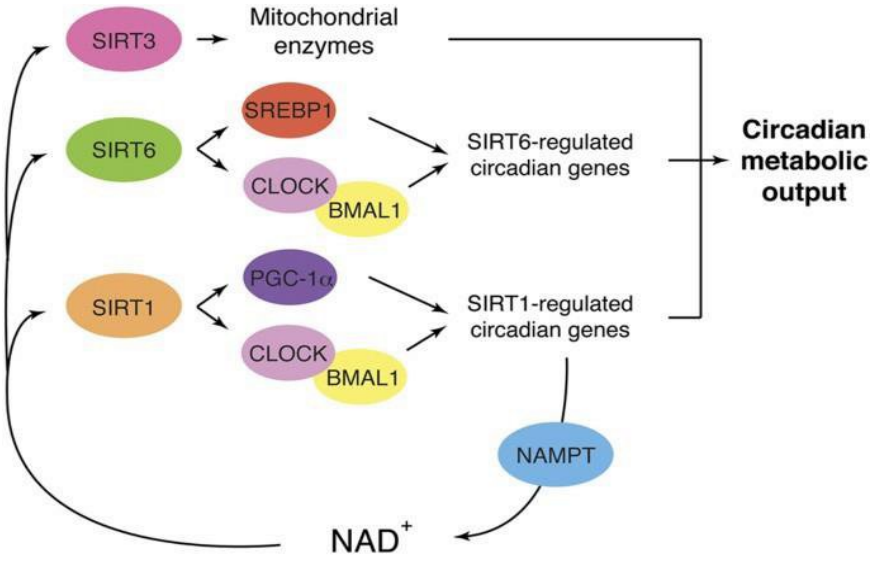

Figure 8. Sirtuins and NAD

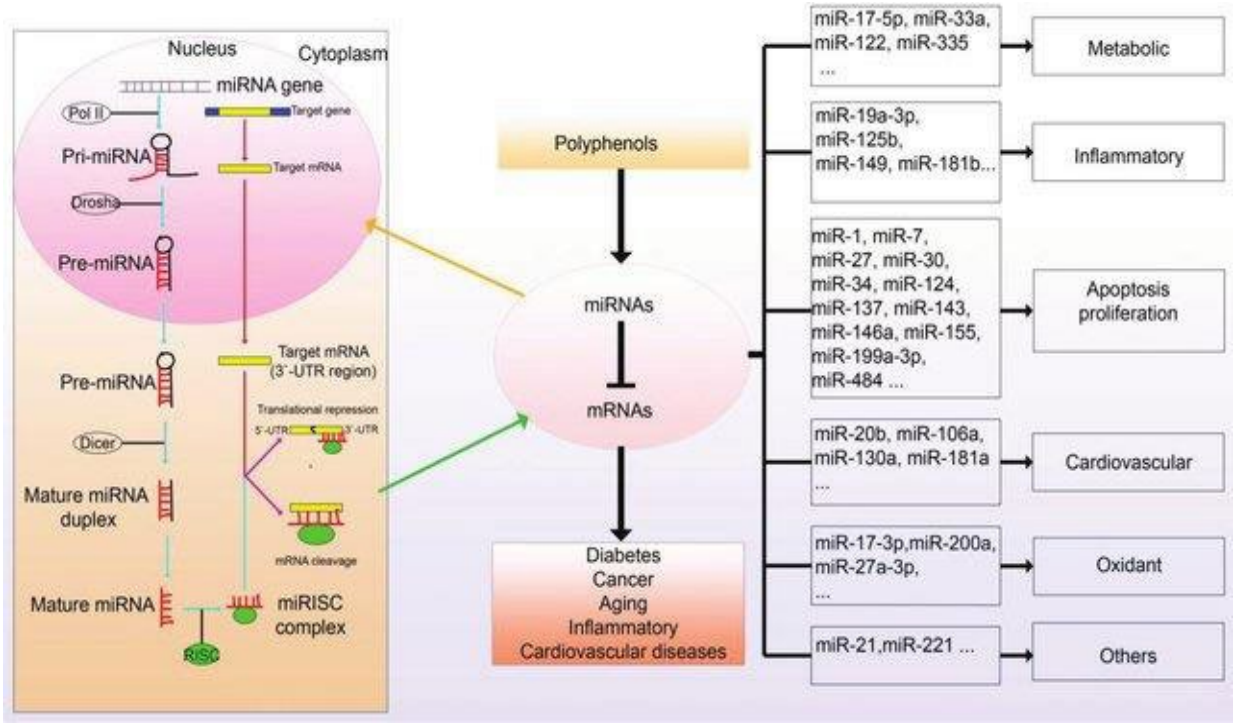

Figure 9. Polyphenols and ncRNAs

Natural Compound	Natural Sources	Pharmacological Effects	Epigenetic Mechanisms of Action
Folate, cobalamin, riboflavin, pyridoxine, methionine	Folate and riboflavin: spinach, asparagus, beans, peas, lentils, sunflower seeds, almonds Cobalamin: fish, shellfish, poultry, milk, eggs Pyridoxine and methionine: grains, nuts, dragon fruit, sesame seeds	Anti-cancer, anti-proliferative, chemoprevention of malignant transformation	Regulation of one-carbon metabolism, SAM/SAH ratio, DNMT and MBD expression; Regulation of MIRNAs (tumor suppressor mIR-122, mIR-34a, mIR-127, and oncogenic mIR-21, mIR-222)
Retinoic Acid	Vietnamese gac, crude palm oil, yellow and orange fruits (mango, papaya), orange root vegetables (carrots), spinach, sweet potatoes	Anti-cancer, anti-proliferative, differentiating, pro-apoptotic	Regulation of DNMTs expression and enzyme activity by affecting p21, AP-1, PTEN, and ERs; Regulation of MIRNAs targeting DNMTs, regulation of tumor suppressor (mIR-15, mIR-16, let-71, let-7c, mIR-43a mIR-342) and oncogenic mIRNAs (mIR-10a); GMNT regulation, histone acetylation
Vitamin D3	Sun exposure, fish, fish liver oils	Anti-cancer, anti-proliferative, differentiating, pro-apoptotic	Regulation of DNMTs expression and enzyme activity by affecting p21, AP-1, PTEN, and ERs; Regulation of histone acetylation, regulation of oncogenic miRNAs (mIR-181a, mIR-181b)
Resveratrol	Roots of hellebore, grapes, mulberries, apricots, pineapples, peanuts	Anti-cancer, antioxidant, anti-proliferative, anti-angliogenesis, anti-inflammatory pro-apoptotic, cardioprotective	Regulation of DNMTs expression and enzyme activity by affecting p21, AP-1, and PTEN, and ERs; activation of deacetylase SIRT1 and p300 HAT; down-regulation of UHRF1; regulation of MiRNAs
Genistein and daidzein	Soybeans, lupin, kudzu, psoralea, fava beans, coffee	Anti-cancer, antioxidant, antihelminthic, anti-metastic, cancer protective	Regulation of DNMTs expression and enzyme activity by affecting p21, AP-1, and PTEN, and ERs; increase in HAT activity; regulation of MIRNAs (tumor suppresor mIR-1296, mIR-16, and oncogenic mIR-27a)
EGCG	Green tea	Anti-cancer, antioxidant, anti-proliferative, anti-angliogenesis, anti-inflammatory pro-apoptotic, cancer protective	Regulation of SAM/SAH ratio by COMT-medicated reactions; direct inhibition of DNMTs by binding to catalytic domain of the enzyme; regulation of tumor suppressor mIRNAs (mIR-16)
Curcumin	Spice turmeric	Anti-cancer, antioxidant, protects against heart failure	Direct inhibition of DNMTs by binding to a catalytic domain of the enzyme; inhibition of MDACs and p300 MAP; regulation of mIRNAs (tumor suppressor mIR-22, mIR-15a, mIR-16, and oncogenic MIR-21, mIR199a)

Figure 10. Epigenetic mechanisms and pharmacological effects of selected natural compounds

Caloric restriction and SIRT SIRT1 activation contribute to caloric restriction (CR) and mediated lifespan extension. CR fails to increase the lifespan of SIRT1 knock-out mice, and these mice do not increase their physical activity, a phenotype typically associated with calorically restricted mice. Similarly, SIRT1 over-expression mimics a caloric restriction phenotype.

The close link between the sirtuin function and cellular metabolism plays a central role in regulating the lifespan. Sirtuins are necessary to activate the life prolonging effect which occurs during the restriction of calories. Studies have shown that in animals, including mammals, a reduced calorie intake leads to a general increase in fitness and prolonged life span. For example, in mice, SIRT1 activity is increased when calorie restrictions are in place [93]. Recently, research has focused on ways to activate these sirtuins without fasting. Foods which stimulate sirt enzymes have come into broader interest (**Figure 5**) [94]. Sirt-foods are plant-based foods composed of polyphenols – secondary plant substances. These substances act on the sirtuins and imitate the 'lack of energy' signal which normally results from fasting, dieting, and/or exercise. In other words, Sirt-foods can activate sirtuins without fasting taking place.

Bluezones and SIRT In the January 1973 issue of National Geographic magazine, the physician Alexander Leaf gave a detailed account of his journeys to countries of purported long-living people: the Hunzas from Pakistan, the Abkhazians from the Soviet Union, and Ecuadorians from Vilcabamba. According to Leaf, there were ten times more centenarians in these countries than in most Western countries. The concept of blue zones have been defined as a rather limited and homogenous geographical area where the population shares the same lifestyle and environment, and its longevity has been proved to be exceptionally high. There is evidence that in these areas, the low incidences of lifestyle-related diseases, including hypertension, obesity, diabetes, fatty liver, and cancer, are also due to higher sirtuin activating diets [95]. In addition, the polyphenols contained in Sirt-foods have been closely linked to positive effects on epigenetic mechanisms and many other genes known for promoting a long and, above all, healthy life [96]. The sirtuin genes, and the resulting sirtuin enzymes, are activated during low energy cycles.

Certain natural plant compounds such as red wine, strawberries, onions, soy, parsley, extra virgin olive oil, dark chocolate, green tea, buckwheat, turmeric, and walnuts are claimed to be able to increase the level of SIRT proteins in the body, and foods containing them have been dubbed "sirtfoods." Sirtfoods have attracted a broad interest in public media: https://www.healthline.com/nutrition/sirtfood-diet#section1 https://www.bbcgoodfood.com/howto/guide/what-sirtfood-diet

ncRNAs Given the broad biological activities of FF and the understanding that multiple ncRNA pathways are involved in the regulation of each biologic activity, one can assume that all FFs address multiple ncRNA, especially miRNA pathways (**Figure 9**) [97].

Clearly, the understanding of the interactions between FF, miRNA responses, and health consequences are at the center of research interest, especially using miRNAS as markers of biological activities. miRNA responses are mostly situation- and tissue specific and often interact with additional epigenetic regulation (**Figure 10**) [98]. For example, **curcumin,** a compound present in turmeric, has been linked to all three major epigenetic modifications (DNA methylation, histone modification and miRNA regulation). A

systematic review indicated that curcumin showed upregulation and downregulation in pancreatic cancer cells (in vitro) and downregulation of oncogenic miRNA in gastric cancers. Curcumin downregulated miR-302, a miRNA that inhibits epigenetic regulators such as DNMT1, and causes DNA demethylation [99]. Curcumin has also been found to be an HDAC inhibitor and modulates the expression of HDACs in mice and cell lines in different cancer types [100].

Mitohormesis concept For a long time, it was generally believed that oxidative stress may cause the observed physiological decline in cellular and organismal functions that occur during aging. However, transgenic mice over-expressing related anti-oxidant genes do not live longer than their wild-type counter-parts, raising some doubts on the universal relevance of this theory as a mechanism of aging [101]. These conflicting data suggest a more complex mode of regulation. Mitohormesis may reconcile many of the seemingly conflicting data related to the role of oxidative stress and health (**Figure 10**) [102]-[88]. Mitohormesis is an application of the theory of hormesis, in which a stressor may have beneficial effects at relatively low doses and deleterious effects at high doses. Conceptually, small and/or transient amounts of reactive oxygen species elicit a protective stress response that may improve lifespan. Relatively large and/or chronic amounts of the same species, however, cause cellular damage or death because they exceed the capacity of the oxidative stress response to maintain homeostasis (Fig. 11) [103], [104].

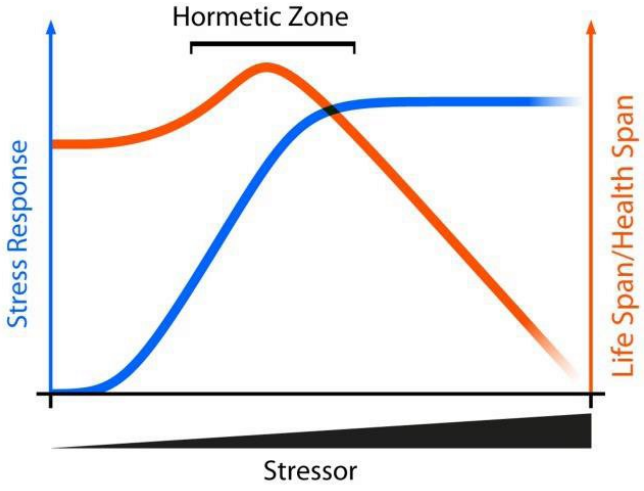

Figure 11. Mithormesis Theoretical curve showing how low doses of a stressor may have beneficial effects by activating intracellular stress response pathways. If the stressor exceeds the capacity of the stress response system to maintain homeo-stasis, then deleterious phenotypes are observed.

Telomere Length and Epigenetic Active FF

Over the past decades, redox research has demonstrated key roles for oxidative stress and inflammation in biological aging and associated diseases. Today, it is commonly accepted that lifestyle and environmental factors largely impact epigenetic regulation and DNA stability through telomere attrition. Further,

epigenetic instability provides a potential record of the cumulative burden of those endogenous and exogenous oxidative stressors encountered over time [105], [106].

Human telomeres are comprised of tandem repeats of a hexametric nucleotide sequence (TTAGGG) that is associated with the shelterin group of proteins. The telomere protein complex is crucial for genomic stability and chromosomal integrity, hence why telomere length has been suggested as a biomarker of biological aging [107]. Conversely, telomeric dysfunction and accelerated attrition have been linked to age-related conditions like cancer, cardiovascular disease, type 2 diabetes, and neurodegeneration. With its specialized ribonucleoprotein structure, the enzyme telomerase is a critical determinant of telomere length as it synthesizes telomeric repeat DNA, consequently slowing down telomere attrition. Human telomerase contains two core components, a catalytic unit called the human telomerase reverse transcriptase (hTERT) and an RNA template (hTERC), along with associated proteins. In adults, most healthy somatic cells express very low telomerase activity in contrast to cells with high replicative demands, including fetal epithelial cells and cells of the immune system [108].

Several phytochemicals such as **curcumin, genistein, or the polyphenol epigallocatechin-3-gallate (EGCG)**, have been shown to positively influence telomere length [109]. These natural bioactive compounds have the potential to act at multiple molecular target sites either directly through their antioxidative capacities or indirectly by affecting signaling pathways, including DNA damage repair, epigenetic mechanisms, or the mitogen activated protein (MAP) kinase pathway [110]. EGCG has not only been described for its marked antioxidative potential, but also for its ability to specifically impair cancer cell progression by blocking signal transduction pathways, and thereby suppressing telomerase activity. These effects have been strongly connected to the inhibition of NF-κB activity, affecting a wide array of processes including MAP kinase-dependent and growth factor-mediated pathways [30]. Next to these findings, EGCG has repeatedly demonstrated anti-proliferative effects by inducing apoptosis and cell cycle arrest in cancer cell studies [111] (**Figure 12**) [112].

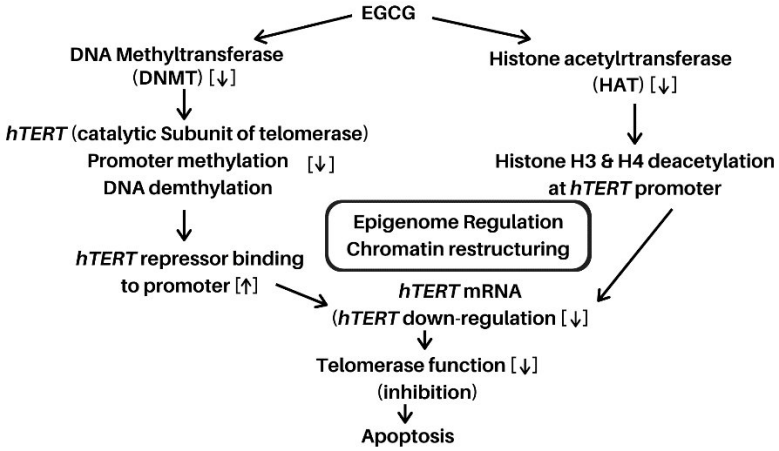

Figure 12. Mechanism of EGCG-induced apoptosis in cancer cells through epigenetic regulation of telomerase. EGCG inhibits both deoxyribonucleic acid (DNA) methyltransferase (DNMT) and histone acetyltransferase (HAT), leading to the DNA demethylation and histones H3 and H4 deacetylation of the

human telomerase– reverse transcriptase (hTERT) promoter, respectively. These events result in the epigenome regulation and chromatin restructuring involving hTERT messenger ribonucleic acid (mRNA) down-regulation and inhibition of telomerase and ultimately apoptosis.

Recently, our group reported from cell experiments that EGCG incubation was associated with telomere shortening and decreased telomerase activity in Caco-2 cells, as well as relatively longer telomeres and increased methylation of six 5'—C—phosphate—G—3' (CpG) sites in the promoter region of human Telomerase Reverse Transcriptase (hTERT) in fibroblasts. At low concentrations, EGCG significantly decreased oxidative damage to lipids in Caco-2 cells and attenuated H2O2 induced oxidation at higher concentrations. These results suggest differential EGCG-mediated telomeric modulation in cancer vs. primary cells and a specific antioxidant activity of EGCG against oxidative damage to lipids in abnormal cells [113].

Aging of the Immune System and Epigenetic Active FF

Aging of the immune system in humans and animals is characterized by a decline in both adaptive and innate immune responses. Aging is also associated with a state of chronic inflammation (“inflammaging”) and an increased likelihood of developing autoimmune diseases. Epigenetic changes in non-dividing and dividing cells, including immune cells, due to environmental factors, contribute to the inflammation and autoimmunity that characterize both the state and diseases of aging. Immune system decline in aging is characterized by a shift from a naive to memory T cell phenotype, type 1 to a type 2 cytokine profile, defective humoral immunity, increased maturation rate of T-cells, chronic low-grade inflammation, and many other changes. Aberrant gene expression in immune system cells resulting from epigenetic changes may contribute to the loss of immune tolerance, inflammation, and autoimmunity [114].

Amongst the various immune cell populations, the T-cell compartment may be most affected by the aging process. A recent human study reported age-related hypermethylated sites primarily located at CpG islands of silent genes and enriched for repressive histone marks of CD8 cells from older individuals [115].

The **thymus**, where T-cells develop, begins to involute at puberty in both mice and humans due to age-related changes that affect both T-cell progenitors and the thymic microenvironment. Similarly, decreased hematopoietic tissue in the bone marrow of mice and humans means B-cell lymphopoiesis also decreases with age. **DNA damage** both promotes cellular senescence to allow **DNA repair** mechanisms and causes activation of the innate immune system to clear damaged cells. The reduced capacity of the aged immune system to clear these cells, therefore, results in an accumulation of genomically damaged and senescent cells within all tissues of the body, including within the immune system itself [116].

Immunomodulatory effects of three classes of nutraceuticals, namely **carotenoids, polyphenols, and polyunsaturated fatty acids (PUFAs)**, are discussed for their interactions with **immunosenscence** [117] (**Figure 13**) [118].

Certainly, changes in gut microbiota with age contribute to the aging of the immune system. Dramatic changes in the structure, abundance, and diversity of gut microbiota with a decline of species producing

anti-inflammatory epigenetic active short-chain fatty acids (SCFAs) have been reported by many groups, including our group [119]–[121]. The use of probiotics [122], prebiotics, and post biotics will be discussed below.

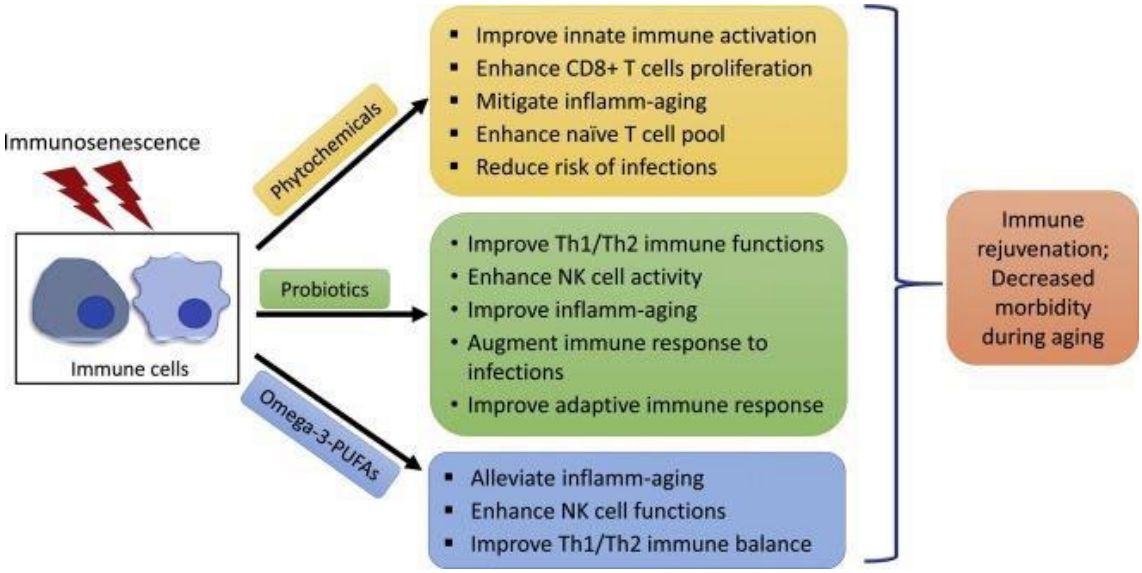

Figure 13. Nutraceuticals-Based Immunotherapeutic Concepts and Opportunities for the Mitigation of Cellular Senescence and Aging

DNA Damage, DNA Repair, and Epigenetic Active FF

Increased formation of mitochondrial ROS was postulated to be a major cause of aging in 1956, when Denham Harman introduced his Free Radical Theory of Aging (FRTA) [190]. According to this concept, increased ROS formation causes an accumulation of damage in the cell with age, resulting in age-related impairment of cellular functions. Consequently, ROS-lowering interventions were widely proposed to be a promising strategy to slow aging in humans. In this regard, natural or artificial substances that can scavenge ROS, so-called antioxidants, were examined intensively.

Nowadays, it seems to be established that enhancement of metabolic rate does not necessarily result in concomitantly increased ROS formation [123] and that the relationship between ROS levels and aging is not linear. Often, trials did not find any health-promoting effects of antioxidant supplementation. Mean-while, it is widely accepted that high levels of ROS cause cellular damage and promote aging, while low levels of ROS may improve systemic defense mechanisms by inducing an adaptive response. This concept has been named mitochondrial hormesis or mitohormesis [103].

At high levels, ROS can lead to impaired physiological function through cellular damage of DNA, proteins, lipids, and other macromolecules. While ROS are deleterious to cells, they also can function as stress-induced signaling molecules in the process of the DNA damage response. Recent reports indicate that DNA damage alone results in increased levels of intracellular ROS. In response to oxidative stress, cells activate both the DNA repair processes and transcription factors [124]. These factors, in turn, modulate levels of expression of ROS-scavenging and processing enzymes. In order to maintain genomic stability under ROS-induced stress, cells have evolved several pathways to repair or respond to the presence of DNA

damage [125]. DNA repair of single-stranded DNA damage includes base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR).

Following any damage to the cellular genome, DNA damage response (DDR) and its key players (DDR sensors) begin to detect and sense DNA lesions and orchestrate the appropriate repair of DNA damage and resolution of DNA replication problems. DNA repair includes regulation by epigenetic mechanisms including chromatin changes, histone modifications, methylation of promotors modulating expression of genes, and miRNAs known to repress DNMT3A, DNMT3B, and DNMT1 expression, which are upregulated upon DNA damage [126].

Considering the important role of epigenetic mechanisms in DNA damage response and DNA repair, it is fair to conclude that functional foods addressing epigenetic mechanisms, including SIRTS, affect DNA repair (**Figure 14**) [90].

In yeasts, it was shown that green tea extract increased the survival rate, decreased the frequency of mutations, and increased the expression of homologous recombination genes, RFA1, RAD51 and RAD52, and nucleotide excision repair genes, RAD4 and RAD14 [127].

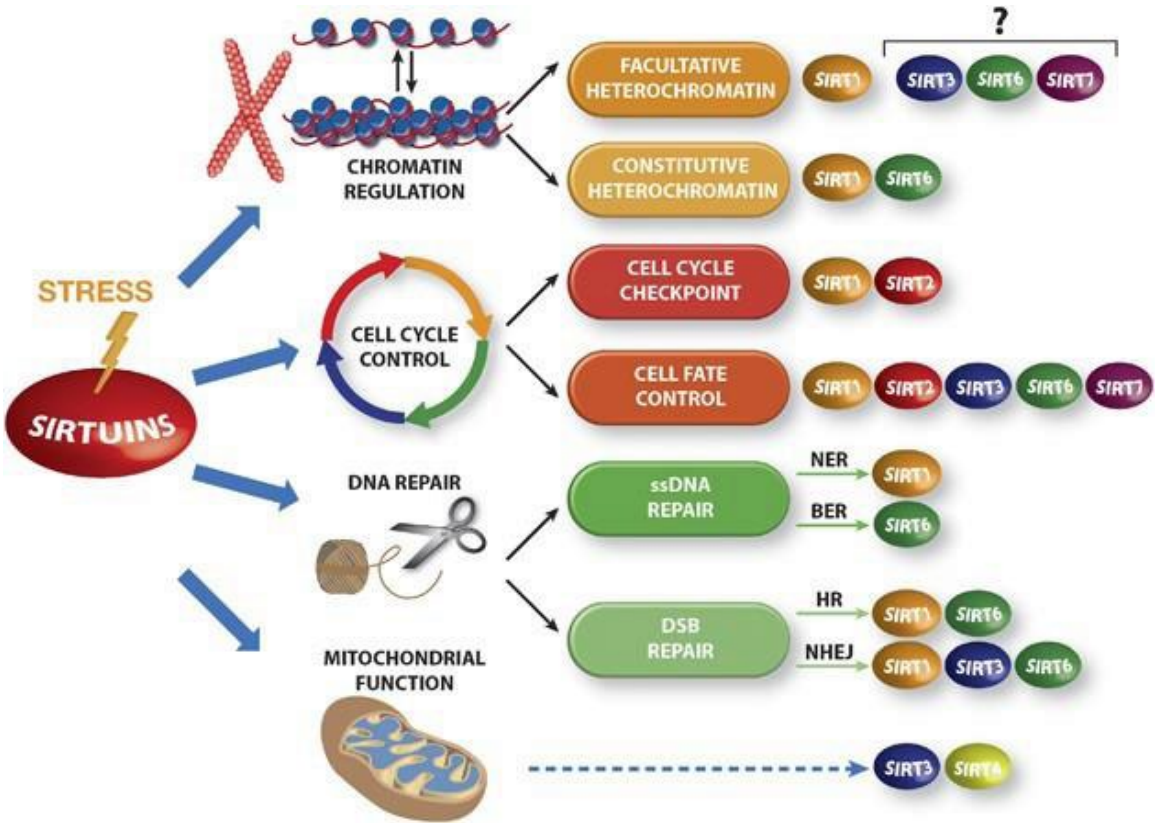

Figure 14. Sirtuins and mechanisms of genomic stability. Sirtuins participate in multiple mechanisms to maintain genomic stability under stress conditions: (a) chromatin regulation at structural and expression levels, (b) cell-cycle control, (c) DNA repair, and (d) mitochondrial-associated functions that promote genomic protection.

Naringenin, a flavanone, could prevent mutagenic changes in prostate cancer cells through increments of the BER pathway. Interestingly, in a study by Silva et al. it was revealed that some polyphenols, such as luteolin and quercetin, act on the intracellular mechanisms responsible for DNA repair, rather than by a direct effect on ROS scavenging. They also found that rosmarinic acid targets OGG1 directly and increases its expression [128].

Our group reported earlier that EGCG and gallic acid decrease high fat induced DNA strand breaks and change expression and DNA methylation of Dnmt1 and MLH1 in mice [129], [130]. Additionally, a vitamin and antioxidant rich diet increase MLH1 promoter DNA methylation in DMT2 subjects [131]. Apoptosis is another important DDR effector with the ability to decrease the risk of cell accumulation with compromised genomes [26]. Induction of apoptosis in cancer cells has been described for natural flavonoids derived from different plants, including Petroselinum crispum, Apium graveolens, Flemingia vestita, Phyllanthus emblica, etc. [132].

Neuroinflammation, Cognitive Decline, and Epigenetic Active FF

Inflammatory processes, especially of the central nervous system and the brain (neuroinflammation), appear to be some of the key processes in neurodegenerative diseases and aging as a whole. On one hand, they are an important tool for the organism to help recover from certain diseases. On the other hand, they often contribute to disease progression itself. In the central nervous system (CNS), microglia cells as part of the innate immune system control inflammatory processes and usually maintain normal CNS function. Upon arrival of adverse stimuli, they are activated and respond according to the external stimulation factors. Microglial cells behave differently according to the amount and type of activation or damage. Under moderate or transient activation, they act as protective mediators for cells, playing an immune resolving, anti-inflammatory part and supporting their surrounding cells by secreting factors that promote cell renewal (TGF-, IL-10, Arginase-1, Ym-1, etc.). In this state, they act as neuroprotective mediators. In contrast, when intensive acute or persisting microglial activation occurs, they secrete different pro-inflammatory cytokines (TNF- , IL-6, IL-1 , COX-2) in combination with reactive oxygen and nitrogen species (ROS, NOS), promoting neuronal damage, disturbing neurotransmitter function, and ultimately leading to irreversible tissue loss. Toll-like receptors (TLRs) of microglia play an indispensable role in cytokine release and pro-inflammatory processes [133],[134].

It is understood that inflammatory processes and persistent microglial activation contribute to cellular aging—a connection, for which the term “inflammaging” has been found. Specifically, systemic, chronic, and low-grade inflammation have shown to be significant risk factors for morbidity and mortality in aging individuals. Systemic inflammatory biomarkers (e.g., IL-6, fibrinogen, and C-reactive protein) are associated with a decline in regional cerebral blood flow, cortical thinning, and poorer abilities in learning and memory function. Activated microglial cells also lose their phagocytic ability, which further contributes to the accumulation of detrimental molecules, which can play a role in AD and other neurodegenerative diseases [135]. Furthermore, the increased presence of senescent cells in different neurodegenerative diseases suggests the contribution of senescence in the pathophysiology of these disorders. DNA damage, oxidative stress, neuroinflammation, and altered proteostasis have been shown to play a role in the onset of senescence. Oxidative stress contributes to accelerated aging and cognitive dysfunction stages, affecting

neurogenesis, neuronal differentiation, connectivity, and survival. During later life stages, it is implicated in the progression of cognitive decline, synapse loss, and neuronal degeneration [136].

It is now generally accepted that chronic neuroinflammation in Alzheimer's disease (AD) patients is not caused primarily by senile plaques and tau tangles, but also by changes in autophagy and senescence functions [137]. Epidemiological evidence linking diet, modifiable environmental factors, and the risk of AD is rapidly increasing. A non-balanced diet appears to impact the risk of neuroinflammation, especially AD and functional [138], [139].

EGCG has become of special interest in neurodegenerative diseases. Several in vivo as well as human observational and intervention studies have shown that there is an inverse link between the amount of green tea catechin intake and cognitive impairment. In an AD mouse model, Rezai-Zadeh et al. showed that high doses of EGCG significantly lowered pathology and plaques and provided a significant cognitive benefit in transgenic mice [140]. EGCG undergoes microbial degradation in the small intestine and later in the large intestine, resulting in the formation of various microbial ring-fission metabolites which are detectable in the plasma and urine as free and conjugated forms. Recently, in vitro experiments suggested that EGCG and its metabolites could reach the brain parenchyma through the blood–brain barrier and induce neurogenesis. These results suggest that metabolites of EGCG may play an important role, alongside the beneficial activities of EGCG, in reducing neurodegenerative diseases [141].

Fisetin, a flavonol compound, was found to prevent oxidative stress-induced nerve cell death, and subsequently, its antioxidant capabilities came into focus. Now, it is available as a dietary supplement in pure form, in doses up to 500 mg, marketed to enhance brain health. In recent years, it has achieved the image of a functional food and is promoted to prolong lifespan and counter various effects of aging. Apart from its antioxidant abilities, fisetin has been studied for its wide-ranging effects on several key pathways involved in cell cycle regulation, apoptosis, the suppression of inflammation, angiogenesis, and metastasis. In vivo, fisetin has been found to enhance long-term memory in non-AD mice [142]. In an A 1–42 mouse-model of AD, Ahmad et al. were able to show that fisetin significantly decreased the A 1–42-induced accumulation of A, BACE-1 expression, and hyperphosphorylation of tau protein. It reversed synaptic dysfunction and had a favorable effect on different proteins involved in AD pathology. Further, it also suppressed various neuroinflammatory mediators. Ultimately, it improved mouse memory when administered intraperitoneally [143].

Spermidine is a biogenic polyamine and intermediate product in the synthesis of spermine (which is also biologically active). Polyamines are essential for cell growth and tissue regeneration, and spermidine was shown to stimulate cytoprotective macroautophagy/autophagy [144]. Due to decreases in the concentration of spermidine in organisms of age, it has been connected to cellular aging [145]. The life span extending properties of spermidine have been studied in vivo, where spermidine was able to prolong the lifespan of Drosophila melanogaster fruit flies and that of mice of up to 25% when administered lifelong. Spermidine was shown to relieve defects in autophagy, as well as increase autophagy markers and life span in mice [146]. The link between spermidine and AD/cognitive decline has been well established. Pekar et al. were recently able to prove the relation of spermidine to age and memory performance [147]. Conclusively, there seems to be a link between the formation of neurodegenerative diseases and corresponding

spermidine levels in the human body. Whether this relationship is causal or a by-product of other detrimental processes remains unclear and needs to be studied further [148].

Isoliquiritin is a flavonoid glycoside compound derived from licorice and chemical synthesis. It shows a broad spectrum of pharmacological activities including antioxidant, anti-inflammatory, anti- cancer, and anti-depression activities. Isoliquiritin ameliorates depression by suppressing NLRP3-mediated proptosis via miRNA-27a/SYK/NF-κB axis [149]. The NLRP3-mediated proptosis, which could be regulated by miRNA-27a, is a key player in the development of depression.

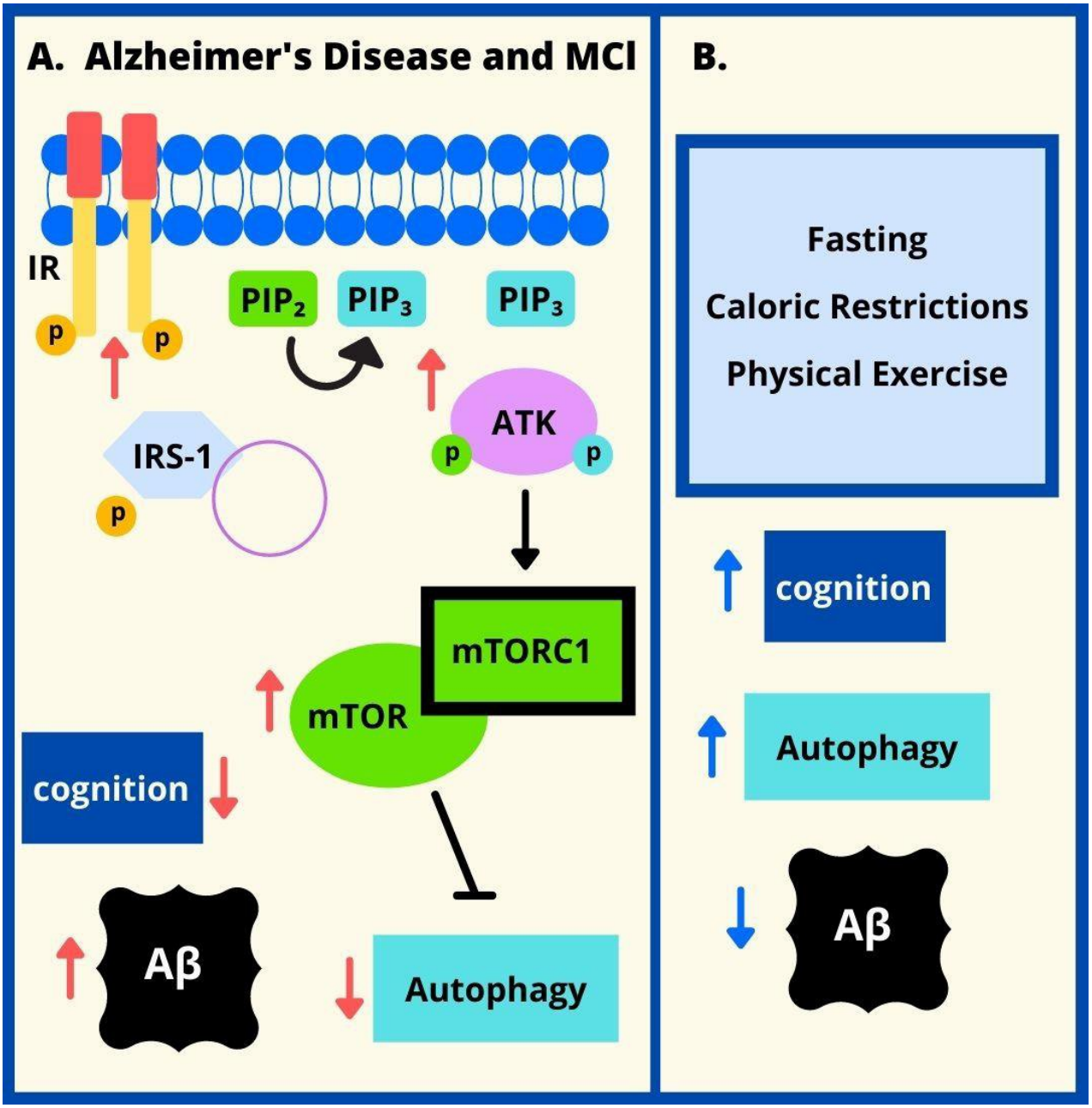

Figure 15. Fasting protocols, caloric restriction, polyphenols, and physical exercise can improve cognitive performance, increase autophagy, and thus decrease Aβ aggregates. Fig composed.

Senescence and Epigenetic Active FF

Cellular senescence describes a state in the cell cycle in which the cell permanently arrests its cell cycle and stops dividing. It was first discovered in fibroblasts, which stop dividing after about 50 cell population doublings before entering "replicative senescence." This "Hayflick limit" is a very robust tool of the organism to stop old or damaged cells from accumulating. It paved the way for the exploration of cellular aging and its underlying mechanisms [23]. Hayflick, who discovered this mechanism in 1961, hypothesized that these now non-dividing cells were involved in the processes of aging because they had lost the ability to participate in repairment and regeneration within tissues, which was later confirmed [24]. After activation of senescence, senescent cells must be cleared from the organism so that they do not accumulate. This can be achieved by either selectively destroying them (as done by senolytic drugs) or inhibiting their function (senostatic drugs) [25]. Senescent cells exhibit the following properties: irreversible replicative arrest, apoptosis resistance, and frequent acquisition of a pro-inflammatory, tissue-destructive senescence-associated secretory phenotype (SASP), where cells produce high levels of inflammatory cytokines, immune modulators, growth factors, and proteases [26]. Additionally, they prevent their apoptotic clearance by using so called pro-survival senescent cell anti-apoptotic pathways (SCAPs)—often targeted in the development of senolytic drugs [27,28] (**Figure 16**) [150].

Senescence can be induced by a variety of intra- and extracellular factors of cellular stress, including abnormal cellular growth, oxidative stress, and autophagias processes. DNA damage, reactive oxygen species (ROS), strong mitogenic signals, depletion of certain tumor suppressors, or mitotic stress also induce senescence [24]. In a normal cell cycle and cellular aging, cells stop replicating after about 50 divisions. This is caused by shortening of telomeres, which occurs because DNA polymerases are not able to completely replicate these sequences. As critically short telomeres can lead to chromosomal instability and tumor formation, the cell enters a state of cell cycle arrest and stops dividing [29]. Disregarding the initiating circumstances, entering the senescent state is coordinated by the p53/p21 and the p16 tumor-suppressive pathways. Uncapped telomeres and DNA double-strand breaks activate a DNA damage response that leads to stabilization of p53 through posttranslational phosphorylation by ATM and ATR serine/threonine protein kinases or by blocking of p53 degradation. Transcription of the cyclin-dependent kinase inhibitor (CDKi) p21 occurs upon p53 stabilization, leading to an initial arrest of the cell cycle. After this initial transient arrest, permanent arrest is controlled by p16INK4A transcriptional upregulation through p38 and/or ERK signaling. Once present, p16INK4A inhibits the activity of both CDK4 and CDK6, thereby leading to RB hypo phosphorylation and permanent blockage of S phase entry [24]. Once the cell enters the state of replicative senescence, this cell cycle state is irreversible, and the cell utilizes SCAPs to stay alive [30]. Additionally, a certain phenotype of senescent cells has been identified that releases proinflammatory cytokines [27], which in turn again promote processes involved in neuroinflammation.

The link between senescence and AD-inducing pathways is well documented. In patients with neurodegenerative diseases, various markers of senescence have been observed. Additionally, it has been shown that senescent cells that express the cell cycle inhibitory protein p16 actively drive age-related tissue

deterioration and shorten healthy lifespan in mice [31]. In 2018, Bussian et al. found a causal link between the accumulation of senescent cells and cognition-associated neuronal loss [30]. The available evidence suggests that there is a link between the cellular mechanism and several age-related diseases such as AD, atherosclerosis, and osteoarthritis [30]. Persisting and accumulating senescent cells not only negatively influence the outlook for neurodegenerative diseases, but have also been identified as a negative factor for other age-related health factors. The combination of Dasatinib, a protein kinase inhibitor drug, and Quercetin was shown to induce apoptosis in senescent cells, described by Zhu et al. in 2015 [32].

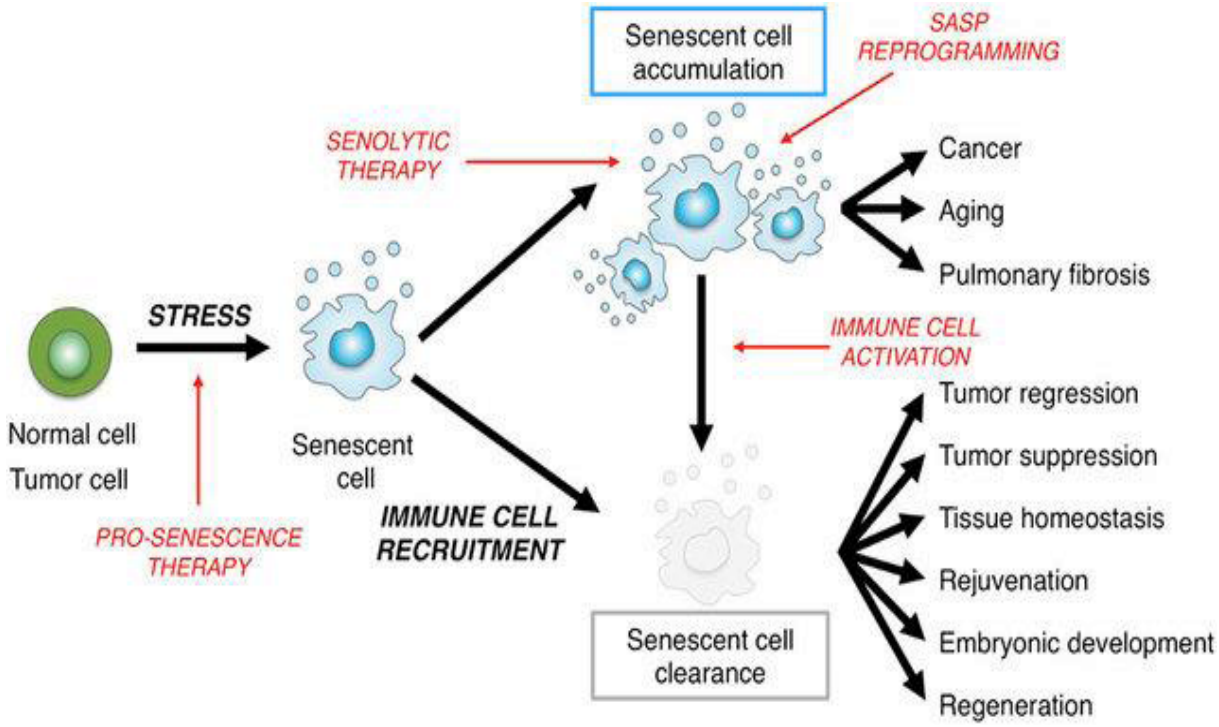

Figure 16. Clearance of senescent cells and therapeutic options. Cellular senescence is more than an anti-proliferative program. Senescent cells secrete factors that constitute the senescence-associated secretory phenotype (SASP). Cellular senescence is followed by senescent cell clearance within those processes that are considered beneficial. However, if the elimination of senescent cells does not occur, senescent cells accumulate and can lead to cancer and aging. Different therapeutic strategies (in red) can be used to exploit the beneficial aspects of cellular senescence and repress the negative ones [150].

Comparing 3T3 preadipocytes and adipocytes, we recently reported that epigallocatechin gallate (EGCG), anthocyanidin, resveratrol, phloretin, and spermidine decrease CDKN1a expression, which is a marker for senescence. EGCG could diminish IL6 and CDKN1a with the strongest effect. Anthocyanidin and EGCG could increase SIRT3 expression. Thus, targeting SIRT3 activating compounds such as EGCG may delay senescence of cells and senescence induced inflammatory processes [151]. Olive phenols hydroxytyrosol and oleuropein have also been shown to inhibit the senescence-associated inflammatory phenotype in fibroblasts [152].

Aging Microbiota and Epigenetic Active FF

The accumulation of bacteria, archaea, and eukaryote that colonizes the gastrointestinal tract is referred to as the "gut microbiota" and has developed over thousands of years with the host into a complicated and mutually beneficial relationship.

Human gut microbiota is composed of several different phyla, including *Bacteroidetes, Firmicutes and Actinobacteria*. However, it has been shown that >90% of the bacterial species are members of *Bacteroidetes or Firmicutes* [153].

According to the 16S ribosomal DNA sequencing data of fecal samples, individual gut microbiotas show distinct profiles, and this inter-individual variation is greater in older adults [154]. The gut microbiota plays a central role in many physiological and immunological processes, such as in the defense against pathogens and in the development of immune and intestinal barrier functions. It is involved in many aspects of metabolism, including the production of bile acids, lipids, vitamins, choline, and polyamine. The intestinal flora is especially involved in the breakdown of indigestible polysaccharides where the fermentation of complex carbohydrates creates short-chain fatty acids (SCFAs) that are involved in many cellular processes and metabolic pathways, as well as strengthens the intestinal barrier function and regulate the immune system and inflammatory reactions [155].

Cross-sectional studies of fecal samples from individuals in different age groups suggest age-related changes in the gut microbiota composition and diversity. Gut microbial diversity inversely correlates with biological age. Furthermore, a co-abundance module consisting of *Ruminococcus, Coprobacillus,* and *Eggerthella* generally becomes abundant with an increase in biological age, independent of chronological age. An interpretation of these results is that as biological age increases, overall gut microbiota richness decreases, while some microbial taxa, associated with unhealthy aging, emerge.

Short-chain fatty acids are products of the breakdown of dietary fibers by the anaerobic gut microbiota. They can easily enter the circulation from the gut and have beneficial roles in energy metabolism.

The most abundant SCFAs are acetate and propionate that are mainly produced by *Bacteroidetes.* Propionate is also produced by *Veilonella*, *Roseburia*, and *Ruminococcus,* whereas butyrate is mainly formed by the phylum *Firmicutes*. Acetate and propionate are absorbed by colonocytes, especially via passive diffusion, pass through them into the portal vein, and are thus transported in peripheral tissues. Butyrate is well known to act in host cells. SCFAs are important energy sources for both gut microbiota and host intestinal epithelial cells [156]. These metabolites exert diverse regulatory functions, and their effects on host physiology, metabolism regulation, inflammation, and immunity have been progressively documented. SCFAs are inhibitors of histone deacetylases and specific ligands for G protein-coupled receptors (GPCRs), such as GPR41 (FFAR3), GPR43 (FFAR2), and GPR109a (HCAR2), located on the surface of epithelial and immune cells [157]. They act as signaling molecules that influence the expansion and function of hematopoietic and non-hematopoietic cell lineages, as well as the maturation and function of microglia. The

maintenance of microglia homeostasis alters gut integrity, changes cell proliferation, and exerts anti-inflammatory, antitumorigenic, and antimicrobial effects [156] (**Figure 17**) [158].

The epigenetic activity of butyrate, as well as the ketone body beta-hydroxybutyrate, positively affect most hallmarks of aging, such as telomerase activity, mitochondrial functions apoptosis, inflammation, senescence, and DNA repair, thus being beneficial in broad areas of inflammaging [159] or aging related neuroinflammation [160].

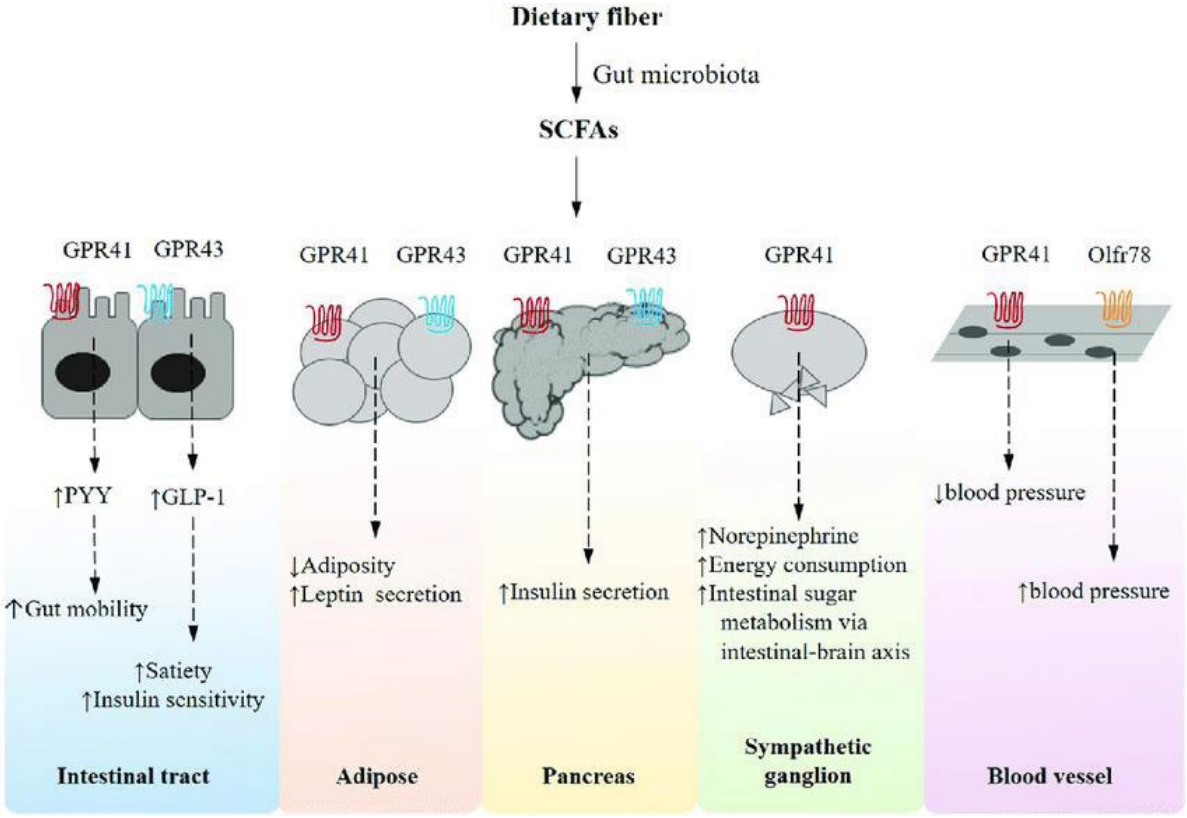

Figure 17. Short-chain fatty acid (SCFA)-receptor-mediated pathways and their effects on host energy metabolism in peripheral tissues. Gut microbes can ferment dietary fiber into SCFAs, which induce an array of G-protein coupled receptor-mediated signaling pathways that are essentially implicated in host energy homeostasis in multiple tissues [158].

Age-related changes in the metagenomics of short-chain fatty acid production have been observed. For instance, frequencies of genes encoding short-chain fatty acid (SCFA) production and those involved in carbohydrate breakdown decrease, while those of genes involved in protein breakdown increase. The reduced frequency of genes for short-chain fatty acid production is also associated with frailty. Thus, the short-chain fatty acids have the potential to modulate healthy aging [161] and strategies to increase the production of SCFAs, especially butyrate, comprised either of butyrate-producing probiotica, **metabolic cross-feeding** of strict anaerobic **butyrate producers** such as *F.prausnitzii, Clostridium leptum, Eubacterium rectale and Roseburia spp.* [162], or prebiotic butyrogenic fibers [163].

Additionally, the scientific interest is shifting from viable probiotic bacteria towards non-viable paraprobiotics and/or probiotic derived biomolecules, so-called postbiotics (**Figure 18**) [165].

Paraprobiotics and postbiotics are the emerging concepts in the functional foods field because they impart an array of health-promoting properties. The postbiotics are the complex mixture of metabolic products secreted by probiotics in cell-free supernatants such as enzymes, secreted proteins, short-chain fatty acids, vitamins, secreted biosurfactants, amino acids, peptides, organic acids, etc. Meanwhile, the paraprobiotics are the inactivated microbial cells of probiotics (intact or ruptured containing cell components such as peptidoglycans, teichoic acids, surface proteins, etc.) or crude cell extracts (i.e., with complex chemical composition). Therefore, postbiotics can include many different constituents including metabolites, short-chain fatty acids (SCFAs), microbial cell fractions, functional proteins, extracellular polysaccharides (EPS), cell lysates, teichoic acid, peptidoglycan-derived muropeptides, and pili-type structures [164] [165].

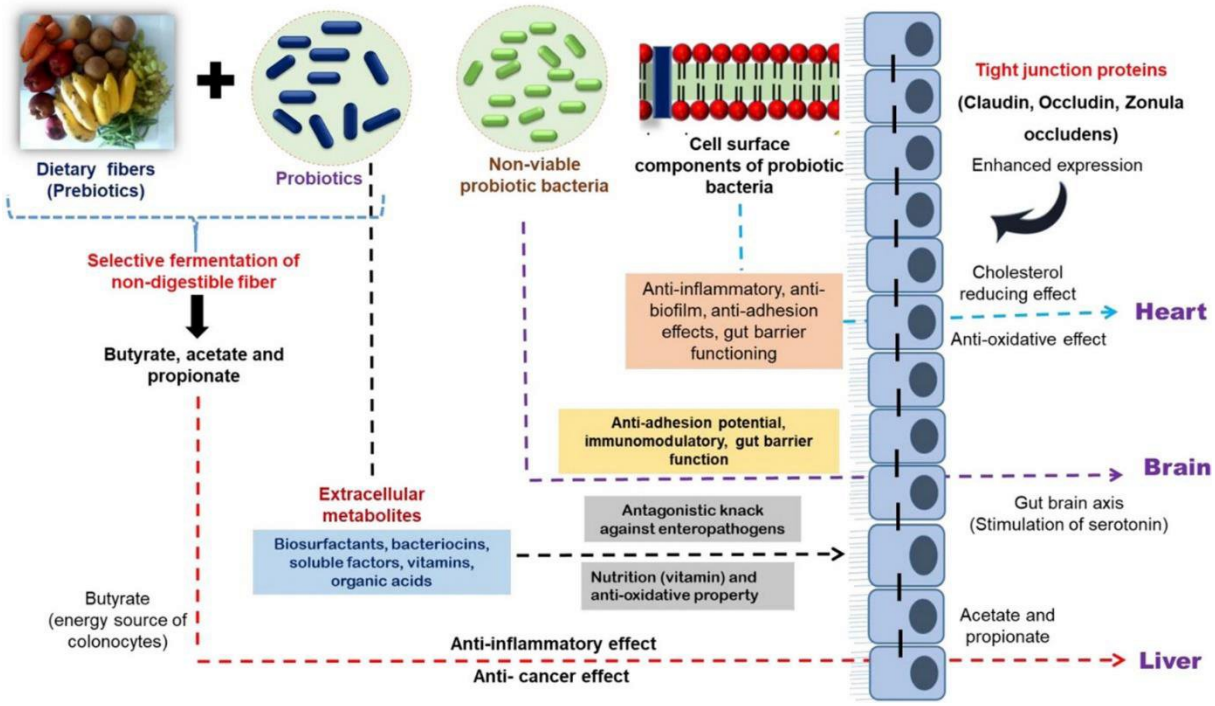

Figure 18. Schematic representation of various health benefits of postbiotic molecules [165]

Caloric Restriction, Fasting, and Fasting Mimetics

To date, caloric restriction (i.e., a reduction in caloric intake without malnutrition) is the only non-genetic intervention that has consistently been found to extend both mean and maximal life span across a variety of species. Key early studies in rodents revealed that mice fed 55–65% caloric restricted diets through their life exhibited a 35–65% greater mean and maximal lifespan than mice eating an ad libitum diet [166]. Although attenuated, these effects remain present even when moderate caloric restriction (20–40%) is implemented in middle-aged mice. Importantly, prolonged caloric restriction has also been found to delay the onset of age-associated disease conditions such as cancer and diabetes in rodents [167] and in nonhuman

primates. Thus, findings from animal studies, including recent primate studies, suggest that prolonged caloric restriction has the potential to extend health-span and thereby increase the quality of life. In recent studies conducted in overweight humans, caloric restriction has been shown to improve a number of health outcomes including cardiac risk factors, as well as improve insulin-sensitivity and enhance mitochondrial function [168], [169]. Additionally, prolonged caloric restriction has also been found to reduce oxidative damage to both DNA and RNA, as assessed through white blood cells [170]. Thus, findings of initial human clinical trials appear to support the promise of caloric restriction demonstrated in animal studies. Several different biological mechanisms may account for the increase in health span and longevity such as downregulation of answers to **oxidative stress, increased autophagy, senolysis, and maintenance of a healthy population of mitochondria** through biogenesis.

CR and Sirtuins Guarente et al. [68] formulated the hypothesis that nutrient-sensing regulators mediate the effects of this diet on aging and diseases based on the findings that sirtuins are NAD+-dependent protein deacetylases and known to counter aging in yeast. Now, years later, a large volume of data begins to illustrate an elaborate set of physiological adaptations to caloric intake mediated by sirtuins. There are many studies that connect sirtuin activation with prevention of aging and diseases of aging in mouse models. It is also clear that other nutrient sensors, such as AMPK, mTOR, and FOXO, are very important in linking diet, metabolism, and aging. SIRT1 and mTORC1 appear to be candidate targets because small molecules such as resveratrol, rapamycin, and other sirtuin activating compounds, STACS, have been described that can alter their activities. One might posit that small molecules that bind to the SIRT1 allosteric site mimic natural endogenous compounds that regulate the enzyme under certain physiological conditions, such as caloric restriction (CR). [171]. More recently, it was found that sirtuins transduce STACs signals through steroid hormone receptors (**Figure 19**) [171] [172].

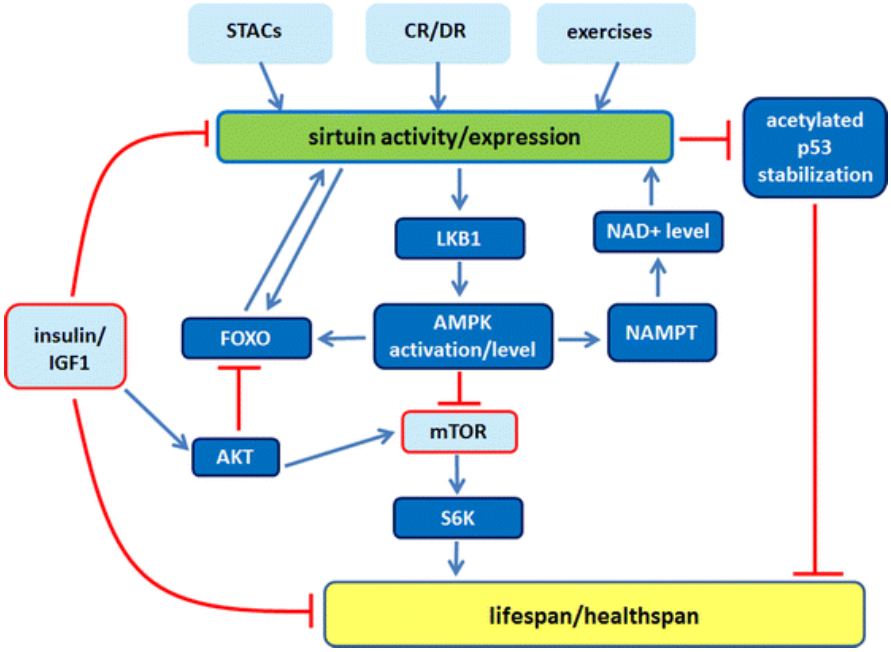

Figure 19. Sirtuins modulate multiple pathways involved in mediating positive effects of some anti-aging interventions, such as calorie/diet restriction (CR/DR) or exercise. Such effects can also be mimicked by sirtuin activating compounds (STACs) [172].

Fasting triggers neuroendocrine responses and adaptations characterized by low levels of amino acids, glucose, and insulin. Down-regulation of the insulin, such as growth factor 1 (IGF-1) signaling pathway and reduction of circulating amino acids, repress the activity of mammalian target of rapamycin (mTOR), resulting in inhibition of protein synthesis and stimulation of autophagy. During fasting, the ratio of AMP to ATP is increased and **AMPK** is activated, triggering repair and inhibition of anabolic processes. Acetyl coenzyme A (CoA) and **NAD+** serve as cofactors for epigenetic modifiers such as SIRTs. SIRTs deacetylate FOXOs and **PGC-1α,** resulting in the expression of genes involved in stress resistance and mitochondrial biogenesis. Collectively, the organism responds to fasting by minimizing anabolic processes (synthesis, growth, and reproduction), favoring maintenance and repair systems, enhancing stress resistance, recycling damaged molecules, stimulating mitochondrial biogenesis, and promoting cell survival, all of which support improvements in health and disease resistance [173].

Glucose and fatty acids are the main sources of energy for cells. After meals, glucose is used for energy, and fat is stored in adipose tissue as triglycerides. During periods of fasting, triglycerides are broken down to fatty acids and glycerol, which are used for energy. The liver converts fatty acids to ketone bodies, which provide a major source of energy for many tissues, especially the brain, during fasting. In the fed state, blood levels of ketone bodies are low, and in humans, they rise within 8 to 12 hours after the onset of fasting, reaching levels as high as 2 to 5 mM by 24 hours [174]–[176].

The metabolic switch from the use of glucose as a fuel source to the use of fatty acids and ketone bodies results in a reduced respiratory-exchange ratio (the ratio of carbon dioxide produced to oxygen consumed), indicating a greater metabolic flexibility and efficiency of energy production from fatty acids and ketone bodies. **Ketone bodies** are not just fuel used during periods of fasting; they are potent signaling molecules with major effects on cell and organ functions. Ketone bodies such as BHB are epigenetically active, and they regulate the expression and activity of many proteins and molecules that are known to influence health and aging [173].

Major theories of the mechanism of how CR interacts with aging include the oxidative damage attenuation hypothesis, glucose-insulin hypothesis, growth hormone and insulin-like growth factor (IGF)-1, and the hormesis hypothesis. Oxidative damage is decreased during caloric restriction (CR) through decreased production of reactive oxygen species and the up-regulation of protective enzymes, resulting in a decrease in DNA damage and increase in genome stability. CR causes decreased levels of circulating insulin and glucose, resulting in decreased cell growth and division, shifting toward maintenance and repair. Levels of growth hormone and IGF-1 decrease in response to CR, which promotes maintenance and repair. CR induces a low level of stress, which enables cells to counteract higher stresses, increasing DNA repair gene expression and favoring maintenance and repair [177]. Additionally, the activity of epigenetic active metabolites induced or elevated due to CR in butyrate and BHB, which contributes to the beneficial effects of CR and fasting. However, long-time CR that results in an individual becoming underweight is obviously not supportive for longevity, as several meta-analyses found a J-shaped association between BMI and mortality, with lowest risks at 24kg/m^2 [178] .

More recently, the involvement of another epigenetic mechanism in CF and fasting was shown: miRNA array analyses revealed that the expression levels of numerous miRNAs changed after 2 days of fasting. These results indicate that components of the miRNA machinery, especially the miRNA-processing enzyme DRSH-1, play an important role in mediating IF-induced longevity via the regulation of fasting-induced changes in gene expression [179]. Expression of miR-29 and miR-30 family members were increased in both CR and fasting in mice. Western blot analysis of the normal liver tissue showed that CR and FA downregulated the IGF-1/Akt pathway, and qRT-PCR showed that the expression of miR-29b, miR-29c, miR-30a, and miR-30b were increased with CR and fasting [180].

Our group analyzed the effects of periodic fasting (PF) on the human gut microbiota, SIRTs expressions, and mitochondrial content in 51 males and females. The participants fasted under supervision for five consecutive days following the Buchinger fasting guidelines. Ketogenesis, selected mRNAs, miRNAs, mitochondrial (mt) DNA, and gut composition were analyzed before and after PF. PF triggered a significant switch in metabolism, as indicated by the increase in ß-hydroxybutyrate (BHB) and pyruvate dehydrogenase kinase isoform 4 (PDK4) expression in the capillary blood. MtDNA, SIRT1, SIRT3, and miRlet7b-5p expression in blood cells were elevated, whereas SIRT6 and miR125b-5p were not affected. Following fasting, gut microbiota diversity increased, and a statistically significant correlation between SIRT1 gene expression and the abundance of *Prevotella* and *Lactobacillus* was detected. The abundance of longevity related C*hristensenella* species increased after fasting and was inversely correlated with age and body mass index (BMI). Thus, this represents the first study showing that fasting not only changes the composition of the gut microbiota, making it more diverse, but also affects SIRT expression in humans [181].

One alternative dietary approach that may produce similar biological changes as caloric restriction that has received increasing interest from the scientific community is **intermittent fasting**. Evidence that this approach may have beneficial effects on longevity was made by Carlson and Hoelzel in 1946 [182]. Although the magnitude of the effect of intermittent fasting on life-span extension is variable (influenced by sex, diet, and genetic factors), studies in mice and nonhuman primates show consistent effects of caloric restriction on the health span [174]. However, a significant increase of epigenetically active ketone bodies seems to be linked to the length of the full fasting time [183].

Although there is no general agreement on definitions, one might differentiate between short term CR and intermittent fasting protocols and long-term CR and fasting by the activation of epigenetic active metabolites, in addition to the activation of SIRTs.

Fasting Mimetics The long time, periodic reduction of calorie intake without malnutrition is the only strategy that reliably extends health span in mammals. However, the strict and life-long compliance with these regimens is difficult, and ways to mimic fasting and caloric restriction mechanisms and benefits have been investigated. Many drugs and nutraceuticals have been studied to act as mimetics of caloric restriction and fasting, which has promoted the emergence of caloric restriction fasting mimetics. Fasting mimetic compounds can be defined as compounds that mimic the protective pathways of caloric restriction and

impact the hallmarks of aging, such as autophagy and senolysis (**Figure 20**) [177]. There is growing evidence that caloric restriction, fasting, and fasting mimetics may trigger senolysis [184].

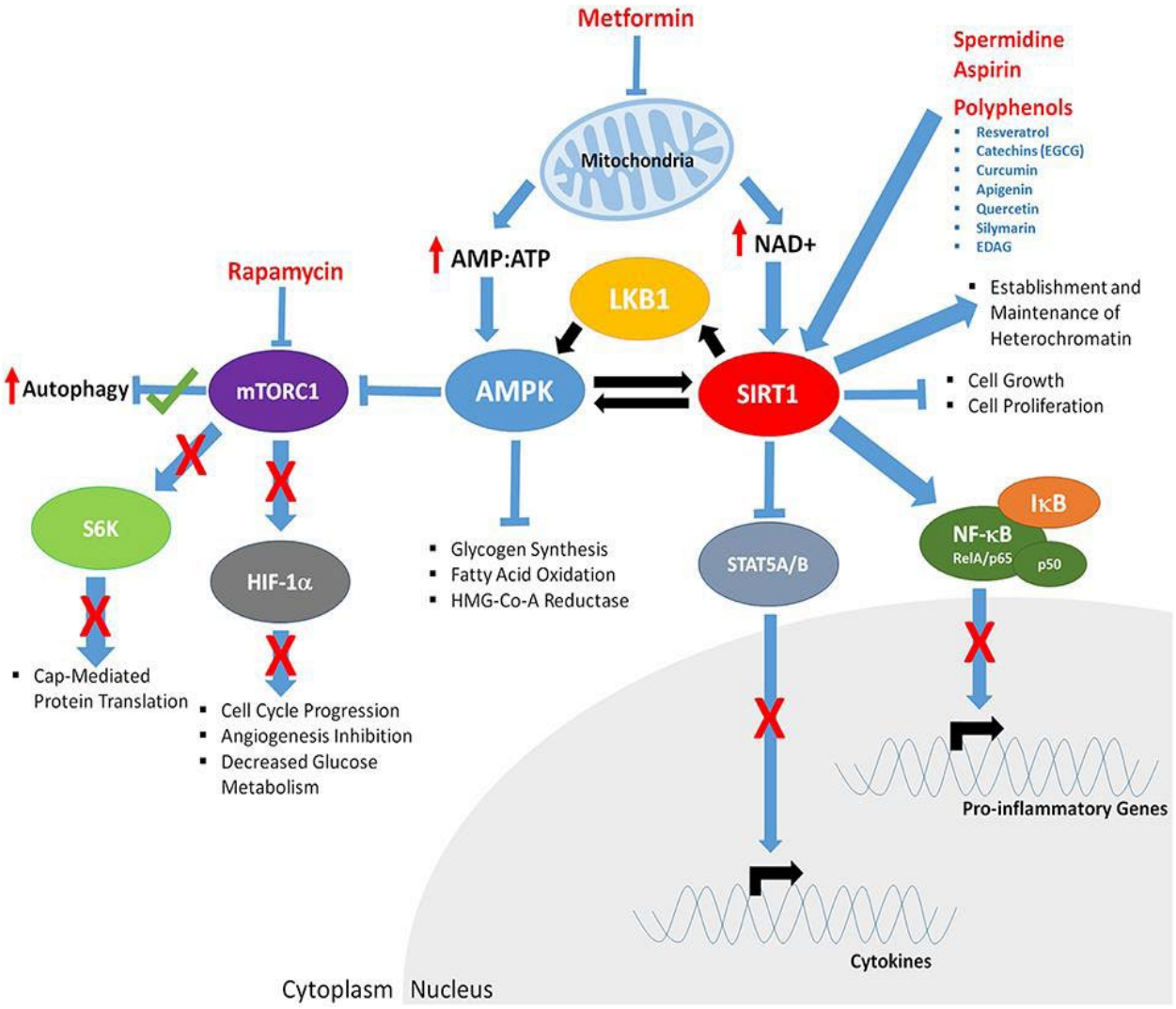

Figure 20. The downstream effects of CR mimetics and nutraceuticals on key aging mediators AMPK and SIRT1 [177].

CR and fasting mimetics mimic several molecular consequences of fasting. The main affected pathways include the SIRT, AMPK, and mTOR pathway. Metformin exposure decreases mitochondrial function, increasing AMP:ATP ratios and levels of NAD+. AMPK inhibits glycogen synthesis, fatty acid oxidation, HMG-Co-A Reductase, and mTOR function. Decreased mTORC1 function results in increased autophagy, decreased S6K activity, and cap mediated protein translation, as well as inhibits HIF-1α, resulting in reduced cell cycle progression, angiogenesis, and glucose metabolism. Rapamycin mTORC1 kinase activity potentially mirrors the effects of Metformin. Through decreased mitochondrial function, Metformin increases NAD+ levels, which promotes SIRT1 activity and results in deacetylation of LKB1 and AMPK, as well as upregulates their respective function. Polyphenols, Spermidine, and Aspirin increase SIRT1

activity, induce deacetylation of the RelA/p65 component of NFκB, prevent degradation of IκB, sequester NF-κB in the cytoplasm, and inhibit proinflammatory gene expression. Cytokine gene expression is repressed through SIRT1-mediated deacetylation and repression of STAT5A/B [177]. There are a number of naturally occurring polyphenols, **including curcumin, resveratrol, catechins (especially EGCG), gallic acid apigenin, quercetin, and the polyamine spermidine** that are discussed as CR/fasting mimetic [177], [185].

Personal Aging and Personalized FF

The hallmarks of aging indicate that different molecular mechanisms contribute to the aging process. Certainly, multiple molecular pathways interconnect these mechanisms, and all elements such as inflammation and senescence are interacting rigorously. Evidence suggests that in different individuals, one or more of these mechanisms contribute primarily to the progression of personal aging. These mechanisms may be seen as drivers in the progression of aging or as personal "Achilles heels" in the development of personal aging-related diseases.

Most recently, multi-omics profiling examined how different types of 'omic' measurements, including transcripts, proteins, metabolites, cytokines, microbes, and clinical laboratory values, correlate with age. Personal aging markers whose levels changed over a short time frame of 2–3 years were found, and different types of aging patterns in different individuals, termed '**ageotypes**', were defined based on the types of molecular pathways that changed over time in a given individual (**Figure 21**) [186]. Ageotypes may provide a molecular assessment of personal aging, reflective of personal lifestyle and medical history, that may ultimately be useful in monitoring and intervening in the aging process [186].

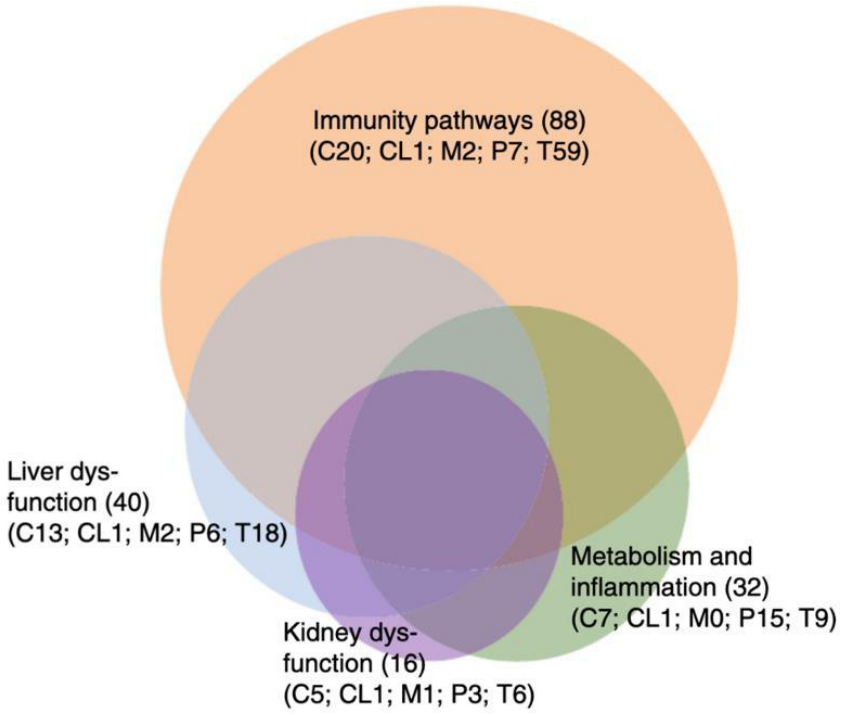

Figure 21. Ageotypes, personalized aging [186]

Individuals of the same age may not age at the same rate. Quantitative biomarkers of aging are valuable tools to measure physiological age, assess the extent of 'healthy aging', and potentially predict health span and life span for an individual [187]. Reliable sets of markers from the areas of genetics, epigenetics, transcriptomics, metabolomics, and microbiota are under development for the analysis of ongoing aging related complex diseases, as well as drivers of personal aging processes (**Figure 22**).

In personalized or precision medicine as well as nutrition, the use of molecular markers enables the detection of ongoing pathological mechanisms and interventions before the onset of symptoms. These developments will result in preventive and personalized health care.

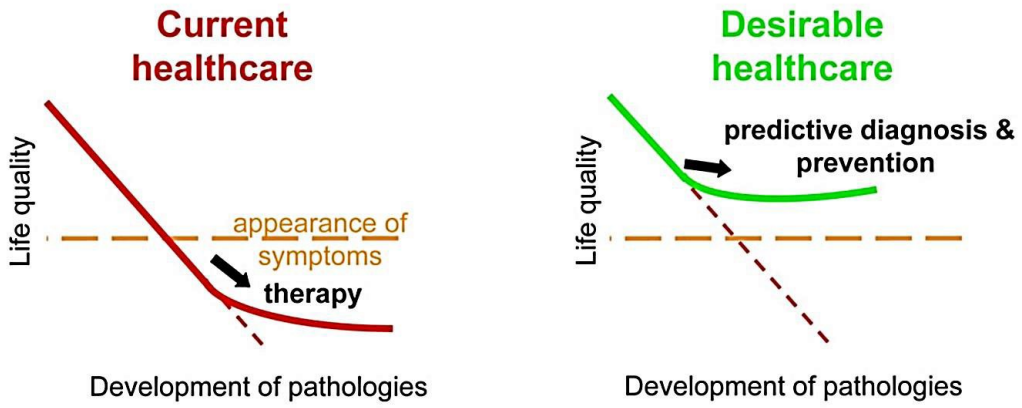

Figure 22. Personalized preventive healthcare, the use of markers in the prevention of diseases.

The use of biomarkers that identify potential mechanisms which drive the accelerated development of aging related diseases will be used as a marker-assisted analysis on a personalized level to prevent or identify accelerated aging. This may come from precision medicine, but also precision nutrition [188], [189] supported by the use of additives, nutraceuticals, and functional foods.

This concept requires the development of algorithms to provide personalized preparations of one or more functional food components in a short time addressing the result of a marker-based screening which considers the requirements of safety, bioavailability, stability, mixability, formulation, and legal requirements.

SUMMARY

- Aging is a multifactorial process including the hallmarks of aging. Epigenetic regulation affects all hallmarks.
- Epigenetic methylation and miRNAs can be used as markers for biological aging and health consequences of lifestyle, nutrition, and functional foods.
- Aging is a personal process with highly personal mechanisms driving accelerated aging, aging-related complex diseases, or resulting in healthy, decelerated aging.
- Nutraceuticals, additives, and functional foods can be used to address functions of each hallmark of aging.

- The use of molecular markers-based analysis of personal aging can be used for the composition of functional food preparations which address mechanisms of concern.

Test Questions

1. Epigenetic mechanisms
a. change the genetic sequence
b. affect the chromatin structure
c. can be passed on to next generations
d. can be affected by polyphenols
e. can be addressed by changes in microbiota structure

2. Polyphenols in physiological concentrations induce
a. epigenetic methylation
b. epigenetic histone acetylation
c. epigenetic miRNAs
d. DNA repair
e. Autophagy
f. Senolysis
g. DNA mutations

3. Sirtuins
a. are epigenetic enzymes
b. are histone deacetylases
c. can be induced by polyphenols
d. are produced by fasting
e. affect aging
f. affect mitochondria
g. functions need to be differentiated according to subclass

4. Butyrate
a. is a metabolite of the microbiota
b. can be produced in fat cells
c. is induced by fasting
d. is epigenetically active
e. affects sirtuins
f. is anti- inflammatory

5. Postbiotics

a. are probiotics of the next generation

b. can contain probiotics, prebiotics and SCFAs

c. affect epigenetic regulation

d. can be produced by fermentation

Answers: 1:(B,C,D,E) 2:(A,B,C,D,E,F) 3:(A,B,C,D,E,F,G) 4:(A,C,D,E,F) 5:(B,C,D)

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