Syed Ibrahim Rizvi *Editor*

Emerging Anti-Aging Strategies

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Foreword

Population aging is one of the main global challenges for sustainable development. The results of fundamental research of the last decade in the field of biogerontology have led to the understanding that the rate of aging can be modified by influencing the basic processes associated with aging, using pharmacological, nonpharmacological, genetic, and gene-therapy interventions, as well as regenerative technologies, with the achievement of a healthier and longer life. There are prerequisites for reducing the burden of both age-associated diseases and geriatric syndromes, primarily frailty syndrome. Managing the aging processes can prevent or at least slow down the onset and progression of these conditions.

This multifaceted book provides an overview of the entire arsenal of modern antiaging technologies.

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Preface

As humankind acquired intelligence during the evolutionary process, its first scientific pursuit involved finding ways to live longer. History is replete with human endeavor of devising ways for a longer lifespan. The ancient Indian text Rigveda (>1000 BC) mentions a drink "**amrita**" that can bestow immortality. Modern science however had little understanding of the mechanism(s) of aging until the 1950s, which is evident from the lecture of Sir Peter Medavar delivered at University College London in 1951, entitled "An Unsolved Problem in Biology."

The last few decades have witnessed tremendous advances in the understanding of molecular events which underline the process of aging. It is therefore a big achievement of science that we now have a clear understanding of the hallmarks of aging. This understanding has provided biogerontologists with putative "targets" which can be exploited for possible antiaging strategies.

The dust is now almost settling on the debate whether human lifespan can be increased. It is being acknowledged that humans have already achieved the highest limit of their lifespan. In this backdrop, antiaging strategies are thus limited to finding ways to increase the healthy lifespan. The major impediment in devising an antiaging strategy is due to the fact that the aging process is highly heterochronic.

Despite the complexities of the aging process, new scientific evidence emerging with extensive research continues to present interesting targets for devising antiaging strategies. The present book is an attempt to provide a compact source of emerging antiaging strategies which offer hope for a longer health span.

Recent evidence provides a strong support to the concept of calorie restriction as a mechanism to derive an antiaging effect. Chapters 4 and 10 provide an update on our current understanding of the effect of CR on aging. The role of nutritional supplements, for example coenzyme Q, curcumin, and spermidine, has been discussed in Chaps. 2, 8, and 11.

Circadian rhythm is being intensively investigated in relation to factors which play a role in aging. The role of melatonin in the aging process and associated disorders is discussed in Chap. 9. Metformin, the common antidiabetic drug, is being extensively investigated for its possible antiaging effects; Chap. 6 details the current status of research on metformin with respect to skin aging. Autophagy induction and sirtuin activation are again promising areas which are being actively investigated (Chaps. 3 and 14).

An interesting Chap. 16 is on the cognitive and emotional aspects of aging. Frequently, research on rodent model of aging is used to derive conclusions for humans. It thus becomes important to understand the age-adjustments with respect to rodents when the same are applied to humans (Chap. 18).

Research on antiaging may sound so exciting to humans but tinkering into nature's design may throw up unknown consequences. With many new technologies finding their way into the human lexicon, there is a need to debate on the ethical issues involved. Chapter 15 is an interesting update on the conceptual and ethical aspects.

I would like to thank all the contributors who provided me with their excellent chapters making possible the compilation of this book.

Allahabad, Uttar Pradesh, India Syed Ibrahim Rizvi

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About the Editor

Syed Ibrahim Rizvi is Professor of Biochemistry and Dean of Research and Development at the University of Allahabad, India. He is a Visiting Professor to Ohio State University, University of Rome, University of Milan, University of Athens, University of Nice, University of Szeged, University of Pisa, Karolinska Institute, Slovak Academy of Sciences, Istanbul University, and Bangladesh Institute of Health Sciences. He has also been an invited speaker to major conferences in the USA, Russia, Hungary, Italy, Saudi Arabia, Kuwait, Egypt, Bangladesh, and Thailand. Prof Rizvi has done seminal research work in the area of mechanism(s) of aging, antiaging interventions and lifespan. He has published extensively in high-impact factor journals. To date Prof Rizvi has published more than 200 research papers. He is on the editorial board of the prestigious Springer journal *Biogerontology*. Prof Rizvi has been a recipient of grants from the International Cell Research Organization, Federation of European Biochemical Societies, European Molecular Biology Organization, Organization for the Prevention of Chemical Weapons, International Foundation for Science, Department of Biotechnology (Govt. of India), and Department of Science and Technology (Govt. of India). Prof Rizvi has been conferred with the Academic Excellence Award of the University of Allahabad in 2016. He has edited a book on Anti-Aging Interventions in 2018 published by Springer Nature. Prof Rizvi has also written a large number of popular science articles which have been published in national newspapers. He is a member of Indian Gerontological Society, Society of Biological Chemists, National Academy of Sciences India, and Asian Network for Research on Antidiabetic Plants.

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Genetics and Epigenetics of Aging and Age-Associated Diseases

1

Anam Naseer and Aamir Nazir

Abstract

Organismal aging is normally associated with a decline in bodily functions and an increase in various disease-related outcomes. Whether aging itself is a disease or is a process encountered as a consequence of biological fatigue by cellular and organismal systems has long been debated. The fact that is affirmed by decades of research is that aging leads to various adverse outcomes including diminished health and vigor, disturbed metabolism, and altered cellular homeostasis thus triggering myriad diseases. Two major factors that drive the process of aging and its associated diseases are (a) "the genetic make-up or the genome" of a person and (b) the "epigenetic events" that are largely impacted by such intrinsic and extrinsic factors of the body and are modulated by the environment/life-style factors, the genome interacts with. The epigenetic events include DNA methylations, histone modifications, and chromatin remodeling among other events that define how organismal function pursues. Effective research within the area towards the identification of specific genetic and epigenetic targets can deliver senolytic compounds and other anti-aging strategies thus promoting healthy aging and slowing the process of decline in health and related frailty.

Keywords

Aging · Genetics · Epigenetics · Histone modifications · DNA methylation

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1.1 Introduction

The term "Aging" is often described as the process of "getting old"; it emanates from the Latin word "*aevum*," signifying "age" or "everlasting time" (Vocabulary 2022). Considering the long-known direct association between declining health and increasing disease burden as a result of age progression, the phenomenon of aging has long been the topic of interest for researchers in various fields who ultimately aim at gaining insights into the science of human aging (geroscience). Aging is an irreversible and time-dependent process that results in the decline in health and vigor of an organism as it gets older. With the progressing age, circadian rhythm breaks and metabolism of the body slows down. Similarly, body stature, behavior, homeostasis, cellular proteostasis, and routine activities are also affected (Stein et al. 2022). Aging also creates a socioeconomic burden on patients and their families (De Magalhães et al. 2017). Since, the advent of the first life form on earth, quest for understanding aging and its associated phenotypes have begun and till date a complete understanding of the process evades us. However, with the advancement in medical facilities and modern technology, the average life expectancy of the world population has been increased to 73.2 years (Worldometer 2022). It is a universal and evolutionarily conserved phenomenon that has not been fully understood and leaves a larger area of research unexplored.

Advancing age expedites decline in functioning of various molecular as well as cellular components and they collectively form the "hallmarks" of aging (Fig. 1.1). These are broadly classified into nine heads namely, genomic instability, telomere attrition, altered intercellular communication, proteostasis disruption, stem cell exhaustion, cellular senescence, mitochondrial dysfunction, nutrient-sensing deregulation, and epigenetic alterations (López-Otín et al. 2013).

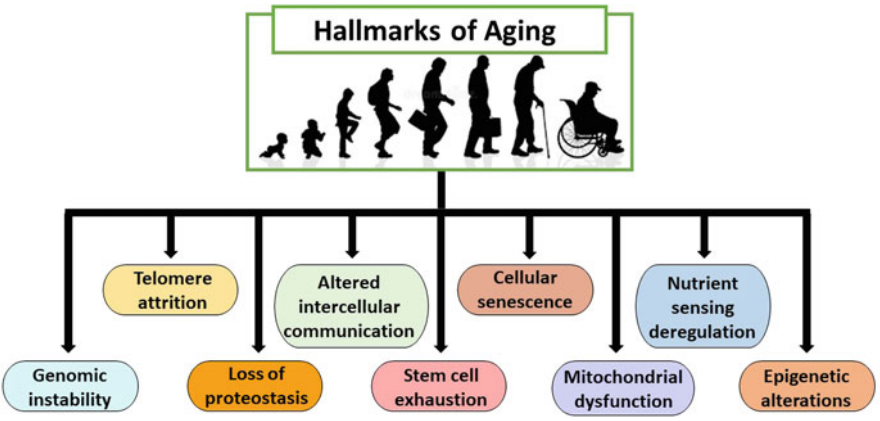

Fig. 1.1 'Hallmarks of aging'

Due to improper functioning and regulation, aging body becomes susceptible to various age-associated diseases such as neurodegenerative diseases, cardiovascular diseases, diabetes, obesity, and cancer, to name a few. These diseases occur either as a result of external environmental factors (epigenetic factors) or they are encoded in the genome of an organism (genetic factors). Genome comprises of the genetic material which codes for the information regarding the organismal development and sustenance. It contains all the information required for the basic functioning of the body at cellular, molecular and organismal levels. It is categorized into two major components: euchromatin and heterochromatin (Tamaru 2010). The active part of the genome is called euchromatin while the inactive part comprises of heterochromatin.

The genome of an organism remains fixed; however, during the course of life, it becomes vulnerable and succumbs to mutations leading to age-associated diseases. Also, with advancing age certain genes, exit initially assigned dormant state (associated with heterochromatin), become active, displaying their effect on health and longevity (Lee et al. 2021). However, the epigenome (comprises of the modifications that occur posttranslationally) is flexible in nature and is influenced by external as well as factors that lead to modifications of the genetic components, such as acetylation, methylation, phosphorylation, sumoylation, and ubiquitinylation. These factors play an important role in regulating the body functions and mechanisms and it is thus vital to study the role of genetics and epigenetics in regulating aging and age-associated diseases.

1.2 Genetics of Aging

Genes form the basis for supporting any lifeform and are arranged as beads on a string, forming the core of hereditary material—DNA or RNA. Genetic material along with histone and nonhistone proteins gives rise to what we call as "genome." The genome codes for two types of sequences: the ones which carry useful information, known as exons, and the others which form the (so-called) "junk" part, known as introns. Interestingly, the introns form the major part of the genome, suggesting that although these are not expressed they play a vital role in the maintenance of genome throughout the life.

According to the genetic theory of aging, lifespan of an organism is decided by the pattern of genes coded onto its DNA. Genes which cap the DNA, known as telomeres, shed and become shorter each time the cell divides and once all the telomeres are shed then there is no room for losing valuable genes or for another cell division and the cell dies. This theory also supports the concept of cellular aging, which states that once the cell reaches its maximum division capacity (known as Hayflick limit) it becomes destined to die by a process known as apoptosis, also called programmed cell death (Shay and Wright 2000).

A large body of work has been done around deciphering the genes involved in aging and age-associated diseases. Certain aging genes and their orthologs have been identified that describe the lifespan in various organisms; for example, a very

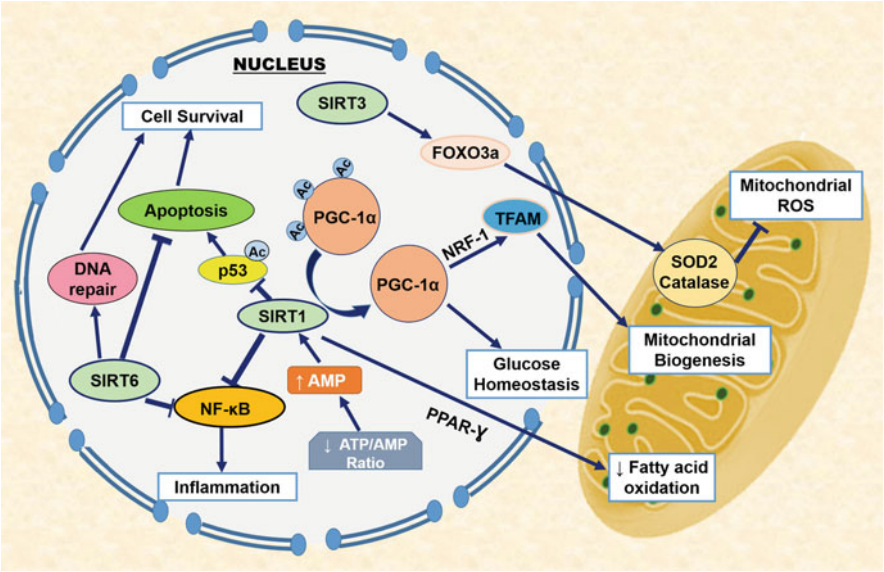

Fig. 1.2 Role of sirtuins in regulating glucose and fat metabolism, mitochondrial function, inflammation and cell survival

well-known class of enzyme, namely "sirtuins," has been long associated with longevity (Hekimi and Guarente 2003) and is evolutionarily conserved thus having its orthologs found in all organisms such as "*SIRT 1–7*" in mammals, *sir-2.1*, *sir-2.2*, *sir-2.3*, and *sir-2.4* in *Caenorhabditis elegans*, *dSir2* in *Drosophila*, *Sir2* in *Saccharomyces cerevisiae* (yeast). Sirtuins are involved in the expression of transcription factors such as AMPK, PGC-1α (Rodgers et al. 2005), p53, and NF-κB (Pillai et al. 2005) and are thus involved in regulating the processes such as glucose and fat metabolism, mitochondrial biogenesis, cell survival, and inflammatory immune response, to name a few (Fig. 1.2).

Also, different mutations resulting in an array of genetic variants, including the mutations in nuclear as well as mitochondrial DNA, help in understanding aging in a better sense. For example, mutation in *clk-1* and *isp-1*, mitochondrial electron transport chain genes, results in decreased oxidative phosphorylation and increased lifespan in *C. elegans*. Thomas Johnson identified the gene *age-1* (class-3 PI3-K) to be the first gene mutation to be involved in increasing the lifespan in *C. elegans*. In the same model as well as in flies and mice, mutation a very well-studied pathway, that is, insulin/IGF-I signaling (IIS) pathway involving genes, such as *daf-2*, results in longer lifespan of the organism (Kenyon et al. 1993). In 1993, Cynthia Jane Kenyon showed that mutation in gene *daf-2*, responsible for dauer larva formation, doubles the lifespan in *C. elegans. daf-2* negatively regulates the gene *daf-16* which encodes for forkhead box O (FOXO) transcription factor that is involved in activities

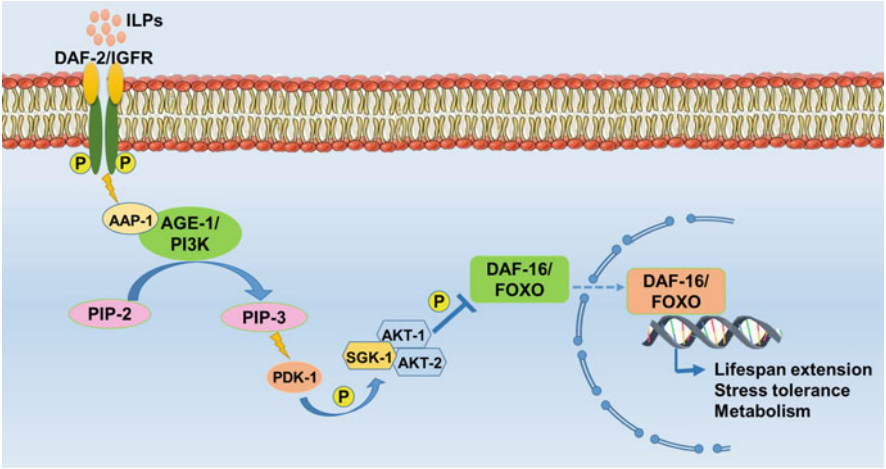

Fig. 1.3 Lifespan extension in *C. elegans* mediated by IIS pathway involving DAF-2 and AGE-1

like stress response, defense mechanisms, and detoxification (Fig. 1.3). Thus, genetic manipulation of genes has long been utilized for promoting longevity.

1.3 Epigenetics of Aging

During the course of evolution, every specie experiences a variety of environmental stressors, geographical barriers, and competition for food and habitat that leads to survival of the fittest. To adjust to the given conditions, an individual undergoes many changes that make them fit for their survival in the existing conditions. These changes become part of epigenetic alterations. These conditions include starvation, exercise, stress (physical or mental), environment (including water and oxygen supply), alcohol consumption, smoking, physical activity, and to some extent deprivation from society. Besides, it has been studied that even monozygotic twins, although they are genotypically similar show variation in their epigenomic profile (Rowbotham et al. 2015; Bell et al. 2012) suggesting a vital role of epigenetics in linking environment with genetics.

Besides, the coded information on the DNA, the genetic code undergoes changes during different biological phenomena such as transcription, translation, and post-translational modifications which lead to the transmission of information in a processed form. These modifications which are not coded by genetic material but expressed in the individuals are categorized into "epigenetic modifications". These include DNA modifications, histone posttranslational modifications, chromatin condensation, and remodeling. Most prominent DNA modification that has been linked with aging is "methylation" (Fig. 1.4).

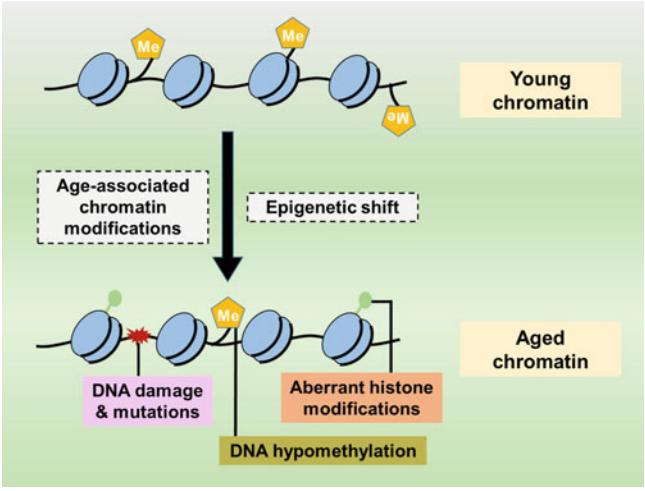

Fig. 1.4 Changes occurring in the structure of chromatin during aging

During the course of aging, the overall DNA becomes hypo-methylated (that is, DNA methylation decreases during aging). However, if we talk about site-specific methylations, there is a variation. Age-specific differentially methylated regions or CpGs show an increase in methylation at specific sites, which involve the promotors of bivalent chromatin domain in precursor or stem cells and in polycomb target genes (Rakyan et al. 2010; Teschendorff et al. 2010; Horvath 2013; Raddatz et al. 2013).

Besides this, sexual disparity also plays an important role in deciding the exposure to age-associated disease. It has been reported and widely accepted that women live longer than men. The global life expectancy on average (in 2016) was 69.8 years for males and 74.2 years for females (World Population Review 2022). Having said that, there is also an increase in DNA methylations with aging and vulnerability towards age-associated diseases for male population. A study identified that with advancing age, there was hyper-methylation of around 36 CpGs. Thus, methylation at Y-chromosome proves to be an important target to study male aging (Li et al. 2022).

DNA methylation has mostly been studied in CpG islands, associated with 5-cytosine residues in CpG islands (5mC). It is found in most eukaryotes including vertebrates. However, for long, DNA methylations were thought to be completely absent in organism like *Caenorhabditis elegans*. Lately, studies have found that instead of the conventional 5mC, methylation at 6-adenine residues (6 mA, which is mostly found in prokaryotes) is prevalent in *Tetrahymena*, *Oxytrichafallox*, *Paramecium aurelia*, *Chlamydomonas reinhardtii* (Fu et al. 2015), *Caenorhabditis elegans* (Greer et al. 2015), *Drosophila melanogaster* (Zhang et al. 2015), and

Table 1.1 Types of histones (Nelson et al. 2008)

S. no.	Histone		Molecular weight (kDa)	Number of amino acids
1.	H1 (Linker histone)		21.1	223
2.	H2A	Core	13.9	129
3.	H2B		13.7	125
4.	H3		15.2	135
5.	H4		11.2	102

plant species like *Arabidopsis thaliana* and *Oryza sativa* (Karanthamalai et al. 2020), to name a few.

In addition to DNA, another most important component that is responsible for getting epigenetically modified is a class of proteins known as "histones." These are a group of positively charged basic proteins that are associated with negatively charged nuclear DNA. These are conserved and play a key role in nucleosome formation and DNA compaction in eukaryotes; however, they are absent in prokaryotes (except Archaea domain). Histones are of five types as described in Table 1.1.

During any biological process, histones undergo various modifications such as methylation, acetylation, and phosphorylation that give rise to either activation or repression of genes associated with them. Their arrangement in the DNA sequence forms a code, known as "histone code" which governs the expressions of associated genes (Strahl and Allis 2000). Over the past few years, specific histone modifications associated with aging have been identified and a large area of research has been done in this field. Most of these include methylation at histone 3 lysine residues which are closely associated with longevity and lifespan regulation. These methylations include:

- H3K4me3
- H3K9me3
- H3K27me3
- H3K36me3

H23K4me3 is the activating modification which regulates the lifespans. In mammals, the complexes that are involved in generating H23K4me3 include (1) Trithorax-related complex, (2) Trithorax complex, and (3) COMPASS complex. In worms, this modification is controlled by a complex consisting of ASH-2, SET-2, and WDR-5. It has been found that decrease in the methyltransferase components promotes longevity whereas decrease in demethylase components, such as knock-down of *rbr-2*, reduces the lifespan and vice versa (Han and Brunet 2012; Yu et al. 2019; Sen et al. 2016; Greer et al. 2010).

Contrastingly, **H3K27me3** is the repressive modification, regulated by UTX-1 (histone demethylase) and PRC2 complex (Polycomb repressive complex 2, which catalyzes H3K27me3). During the course of aging, the level of H3K27me3 elevates in worms and flies (Yu et al. 2019; Sen et al. 2016).

H3K9me3, another histone methylation responsible for transcriptional silencing, is regulated by MET-2, which prevents DNA damage and instability in genome during aging (Yu et al. 2019).

H3K36me3 is associated with activating transcription and is also one of the most important histone modifications. In yeast, it has been found that loss of a histone demethylase coded by gene *Rph1* results in lifespan extension by increasing the levels of H3K36me3 (Sen et al. 2015). Also, in *C. elegans* it has been studied that knockdown of *met-1*, a H3K36me3 methyltransferase, results in global decrease in H3K36me3 levels thus reducing the lifespan (Pu et al. 2015). These results suggest that this modification is important for maintaining longevity.

Second most commonly occurring modification at lysine residues of histone tail after methylations is acetylation which is thought to epigenetically regulate longevity. It involves the addition of acetyl group with the help of enzyme histone acetylases (HATs) or the removal of the added acetyl group via histone deacetylases (HDACs). The common acetylation marks associated with aging includes H3K23ac, H4K12ac, and H3K9ac. However, the response associated with these modifications vary and are dependent on the position of the lysine residue that is acetylated. For example, in *Drosophila,* reduction in H3K23ac levels results in impaired neuronal behavior, learning, and courtship activities (Li et al. 2018). However, decrease in H4K12ac prolongs the lifespan (Peleg et al. 2016).

In addition to HATs, the role of HDACs is also proven to be very critical in maintaining lifespan. For example, a very well-known class of NAD+ dependent class III HDACs called "sirtuins" have been extensively studied to be involved in promoting longevity. The pro-longevity effects of these conserved proteins (Kaeberlein et al. 1999; Imai et al. 2000; Tissenbaum and Guarente 2001) were described by Leonard Guarente for the first time in 1991.

Apart from genetic manipulation, another important phenomenon, known as "calorie restriction" increases longevity. It is the process by which the intake of calories is restricted to an extent that does not cause lethality. According to studies conducted in various model organisms, restriction of diet or calories (to around 30%) has proved to be beneficial. It epigenetically modifies the genome and increases the longevity in mice as well as flies and worms and to some extent (about 50%) in lemurid primate (*Microcebus murinus*) (Pifferi et al. 2018).

However, calorie restriction when applied in combination with genetic manipulation results in additive response towards lifespan extension.

Apart from DNA and histones, chromatin structure also undergoes transient changes with aging. During aging, the chromatin loosens and loses its original form and as a result the once silent genes associated with heterochromatin become active and begin to express themselves resulting in aging anomalies. One of the important and most studied chromatin remodeling complex includes SWI/SNF (switch/sucrose non-fermenting complex) (Zhou et al. 2019). It is responsible for activating transcription while another chromatin remodeling complex, that is, polycomb repressive complex (PRC) silences transcription (Bracken et al. 2019; Stanton et al. 2017). This contrasting role of these complexes along with other

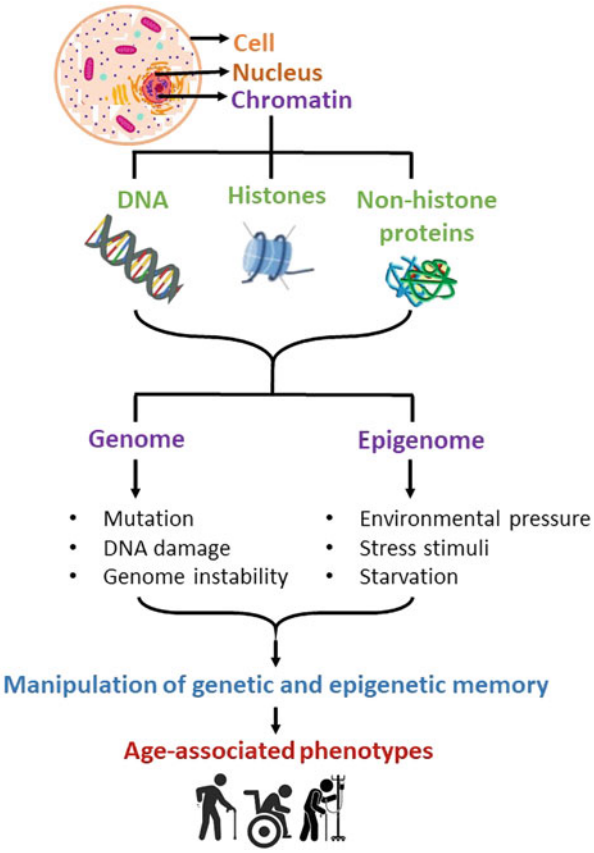

Fig. 1.5 Role of genome and epigenome in aging

mentioned modifications is important for chromatin regulation, which gets disturbed during aging.

Thus, synergism between genome and epigenome either via mutations or through environment-induced epigenomic interventions leads to manipulation of genetic and epigenetic memory hence leading to aging-associated phenotypes (Fig. 1.5).

1.4 Case Studies of Age-Associated Diseases

The field of aging biology research is expanding with every passing second and a tremendous amount of novel work is being conducted every day. However, till date, no direct measurement of aging has been developed, meaning there is a lacuna for standard measuring techniques with respect to aging. However, other parameters associated with aging, such as quantification of cancer-associated genes, DNA damage proteins, behavioral changes, etc., can be estimated easily, forming the basis for analyzing the phenomenon of aging. Different strategies have been

employed to prevent the early onset of aging and also to delay the symptoms of age-associated diseases.

One of the important methods to delay early aging is to adopt a healthy lifestyle. It plays a very essential role in framing one's epigenome expression of metabolism boosting and immune responsive genes and vice versa. Thus, the proverb is truly stated that "*we are, what we eat*." Eating healthy and staying fit is the key to delay the signs of aging. Besides this, genetic manipulation has also been observed to yield good results in terms of alleviating the harmful effects of age-associated diseases. It is well demonstrated in case of obesity, type 2 diabetes, neurodegeneration, cancer, and cardiovascular diseases (discussed ahead).

1.4.1 Obesity

Obesity has recently been addressed as public health concern globally. This condition involves higher body to mass ratio (known as body mass index, BMI). A person is said to be obese if his/her BMI is 30.0 or more. Furthermore, obesity is subcategorized into: class 1 (BMI: 30–<35), class 2 (BMI: 35–<40), and class 3 (BMI: 40 or more) obesities. Class 3 obesity is also known as "severe obesity" (Centers for Disease Control and Prevention 2022). According to WHO, around 13% of the world's population was estimated to be obese in 2016 (World Health Organization 2022a), suggesting a rapid increase in reduced life expectancy. Studies have shown that due to obesity, life expectancy in men and women after 40 years of age, has been reduced by 5.8 and 7.1 years, respectively (Tam et al. 2020). Thus, obesity causes early onset aging-associated symptoms and also increases the risk of other age-associated diseases like hypertension, CVDs, ischemic heart disease, and stroke, to name a few.

One of the major causes of this condition can be attributed to a sedentary lifestyle. The absence of physical exercise and loads of processed food intake results in extra calories that are not metabolized thus it keeps on depositing in the form of extra fat leading to obesity. Mechanistically, obesity targets multiple pathways genetically and epigenetically. Excess amount of calories causes increased ROS production, damage to nuclear machinery and endoplasmic reticulum, altered Ca^{2+} flux, stressed mitochondrial DNA, impaired autophagy, reduced homeostasis, cellular senescence, and an increase in epigenetic age (López-Otín et al. 2013). Obesity also reduces the telomere length in white blood cells (Valdes et al. 2005; Kim et al. 2009; Buxton et al. 2011) thus causing early aging.

1.4.2 Type 2 Diabetes

Type 2 diabetes is a disease resulting from increased blood glucose levels due to either inactive lifestyle, obesity, or progressing age. Aging poses a severe risk factor for the onset of this disease. Also, people with obesity (another age-associated disease) have a high risk of developing type 2 diabetes. With increasing age, there

is a decline in metabolic activity, increased oxidative stress, neuroinflammation, impaired insulin secretion, and sensitivity (Chia et al. 2018).

According to the international diabetes federation, by the year 2021, the total population of adults suffering from diabetes was around 537 million. It is estimated that by the year 2030 the world population suffering from diabetes will reach 643 million (International Diabetes Federation 2022). Out of the total diabetic population, around 95% account for type 2 diabetes (World Health Organization 2022b).

1.4.3 Neurodegeneration

Neuronal damage and degeneration increase with advancing age. Moreover, the genomic and epigenomic composition might enhance or suppress the event of neurodegeneration. In elderly individuals, the most prominent age-associated neurodegenerative diseases include Alzheimer's disease (AD) and Parkinson's disease (PD). AD involves decrement in memory and learning ability, reduced cognition, and dementia. It is associated with the deposition of protein aggregates of amyloid-β (Aβ) (plaques) and hyper-phosphorylated tau (forming neurofibrillary tangles). PD involves decreased coordination, motor function dysregulation, tremors, shaky movements, and dementia, caused due to aggregation of misfolded α-Synuclein protein (Hou et al. 2019). It is estimated that by the year 2050, the global rate of dementia will rise to around 152.8 million (Nichols et al. 2022).

Genetic mutations in apolipoprotein E (*APOE*) ε4 allele, *PRESENILIN 1/2*, APP pathway, *SNCA*, *DJ-1*, *PINK1*, or *PRKN* genes form the genetic basis of neurodegenerative diseases; while, environmental pressure such as diet or heavy metal toxicity (including aluminum, copper, zinc, etc.) (Modgil et al. 2014) form the epigenetic basis of neurodegeneration. Both genetic and epigenetic factors have a combinatorial effect like misfolding of aggregated proteins, decrease in protein clearance, impaired autophagy and apoptosis, increased production of ROS, and cellular senescence. These signs overlap with those of aging.

1.4.4 Cardiovascular Diseases (CVDs)

For the older population, that is, above 65 years of age, cardiovascular diseases have been one of the most common causes of death worldwide (Chiao et al. 2016). It is estimated that by the year 2030, CVDs will account for 40% of total diseases in the elderly and will become the leading cause of death in them (Groff and La Vigne 2002).

CVDs include diseases like hypertension, stroke, myocardial infarction, and atherosclerosis. With aging, the cardio-vasculature becomes inefficient thus making the body vulnerable to these diseases. Conditions such as hypertrophy, stiffness of arteries, and deregulated heart rate result in the weakening of vasculature and blood circuitry. These result in increased oxidative stress, increased inflammatory

cytokines, DNA damage, and cardiomyocyte senescence (North and Sinclair 2012). Thus, apart from excess fatty acids and cholesterol, aging is another risk factor for developing CVDs.

1.5 Therapeutic Strategies to Modulate Aging

Epidemiologic and predictive modeling studies reveal that the aged population (60 years and above) would be at a whopping 2.1 billion by the year 2050 (World Health Organization 2022c) thus making a larger chunk of population prone to age-associated diseases. Proactive measures by researchers as well as healthcare providers will include having efficient strategies in place towards countering such burden and promoting "healthy aging." Various therapeutic strategies that are being researched and employed, delay the signs of aging and improve health span; this includes the development of potential anti-aging drugs and altered dietary regimes, that will aid in modulating aging, genetically or epigenetically. Drugs such as metformin, rapamycin, and resveratrol are involved in targeting multiple pathways that are hampered during aging. For example, metformin is tested to increase longevity in *C. elegans* and also it has positive effects on learning, memory, and tau protein clearance in SAMP8 mice. It functions by decreasing insulin signaling (Liu et al. 2011) and mTOR pathways (Nair et al. 2014) and activating AMPK signaling (Cho et al. 2015) thereby maintaining glucose homeostasis, decreasing ROS (Batandier et al. 2006), repairing DNA damage (Algire et al. 2012; Cabreiro et al. 2013), and enhancing tumor suppression. Similarly, a widely known inhibitor of mTOR pathway has been shown to increase the average lifespan in case of yeast, worms, and flies (Johnson et al. 2013). Also, a phytoalexin known as resveratrol is found to improve health span and longevity in case of yeast and worms. This compound functions by activating sirtuin proteins (Cao et al. 2018; Alcaín and Villalba 2009; Price et al. 2012; Wood et al. 2004). Apart from these FDA-approved drugs, certain senolytic compounds, such as quercetin and dasatinib, have also been implicated to have a potential in regulating health span and longevity by selectively clearing the senescent cells thus inducing apoptosis (Tse et al. 2008). Besides, several dietary regimes such as calorie restriction or intermittent fasting also improve lifespan and health (Vera et al. 2013) by regulating energy-sensing pathways, involving AMPK, NAD+/NADH, and sirtuins. In a recent human study, it has been found that 14% calorie restriction improves immunometabolism (Spadaro et al. 2022).

In summary, it appears prudent to identify such pharmacological modulators that specifically target genetic and epigenetic changes associated with aging. Research in this direction can aid in identifying such specific targets and further design of new chemical entities or repurposing of existing drugs can be tested against such targets. The future direction should be having newer molecules in this space, which promote healthy aging and better the quality of life in old age.

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Coenzyme Q as an Antiaging Strategy

2

Guillermo López-Lluch

Abstract

Aging is a very complex process in which many factors are involved. As a common factor the accumulation of damaged cell and tissue structures, impair cell activity at different levels ending in the malfunction of organs and cell death. In this process, dysfunctional mitochondria play a very important role. Coenzyme Q is a key factor in the activity of mitochondria and in the protection of cells against lipid peroxidation. Its levels decrease during aging and supplementation improve cell functionality and delay the progression of age-related diseases. In this chapter, the importance of CoQ in aging and the strategies to restore levels in the cell and organs are shown. These strategies can help to improve health during aging and demonstrate that the maintenance of CoQ levels can be considered a good antiaging strategy.

Keywords

Coenzyme Q · Aging · Mitochondria · Metabolism · Antioxidant

2.1 Introduction

Aging is a complex process that must not be considered a disease. As Suresh Rattan indicated in 2014, aging is the consequence of the loss of the capacity to maintain the capacity to respond to stress and buffer the damage produced by internal and external injuries (Rattan 2014). The decay in the capacity to respond to stress is associated

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with the accumulation of cell and tissue damages that are associated with many of the diseases associated with aging. As Suresh Rattan indicates, a situation of control of the physiological homeostasis known as homeodynamic space is being reduced and substituted by the increase of a vulnerability area in which any age-associated disease is accompanied by a decay in the physiological function of nearly all the organs.

To understand aging we have to revise the events that are associated with this process. These events are molecular changes such as DNA damage that produce genomic instability, reduction of the length of telomeres, alterations in epigenetic marks, and a decay in proteostasis. All these changes are considered as primary hallmarks that cause damage (López-Otin et al. 2013). In response to the damage, cells try to maintain the capacity to respond to stimuli such as nutrient sensing that are considered key in the control of metabolism, mitochondrial capacity, and cell physiology and in this response they accumulate a deregulated nutrient sensing, dysfunctional mitochondrial and a situation known as senescence that has been associated with age phenotype. Finally, this produces exhaustion of stem cells reducing the capacity to maintain the reparation of tissues and alters intracellular communication impairing the capacity of cells to respond in a balanced way (López-Otin et al. 2013).

In all this process, mitochondria play a key role (Sun et al. 2016). Mitochondrial dysfunction has been associated with most of the chronic metabolic diseases and with the progression of the systemic deterioration associated with aging (Hernandez-Camacho et al. 2018; López-Lluch 2021). The accumulation of dysfunctional mitochondria not only reduces the capacity of cells to generate ATP and regulate many anabolic and catabolic processes located at mitochondria (López-Lluch et al. 2018) but also produces high levels of reactive oxygen species (ROS) that cause oxidative damage in cells and organs (Miquel 1998).

Under unbalanced metabolic conditions, high ROS production can overcome antioxidant protection in cells increasing oxidative damage and impairing yet more the dysfunctional activity of mitochondria and metabolic systems. As a key factor in both, metabolic activity in mitochondria and antioxidant protection of cell membranes, coenzyme Q (CoQ) can be considered essential for the maintenance of homeostasis in cells during aging (López-Lluch 2020, 2021).

This chapter shows the importance of CoQ in aging progression and the probable use of supplementation with this compound or the induction of its synthesis in the prevention of aging progression.

2.2 Importance of CoQ in Aging

CoQ is a molecule found in living organisms. It is a lipid molecule obtained after the combination of a phenolic ring modified from p-hydroxybenzoic acid with an isoprene tail. The family of molecules of CoQ differ in the length of the tail. The number of isoprene units can vary from six (CoQ_6) in *S. cerevisiae*, eight (CoQ_8) in *E. coli*, nine (CoQ_9) and ten (CoQ_{10}) in rodents, and ten (CoQ_{10}) in humans.

2.2.1 Mitochondrial Functions

It is widely known that the main function of CoQ is as an electron transport molecule through the mitochondrial respiratory chain. CoQ transport electrons from complexes I and II to complex III (López-Lluch et al. 2010). However, this molecule has also structural function stabilizing and controlling the assembling of mitochondrial complexes in more efficient structures known as supercomplexes (Scialo et al. 2016). In these structures, CoQ permits the channeling of electrons directly from complex I to IV avoiding the leakage of electrons to oxygen and reducing the production of ROS (Lapuente-Brun et al. 2013). Probably a decrease in CoQ levels in mitochondria can reduce the capacity of assembling of mitochondrial complexes during aging contributing to the increase of ROS production in aged mitochondria (Gomez et al. 2009; Gomez and Hagen 2012). Further, CoQ is also an internal component of complexes I and III and its deficiency probably affects its assembly in stable complexes (Santos-Ocana et al. 2002). We can assume that depletion of CoQ levels contributes to the destabilization of both, complexes such as III and also I and supercomplexes during aging.

CoQ not only plays an essential role in the activity of the mitochondrial electron transport chain (mtETC) but also acts as an electron acceptor of other dehydrogenases involved in many other different metabolic processes (López-Lluch 2021). These dehydrogenases are dihydroorotate dehydrogenase involved in the biosynthesis of nucleotides (Evans and Guy 2004); mitochondrial glycerol-3-phosphate dehydrogenase (Mracek et al. 2013; Rauchova et al. 1992); the electron transport flavoprotein dehydrogenase, that intervenes in fatty acid β-oxidation and the catabolism of branched-chain amino acids (Watmough and Frerman 2010); and enzymes involved in the metabolism of amino acids such as proline dehydrogenase (Erecinska 1965) and sulfide-quinone oxidoreductase (Ziosi et al. 2017). The reduced form of CoQ, ubiquinol, generated by the activity of these dehydrogenases is reoxidized to ubiquinone by complex III at the mETC contributing to the generation of the proton motile force needed to synthesize ATP even when complexes I or II are affected.

Mitochondrial CoQ is also involved in the regulation of mito/autophagy. It has been demonstrated that the deficiency in CoQ_{10} induces the degradation of mitochondria by mitophagy (Rodriguez-Hernandez et al. 2009) aggravating the pathophysiology found in patients suffering CoQ_{10} deficiency (López et al. 2014; Monzio Compagnoni et al. 2018).

2.2.2 Cell Membrane CoQ_{10}, the Forgotten Key Function

Although the role of CoQ in mitochondria is the most known function of CoQ, this molecule is also present in all cell membranes and in plasma lipoproteins. In all cell membranes, including mitochondrial membranes, and lipoproteins, CoQ is the main antioxidant that prevents lipidic peroxidation and ferroptosis. In cell membranes, CoQ protects phospholipids against peroxidation by disrupting lipid peroxidation

chain directly (Ernster et al. 1992), or by maintaining α-tocopherol or ascorbic acid in their respective reduced and antioxidant active form (Fernandez-Ayala et al. 2000).

To perform its antioxidant activity, CoQ is maintained in a redox cycle by reductases that transfer electrons from cytosolic NAD(P)H to ubiquinone. Among these are reductases, $CytB_5R_3$ (Arroyo et al. 2000; Villalba et al. 1997) and NQO1 (Beyer et al. 1996). This system has been associated with many other activities in the plasma membrane and received the name of plasma membrane redox system (PMRS) (Rodriguez-Aguilera et al. 2000). Among these activities, these reductases can regulate the levels of NAD+ locally modulating biological activities associated with aging such as sirtuins (Olgun 2009) or cell signaling pathways such as cyclic AMP (cAMP) (López-Lluch et al. 1998). CoQ_{10} in cell membranes also prevents apoptosis mediated by ceramides (Barroso et al. 1997b). In this role, CoQ_{10} acts in combination with known antioxidants such as α-tocopherol or ascorbate (Barroso et al. 1997a).

The reduction of lipid peroxidation by CoQ is considered a key process to avoid the non-apoptotic cell death associated with the accumulation of lipid peroxidation known as ferroptosis (Sheng et al. 2017). Ferroptosis has been associated with age-related diseases such as Parkinson's (Do Van et al. 2016) or Alzheimer's (Zhao 2019), frailty (Larrick et al. 2020), inflammation, and cancer (Toyokuni et al. 2020). Recently, ferroptosis suppressor protein 1 (FSP1), a CoQ-dependent oxidoreductase has been shown to play a key role in the inhibition of ferroptosis (Bersuker et al. 2019). FSP1 was formerly known as a flavoprotein called apoptosis-inducing factor mitochondria-associated 2 (AIFM2) (Doll et al. 2019). The mechanism by which FSP1 blocks apoptosis seems to be through migrating from mitochondria to the cell membrane to reduce CoQ_{10} at plasma membrane using NAD(P)H (Doll et al. 2019).

2.2.3 Plasma Low Density Lipoprotein (LDL) Protection

In plasma lipoproteins, CoQ_{10} clearly protects against oxidative damage (Stocker et al. 1991; Tribble et al. 1994). Oxidation of LDLs strongly depends on the levels of ubiquinol and α-tocopherol (Tribble et al. 1994). However, ubiquinol is much more efficient in inhibiting LDL oxidation than other antioxidants (Kontush et al. 1995; Stocker et al. 1991). In fact, the multiple-modified cholesteryl LDLs, responsible for the accumulation in atheroma plates (Nikiforov et al. 2017), show high levels of oxidized CoQ_{10} and lower concentration of α-tocopherol (Tertov et al. 1998), indicating the importance of ubiquinol in the protection against atherosclerosis. Further, low levels of CoQ_{10} in plasma and the increase of oxidized CoQ_{10} fraction have been associated with diseases such as breast cancer and sporadic ALS respectively indicating a putative role of plasma CoQ10 levels with, at least, the impairment of these diseases (Cooney et al. 2011; Sohmiya et al. 2005).

Interestingly, a new NADH-oxidoreductase responsible for the reduction of CoQ_{10} in lipoproteins has been recently discovered in the outer surface of the plasma

membrane of hepatocytes (Takahashi et al. 2019). However, we cannot discard the existence of these reductases in other cells since the reduction of CoQ_{10} in LDLs was already discovered in 1993 in both, liver and red blood cells (Stocker and Suarna 1993).

The decrease of the levels of CoQ_{10} in plasma has been proposed as a biomarker of aging (López-Lluch et al. 2010; Niklowitz et al. 2016) and recently we have found that the plasmatic levels of CoQ_{10} are related with the physical capacity and cardiovascular risk in elderly people (de la Bella-Garzon et al. 2022) whereas sedentarism seems is related with lower CoQ_{10} levels and a higher LDL oxidation in plasma (Del Pozo-Cruz et al. 2014). Since levels of CoQ_{10} in plasma in humans show a wide range of concentration, longevity studies of these levels and their relationship with the evolution of age-related chronic diseases would clarify the importance of these levels in aging and these diseases.

2.2.4 Secondary CoQ Deficiency Is Associated with Aging and Age-Related Diseases

In 1989, Kalen et al. (1989) demonstrated that the levels of CoQ in tissues and organs decrease in aged individuals affecting both rats and humans (Fig. 2.1). The study of Kalen et al. showed the results in the levels of CoQ in relationship with the wet weight. Probably the difference in the methodology can explain some minor discrepancies with other studies in rats that use protein levels as reference (Beyer et al. 1985). It is now assumed that in elderly humans (77–81 years) the levels of

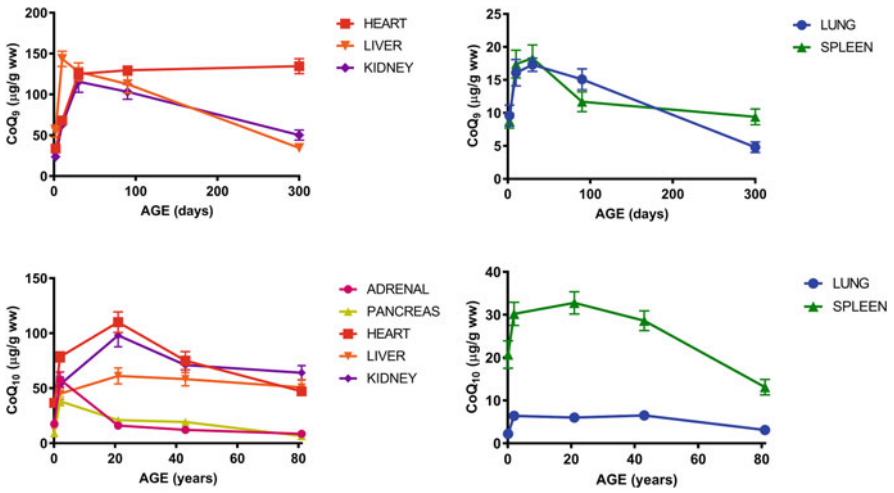

Fig. 2.1 CoQ_9 levels in (µg/g wet weight) in different organs and tissues in rat along life (upper line) and CoQ_{10} levels in the same organs in humans (lower line). (Figures performed from the data published in Table 1 by Kalen et al. (1989) with permission of the published (Kalen et al. 1989))

CoQ_{10} are lower than in young and mature individuals. This effect was found in most of the organs studied (Kalen et al. 1989) (Fig. 2.1).

The evolution of CoQ_{10} levels during aging in brain show conflicting results, some studies indicated a decrease (Edlund et al. 1994), whereas others did not find any change after the levels reached stability in young organisms (Beyer et al. 1985; Zhang et al. 1996).

In relationship with mitochondrial dysfunction, the deficiency of CoQ has been associated with many age-related diseases (Mantle and Hargreaves 2019) such as type 2 diabetes or insulin resistance (Fazakerley et al. 2018), cardiovascular disease (Shimizu et al. 2017), neurodegeneration (Ogawa et al. 2002), chronic kidney disease (Gvozdjakova et al. 2020), liver disease (Chen et al. 2019a), inflammaging, and immunosenescence (Fuller et al. 2006) or sarcopenia (Guescini et al. 2017). Further, we recently have associated mitochondrial dysfunction and CoQ_{10} deficiency with the unbalanced response of the immune system to viral infections such as COVID-19 (Moreno Fernandez-Ayala et al. 2020). In any case, this relationship indicates an important role of CoQ_{10} in the control of ROS production and the onset and severity of the diseases (Navas et al. 2021).

The relationship between the decrease of CoQ levels during aging and the decay in the functionality of mitochondria is not clear. Probably both processes feed each other in a vicious cycle in which deficient mitochondria show reduced levels of the CoQ synthesis complex and the dysfunction of this complex impairs yet more the activity of these mitochondria. For this reason, some studies suggest that secondary CoQ deficiency associated with aging can appear in response to the dysfunction of the components of the mtETC (Kuhl et al. 2017). Thus, defects in the expression of mtDNA affect the activity of mtETC complexes that correlates with the downregulation of the members of the CoQ-synthome and in the decrease of mitochondrial CoQ levels (Kuhl et al. 2017).

On the other hand, the importance of CoQ deficiency in aging shows some paradoxical aspects. Defects in the CoQ-synthome have been associated with the increase in lifespan in mice in an effect attributed to the activation of protective responses by mitohormesis and the increase of mitophagy and mitochondrial turnover (Diaz-Casado et al. 2019). However, in other cases, this effect has been attributed to other mechanisms different from mitohormesis, at least, in liver (Rodriguez-Hidalgo et al. 2018). Probably, in these cases, moderate CoQ deficiency activates mitophagy that removes dysfunctional mitochondria improving by this mechanism cell physiology and reducing the accumulation of oxidative damage (Rodriguez-Hernandez et al. 2009).

In aging, the disruption of the mito/autophagy mechanisms together with the reduction of CoQ_{10} levels and mitochondrial dysfunction can accelerate the deterioration of metabolism and cell activities ending in cell death and the dysfunction of organ activities.

2.3 Supplementation with CoQ_{10}, a Great Problem

Dietary supplementation with CoQ_{10} improves many dysfunctions associated with aging and age-related diseases. Dietary CoQ_{10} is highly incorporated by white blood cells, spleen, thymus, liver, adrenal, ovaries, and heart (Bentinger et al. 2003). However, it is incorporated at very low levels in muscle, kidney, or brain, the organs that suffer high levels of dysfunction when the synthesis of CoQ_{10} is affected by mutations in the members of the CoQ-synthome (Alcazar-Fabra et al. 2021; Navas et al. 2021). Further, the bioavailability of CoQ_{10} in humans show very high differences among the individuals even with preparations that show high bioavailability, a dissimilar response that can severely affect the conclusions of the studies (López-Lluch et al. 2019).

Moreover, an important issue must be taken into consideration. If the cause of the reduction of CoQ levels in aged mitochondria is the damage of the mtETC, the supplementation with CoQ_{10} can be useful for those mitochondrial structures that are not severely affected but not useful for highly damaged mitochondria. For this reason, the studies about the effect of supplementation with dietary CoQ_{10} and mitochondrial activity in age-related diseases in aged organisms must be accompanied by the determination of the initial mitochondrial damage and CoQ levels in tissues and organs. In the case of humans, these studies must include the determination of the right CoQ_{10} levels that reach plasma and the net increase obtained after supplementation.

Regardless of these considerations, supplementation with CoQ_{10} has been suggested as a successful therapy to delay the progression of aging or age-related diseases (Hernandez-Camacho et al. 2018). Its use as a therapeutical compound has been suggested for the treatment of the earlier phases of neurodegeneration (Takahashi et al. 2016) or cardiovascular diseases found in aged individuals (Yang et al. 2015). In the case of cardiovascular diseases, CoQ_{10} shows very interesting results as many studies have demonstrated that treatment with CoQ_{10} and selenium improves cardiovascular capacity even in elderly people (Alehagen et al. 2018, 2019).

On the other hand, plasma CoQ_{10} can directly interact with vascular endothelial cells. Atherosclerosis is directly related to vascular senescence and in this process, mitochondrial dysfunction plays an essential role (Salazar 2018). It has been shown that CoQ_{10} prevents senescence in these endothelial cells (Huo et al. 2018). In fact, it is being considered for the treatment of endothelial cell dysfunction (Gao et al. 2012). We can consider that the protection against oxidative damage and mitochondrial dysfunction of endothelial cells can be considered a key antiaging activity of CoQ_{10} (Duran-Prado et al. 2014; Xue et al. 2013). In fact, recently the combination of NOX4 and CoQ_{10}, through the activity of CYB_5R_3, has demonstrated a protective effect against oxidation in endothelial cells (Yuan et al. 2021).

All these studies and many others (Hernandez-Camacho et al. 2018) indicate that supplementation with CoQ_{10} could be considered as a therapeutic procedure to prevent or, at least, delay age-related mitochondrial dysfunction and also maintain the antioxidant capacity affecting cell membranes in age-associated diseases.

However, preclinical and clinical studies must be performed rigorously in order to assure a good CoQ_{10} bioavailability and control the right increase in plasma CoQ_{10} reached after supplementation even in the elderly (Pravst et al. 2020).

2.4 Use of CoQ-Derived Mitochondrial-Targeted Antioxidants

Due to the low absorption of CoQ_{10} and the variability of bioavailability among individuals, the use of CoQ-derived compounds such as MitoQ, idebenone, or others has been considered (Zinovkin and Zamyatnin 2019). These compounds are considered safe and well-tolerated and are being used in the treatment of some diseases, especially, mitochondrial complex I-deficient diseases in a NQO1-dependent mechanism in the case of idebenone (Haefeli et al. 2011). It seems that the protective capacity of idebenone resides in its capacity to reduce lipid peroxidation in complex I-defective conditions (Erb et al. 2012).

Both, idebenone and MitoQ, prevent oxidative damage in mitochondrial membranes impairing by this mechanism the progression of mitochondrial dysfunction (Dai et al. 2014). In fact, the use of MitoQ has been proposed also for the treatment of diseases associated with the impairment of mitochondrial complex I and the accumulation of mtDNA damage, two of the factors that are involved in aging (Plecita-Hlavata et al. 2009). Further, the use of MitoQ has been proposed in the treatment of neurodegenerative diseases such as Alzheimer's disease (Manczak et al. 2010; Reddy 2006). In an accelerated Alzheimer's disease model of mice, MitoQ has been shown to avoid memory loss and extends lifespan (Young and Franklin 2019). The treatment with MitoQ induces mitochondrial biogenesis and turnover in a model of Huntington's disease (Yin et al. 2016).

The most promising role of MitoQ resides in the treatment of age-related endothelial dysfunction as a putative therapeutic compound in the prevention of atherosclerosis (Gioscia-Ryan et al. 2014). Treatment with MitoQ has shown the capacity to revert in vivo aortic stiffness in old mice (Gioscia-Ryan et al. 2018) or age-related vascular dysfunction in skeletal muscle-fed arteries (Park et al. 2018). In the case of idebenone, this compound prevents the oxidation of LDL reducing endothelial and mitochondrial dysfunction (Lin et al. 2015).

Interestingly these compounds have shown also important anti-inflammatory effects. Idebenone is able to reduce the release of proinflammatory cytokines in spontaneous chronic murine colitis by its antioxidant capacity (Shastri et al. 2020). In this mechanism, the reduction of ROS levels after the treatment with idebenone can decrease the activation of the inflammasome and the release of proinflammatory cytokines such as IL-1β and TFN-α (Akpinar et al. 2021). Other proinflammatory cytokines such as IL-17A and IL-18 have been found to be reduced in a murine model of lupus erythematosus (Akpinar et al. 2021). The same anti-inflammatory effect has been suggested for MitoQ that reduces the release of proinflammatory cytokines at the same time that increased the release of the anti-inflammatory IL-10 cytokine (Lowes et al. 2008).

Nearly all the studies carried out with MitoQ, idebenone, or other mitochondria-targeted antioxidants have demonstrated that their antioxidant activity in reducing ROS and oxidative damage is key in their age-related protective effect (Braakhuis et al. 2018; Feniouk and Skulachev 2017). The maintenance of the endogenous CoQ_{10} levels will produce the same effect; however, in the case of CoQ_{10} deficiency, probably the use of more soluble and with a higher bioavailability and ability to reach tissues and organs such as muscle or brain can help in the reduction of damage associated with aging. More studies are needed in order to understand the security and physiological effect of these promising compounds.

2.5 Induction of CoQ_{10} Synthesis

We can consider that one of the most promising strategies to maintain CoQ_{10} homeostasis in cells is to use strategies able to activate endogenous synthesis even during aging. Interestingly, the studies performed in many models have demonstrated that some of the most promising prolongevity strategies also modulate CoQ levels in many organisms. In this section, a resume of the different findings that relate these strategies with CoQ and aging are shown.

2.5.1 Nutritional Interventions

It is widely known that caloric restriction (CR) is the most powerful nongenetic mechanism able to increase longevity in many organisms (López-Lluch and Navas 2016). Among other effects, CR induces mitochondrial biogenesis and improves metabolic efficiency by modulating mitochondrial activity and turnover (López-Lluch et al. 2006). Through these changes, CR reduces ROS levels and increases antioxidant activity ending in the reduction of oxidative damage.

Regarding CoQ, CR seems to be able to maintain the levels of CoQ in mature and old rat hearts reducing significantly the rate of lipid peroxidation (Armeni et al. 1997). CR also reverted the decrease of CoQ_9 in skeletal muscle mitochondria (Lass et al. 1999). The same effect was found in other organs such as rat kidney and heart in which aged mitochondria showed a decrease in CoQ_9 levels that were restored by 40% CR (Kamzalov and Sohal 2004).

Interestingly, CR induces clear changes in the activity of the dehydrogenases associated with plasma membrane that are members of the system known as plasma membrane redox system (PMRS). We have found that CR modulates the activity of the components of this system depending on the age of the animal (De Cabo et al. 2004; López-Lluch et al. 2005). In plasma membrane of liver from rats and mice fed under CR conditions, the activities of $CytB_5R_3$ and NQO1 increased but only in old animals (De Cabo et al. 2004; López-Lluch et al. 2005). The same effect was found in the plasma membrane of the brain in which oxidative damage was reduced by CR (Hyun et al. 2006). Increases in CoQ levels and activation of CoQ-dependent

antioxidant activities and prevention of oxidative damage have been also found in the muscle of CR-fed old mice (Rodriguez-Bies et al. 2015).

The induction of these activities in old animals produced a greater resistance against oxidative damage. Interestingly, the rise of CoQ-dependent antioxidant activities was accompanied by a higher presence of CoQ_{10} in the rodent plasma membrane (De Cabo et al. 2004; López-Lluch et al. 2005). The rise in CoQ_{10} levels can be directly related to the induction of the activity of the antioxidant enzymes contributing importantly to the prevention of lipid peroxidation and ferroptosis (Bello et al. 2005).

Taken into consideration the protection against ferroptosis associated with FSP1, a new CoQ-dependent oxidoreductase (Bersuker et al. 2019; Dai et al. 2020), and the role of this mechanism of cell death in the progression of age-associated diseases (Larrick et al. 2020; Stockwell et al. 2020; Toyokuni et al. 2020), the role of CoQ-dependent antioxidants activities in cell membranes can be key in the progression of aging and chronic diseases associated with age. The study of these activities and the mechanisms involved in the transport (Fernandez-Ayala et al. 2005) and control of levels of CoQ in plasma membrane are necessary to determine if this protective mechanism can be modulated.

2.5.2 Physical Activity and Exercise

Physical activity and exercise are also considered prolongevity effectors. Again, oxidative damage seems to be at the center of the effect of exercise on the prevention of physiological disorders. In sedentary aged individuals, lipid peroxidation levels are elevated indicating a deficiency in antioxidant protection (Bailey et al. 2010). As CR, exercise induces adaptive changes in the levels of CoQ in muscle (Gohil et al. 1987). Further, exercise also induced changes in CoQ levels and activity of CoQ-dependent antioxidants depending on the age of the animals (Wang et al. 2015). The effect of exercise on CoQ levels was organ- and tissue-dependent (Quiles et al. 2001; Tung et al. 2015). This effect can be associated with the induction of mitochondrial biosynthesis since endurance exercise increases mitochondrial biogenesis and mitochondrial CoQ_9 levels in muscle.

We have found that in human blood plasma the levels of CoQ_{10} increase in old active individuals whereas decrease in sedentary participants (Del Pozo-Cruz et al. 2014). Further, plasma CoQ10 levels in elderly people are highly associated with the aerobic capacity of individuals whereas it is not related to strength capacity indicating a relationship with mitochondrial-dependent physical activity (de la Bella-Garzon et al. 2022).

On the contrary, supplementation with CoQ_{10} improves the physical activity of patients suffering from mitochondrial diseases. In these patients, the treatment with CoQ_{10} improved mitochondrial function permitting a higher physical performance (Bendahan et al. 1992). In animal models of accelerated senescence, the combination of ubiquinol with exercise improved the activity of skeletal muscle preserving mitochondrial structure (Andreani et al. 2018). Furthermore, recent studies have

demonstrated that supplementation with MitoQ can improve muscle performance and increase mitochondrial biogenesis markers in untrained middle-aged men (Broome et al. 2022) and in trained individuals (Broome et al. 2021) and the use of CoQ_{10} as supplementation for optimizing exercise performance in athletes is worth to be considered and studied with well designed scientific procedures (Drobnic et al. 2022).

As a hypothesis, the release of myokines from muscle can influence liver activity and increase the activity to package CoQ_{10} into lipoproteins in hepatocytes. This could explain the higher levels of CoQ_{10} bound to lipoproteins that end in the protection of these particles against oxidative damage (Carneros et al. 2020).

2.5.3 Bioactivenutritional Compounds: Nutraceuticals

Many nutritional bioactive compounds prevent the progression of age-related chronic diseases (Ferrari 2004). The mechanisms of these compounds are based on the induction of metabolic sensors such as AMPK and Sirtuins and the induction of antioxidant activities (Chen et al. 2019b; Li et al. 2021). Regarding CoQ_{10} levels, the screening of compounds able to delay aging progression by modifying mitochondrial activity and turnover and increase CoQ_{10}-associated antioxidant activities may help to maintain a balanced mitochondrial activity and CoQ levels during aging (Collins et al. 2006; Fernandez-Del-Rio and Clarke 2021; Fernández-Del-Río et al. 2017).

Among these compounds, resveratrol (RSV) and coumarate could be donors of the aromatic ring for CoQ synthesis in mouse and human cells (Xie et al. 2015). In the case of deficiency of the *p*-hydroxybenzoic acid production, the natural aromatic ring precursor for CoQ synthesis, the use of these compounds as secondary precursors could be interesting. However, the low bioavailability of these compounds could block their use in physiological conditions (Ramírez-Garza et al. 2018).

We have demonstrated that RSV can induce the synthesis of CoQ in cultured cells. The effect of RSV can be associated with the induction of mitophagy and mitochondrial synthesis instead of the direct induction of CoQ synthesis. Further, RSV also increased the levels of the antioxidant enzymes that use CoQ as an electron acceptor. $CytB_5R_3$ levels were increased in brain, kidney, and liver in RSV-supplemented animals whereas the protein levels of NQO1 were induced only in muscle whereas decreased in liver of aged mice (Tung et al. 2015). On the other hand, the activity of NQO1 was increased in brain, heart, and liver (Tung et al. 2015).

RSV can also modulate the levels of mRNA of the components of the CoQ-synthome. In animals fed with a high-fat diet (HFD), RSV was able to revert the inhibitory effect of this diet in the levels of many of the mRNA transcripts for CoQ-synthome in liver (Meza-Torres et al. 2020). This indicates that RSV can modulate, in some way, the regulatory mechanisms involved in the induction of

the expression or the maintenance of mRNA codifying for the proteins involved in CoQ synthesis.

All these studies indicate that polyphenols can affect CoQ synthesis both directly and indirectly. However, the exact role and the mechanisms involved in this effect remains unclarified and further research is needed.

2.5.4 Use of Pharmacological Compounds

Induction of CoQ_{10} synthesis by pharmacological compounds could be a very interesting strategy to reestablish the endogenous synthesis of CoQ_{10} during aging. Its induction by inducers of the peroxisome did not show results in aged animals (Turunen and Dallner 1998) probably indicating external factors to the CoQ-synthome that block CoQ synthesis. However, some squalene epoxides, intermediates of cholesterol synthesis, can be useful for stimulating CoQ biosynthesis (Bentinger et al. 2014). Interestingly, recent studies have demonstrated that the stimulation of the mevalonate pathway is important to maintain CoQ biosynthesis needed for the pyrimidine synthesis through de dihydroorotate dehydrogenase in p53-null tumoral cells (Kaymak et al. 2020).

If it is important for growth active tumoral cells, the maintenance of this pathway is also essential to keep CoQ synthesis in normal cells. For this reason, the effect of statins treatment on CoQ_{10} levels in plasma and tissues in elderly individuals suffering hypercholesterolemia must be carefully controlled. Probably the determination of CoQ_{10} in plasma is not enough to understand the effect on other tissues that cannot get CoQ_{10} from plasma such as muscle, kidney, and brain. Many studies indicate that statins can produce important side effects on these organs mainly affecting mitochondria, metabolism, and levels of CoQ10 (Dohlmann et al. 2019; Langsjoen et al. 2019; Prajapati et al. 2017; Vaughan et al. 2013). Taken into consideration the importance of the maintenance of the mevalonate pathway in the synthesis of CoQ_{10}, the studies about the effect of statins on CoQ10 levels must be performed carefully.

Regarding pharmacological compounds associated with CoQ homeostasis, rapamycin is considered an interesting factor resembling many CR effects (Karunadharma et al. 2015). Rapamycin affects the response to mitochondrial stress and for this reason has been also associated with the regulation of CoQ homeostasis (López-Lluch and Navas 2016). However, the direct effect on CoQ synthesis of this inhibitor of mTOR have not been studied. However, it seems that this compound needs to induce CoQ synthesis in some way since in COQ_9 mutants the effect of this compound was null (Barriocanal-Casado et al. 2019).

More promising effects have produced the biguanide metformin. This molecule is widely used for treating type II diabetes (Kulkarni et al. 2020). Although the mechanism of action of this compound is not clear, it seems that it accumulates in mitochondria and inhibits complex I stimulating ROS production (Bridges et al. 2014). On the contrary, in macrophages, metformin reduced the production of ROS from complex I reducing the inflammatory response (Kelly et al. 2015). Metformin

activates the AMP-activated protein kinase (AMPK)/mTORC1 signaling pathway involved in the induction of mito/autophagy. This could explain why metformin can induce the synthesis of CoQ_{10} through the induction of mitochondrial turnover as CR and polyphenols already do (Yan et al. 2017).

On the other hand, recent studies have found natural ubiquinone derivatives such as antroquinonol as inducers of mitochondrial activity and mito/autophagy (Chiang et al. 2010; Yu et al. 2012). In the same manner as rapamycin and metformin, another ubiquinone form, 4-acetylantrocamol LT3, also inhibits mTOR and activates AMPK and autophagy (Chen et al. 2020). In any case, the effect of these compounds on the synthesis of CoQ remains to be elucidated.

All these compounds show the same common factor. They show a prolongevity effect at the same time that induce mito/autophagy and induce mitochondrial biosynthesis. The induction of the renovation of mitochondria needs the production of new CoQ molecules that are needed for mtETC complexes I and III assembly and the organization in supercomplexes. Thus, the effect of these compounds could be only a secondary effect associated with mitochondrial turnover. However, more studies are needed to understand the role of AMPK activation in CoQ induction and maintenance of CoQ homeostasis.

2.6 Conclusions

Secondary deficiency of CoQ_{10} in aging can be considered an important factor in the acceleration of the accumulation of mitochondrial damage during this process. As in a vicious cycle, the decay in CoQ_{10} levels can be involved in mitochondrial dysfunction and in higher ROS production that accelerate oxidative damage. In absence of CoQ_{10}, essential CoQ-dependent antioxidant enzymes cannot block lipid peroxidation at the membranes increasing ferroptosis.

Many studies have demonstrated that supplementation with CoQ_{10} can, in part, reduce oxidative damage and increase mitochondrial activity. Finding nutritional or pharmacological compounds able to induce endogenous synthesis of CoQ_{10} and/or levels and activities of CoQ-dependent antioxidant enzymes will be essential in the reduction of oxidative damage and maintenance of mitochondrial activity.

More research is needed, especially in humans in which most of the studies are performed in blood plasma. Plasma CoQ_{10} levels cannot reflect the right conditions of CoQ_{10} in organs and tissues. In any case, in studies performed to determine if the supplementation with CoQ_{10} can to some extent delay or improve the evolution of age-related chronic diseases, determinations of the right levels reached in blood after supplementation are needed. Not all the compounds found in the market present the same bioavailability and, in some cases, the effect of the supplementation is so low (López-Lluch et al. 2019) that the null effect of supplementation on disease markers is clearly foreseeable.

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3 Autophagy as a Promising Therapeutic Target in Age-Associated Neurodegenerative Disorders

Iipsha Bhaduri, Anchal Trisal, and Abhishek Kumar Singh

Abstract

Aging, and the reason for the process, has been an area of research where strides can improve the healthspan and lifespan of people all over the globe. Cellular senescence and cell death due to oxidative stress-mediated damage to macromolecules are the major reasons for aging as a process but certain factors increase and decrease the rate of aging. The process of autophagy contributes to the decrease of the rate of aging and also alleviates and prevents the symptoms of aging-induced neurodegenerative diseases. Therefore, targeting autophagy process and increasing its frequency can improve the pathologies of many age-associated neurodegenerative disorders; especially Alzheimer's disease (AD) and Parkinson's disease (PD). Practices like intermittent fasting and caloric restriction have shown to increase the rate of autophagy and, in turn, have shown promising results as therapeutic interventions in AD and PD. Administration of caloric restriction mimetics like spermidine, rapamycin, metformin, and fisetin have shown to improve cognitive functioning in AD and PD through autophagy activation.

Keywords

Autophagy · Aging · Alzheimer's disease · Parkinson's disease · Caloric restriction · Intermittent fasting

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3.1 Introduction

Aging refers to a series of biological processes leading to changes in bodily homeostasis. It is the result of many events that coalesce to result in a single phenomenon. Aging processes involve cellular and molecular changes that impact the overall physiological conditions. Some common complications that arise with progressing age are the thickening of blood vessels, decreased metabolic rate, and decline in memory and cognition. The question of how organisms age, or rather, of how aging mechanisms can be regulated has been of great prevalence since the late twentieth century when it was first discovered that aging, and, in turn, longevity can be modulated

Many theories of aging such as telomere, genetic, epigenetic, and free radical theories have been proposed so far but none of them individually has the ability to explain the underlying mechanism to its full extent. Of these theories, oxidative stress/free radical is the most studied theory (Fig. 3.1). The free radical theory of aging proposes that the generation and subsequent accumulation of reactive species is a factor driving the aging mechanism. Reactive oxygen species (ROS) generated in our body as a result of normal cellular processes play an integral role in cellular signaling. ROS is majorly produced from mitochondria via the complexes involved

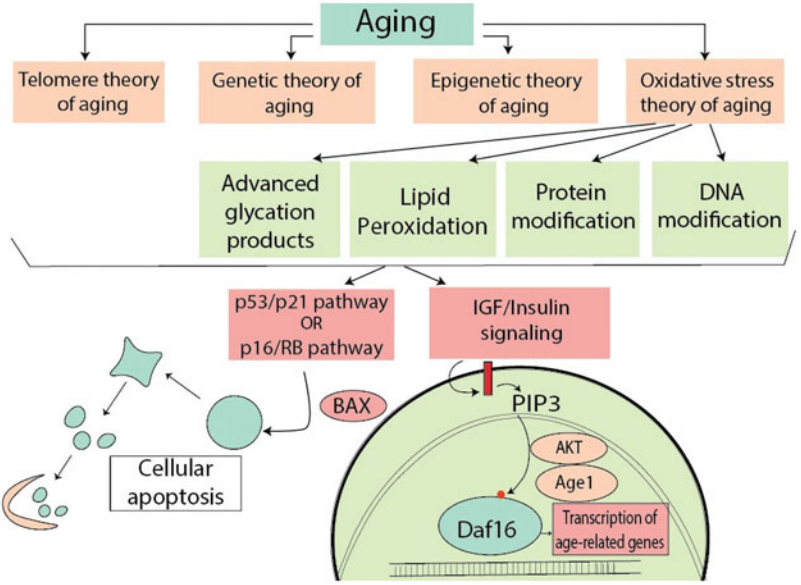

Fig. 3.1 Theories of aging which include (1) Telomere theory of aging; (2) Genetic theory of aging; (3) Epigenetic theory of aging; (4) Oxidative stress theory of aging (lipid peroxidation, protein modification, DNA modification, advanced glycation products); which further results in either (a) cellular apoptosis via either p53/p21 pathway or p16/RB pathway; (b) transcription of age-related genes via IGF/Insulin pathway

in the electron transport chain. Specifically, complex-1 is more prevalent for the generation of ROS in the brain (McLennan and Degli Esposti 2000).

Certain enzymes, under normal physiological conditions, act as antioxidant defense including superoxide dismutase (SOD) which converts O_2 to a less toxic H_2O_2 and water. Glutathione peroxidases are a family of isoenzymes that are responsible for the reduction of H_2O_2 using glutathione (GSH) as an electron donor (Kim et al. 2015). Nuclear factor E2-related factors (Nrfs) have been found to play a significant role in the reduction of oxidative stress. Nrf2 causes upregulation of antioxidant response element (ARE) genes which are constituents of antioxidant enzymes like NADPH oxidoreductase and glutathione-*s*-transferase (Tonelli et al. 2018). Nrf1 is responsible for the modulation of stressors that are generated in homeostatic conditions. The depletion of Nrf1 is believed to be an indicator of cellular oxidative stress. With aging, a disruption in antioxidant pathways is observed. The level of SOD is seen to decrease in an age-dependent manner which disrupts the ratio of oxidized to unoxidized macromolecules.

These radicals have a high reducing power thus they are prone to causing oxidative damage to biomolecules. The accumulation of oxidized biomolecules interrupts several cellular processes, tampering with cellular functions. Lipids are the most vulnerable to oxidation forming lipid peroxides which are constituent of lipofuscin, a morphological marker of aged cells. Free radicals can also cause modifications in proteins causing misfolding and accumulation in the cells which is the hallmark of several age-related neurodegenerative diseases.

Autophagy is a highly conserved catabolic process by which damaged biomolecules and cell organelles are engulfed, digested, and recycled. During advancing of age, autophagy process declines and, in its absence, there is excessive deposition of toxic materials that tamper with cellular functions. This disruption is associated with cellular death and loss of function which are the key characteristic of aging. Many studies have been able to draw correlations between the aging and autophagy process. While aging does not definitively mean the onset of a disease, it does increase the susceptibility to certain age-related diseases. Neurodegenerative diseases are a pertinent example of these kinds of diseases. Alzheimer's disease and Parkinson's disease are highly debilitating disorders that significantly alter the quality of life. Ventures aimed at formulating therapies for these disorders have drawn parallels between alleviation of symptoms and induction of autophagy. Therefore, anti-aging strategies that upregulate autophagy are considered to be a promising therapeutic approach for neurodegenerative disorders.

3.2 Molecular Mechanisms Governing the Aging Process

3.2.1 Insulin Signaling Pathway and Aging

Insulin and Insulin-like growth factor (IGF) play a significant role in moderation of aging processes (Fig. 3.1). Increased IGF signaling or enhanced insulin resistance promotes cellular senescence and development of senescence-associated secretory

phenotype (SASP). IGF-1 interacts with several aging genes like Age-1 and Daf-16. IGF-1 signaling generates PIP3 and causes phosphorylation of Daf-16 by Age-1 and other age-associated genes in an AkT-dependent manner (Evans et al. 2008). Moreover, insulin and IGF-1 also interact with mTOR to promote senescence (Frederik Nijhout and Callier 2013).

3.2.2 mTOR/AMPK Signaling Pathway and Aging

Mammalian target of rapamycin (mTOR) is a serine-threonine-containing protein that belongs to PI3K kinase family. mTOR forms two complexes: mTORC1 consisting of raptor protein and mTORC2 consisting of rictor protein. mTORC1 is sensitive to amino acids and is important in the regulation of diet-induced senescence and aging. A high-fat diet elevates mTOR activity that accelerates senescence (Cota et al. 2006). mTOR has been observed to contribute to several hallmarks of aging. Moreover, mTOR interacts with MAPK to produce SASP (Herranz et al. 2015). SASP involves the release of soluble (interleukins, cytokines, and growth factors) and insoluble factors that cause membrane dissociation and impaired processing of cell organelles (Coppé et al. 2010). Increased mTOR activity is also associated with an imbalance in proteostasis, another major indicator of aging. Imbalanced ubiquitin-proteasome system due to increased mTOR causes burden of damaged or misfolded proteins on the cell (Zhao et al. 2015). On the contrary, mTORC1 has also shown to increase the activity of the ubiquitin-proteasome system, causing the degradation of healthy proteins, inducing cellular stress (Zhang et al. 2014). AMPK is a regulator of mTOR. It causes phosphorylation and subsequent inactivation thus indirectly affecting the aging process.

3.2.3 Sirtuin Pathway and Aging

Sirtuins are a family of histone deacetylases that also contribute to the regulation of aging processes. Sirtuins are primarily antiaging molecules as they inhibit certain processes that lead to aging. Knock-out experiments in mice have shown that sirtuin specifically Sirt1 helps to mitigate DNA damage due to aging and significantly decreased the expression of p16. Sirt3, with conclusive proof, has been shown to increase longevity. Mutants lacking Sirt3 show decreased oxygen utilization and increased production of ROS thus contributing to the aging process. Sirt6 downregulates the IGF signaling pathway, preventing cellular senescence (Grabowska et al. 2017).

3.3 What is Autophagy?

Autophagy is the cell's self-eating mechanism responsible for the degradation and recycling of damaged or weathered cytoplasmic elements in the event of stress or during development. Autophagy process involves the formation of double-

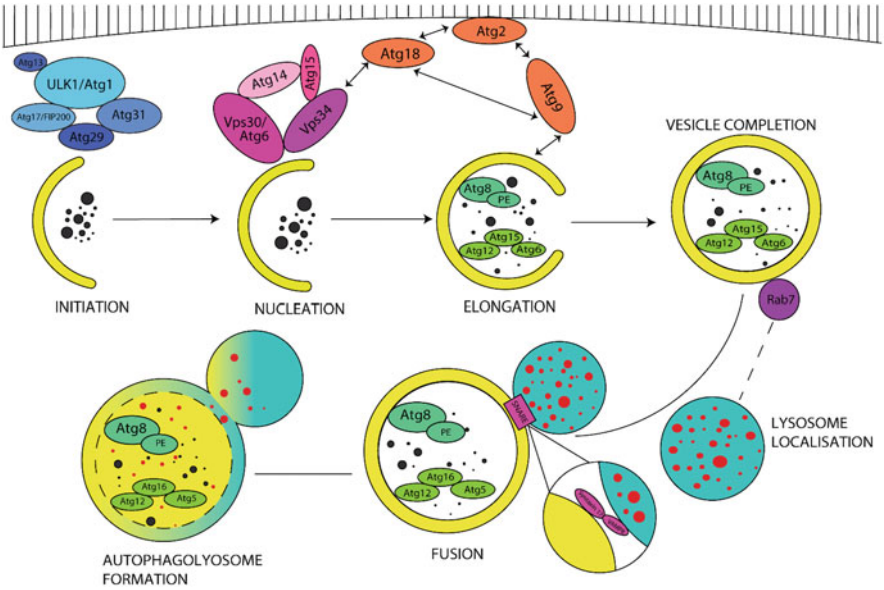

Fig. 3.2 Process of autophagy: (1) The ULK1/Atg complex initiates the formation of the autophagosome, which is regulated by the phosphorylation of Atg13; (2) The Atg complex combines with the PtdIns3K class III complex (a lipid kinase involved in vesicle nucleation) and contributes to nucleation of the autophagosome, and the concurrent interaction between PtdIns(3)P and Atg18 is indispensable to the autophagic process; (3) The elongation of the autophagosome is facilitated by two complexes namely Atg8/PE and Atg15/Atg12/Atg6; (4) The vesicle is completed by the interaction between Atg1 kinase and Atg9; (5) The migration of autophagosomes to the lysosome is performed by Rab7, a GTP-binding protein, localized on the autophagosome membrane, linking to microtubules, aiding in movement; (6) SNARE proteins are required for the fusion of the outer membrane of the autophagosome to the lysosomal membrane; (7) Autophagolysosome formation initiates destruction of cell debris and clearance of buildup

membraned vesicles called autophagosomes, that enclose the substrates to be broken down including superfluous or damaged organelles, cytosolic proteins, and invasive microbes (Feng et al. 2014). The autophagosomes are further transported to the lysosome where they fuse to form autolysosomes in which the inner membrane of the autophagosome is degraded along with the sequestered substances. They are broken down into simple molecules like amino acids which are recycled to the cytosol (Wesselborg and Stork 2015). Different steps of autophagy process are regulated by the Autophagy Related Genes (ATG) and the regulatory mechanism is highlighted in Fig. 3.2.

3.3.1 Regulators of Autophagy

3.3.1.1 mTOR/AMPK

There has been extensive research on the role of mTORC1 activity in autophagy. Activation of mTORC1 is dependent on Ras family of genes which is typically

found in association with GDP and a TSC1/2 domain. The presence of growth factors activates either PI3K/AkT or Ras/Raf/ERK pathway that allows phosphorylation of TSC1/2 and further activation of mTORC1. Moreover, amino acids also activate mTORC1 in a Ras-independent manner. mTORC1 negatively regulates autophagy at different stages of autophagosome formation. As discussed above, mTORC1 phosphorylates ULK1 and Atg13 preventing the initiation of autophagy in nutrient-rich conditions (Hosokawa et al. 2009). mTORC1 also influences the nucleation process via phosphorylation of ULK1. In addition, mTORC1also directly regulates the PtdIns3K complex by phosphorylation of its components. Atg14 phosphorylation impairs the transport of the complex to the pre-autophagosomal structure (PAS). AMBRA1 is a protein that attaches the PtdIns3K complex to the cytoskeleton. Decreased level of mTOR causes dissociation of AMBRA1, allowing the recruitment of PtdIns3K to the PAS (di Bartolomeo et al. 2010). WIPI2 (mammalian homolog of Atg18) is also phosphorylated by mTOR in nutrient-rich conditions leading to its ubiquitination and proteasomal degradation (Wan et al. 2018). AMPK in an inhibiter of mTOR and is responsible for the induction of autophagy. Moreover, AMPK phosphorylates ULK1 to induce autophagy. The balance between mTOR and AMPK acts as a sensor, only allowing for autophagy induction in severe nutritional deficit (Kim et al. 2011).

3.3.1.2 Sirtuins

Sirtuins are a family of class III histone deacetylases. They mainly influence various cellular functions including apoptosis and autophagy. They act as positive regulators of autophagy. A decline in nutrition status or an upregulation of cytoplasmic NAD+ increases the levels of sirtuins. Sirt1 deacetylates Atg5, 7, and 8 in an NAD+-dependent manner thus inducing autophagy (In et al. 2008). Sirtuins also deacetylate certain transcription factors that contribute to autophagy, including members of the FOXO family, FOXO1 and FOXO3. FOXO1 deacetylation by sirt1 upregulates autophagy by increasing the flux of Rab7 (Hariharan et al. 2010). Sirt2 also plays a significant role in the regulation of FOXO genes. The cytoplasmic p53 is a repressor of autophagy, and its deacetylation by FOXO via Sirt2 regulation promotes autophagy process. Moreover, Sirt2-mediated regulation of FOXO also activates Atg7 (Zhao et al. 2010).

3.3.2 Correlation Between Autophagy and Aging

A disruption in autophagy has been shown to cause significant senescence-promoting activity in cells. Moreover, absence of autophagy interrupts cell cycle progression at the G2/M phase due to increased aneuploidy and DNA replication errors (Matsui et al. 2013). UV radiation resistance-associated gene (UVRAG) is of significant importance to DNA repair enzymes and, in turn, regulates genomic stability. Downregulation of autophagy causes a simultaneous decrease in UVRAG levels, making the genome unstable and thus accelerating the aging process (Zhao et al. 2012).

Furthermore, increased level of mTOR can contribute to accelerated aging through decline in autophagy. The phosphorylating activity of mTOR has shown to promote cellular senescence. Increased insulin activates Akt which is a positive regulator of mTOR. Thus, autophagic decline is a consequence of increased insulin signaling. Sirtuins signaling is disbalanced with the progression of age. As mentioned above, impairment of Sirt2 signaling causes an upregulation of p53 thus driving senescence.

3.4 Role of Autophagy in Age-Associated Neurodegenerative Disorders

Aging is a risk factor for age-related neurodegenerative diseases due to the increased production of ROS and impaired redox homeostasis. Declined or dysfunctional autophagy during advancing of age further contributes to onset and progression of neurodegenerative diseases.

3.4.1 Alzheimer's Disease

Alzheimer's disease (AD) is the most common progressive neurodegenerative disease primarily hallmarked by the accumulation of senile plaques and neurofibrillary tangles. Senile plaques are aggregates of amyloid-β protein caused by improper cleavage of amyloid precursor protein (APP) by β-secretase. According to Hardy and Higgins, the accumulation of Aβ protein is characteristic in AD and it predicates vascular damage, oxidative damage, and the formation of neurofibrillary tangles (NFTs) by triggering a downstream signaling cascade. Neuronal death is triggered by the free radicals-mediated oxidation of biomacromolecules. AD is also linked with excessive accumulation of autophagic vacuoles (AVs), which are observed to contain APP. Increased amounts of AVs is hypothesized to occur due to impaired maturation of autophagosomes (Yu et al. 2005). These observations may conclude that AVs may act as sites for Aβ synthesis (Pasternak et al. 2003). Deposition of intracellular Aβ causes an increased dysfunction in autophagy pathways and has deleterious effects due to impaired clearance of dead organelles and oxidized biomolecules.

NFTs are aggregates of hyperphosphorylated tau protein. Neurons expressing NFTs also have increased levels of ROS. There is also a significant reduction in antioxidant pathways suggesting impaired redox homeostasis. Santagata et al. demonstrated that oxidative stress does not act directly to phosphorylate tau, but instead, oxidative stress may act downstream to the formation of NFTs, further exacerbating NFT-mediated neurotoxicity. Induction of autophagy by NFT deposition can activate the JNK pathway, leading to apoptosis (Dias-Santagata et al. 2007). Tauopathy in AD is also associated with a decline in autophagic processes. Increased activities of Akt and mTOR are observed and inferred to contribute to hyperphosphorylation of tau protein. Tauopathy can also be upregulated due to the

increased amount of mTORC1 and mTORC2 activity in AD. Moreover, mTOR assists in the aggregation of NFTs after tau hyperphosphorylation (Tang et al. 2013).

3.4.2 Parkinson's Disease

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease that is characterized by dyskinesia and may also cause cognitive deficits. The pathophysiology of PD is strongly associated with the accumulation of α-synuclein protein as Lewy bodies in dopaminergic neurons of substantia nigra (Singleton et al. 2013). Alpha-synuclein is a 140-amino acid protein, which is natively unfolded, thought to be a synaptic modulator. The oligomerization of α-synuclein, which is the main etiopathology attributed to PD, is brought mainly by oxidation and nitrosylation caused by ROS and RNS. Dopamine is also a contributing factor that leads to oxidization of α-synuclein. Oxidative stress is one of the major causes of PD, especially the sporadic form. The oligomerization and coalescence of α-synuclein due to ROS can be directly traced to aging and the process of accumulation of aging factors inside the cell itself. The mitochondrial stress further aggravates intracellular deposition of α-synuclein

Native α-synuclein is cleared by the activation of CMA-induced autophagy pathways, which does not occur in cases of oligomers and excess deposition. As a result, there is an increase in active macroautophagy due to the activation of Atg1/ULK1 complex via the mTOR pathway. While induction of autophagy has seen to have some beneficial effects in PD, excessive autophagy can lead to excessive cell death (Lynch-Day et al. 2012). Another direct effect of α-synuclein deposition is the interruption in the autophagic pathway caused by the mislocalization of Atg9 leading to disruption in the formation of the autophagosome. This leads to the formation of autophagic vacuoles, predisposing the cells to senescence and death (Cheung and Ip 2009; Pan et al. 2008).

3.5 Strategies to Induce Autophagy as a Therapeutic Approach to Mitigate Neurodegenerative Disorders (NDDs)

The mechanism(s) of autophagy activation through different strategies such as caloric restriction, intermittent fasting, and caloric restriction mimetics for the management of neurodegenerative disorders have been discussed below and highlighted in Fig. 3.3.

3.5.1 Caloric Restriction as a Therapeutic Approach for NDDs

As the name suggests, caloric restriction (CR) involves limiting calorie intake to as low as 50% of the body's needs, without causing malnutrition. CR plays a significant

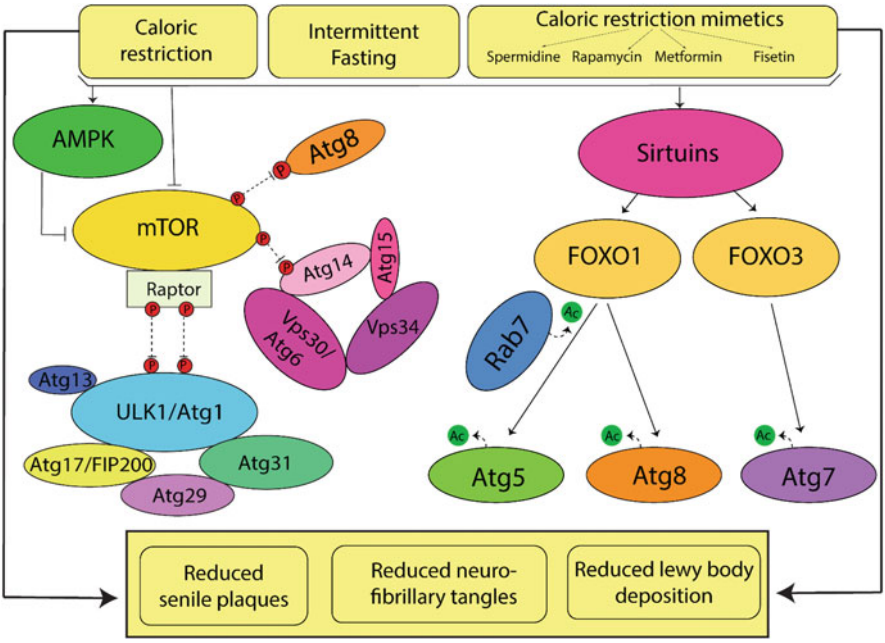

Fig. 3.3 Caloric restriction, intermittent fasting, caloric restriction mimetics (spermidine, rapamycin, metformin, etc.) upregulate autophagy by (1) upregulating AMPK pathway, which inhibits mTORC1, resulting in the formation of ULK1/Atg complex thereby upregulating autophagy; (2) upregulating sirtuins (FOXO1, FOXO3) to trigger Atg complex and Rab which facilitate autophagolysosome formation; which results in clearance and recycling of senile plaques, neurofibrillary tangles, and Lewy body

role in the induction of autophagy. As mentioned above, amino acid levels regulate mTORC1 signaling. Moreover, an increased level of NAD+ causes activation of sirtuin-mediated autophagy. It is also hypothesized to alleviate oxidative stress. Only a 25% restriction in calorie intake has shown to cause a remarkable decline in metabolic rate and a decrease in the cost of physical activity. Therefore, the generation of oxygen free radicals is mediated by CR (Buchowski et al. 2012). IGF-1 signaling is also regulated by CR. Reduced insulin level or increased insulin sensitivity allows for a decline in aging mechanisms and ROS generation.

CR has been found to be an effective strategy to reduce the rate of onset of neurodegenerative disease. In several studies, it has been demonstrated that a low-calorie diet effectively reduces the susceptibility of the subject to develop AD. It has a potential neuroprotective effect on the hippocampal and cortical neurons, and it effectively regulates excitotoxicity. CR is also found to be effective in PD where it confers neuroprotection to dopaminergic neurons in the substantia nigra, preventing their degeneration. There is a significant improvement in motor functions in PD patients who were on a low-calorie diet (Mattson 2003). Moreover, dietary restrictions also upregulate the levels of neurotrophic factors in the brain,

specifically brain-derived neurotrophic factor and nerve growth factor. This causes increased neurogenesis and compensates for the loss of neurogenesis during aging thus reducing NDDs (Mattson 2003).

3.5.2 Intermittent Fasting as a Therapeutic Approach for NDDs

Intermittent fasting is a technique that assigns meal timings and is characterized by periods of voluntary fasting followed by a non-fasting period. Intermittent fasting has effects similar to those seen in caloric restriction. Intermittent fasting has been observed to attenuate oxidative stress as several biomarkers associated with oxidative stress are downregulated. Lipid peroxidation is significantly decreased. A causal upregulation in antioxidant pathways is also observed during intermittent fasting (Sharsher et al. 2022). A mild elevation in Sirt3 level is observed after significant periods of intermittent fasting. Modulation of autophagy induction by Sirt3 shows promising results (Wegman et al. 2015). Moreover, decreased levels of insulin/IGF-1, as in caloric restriction, also contributes to reduced oxidative stress and elevated autophagy induction.

Intermittent fasting shows a positive regulatory effect on neurodegeneration. It is associated with decreased susceptibility to vascular dementia. Moreover, intermittent fasting decreases the levels of circulating interleukins and tumor necrosis factors, while upregulating brain growth factors for increased neurogenesis. It causes an improvement in neural plasticity in a cAMP-dependent manner which helps in learning and memory formation in AD. Memory formation is also enhanced by the regulation of IGF signaling in the hippocampus (Shin et al. 2018; Yoon and Song 2019). A decreased alpha-synuclein burden was observed after intermittent fasting thus regulating motor as well as cognitive symptoms of PD. The mitochondrial degradation due to α-synuclein buildup is regulated by intermittent fasting (Neth et al. 2021).

3.5.3 Caloric Restriction Mimetics as a Therapeutic Approach for NDDs

The advantages of caloric restriction are manifold, but the maintenance of a low-calorie diet poses a serious challenge. It is difficult to enforce such a diet regime in humans. Moreover, continued caloric restriction in late adulthood can cause reduction in bone density and muscle mass. To combat these difficulties, attention is being paid to the use of caloric restriction mimetics (CRMs). CRMs are compounds that mimic the similar molecular, biochemical, hormonal, and physiological effects as produced by caloric restriction.

There are different classes of CRMs, each having different mechanisms of action. However, they are beneficial as therapies for NDDs by upregulating autophagic processes and alleviating oxidative stress. Some common CRMs are mentioned in Table 3.1 with their mechanisms of action and their potential therapeutic effect in mitigating the symptoms of AD and PD.

Table 3.1 CRMs mediated mechanism of action to manage neurodegenerative disorders

Caloric restriction mimetic	Mechanism of action	Role in neurodegenerative disorders	References
Spermidine	Induces autophagy by • Upregulation of Atg3 and Beclin1 • Upregulation of AMPK • Activates FOXO3 signaling Prevents oxidative stress by • Protection of mitochondria • Antioxidant property; causes utilization of oxygen	Parkinson's disease • Neuroprotection of dopaminergic neurons in substantia nigra • Reduced alpha-synuclein neurotoxicity • Parkin1-dependent mitophagy regulation Alzheimer's disease • Mediates levels of plasminogen activators; prevents stabilization of Aβ plaques	Hofer et al. (2021), Yang et al. (2017, 2020), Yan et al. (2019), Sharma et al. (2018), Büttner et al. (2015), Singh et al. (2020)
Rapamycin	Induces autophagy by • Inhibition of mTOR • Upregulation of Sirt1 Prevents oxidative stress by: • Regulating activity of Complex 1 of mitochondria • Prevents fragmentation of mtDNA • Reduces neuroinflammation by preventing microglial activation	Parkinson's disease • Prevents transcription of RTP801 Alzheimer's disease: • Reduced Aβ generation due to reduced AVs • Prevents hyperphosphorylation of tau protein	Tang et al. (2013), Lesniewski et al. (2017), Martínez-Cisuelo et al. (2016), Malagelada et al. (2010), Feng et al. (2019), Spilman et al. (2010), Singh et al. (2016a, b, 2019a)
Metformin	Induces autophagy by • Upregulation of Beclin1 and LC3 • Upregulation of Sirt1 Prevents oxidative stress by • Regulation of proton gradient in mitochondria • Inhibition of NADPH oxidase	Parkinson's disease: • Inhibits phosphorylation of alpha-synuclein • Neuroprotection of dopaminergic neurons. Alzheimer's disease: • Neuroprotective effect on cells containing APP and Aβ deposits • Facilitates neuronal repair and enhances neurogenesis	Chiang et al. (2016), Ou et al. (2018), Garg et al. (2017a, b)

(continued)

Table 3.1 (continued)

Caloric restriction mimetic	Mechanism of action	Role in neurodegenerative disorders	References
	• Enhanced antioxidant enzyme activity		
Fisetin	Induces autophagy by • Upregulation of Atg3 and Beclin1 • Upregulation of Sirt1 and Sirt2 Prevents oxidative stress by • Increased glutathione activity • Activation of PMRS (plasma membrane redox system)	Parkinson's disease • Reduced alpha-synuclein inclusions • Upregulation of SNCA • Increased activity of dopamine transporter (DAT) Alzheimer's disease • Regulation of BACE enzyme activity • Interacts with acetylcholinesterase inhibitor to decrease ACh levels.	Rosado-Ramos et al. (2021), Kumar et al. (2020), Chen et al. (2020), Currais et al. (2014), Singh et al. (2018, 2019b)

3.6 Conclusion

Aging, a natural process, is the major risk factor for neurodegenerative diseases (NDDs). Understanding the role of autophagy that may ameliorate or improve the symptoms of these age-associated NDDs has been part of recent scientific research. The role of autophagy in NDDs is of great significance when considering therapeutic approaches. The upregulation of autophagy is something that is inbuilt within the body's defenses, and previously thought to be an impossible process of clearing protein aggregation from neuronal environments can be achieved by introducing natural compounds to the body. Autophagy provides new, relatively risk-free therapeutic approaches against NDDs that affect much of the human population today and reaffirms the quality of life for them.

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Ethical Statement None

Conflict of Interest The authors declare that they have no conflict of interest.

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Glycolytic Inhibitors as Caloric Restriction Mimetics (CRM)

4

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Abstract

Aging is the loss of the physiological integrity of the living system over time, resulting in reduced function and a higher risk of death. In view of the increase in the human lifespan in the last couple of centuries, it is critical to develop effective ways for preventing or delaying the onset of severe age-related disorders. Many intriguing and exciting antiaging strategies are being developed and constantly being put under scientific scrutiny. Caloric restriction has shown great promise as an alternative health-span intervention. Caloric Restriction Mimetic (CRM) research aims to develop drugs that target Caloric Restriction (CR)-affected metabolic and stress response pathways without restricting caloric intake. 2-Deoxyglucose (2-DG), the first viable CRM, was investigated in 1998, and the number of possibilities has grown significantly since then. Upstream CRMs regulate energy metabolism, whereas downstream CRMs regulate intracellular signaling. Metformin (an anti-diabetic medicine that activates AMPK), Rapamycin (an immunosuppressive drug that inhibits mTOR), Resveratrol (a polyphenol that activates Sirtuin), and other downstream drugs are just a few examples. Glycolytic inhibitors (2-deoxy-D-glucose, D-allulose, D-glucosamine) and Acarbose (an anti-diabetic medication and glycosidase inhibitor) are examples of upstream subtypes. The focus of this chapter is on glycolytic inhibitors that can be used as CRM. The main idea is that by slowing down cellular energy metabolism, the cell would be stimulated to elicit CR-like responses.

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Keywords

Aging · Caloric restriction · Caloric restriction mimetics · Glycolytic inhibitors

4.1 Introduction

Aging is defined as a gradual loss of physiological integrity, which results in diminished function and an increased risk of death. In recent years, aging research has progressed at an unforeseen rate, and nine hallmarks of aging have been identified at the molecular and cellular levels as a result of this rising knowledge. Genomic instability, telomere attrition, epigenetic changes, proteostasis loss, unregulated nutrition sensing, mitochondrial failure, cellular senescence, stem cell fatigue, and altered intercellular communication are some of the hallmarks (López-Otín et al. 2013).

As a result, finding effective strategies to slow down aging and prevent or delay the onset of devastating age-related diseases is critical. Many antiaging strategies are being developed that appear promising and have the potential to attract scientific attention in the coming years. Caloric restriction and its mimics, telomerase activators, autophagy inducers, plasma membrane redox system activators, senolytic therapeutics, epigenetic modulators, and stem cell therapies are among them (Saraswat and Rizvi 2017).

Dietary restriction, or more precisely Caloric Restriction (CR), has demonstrated tremendous promise among alternative healthspan therapies. Caloric restriction (CR) is the practice of reducing energy (food) intake without becoming malnourished. It slows the aging process, extends the era of youth, delays the onset of age-related disorders, and increases the lifespan of animals of various phylogenetic groups. It has been a popular concept in gerontology for decades (Lee and Min 2013). In the vast majority, if not all, living species, CR, combined with intermittent fasting, is the best-known technique for promoting health and lifespan (Maduro et al. 2021).

In 1935, McCay proposed that improved longevity was due to a reduction in calories rather than particular nutrients. Since then, similar findings have been validated in a variety of model organisms ranging from yeast to invertebrate species like *Caenorhabditis elegans* and Drosophila melanogaster to rodents and primates (Yessenkyzy et al. 2020). Reduced body temperature and plasma insulin levels are the two important biomarkers of CR (Ingram et al. 2006).

Long-term CR demands a significant amount of willpower, and the long-term adverse repercussions are unknown. First, there is the issue of long-term dieting, which may require calorie intake to be lowered by 30–40% below baseline levels. Second, there are unfavorable side effects, such as decreased body temperature and libido, which have been documented. Third, the long-term health risks associated with CR, such as reduced bone mineral density or slow wound healing, have yet to be thoroughly determined (Ingram and Roth 2011).

The continued focus of research on the antiaging mechanisms caused by CR has resulted in a novel technique that is based on CR mimetics (CRM). The goal is to find compounds that imitate CR by targeting metabolic and stress response pathways that are altered by CR, but without limiting caloric intake, at least in the early stages of the intervention. The first possible CRM, 2-deoxyglucose (2-DG), was proposed in 1998. Since then, the number of candidates has risen exponentially (Ingram and Roth 2015).

Any candidate for CRM should have the following characteristics: It must have metabolic, hormonal, and physiological effects similar to CR; it must have little effect on long-term food consumption; it should protect against a variety of stressors by activating stress response pathways similar to CR; and it must display CR-like benefits on longevity, age-related illness prevention, and maintenance of function (Ingram et al. 2006).

CRMs are classified into numerous groups, including:

1. *Glycolytic inhibitors*: 2-Deoxy-D-glucose, 3-bromopyruvate, D-allulose, D-glucosamine, mannoheptulose, astragalin, chrysin, genistein, etc.
2. *Polyamines*: Putrescine, spermidine, spermine, etc.
3. *Polyphenols*: Curcumin, quercetin, resveratrol, gallic acid, etc., others include hydroxycitric acid, salicyclic acid, NAD+ precursors, etc. (Hofer et al. 2021) (Fig. 4.1)

CRMs affect both downstream and upstream targets. Upstream-type CRMs limit energy metabolism, particularly glucose metabolism modulation, whereas downstream-type CRMs work on an intracellular signaling route. Metformin (an anti-diabetic drug that stimulates AMPK), Rapamycin (an immunosuppressive drug that suppresses mTOR), Resveratrol (a polyphenol that activates Sirtuin), and other downstream drugs are examples. The upstream subtype includes Glycolytic inhibitors (2-deoxy-D-glucose, D-allulose, D-glucosamine, etc.) and Acarbose (an anti-diabetic drug for Glycosidase inhibition) (Shintani et al. 2018).

This chapter will focus on glycolytic inhibitors which may be employed as CRM. The basic assumption was that by slowing down cellular energy processing, the cell would be stimulated to produce responses similar to true CR. In the glycolytic process, several areas of intervention can be recognized, most notably inhibition of enzymes involved in the glucose to ATP conversion steps (Ingram and Roth 2011).

Although there are many possible targets, practically all of the research in this field has concentrated on inhibitors of hexokinase and phosphoglucose isomerase, the first two steps in the glycolytic cascade. Hexokinase (HK) isozymes HK-1, HK-2, HK-3, and HK-4 have been found in animals. Depending on the substrate and context, each has a different location, physiological function, and kinetics (Ingram and Roth 2021) (Fig. 4.2).

The majority of the possible drugs mentioned below here are HK-2 inhibitors.

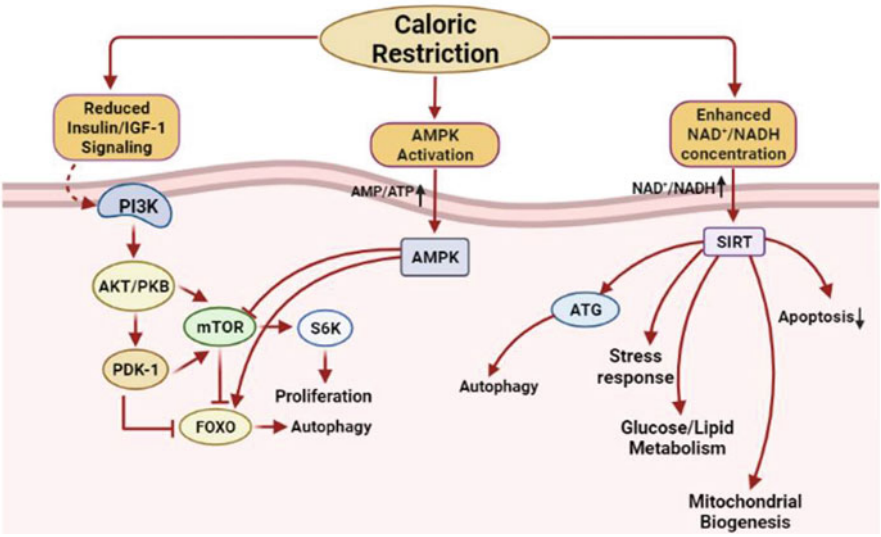

Fig. 4.1 Molecular mechanism of caloric restriction. Caloric restriction inhibits insulin signaling and promotes autophagy by suppressing the PI3K and AKT pathways. CR reduces mitochondrial function while raising the AMP:ATP ratio and NAD+ levels. AMPK is activated when the AMP:ATP ratio rises. AMPK (indirectly through phosphorylation of the TSC1/2 complex; not shown) inhibits mTOR action. Increased autophagy and decreased S6K activity result from decreased mTOR function. SIRT also inhibits apoptosis and promotes autophagy, stress response, glucose/lipid metabolism, and mitochondrial biogenesis. *IGF 1* insulin growth factor 1, *S6K* S6 kinase, *ATG* autophagy related gene, *SIRT* sirtuin

4.2 Glycolytic Inhibitors

4.2.1 2-Deoxy-D-Glucose (2-DG)

2-Deoxy-D-glucose (2-DG) is a glucose derivative with a hydrogen atom in place of the 2-hydroxyl group. 2-DG, the first proposed dietary restriction mimic, is not metabolized by glycolysis. By inhibiting glycolytic activity, it is expected to delay age-related dysfunctions and extend lifespan (Shintani et al. 2018). 2-DG inhibits the phosphoglucose isomerase-mediated synthesis of glucose-6-phosphate from glucose. 2-DG enters cells through a glucose transporter but then acts at the second step of glycolysis, resulting in intracellular accumulation of 2-deoxy-D-glucose-6-phosphate (2-DG6P), which inhibits the action of glucose-6-phosphate isomerase. The rationale for the use of this compound was to reduce cellular energy, and ATP (Ingram and Roth 2021).

A study has shown that increased glucose availability shortens *Caenorhabditis elegans* life span and impaired glucose metabolism by 2-DG increases life expectancy by inducing mitochondrial respiration. Reduced glucose availability promotes the generation of reactive oxygen species (ROS), stimulates catalase activity, and

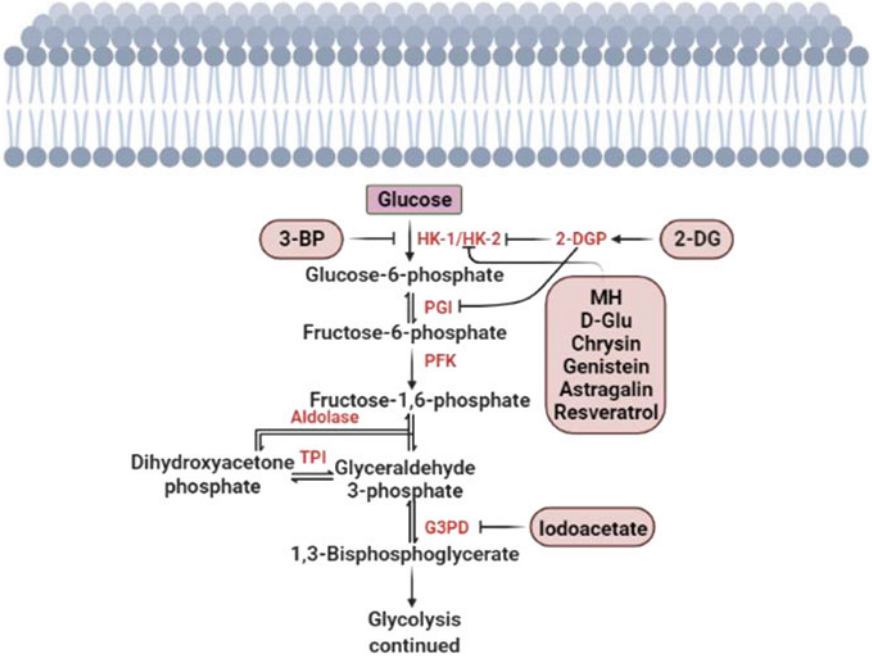

Fig. 4.2 Mechanism of action of CRMs. 2-DG, in the form of 2-DGP, inhibits the formation of glucose-6-phosphate from glucose at the second step, PGI, as well as hexokinase, which is the first step in glycolysis. D-Glu inhibits HK-1.3-BP, Chrysin, Genistein, Astragalin, Resveratrol inhibits HK-2. MH inhibits both HK-1 and HK-2. Iodoacetae inhibits G3PD. *HK-1/HK-2* hexokinase 1/hexokinase 2, *PGI* phosphoglucoisomerase, *PFK* phosphofructokinase, *TPI* triose phosphate isomerase, *G3PD* glyceraldehyde-3-phosphate dehydrogenase, *2-DGP* 2-deoxy-D-glucose-6-phosphate

boosts oxidative stress tolerance and survival rates. The findings support a speculative concept known as mitochondrial hormesis, or "mitohormesis." Through AMPK, 2-DG stimulates the utilization of stored fat and mitochondrial respiration by reducing glycolysis (Schulz et al. 2007). Long-term 2-DG consumption, on the other hand, caused cardiac vacuolation in rats and increased mortality (Minor et al. 2010).

2-DG treatment in rats leads to decreased plasma insulin levels and body temperature, so designating it as a potential CRM. 2-DG has been proven to protect against a variety of diseases, including neurodegeneration, cancer, and epilepsy (Kumar et al. 2020). In the case of Alzheimer's disease, dietary 2-DG therapy increased ketogenesis and ketone metabolism, improved mitochondrial bioenergetic capability, decreased β-amyloid formation, and promoted mechanisms of β-amyloid clearance (Yao et al. 2011).

Several studies imply that intermittent fasting plus dietary supplementation with 2-DG can achieve blood pressure, heart rate, and insulin level reductions comparable to or greater than those obtained with regular physical exercise programs through a

mechanism involving stress reactions (Wan et al. 2003). The 2-DG diet has a considerable impact on cardiovascular function. For 6 months, rats fed a 2-DG diet had a considerably decreased heart rate (HR) and mean blood pressure (BP). As a result, long-term intermittent food supplementation with 2-DG leads to lower resting BP and HR, lower plasma insulin and glucose levels, and improved cardiovascular and neuroendocrine adaptation to stress in rats (Wan et al. 2004).

2-DG has proven to be quite efficient against cancer cells. Doxorubicin (an antitumor medication) cytotoxicity was significantly reduced in newborn rat cardiomyocytes treated with 2-DG. Treatment reduced intracellular ATP levels by 17.9%, but it avoided DOX-induced severe ATP depletion. This is accomplished in a variety of ways, including ATP level preservation and AMPK activation (Chen et al. 2011).

Because of its capacity to block glycolysis and ATP generation, 2-DG appears to be an effective cytotoxic agent, which has been found mostly in cancer cells. It has a distinctive ability to harm cancer cells in hypoxic conditions. It could be used in conjunction with other medicines, bioactive substances, and radiotherapy (Pajak et al. 2019).

In mice, dietary 2-DG dramatically reduced tumor incidence, delayed its incidence, and hampered tumor progression. Reduced levels of critical metabolic pathway actors such as phosphatidylinositol 3-kinase (PI3K), phosphorylated-Akt, and hypoxia-inducible factor-1 alpha (HIF-1α) were also found in 2-DG-fed mouse tumors. Furthermore, the decrease in CD4+/CD8+ ratio and T-regulatory cells found in 2-DG fed mice revealed that antitumor immunity and T cell effector activity was improved (Singh et al. 2015).

In Hs68 cells, the drug 2-DG increased intracellular NAD+ levels at noncytotoxic concentrations in a time- and concentration-dependent manner, as well as SIRT1 activities. It also significantly improved cell longevity, with increased population doubling levels and lower activity of senescence-associated β-galactosidase (as an aging biomarker) (Yang et al. 2011).

Combining 2-DG with nonthermal plasma could be used as a therapy for blood cancer cells. 2-DG improves the efficacy and selectivity of plasma while also inducing synergistic suppression of cancer cell development via glycolysis and death (Kaushik et al. 2015).

According to a study, combining 2 DG with lovastatin (a mevalonate pathway inhibitor) lowered cell viability, halted cells in the G2/M phase, and triggered apoptosis in colorectal cancer cells. The combination treatment also reduced cellular oxygen consumption and the rate of extracellular acidification, resulting in lower ATP generation and steady-state ATP levels (Huang et al. 2021).

There are some dependent toxicity reports. Young male rats were fed diets supplemented (by weight) with 0.2%, 0.4%, or 0.6% 2-DG, with the 0.6% dose appearing to be toxic, as evidenced by dramatically reduced body weight, decreased appetite, and a few deaths. As a result, the effective dose range for 2-DG seemed to be between 0.2% and 0.6%, with 0.4% being the most effective amount. At the 0.4% dose, plasma insulin and body temperature were both reduced (Lane et al. 1998). A study found that 2-DG administered in the diet causes cardiac toxicity in rats

(0.04–0.6% 2-DG by weight in the diet) and increases mortality at doses greater than 0.2 g/kg (0.4% in the diet). The latter happens as a result of heart failure, despite apparently favorable reductions in blood glucose, insulin, and body temperature, all of which are biomarkers of an efficient CRM (Minor et al. 2010).

As a result, we may conclude that by suppressing glycolytic activity, 2-DG is expected to delay age-related dysfunctions and prolong longevity, and it looks to be an effective cytotoxic agent, which has primarily been detected in cancer cells. It has been shown to protect against neurodegeneration, cancer, and epilepsy, among other disorders. It may be used with other medications, bioactive compounds, and radiation.

4.2.2 3-Bromopyruvate (3-BP)

The brominated derivative of pyruvic acid is 3-bromopyruvate (3BP), which is a simple lactic acid analog. The suppression of Hexokinase-2 (HK-2), the first stage in glycolysis, is what draws people's attention to 3BP. 3BP penetrates tumor cells by lactic acid transporters and blocks HK-2 from binding to mitochondria (Ingram and Roth 2021).

It is thought that CR can reduce oxidative damage by slowing down the flow of energy and metabolism, or "pace of life" thereby slowing down the aging process (Redman and Ravussin 2011). There are significant studies that show an increase in oxidative stress as humans age, which has been connected to an increase in mitochondrial ROS production (Sena and Chandel 2012). Increased ROS levels can operate as a signaling molecule, lowering or delaying the onset of a range of chronic diseases and, in turn, extending a human's lifespan (Schieber and Chandel 2014). The production of reactive oxygen species (ROS) increased significantly in 3-BP-treated rats, according to research (both young and old). Short-term 3-BP therapy can protect against oxidative stress by inducing a hormetic response in response to an increase in ROS, resulting in the activation of a protective defense mechanism (Arya et al. 2020).

In *Leishmania amazonensis*, 3-BP dramatically inhibited the activity of glycolytic enzymes (Glucose kinase, Glyceraldehyde-3-phosphate dehydrogenase, and enolase), potentially depleting critical sources of ATP via substrate-level phosphorylation. As a result, ATP production and O_2 consumption decreased, confirming 3-BP's energy depletion impact (Gomes et al. 2021).

Although there has been little research on this compound's efficacy as a CRM, several studies have tested its efficacy in a range of tumor models. This combination of glycolysis and translation inhibition is uncommon with other energy blockers. Another crucial feature is 3-BP's ability to disrupt cellular redox balance by depleting antioxidants and boosting free radical production, which would trigger apoptosis and eventually lead to cancer cell death (Ganapathy-Kanniappan et al. 2010).

Kanniappan discovered that 3-BP is selectively taken up by tumor cells via monocarboxylate transporters (MCTs), which are typically overexpressed in cancer cells, in a follow-up study (for the export of lactate produced during aerobic

glycolysis). The potency of 3-BP as a potent and promising anticancer drug is highlighted by the specificity of molecular Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) targeting and selective uptake by tumor cells (Ganapathy-Kanniappan et al. 2013).

In treatment with 3-BP, cellular ATP was depleted, and mitochondrial depolarization was produced, all without altering levels of reducing equivalents. 3-BP induced substantial cell death when coupled with cisplatin or oxaliplatin. In resistant p53-deficient cells, the antiproliferative effects of low-dose platinum were dramatically amplified (Ihrlund et al. 2008).

Another study found that intra-arterially administered 3-BP destroyed tumors while not affecting the normal hepatic parenchyma. In an era when pharmaceuticals with lesser toxicity are being developed for the treatment of liver cancer, 3-BP-mediated suppression of tumor metabolism appears to be a very appealing potent potential therapeutic option (Liapi and Geschwind 2010).

In the well-established RMT rat breast cancer model, 3-BP preferentially targets tumor cells. When compared to uptake in the tumor tissue of control rats following 3-BP treatment, selective targeting was demonstrated by significantly lower 18F-deoxyglucose absorption in the tumor tissue (Buijs et al. 2009).

The conjugation of GSH with serum proteins at thiol groups causes 3-BP to be rapidly inactivated (Sadowska-Bartosz et al. 2016). Both yeast and human cancer cells are affected by 3-BP therapy, which causes DNA damage. To begin, 3-BP activates the DNA damage checkpoint pathway, resulting in phosphorylation of the H2A histone (H2A.X in humans), a sensitive marker of both single- and double-strand breaks (Cal et al. 2020).

3-BP Reduced mitochondrial activity in yeast causes mitochondrial-dependent ROS generation, which leads to increased sensitivity to 3-BP, most likely as a result of increased ROS mitochondrial DNA damage after 3-BP treatment (Cal et al. 2021).

As a result, we may deduce that 3-BP is a glycolytic inhibitor that causes an increase in oxidative stress, which is linked to ROS generation, leading to defense mechanism activation and therefore serving as an effective CRM. Suppression of tumor metabolism by 3-BP also appears to be a powerful prospective treatment strategy. As a result, 3-BP has the potential to be a powerful and promising CRM.

4.2.3 D-Allulose

D-Allulose (D-Alu; D-psicose), a C-3 epimer of D-fructose and a rare hexose sugar found in small amounts in nature, is a C-3 epimer of D-fructose. This molecule, on the other hand, is advertised as a calorie-free functional sweetener that is simple to make at high yields from D-fructose. D-Alu enters cells via glucose transporters and suppresses glycolysis, increasing stored fat metabolism and mitochondrial respiration through AMPK. Increased respiration generates a brief rise in ROS generation, resulting in increased antioxidant activity, tolerance to oxidative stress, and survival rates (Shintani et al. 2018). The molecule was initially discovered in wheat more than seven decades ago and is found in trace amounts in a variety of foods. The

chemical is only slightly degraded after ingestion and is mostly unaltered when excreted. D-Alu serves as a mild inhibitor of α-glucosidase, α-amylase, maltase, and sucrase in the intestine (Ingram and Roth 2021).

It is a low-energy monosaccharide sugar found in trace amounts in a variety of fruits, such as kiwis (9.4 mg/100 g food), figs (29.6 mg/100 g food), and raisins (38.7 mg/100 g food). D-Alu has a 70% relative sweetness to sucrose and a calorie content of 0.2 kcal/g, which is 95% less than sucrose (Mooradian 2019). Nonenzymatic reactions during the cooking of high-sugar products, such as seasoning sauces and confectionery, can result in higher, detectable levels of D-Alu (e.g., 0.5 mg/100 g in coffee, 130.6 mg/100 g in Worcester sauce) (Oshima et al. 2006).

D-Alu enters and exits intestinal enterocytes via the glucose transporters GLUT5 and GLUT2, according to a cell culture study (Hossain et al. 2015). D-Alu enhances lifespan in *C. elegans* via increasing oxidative stress resistance, most likely through a system that is activated by at least one sort of AMPK-dependent dietary restriction mechanism. At a concentration of 10 mM, D-Alu raised the mRNA expression and enzyme activity of superoxide dismutase (SOD) and catalase by 1.4 and 2.6 times, respectively (Shintani et al. 2017a).

In a trial conducted by Franchi et al., D-Alu given in addition to a typical sucrose load resulted in a dose-dependent reduction in plasma glucose at 30 min when compared to the control. This was the largest study of D-Alu effects in Westerners (White and African American patients), showing an early dose-dependent reduction in plasma glucose and insulin levels, as well as a reduced postprandial glucose and insulin excursion in subjects without Diabetes mellitus (Franchi et al. 2021).

After consumption of D-Alu without any sugar as a control, 13 healthy men and women fasted overnight and ate a standardized meal in a random study. D-Alu increased postprandial fat oxidation while decreasing carbohydrate oxidation at a modest dose. Aside from insulin, total cholesterol, and triacylglycerol, no other parameters were changed. This suggests that D-Alu could be used as an anti-obese sweetener in humans (Kimura et al. 2017).

When rats on a high-fat diet were given D-Alu, they had less weight growth and fat buildup (Chung et al. 2012a). D-Alu preserves body weight and inhibits abdominal and hepatic fat buildup in obese mice, and is thus likely to be approved for commercial usage as a sugar alternative in diets to prevent obesity and obesity-related disorders such as hepatic steatosis and diabetes. D-Alu supplementation in the diet has a particularly positive effect on postprandial hyperglycemia (Itoh et al. 2015).

The activation of genes involved in fatty acid production inhibited fat formation. D-Alu can decrease the expression of genes involved in fatty acid production at the same time. D-Alu is a low-calorie sweetener that can give enough sweetness without causing lipid metabolic problems or major weight gain, which helps to justify its usage in foods as a natural sweetener (Chen et al. 2019).

In hyperglycemic mice fed chow or the High-Fat Diet, oral treatment of D-Alu enhanced Glucagon-like peptide-1 receptor (GLP-1) release, decreased food intake, and improved glucose tolerance. D-Alu increased insulin release and activity while reducing glucose generation, promoting glucose tolerance. Chronic D-Alu corrects

arrhythmic overeating, obesity, and diabetes in a time-dependent manner, implying that chronotherapeutic regulation of vagal afferent GLP-1R signaling may benefit the treatment of metabolic illnesses (Iwasaki et al. 2018).

Shintani et al. postulated that nuclear transport of glucokinase (HK-1) in the liver was responsible for the improvements in body composition and glucose responses (Shintani et al. 2017b).

In dogs, long-term D-Alu treatment at a dosage rate of 0.2 g/kg/day is well tolerated. Except for lipids that showed antihyperlipidemic effects, D-Alu treatment did not elicit clinical symptoms or changes in hematological or biochemical levels. The administration of D-Alu did not affect body weight. In healthy dogs, D-Alu had no cumulative impact on glucose metabolism. In conclusion, long-term D-Alu treatment has no negative consequences in dogs (Nishii et al. 2017).

A randomized controlled trial involving placebo control, low D-Alu, and high D-Alu groups revealed a significant decrease in not only body mass index (BMI), but also total abdominal and subcutaneous fat areas when compared to the placebo group. Low-dose D-Alu reduced total abdominal fat area only (Han et al. 2018a).

Natsume et al. employed the hyperinsulinemic-euglycemic clamp (HE-clamp) method to establish for the first time that D-Alu increases systemic and muscular insulin sensitivity in conscious rats suffering from a high-sugar diet. D-Alu also lowered plasma TNF-α levels and restored the insulin-induced inhibition of Akt phosphorylation in the soleus muscle and epididymal fat tissues (Natsume et al. 2021).

D-Alu increased hepatocyte HDL-cholesterol uptake while also increasing hepatic SR-B1 protein levels. D-Alu was also discovered to increase Reverse Cholesterol Transport, lowering HDL-cholesterol levels. This study raises the possibility that D-Alu could be used as a sweetener to help prevent the start of atherosclerosis (Kanasaki et al. 2020). In prediabetic rats, D-Alu reduced peripheral insulin resistance, hippocampus apoptosis, hippocampal insulin resistance, brain oxidative stress, brain mitochondrial ROS generation, brain insulin insensitivity, and hippocampal synaptic dysfunction, resulting in improved learning (Pratchayasakul et al. 2020).

Insulin sensitivity was improved, cardiac mitochondrial dysfunction was reduced, and mitochondrial dynamics were improved by D-Alu. These results indicated that D-Alu had cardioprotective properties (Pongkan et al. 2021).

To determine the maximum single dose for occasional administration, the D-Alu dose was gradually increased in stages of 0.1 g/kg BW. Until a dose of 0.4 g/kg BW for regular administration, no incidences of severe diarrhea or gastrointestinal (GI) symptoms were reported. When total daily D-Alu intake was gradually increased to 1.0 g/kg BW for regular ingestion, severe nausea, stomach pain, headache, anorexia, and diarrheal symptoms occurred. As a result, Han et al. determined that the maximum single dose of D-Alu for infrequent ingestion is 0.4 g allulose/kg BW, while the maximum total daily consumption for this sugar substitute is 0.9 g allulose/kg BW for regular ingestion (Han et al. 2018b).

A study was performed to determine if a low oral dose of D-Alu (3%) may produce toxicity. The relative weights of the liver and kidneys increased after

long-term treatment in rats. However, no data indicating observable D-Alu treatment-related toxicity were found in hematological, chemical, or histological studies (Yagi and Matsuo 2009). D-Alu orally had an LD50 of 16 g/kg in rats, according to previous research (Matsuo et al. 2002).

4.2.4 Mannoheptulose

Mannoheptulose (MH) is a rare 7-carbon sugar that inhibits both Hexokinase (HK-1 and HK-2), the first step in intracellular glycolysis, and the related liver isozyme, glucokinase (HK-4), which is the first step in the cellular metabolism of glucose to ATP and pyruvate (Ingram and Roth 2021). It is indeed considered that halting glucose metabolism triggers a biological reaction similar to what happens when we are on a diet. Specific genes are activated, which leads to greater cellular metabolism efficiency and the induction of defensive systems. MH is a primary soluble sugar found in phloem sap, leaf petiole exudates, seed, and mesocarp of avocados, and it is thought to be created during photosynthesis (Ingram et al. 2021).

MH was proposed as a therapy for hypoglycemia in investigations conducted during the 1970s. MH produced massive glucose rises with a substantial reduction in insulin when given at high doses. The suppression of glycolysis in pancreatic β-cells was thought to be the source of this activity. In rodent tumor models, high dosages of MH are effective (Ingram and Roth 2015).

Ingram et al. 2021 described a method for producing unripe avocado extract (AvX) that is high in the glycolytic inhibitor MH. The weight of MH in AvX is 14%. When mice and dogs were fed the AvX, they were able to validate the bioavailability of MH in urine and plasma. Finally, scientists found that giving AvX to mice and dogs lowered blood glucose and insulin levels (Ingram et al. 2021).

McKnight et al. discovered that in the presence of high dietary carbohydrate (CHO) relative to fat, MH increased fasting Respiratory Quotient and postprandial Energy Expenditure (5–10 h) in adult Beagle dogs, implying that MH elicits changes in energy-sensing pathways and macronutrient fuel selection. Surprisingly, the current investigation discovered that acute intake (14 days) of a low-CHO diet enhanced the incremental area under the curve (AUC) for glucose (irrespective of MH). Despite the dog's ability to adapt macronutrient oxidation to diet composition, this result remained consistent (McKnight et al. 2014).

Fasting serum glucagon-like peptide-1 and postprandial serum ghrelin were both considerably enhanced by MH (168 mg/kg). MH decreased physical activity while increasing fasting serum gastric inhibitory peptide. These results suggested that dietary MH can increase fullness while lowering daily energy expenditure (McKnight et al. 2015a).

Bodyweight, resting Energy Expenditure, or phosphorylation of skeletal muscle AMPK was not affected by the MH diet. The postprandial Respiratory Quotient and fat-to-lean-body-mass ratio were much lower in dogs fed MH. On weekends, but not on weekdays, dogs fed MH had lower physical activity during light periods (but not dark). These findings imply that MH has an effect on adult dogs' energy balance, but

that these effects are dose-independent and unrelated to physical activity (McKnight et al. 2015b).

Finally, McKnight et al. discovered no profound impacts on glucose responses or lipolysis in Labrador retrievers fed an AVX diet (6 mg/kg) for 2 weeks in a cross-over design utilizing isotope tracking methods (McKnight et al. 2018).

4.2.5 D-Glucosamine

D-Glucosamine (GlcN; 2-amino-2-deoxy-D-glucose) is a constituent of chitosan and chitin generated by arthropods, fungi, and cephalopods in nature. GlcN is produced industrially by hydrolysis of crab exoskeletons, which are primarily made up of chitin (Shintani et al. 2018). Glucosamine sulfate's long-term combination of structure-modifying and symptom-modifying effects showed that it could be a disease-modifying agent in osteoarthritis (Reginster et al. 2001). GlcN, in its phosphorylated form, GlcN-6-phosphate, is a potent hexokinase inhibitor that targets glucokinase, or HK-1, the isoform with the highest concentration in the liver (Ingram and Roth 2021).

GlcN extends *Caenorhabditis elegans* life span by affecting glucose metabolism, which activates AMPK and enhances mitochondrial biogenesis, independent of the hexosamine route. Unlike 2-DG, GlcN enhances the life span of aged mice (100-weeks old) by inducing mitochondrial biogenesis, lowering blood glucose levels, increasing amino-acid catabolism, and having no effect on food intake, body composition, or energy expenditure (Weimer et al. 2014). In a study it was discovered that GlcN causes a mitohormetic effect by causing a transitory rise in ROS, implying that GlcN could be an effective CRM (Kumar et al. 2021).

By reducing fat deposition weight and serum leptin levels, glucosamine treatment in rats was able to partially or suppress some of the effects of the High-Fat diet, resulting in a reduced rise in the insulinemic response to a glucose injection and reduced postabsorptive glycemia. GlcN-treated rats showed lower hepatic glycogen levels and only modestly decreased glucose tolerance (Barrientos et al. 2010).

Glucosamine activated autophagy via an mTOR-independent mechanism at doses ranging from 500 M to over 40 mM. Glucosamine-induced autophagy was found to be effective in removing ubiquitin-conjugated proteins and 79-glutamine repeats. As a result, glucosamine taken orally may aid in the prevention of neurodegenerative illnesses and the promotion of antiaging effects (Shintani et al. 2010). By blocking the mTOR pathway, glucosamine protects Nucleus Pulposus cells and increases autophagy (Jiang et al. 2014).

GlcN governs autophagy pathway molecular targets in vitro and in vivo, with the enhancement of autophagy mainly dependent on the Akt/FoxO and mTOR pathways. These findings suggest that GlcN is an effective autophagy activator and motivates future research on its efficacy in modifying aging-related cellular changes and supporting joint health (Caramés et al. 2013).

Katoh et al. study was the first to show that glucosamine enhances vascular endothelial function via regulating intracellular redox equilibrium in people. Thus,

glucosamine may have anti-atherosclerotic characteristics, probably due to the antioxidant capacity's improvement of endothelial function. For 4 weeks, oral glucosamine dramatically enhanced Flow Mediated Vasodilation (Katoh et al. 2017).

Takahashi's study found that feeding GlcNAc (*N*-acetylglucosamine) to F344 rats for 52 and 104 weeks had no negative effects on clinical signs or mortality, except for a slight reduction in body weight gain of more than 2.5%, which could be due to a reduction in caloric intake due to the test compound's high concentration rather than a toxic effect (Takahashi et al. 2009). In a cohort study of Washington State adults aged 50–76 years, glucosamine use was linked to lower total mortality (Pocobelli et al. 2010). Lin et al. discovered several GlcN compounds that limit HK-2 activity (Lin et al. 2016).

Because of its promising results in inhibiting biochemical pathways disrupted in a broad range of cancer cells while having no adverse effects on normal cells, glucosamine could be a unique and promising anticancer therapy (Zahedipour et al. 2017). Oral glucosamine administration had no negative effects on the blood, urine, or fecal parameters in human subjects, and it had no mutagenic or neurotoxic effects (Anderson et al. 2005). Gastrointestinal problems, such as discomfort, diarrhea, nausea, and pyrosis, are the most prevalent side effects observed with glucosamine supplementation (Salazar et al. 2014).

Humans tolerate glucosamine well and it has no significant negative impact on glucose homeostasis or metabolism. Glucosamine and its derivatives have a wide range of biochemical and pharmacological effects, with anti-inflammatory and antioxidant qualities accounting for the vast majority of their applications. In addition to its therapeutic uses, glucosamine has been shown to improve health markers in inactive women and to reduce the risk of overall and cause-specific death (Dalirfardouei et al. 2016).

4.2.6 Iodoacetate

Other chemicals inhibit glycolysis therefore there could be a lot of CRM candidates. Inhibiting a specific step in the glycolysis pathway could be one of their objectives. Glyceraldehyde-3-phosphate dehydrogenase, for example, is inhibited by iodoacetate (Mattson et al. 2001).

Guo et al. found that iodoacetate had a considerable cytoprotective effect in cultured hippocampus neurons under conditions that are significant to the pathophysiology of numerous neurodegenerative diseases. Pretreatment of cultured hippocampal neurons with Iodoacetate provides protection from cell death caused by glutamate, iron, and trophic factor withdrawal, suppresses oxyradical production, regulates mitochondrial function in neurons after oxidative insults, and upregulates heat-shock proteins HSP70 and HSP90, as well as the anti-apoptotic protein Bcl-2 in neurons. Interestingly, iodoacetate concentrations far lower than those required to block glycolysis provided protection (Guo et al. 2008).

4.2.7 Chrysin, Genistein, Astragalin, and Resveratrol

Several phytochemicals have been studied as HK-2 inhibitors and anticancer agents (Akins et al. 2018), with a few of them brought up below.

Chrysin (5,7-dihydroxyflavone) is a type of natural polyphenol found in honey, propolis, and a variety of medicinal plants and fruits, including bitter melon (*Momordica charantia*) and wild Himalayan pear (*Pyrus pashia*) (Stompor-Gorący et al. 2021). Wojnar et al. found that chrysin lowered oxidative stress in the lenses of male type 1 diabetic rats by reducing antioxidative enzyme activity, lowering levels of oxidative damage indicators, and enhancing the oxidative stress index (Wojnar et al. 2020).

By suppressing the activities of Alcohol Dehydrogenase, Cytochrome P450 2E1, Xanthine Oxidase, and catalase, chrysin protects the liver and kidney of Wistar rats against oxidative damage caused by prolonged ethanol use (Tahir and Sultana 2011). Chrysin enhanced collagen I secretion and showed anti-photoaging activity by reducing collagen I degradation, repairing oxidation damage, and lowering the rate of senescence in Human Dermal Fibroblasts, suggesting that it could be used as a functional cosmetic agent due to its anti-photoaging and anti-melanogenesis properties (Zhu et al. 2016).

The use of chrysin may help to slow tumor growth. The mechanism was ascribed to decreased glycolysis and apoptosis driven by the reduction of HK-2 expression (Xu et al. 2017). In diabetic mice, oral administration of chrysin resulted in considerable control of proteinuria and aberrant alteration of glomerular ultrastructure. It also enhanced the induction of slit diaphragm protein (podocin/nephrin) in diabetic glomeruli (Naz et al. 2019). In terms of metabolic effects, employing the Streptozotocin model of diabetes in mice (Ramírez-Espinosa et al. 2017) and rats (Samarghandian et al. 2016), a few studies have indicated favorable benefits for glucose and insulin parameters as well as oxidative stress. It is promoted as a dietary supplement, although it has low oral bioavailability and does not cause any harmful side effects at the levels tested (Hofer et al. 2021).

Another phenolic glycolysis inhibitor, **Genistein** (4′,5,7-trihydroxyisoflavone), is found in a variety of foods, including soy-based products (mature soybeans contain 5.6–276 mg/100 g), legumes (0.2–0.6 mg/100 g), fruits, nuts, and vegetables. It has a high solubility in polar solvents but low solubility in water, has a bitter taste, and has poor oral bioavailability (Spagnuolo et al. 2015) which can be alleviated by utilizing genistein's glycoside form (Kwon et al. 2007).

Tao et al. examined the anticancer properties of genistein and Gen-27, a flavonoid that was recently synthesized. They found that applying Gen-27, but not genistein, to a range of human breast cancer cell lines suppressed the growth and proliferation of the cultures in a concentration and time-dependent manner (Tao et al. 2017).

In US, men and women who generally consumed low to moderate amounts of soy foods, a prescribed amount of Genistein intake was linked to a decreased risk of type 2 diabetes (Ding et al. 2016; Rienks et al. 2018). Oral genistein administration showed no effect on hypertension in adults. However, genistein administration for 6 months or longer greatly increased the effectiveness of genistein in metabolic

syndrome people (Hemati et al. 2020). Higher levels of genistein were linked to a decreased risk of breast cancer and diabetes (Rienks et al. 2017).

Pokeweed and ferns contain **Astragalin**, which is a 3-O-glucoside of kaempferol. In Chinese herbal medicine, it is a common ingredient. In a variety of animal models, the chemical has proved to be effective against cancer (Ingram and Roth 2021). Although astragalin is abundant in the root of Astragalus membranaceus (Kwon and Park 2012), the non-glucoside form kaempferol has been weakly linked to anticancer properties (Mohammadi et al. 2016). Li et al. investigated whether Astragalin may limit Hepatocellular Carcinoma (HCC) cell growth in vitro and in vivo, revealing significant glycolytic inhibition via HK-2 reduction. They found that applying astragalin to HCC cells suppressed their proliferation significantly in cell culture experiments. Similarly, in mice models using HCC xenografts, that given astragalin had minimal tumor growth (Li et al. 2017).

Astragalin reduces the enhanced expression and activity of Protein Tyrosine Phosphatase 1B in the skeletal muscles of streptozotocin-treated diabetic rats, allowing it to have insulin-sensitizing and hypoglycemic effects (Wu et al. 2005). Its kaempferol form is also said to have anti-diabetic properties (Munhoz and Frode 2017). Although the authors of this meta-analysis indicated that more high-quality trials are needed to prove the clinical efficacy and safety of Astragalus in the treatment of diabetes, as adjuvant therapy, it may be beneficial for blood glucose management in individuals with Type 2 Diabetes Mellitus (Tian et al. 2016).

In the diabetic mouse model (KK^{ay}), astragalin can control part of the insulin signaling in insulin-resistant skeletal muscle, suggesting that it could be a promising insulin sensitizer for the treatment of type 2 diabetes (Liu et al. 2010).

Resveratrol (3,40,5-trihydroxytrans-stilbene) is a phytoalexin present in a variety of plants, notably grapes and berries, that has potent anticancer properties (Brimson et al. 2021). It was one of the first and most widely studied potential CRMs, with a variety of antiaging benefits but no substantial impact on longevity in mice fed a high-fat diet. The interaction of this chemical with sirtuin genes is the mechanism behind many of its positive effects (Ingram and Roth 2021).

Resveratrol has also been shown to inhibit HK-2, implying that it is a glycolytic inhibitor. Resveratrol, for example, made aerobic glycolytic HCC cells more susceptible to apoptosis, reducing cell growth and proliferation (Dai et al. 2015). Another study found that resveratrol inhibits tumor growth in a xenograft mice model by targeting HK-2-mediated glycolysis (Li et al. 2016). Resveratrol is a strong CR mimic with anti-diabetic and cardiovascular effects in mice and humans (Chung et al. 2012b) (Fig. 4.3).

4.3 Conclusion

CRM research is quickly increasing to study a wide range of prospective candidates with the potential to improve both healthspan and lifespan without the calorie restrictions imposed by CR. Until now, almost all CRM candidates have been focused on inhibitors of hexokinase and phosphoglucose isomerase, the first two

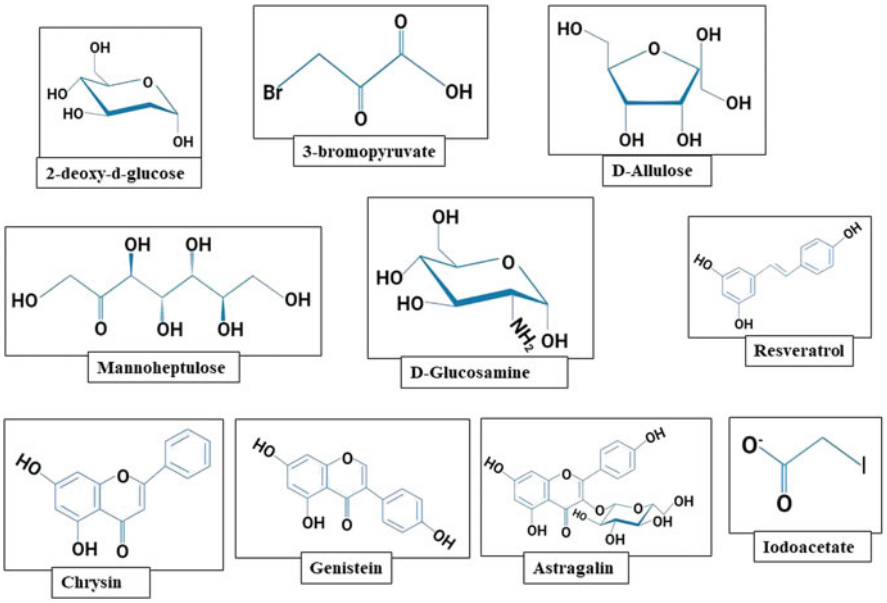

Fig. 4.3 Chemical structures of CRMs

steps in glucose metabolism. A few well-known natural chemicals, including glucosamine, resveratrol, mannoheptulose, and D-allulose, have been proposed as candidates, while others, such as 2-deoxy-D-glucose and 3-bromopyruvate, are synthetic molecules. A few options, such as D-allulose and glucosamine, have been tested in clinical trials with modest success; nevertheless, no candidate has emerged as a commercial product. More clinical trials will undoubtedly be required to assess the efficacy and safety of prospective drugs. In addition, numerous more options targeting different processes in intracellular glycolysis are anticipated to emerge.

In our review, we focused on glycolytic inhibitory targets because we believe that such therapies will most closely resemble the metabolic activities of CR, namely, eliciting cellular responses to a perceived reduction in energy output.

Finally, as more preclinical and clinical evidence accumulates, CRMs emerge as a promising future subject of research that clinical and nutrition experts alike should investigate in depth. We believe that the concept of CR mimetic has opened up a slew of new pro-longevity options. As these approaches grow more popular and research geared toward this notion intensifies, we expect many new approaches to be explored and potential candidates to be identified in many laboratories.

4.4 Future Perspective

Although no single prospective mimic appears to generate all of the positive effects of genuine CR, cocktails comprising a combination of these compounds appear to be viable and may ultimately be the most effective strategy. It will be fascinating to examine how the substances mentioned here contribute to the positive impacts of well-studied healthy diets. Finally, amounts of dietary CRM candidates might be improved in both existing and freshly established healthy diet plans.

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Anti-aging and Rejuvenation Based on Stem Cell Therapy

5

Mario F. Muñoz, Francesco Marotta, Amir Moghadam Ahmadi, Azam Yazdani, Fang He, Roberto Catanzaro, Cristina Garzón-Rodriguez, and Antonio Ayala

Abstract

Stem cells are present in the tissues and organs. These cells remain in a quiescent and undifferentiated state until it is physiologically necessary to produce new descendant cells or when a disease or tissue damage activates their proliferation with the aim of repairing the tissue. In response to damage, stem cells secrete components of the extracellular matrix components, paracrine factors, and extracellular vesicles, primarily exosomes, and can also increase their own pool and/or differentiate. The pluripotency and immunomodulatory features of stem cells

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could potentially be an effective tool in cell therapy and tissue repair for both autologous and allogeneic use. In the specific case of mesenchymal stem cells of adipose tissue, these can be isolated in large amounts from the biological material obtained by liposuction.

Aging affects the capacity for self-renewal and differentiation of stem cells. This is why there is a decrease in the potential for regeneration. Aging involves the loss of optimal functions of the organism over time. Cell therapy could potentially be one of the most promising therapies to control aging due to the fact that single stem cell transplantation can regenerate or substitute the injured tissue. Stem cells can be used well both to prevent diseases and for the treatment of any of their clinical stages due to the ability of stem cells to recognize and migrate to sites of damage from the circulation in response to signals that are activated in damaged tissues. To understand the involvement of stem cells not only in tissue maintenance and disease but also in the control of aging it is important to know and identify their properties, functions, and regulation in vivo, which are addressed in this chapter.

Keywords

Adipose tissue · Stem cells · Aging · Rejuvenation

5.1 Introduction

Several years have passed since stem cells (SCs) began to be discussed but it has been in the last decade that there have been real advances in the application of cell therapies. This progress has been marked by the development of the techniques and regulations of each country. Practically, all medical specialties have considered the use of SCs, especially those that can use a local injection of SCs. In 2012, there were 220 registered clinical trials and 1138 in 2020. Unfortunately, very few translate into published results (Liu et al. 2020). The challenge of cell therapies is to demonstrate that they produce better results than conventional therapies and those future treatments are less empirical.

5.2 Definition and Classification of the SCs

Stem cell is a general concept that refers to the undifferentiated cells present in the tissues and organs of living beings, which can proliferate more or less actively depending on their location, maintaining their undifferentiated state, or becoming, in response to certain physiological or experimental conditions, in certain cell types of the tissue where they are found naturally or in tissues where they are implanted. Therefore, when divided, SCs have the ability to remain as a stem cell or differentiate into some specific type of cell.

In most tissues, there are mechanisms of self-repair/regeneration carried out, mainly, by these resident stem cells with the ability to differentiate and give rise to new cells to replace those that have been damaged, which are lost by apoptosis or necrosis, naturally, by damage or disease thus contributing to the tissue repair where they reside.

The classification of stem cells can be made according to different criteria (Zakrzewski et al. 2019). For example, they can be classified according to their potency (differentiation capacity) into totipotent, pluripotent, multipotent, oligopotent, and unipotent as they can be differentiated into any, a limited range, or into a single cell type.

It can also be grouped according to its source into embryonic, fetal, and adult. In the embryo, stem cells produce many types of cells that give rise to different tissues and organs. Adult stem cells, which are also called somatic stem cells, reside in most mammalian tissues. Their presence in almost all tissues of the body is associated to their perivascular localization. On the other hand, there are the so-called induced pluripotent stem cells (IPSCs) that are obtained in vitro from somatic cells.

According to their lineage, SCs are mainly classified into hematopoietic and mesenchymal.

1. Hematopoietic cells are multipotent cells that can be differentiated into different types of blood cells, including myeloid and lymphoid lineage cells. They can be found in various organs such as peripheral blood, bone marrow, and umbilical cord blood.
2. Mesenchymal stem cells (MSCs), also known as multipotent stromal cells or mesenchymal stromal cells, are multipotent no hematopoietic adult stem cells that are present or reside in the stroma of virtually all tissues, including bone marrow, adipose tissue, and Wharton's jelly. Their physiological role is to support the repair and regeneration of tissues.

They are called *mesenchymal cells* because they can be differentiated into various mesoderm-type cells, such as osteoblasts, chondrocytes, and adipocytes, depending on the microenvironment in which they are and their signaling and interaction with the extracellular matrix.

These cells remain in a quiescent and undifferentiated state until it is physiologically necessary to produce new descendant cells or when a disease or tissue damage activates their proliferation with the aim of repairing the damaged tissue in which they reside. Since these repair mechanisms are not always sufficient, the pluripotency and immunomodulatory features of MSC could potentially be an effective tool in cell therapy and tissue repair for both autologous and allogeneic use.

These cells have the ability to self-renew in vitro stably through several passages, adhere to plastic, and are phenotypically well described, expressing CD29, CD73, CD90, and CD105, while negative for hematopoietic markers such as CD14, CD34, and CD45 (Rastegar et al. 2010). They can also modulate the immune system, secrete trophic factors, and selectively migrate to injured tissues.

5.3 The Biological Role of Stem Cells and Metabolic Features of SCs

To understand the involvement of SCs in tissue maintenance and disease, it is important to know and identify their properties, functions, and regulation in vivo, which may be different from culture conditions. For example, SCs can differentiate in vitro into different cell types, but it is not clear that this occurs in vivo. Therefore, it is necessary to distinguish between its physiological and nonphysiological properties (Joseph and Morrison 2005).

In the specific case of mesenchymal SCs of adipose tissue, these can be isolated in large amounts from the biological material obtained by liposuction, unlike those that are located in other tissues in very small quantities. Adipose tissue is basically composed of adipocytes that represent 90% of the total volume (Cohen and Spiegelman 2016). In addition, it contains different types of cells collectively called stromal vascular fraction (SVF) which include MSCs (in this case also called adipose tissue stem cells, ADSC), endothelial precursor cells, endothelial cells, macrophages, smooth muscle cells, lymphocytes, pericytes, and preadipocytes. All SVFcells are held together by the extracellular matrix. ADSCs are obtained from different abdominal regions, and the performance will depend on the area where adipose tissue was obtained. When obtained by a standard liposuction procedure, SVF is composed of about 5 million cells, of which 2–3% are MSCs. In relation to the localization of ADSC, some authors believe that they are in the vasculature of adipose tissue, while for others they reside in perivascular localization (Si et al. 2019).

In clinical applications, this SVF is generally used for different treatments. In research, they usually go further and SCs are specifically isolated using a magnetic separation system (Muñoz et al. 2018).

Once isolated, it is necessary to ensure that the cells meet the criteria of International Society of Cellular Therapy (ISCT) and that they are capable of differentiating into different cell types (Muñoz et al. 2018). We must bear in mind that, in vivo, these ADSCs will differentiate only in adipocytes and not in osteoblasts, for example. The involvement of these ADSCs in the maintenance of adipose tissue is important. Not long-ago adipose tissue was considered simply a mere deposit of fat, a thermal insulator or energy reserve, or for the mechanical protection of tissues and organs. Now, we know that it interacts with different tissues and systems and plays a central role in systemic metabolism and physiology. Thus, adipose tissue secretes many molecules of metabolic importance, including TNF alpha, adiponectin, resistin, and RBP4 among others. In addition, a healthy adipose tissue is necessary for proper metabolism control (Choe et al. 2016).

On the other hand, it should be taken into account that although they have been identified in many tissues, SCs are not necessarily required for their maintenance. For example, most of the liver's regenerative capacity is carried out from differentiated hepatocytes, which retain tremendous proliferative potential. This does not exclude the possibility that SCs also participate in tissue repair in certain

types of damage. However, if the goal is purely therapeutic, the origin and physiological significance of SCs are not too much relevant.

Regarding the metabolic characteristics of SCs, there are some studies showing that these cells have a metabolic profile different from specialized cells (Meacham et al. 2022). This seems reasonable since, supposedly, the main role of these SCs is to maintain and repair the tissue in which they reside. Then, the SCs that are responsible for tissue regeneration have to be resistant to signs of damage and be specifically activated by factors that are related to tissue damage to promote regeneration through different mechanisms.

In response to damage, SCs secrete components of the extracellular matrix components, paracrine factors, and extracellular vesicles, primarily exosomes, and can also increase their own pool and/or differentiate. Another important SCs response to damage is the activation of an immunomodulation program.

It has been suggested that autophagy activation mechanisms in SCs could confer protection against cell death under tissue damage conditions (Meacham et al. 2022; Sagaradze et al. 2020). Moreover, it has been shown that SCs contain high glutathione levels which is also responsible for the resistance to cytotoxic substances accumulated during harmful events (Sagaradze et al. 2020). It has also been reported that ADSCs have telomerase activity to some degree (Hiyama and Hiyama 2007).

5.4 Intracellular Signaling Pathway in Stem Cells (Self-Renewal and Differentiation)

Undoubtedly, one of the most well-tuned processes in SCs is the intracellular signaling pathways related to self-renewal and differentiation. It is necessary that SCs maintain pluripotency along the time with continuous self-renewal divisions. At the same time, they have to be ready to differentiate under specific biological conditions.

The aim of the self-renewal process is to perpetuate the pool of SCs for life. It is a cell division with the maintenance of an undifferentiated state. The self-renewal capacity is essential for stem cells to expand their numbers during development, and restore the pool of stem cells after injury, since defects in self-renewal mechanisms could lead to developmental defects, such as premature aging phenotypes or cancer. Although stem cells have extensive self-renewal potential, they can only undergo a limited number of divisions (Foudi et al. 2009; Kiel et al. 2007; Wilson et al. 2008).

Unlike embryonic stem cells, adult stem cells have a large but limited potential for self-renewal. This requires a new set of tissue-specific self-renewal programs to control cell cycle. In many cases, these programs must perpetuate stem cells throughout life, in response to developmental changes and/or tissue regeneration in future physiological events. They will need mechanisms that give them the possibility of having repeated periods of quiescence and reentry into the cell cycle. This implies a more complex regulation of the cell cycle in adult SCs with more control and regulation mechanisms than in ES cells (He et al. 2009).

Most adult SCs reside within specialized microenvironments known as niches (Morrison and Spradling 2008). These niches provide an anchor site, produce signals that regulate cell survival, cell cycle status, and differentiation. But its main goal is to keep SCs undifferentiated. For unknown reasons, many SCs require intermittent periods of quiescence for maintenance. Loss of quiescence signaling leads to increased proliferation and depletion of SCs (He et al. 2009; Kobielak et al. 2007).

It has been described three pluripotency factors (Klf4-Sox2-Oct4) and one proliferative factor (cMyc) that confer to SCs the status of pluripotent. These factors are also known as "Yamanaka-Factors." However, over the years, other proteins have taken relevance in maintaining pluripotency, such as Nanog, which means "earth forever young" in the ancient Celtic language. Indeed, Nanog-deficient mice are prone to differentiate spontaneously in their ESCs (Chambers et al. 2003; Mitsui et al. 2003). The Oct4-Sox2-Nanog network forms the core of a regulatory circuit that promotes the expression of genes that maintain pluripotency, while inducing the repression of these genes promote differentiation. Oct4, Sox2, and Nanog regulate their self-expression, as well as the others, forming a positive feedback loop. The network needs to be adjusted for positive and negative regulation because a slightly hyper or hypoactivation of any of these factors can interrupt the state of pluripotency (Niwa et al. 2000).

Another critical transcription factor is the Ronin protein. Ronin suppresses ES cell differentiation by binding directly to differentiation-inducing genes including GATA4 and GATA6. During the differentiation process, Ronin (Dejosez et al. 2008) and Nanog can be inactivated by proteolysis through the action of caspase-3 (Fujita et al. 2008).

Although this process has been simplified, other proteins are involved in these pluripotency and differentiation signaling pathways. SIRT1 mediates the deacetylation of Sox2, helping to maintain the activity of this transcription factor in the nucleus and allowing delay of cell senescence (Yuan et al. 2012). Using small interference RNA for *SIRT1* expression, acetylation of Sox2 is induced, promoting its cytosolic translocation from the nucleus and posterior ubiquitination, leading to its degradation in the proteasome (Yoon et al. 2014). SIRT1 activation by resveratrol can reverse these effects by enhancing colony-forming ability and increasing differentiation potential into osteogenic and adipogenic lineages in a dose-dependent manner (Yoon et al. 2014; Choi et al. 2019). Levels of SIRT1 decrease during differentiation processes (Shakibaei et al. 2012; Simic et al. 2013) and age (Muñoz et al. 2020). Based on these, SIRT1 is shown to play a crucial role in maintaining the self-renewal state in SCs, losing the ability of keeping the stemness in SCs with aging.

Despite the importance of Sox2 expression in maintaining quiescent and self-renewal states in MSCs, it seems that Sox2 also plays a dual role in osteogenic and adipogenic differentiation. Induction of adipogenesis requires moderate levels of Sox2 and YAP expression which acts as a rheostat to regulate the determination of MSC fate, while the SOX2 gene must be suppressed to induce Wnt/β-Catenin signaling pathway activation for osteogenesis induction (Seo et al. 2011).

If there are low levels of Sox2 or YAP1, adipogenic differentiation and PPARγ induction are prevented; however, Sox2 overexpression may compensate for the decrease in YAP1 and allow adipogenesis, suggesting that Sox2 and YAP1 could have overlapped functions (Seo et al. 2013). YAP1, similar to Sox2, blocks osteogenic differentiation and antagonizes the Wnt signaling pathway through β-Catenin binding and Dkk1 protein induction that inhibit the Wnt pathway. Although Sox2 can also directly induce Dkk1 expression (Park et al. 2012), suggesting that Dkk1 is synergistically regulated by Sox2 and YAP1. Some authors suggest that Sox2 regulates the fate of the osteoadipo fate lineage in MSCs by promoting adipogenesis that regulates YAP1 expression, a transcriptional effector that is restricted by the Hippo pathway (Seo et al. 2013); however other authors state that Sox2 is a potent inhibitor of both adipogenic and osteogenic differentiation (Schönitzer et al. 2014). Regarding chondrogenic differentiation, it is necessary suppress Sox2 expression and activate other components of the Sox family, like Sox9, Sox5, and Sox6, as well as Twist1, TCF1, FOXO3A, Dlx4, Nessy, Sox13, and Tbox6 (Park et al. 2012; Robert et al. 2020; Razmara et al. 2021).

Other proteins of prominent relevance in aging also play an important role in differentiation signaling pathways. SIRT1 is negatively correlated with adipogenesis in preadipocytes. Considering that adipocytes differentiate from MSCs, differentiation may be influenced by SIRT1. In vitro activation of SIRT1 activation in vitro could alter the differentiation fate of MSCs, promoting osteogenic versus adipogenic differentiation (Yuan et al. 2012; Shakibaei et al. 2012; Peltz et al. 2012). In a SIRT1+/− mouse model, bone formation is decreased and adipogenesis is increased, resulting in an evident reduction of bone mass (Zhang et al. 2010) besides reduced differentiation toward osteoblasts and chondrocytes (Simic et al. 2013).

Part of the intervention of SIRT1 in differentiation processes may be due to its deacetylation capacity, acting on Runx2 (Shakibaei et al. 2012) and β-Catenin (Gaur et al. 2005). Although, it seems that PPARγ could also be deacetylated by SIRT1 (Han et al. 2010). A recent study suggests that deacetylating PPARγ at Lys293 and Lys268 by SIRT1 induces brown adipose tissue (Qiang et al. 2012). Resveratrol (3,4′,5-trihydroxystilbene) has been described as capable of promoting both dose-dependent osteogenic differentiation and time-dependent adipogenic differentiation (Pacholec et al. 2010). Resveratrol-inhibited adipogenic differentiation at a high dose (10 mM) during short-term exposure, but increased adipogenic potential at a lower dose (0.1–5 mM) when cells were exposed for longer periods (Peltz et al. 2012). Although, other authors suggest that resveratrol improves both osteogenic and adipogenic potential because SIRT1 directly regulates Sox2 to maintain self-renewal and multipotency (Yoon et al. 2014).

Another protein that appears to be involved in promoting osteogenic differentiation and suppressing adipogenic differentiation is AMPK (Kanazawa et al. 2009; Vingtdeux et al. 2011). There are different processes by which AMPK can be involved in these pathways. For example, compound C, an AMPK inhibitor, promotes lipid formation as a consequence of activation of specific adipogenic genes, and a decrease in mineralized matrix deposition by inhibition of specific osteogenic genes during MSC differentiation (Kim et al. 2012) indicating that

AMPK favors osteogenic differentiation at the expense of adipogenic differentiation. Several signaling pathways could be involved in AMPK-mediated differentiation of MSCs. One signaling pathway is through ERK, its activation could favor the differentiation of MSCs toward the osteogenic lineage at the expense of the adipogenic lineage (Jaiswal et al. 2000). Furthermore, AMPK could regulate osteogenic differentiation by also inhibiting the mTOR pathway (Pantovic et al. 2013). It even seems that it could also regulate the transcription of β-Catenin (Zhao et al. 2011). Recent studies also show that AMPK is preferentially associated with osteogenic commitment inducing RUNX2 phosphorylation, whereas the lack of this phosphorylation leads to adipogenesis (Chava et al. 2018).

It is necessary to take into account that SIRT1 and AMPK can interact with each other in differentiation processes. AMPK activity could be regulated by LKB1, CaMKKβ, and TAK1, which could also be modulated by SIRT1 activity (Carling et al. 2008; Herrero-Martín et al. 2009). During differentiation, SIRT1 could deacetylate LKB1 while AMPK could positively regulate Nampt expression and activity, an enzyme necessary for SIRT1 to exert its deacetylase activity (Cantó et al. 2009).

5.5 Effect of Aging on Stem Cells

Aging affects the capacity for self-renewal and differentiation of SCs (Ermolaeva et al. 2018). This is why there is a decrease in the potential for regeneration. As we have already seen, many molecules support the biochemistry of SCs. Two of the most relevant to maintain the capacity for self-renewal and pluripotency are Nanog and Sox2. In addition, SIRT1 and AMPK, critical regulators of a large number of cellular processes, play an important role in the differentiation of SCs.

Both transcription factors and the two longevity pathways tend to decline with age. While this is the trend, there is great inter-individual variability (Muñoz et al. 2020). Thus, we found older patients still retain better levels of these proteins than younger patients, so it would be necessary to determine the levels of these proteins individually in SCs of each donor before their use in Regenerative Medicine.

Oxidative stress is one of the factors involved in the biological decline of MSCs (Denu and Hematti 2016). Therefore, it is important to know whether the viability of the SCs is affected by oxidant compounds, since the location where they will be injected is usually a site with high levels of oxidation and inflammation. Previous studies have shown that the sensitivity of SCs is different in each patient and that small variation in the degree of oxidative stress greatly affect the viability of SC in some patients, but not in all of them (Muñoz et al. 2020).

CSCs also undergo the process of cellular senescence, which leads to alteration of its functions and a progressive decrease in tissue maintenance and regeneration of tissues (Sagaradze et al. 2020). This senescence affects the biology of SCs. For example, MSCs senescence implies reduced osteogenic capacity, which may contribute, for example, to osteoporosis observed in old organisms.

In addition, it has been observed that MSCs derived from old donors showed dysfunction to stimulate vascularization because of a lack of proangiogenic factors production (Efimenko et al. 2014). Another fact is SCs derived from obese donors have low self-renewal capacity induced by oxidative stress, affecting mitochondria, producing DNA damage, telomere shortening, low proliferative rates, and apoptosis (Pérez et al. 2015).

5.6 Stem Cell Therapeutic Strategies

MSCs have the ability to easily expand in vitro when isolated from their niche in vivo, selectively migrate to injured tissues, modulate, and evade the immune system, and secrete trophic factors that aid tissue repair (Sagaradze et al. 2020).

Considering aging as the loss of optimal functions of the organism over time, cell therapy could potentially be one of the most promising therapies to control aging due to the fact that single stem cell transplantation can regenerate or substitute the injured tissue. This suggests the possibility of having body replacements on demand as we age. From a clinical point of view, we are far from that utopia yet; however, some clinical applications to treat age-related diseases with stem cell therapy have been successful.

The most versatile cells are embryonic stem cells (ESCs) (Burns et al. 2009; Ilic and Ogilvie 2017). These cells can differentiate into all types of adult cells (Ilic and Ogilvie 2017; Scheffler et al. 2003). However, its application has some ethical implications such as the destruction of human embryos (Bajada et al. 2008; Hipp and Atala 2008) and the potential to elicit an immune response because its use implies an allogeneic source (Hipp and Atala 2008). iPSCs, on the contrary, solve part of these problems because they do not have ethical issues and autologous transplantation is possible (Hanatani and Takasu 2021). However, both have been described to have a high degree of tumorigenesis, especially due to the formation of teratomas in vivo after transplantation (Bajada et al. 2008; Hipp and Atala 2008). Currently, this inconvenience has been mitigated by differentiating ESCs and iPSCs to target cells in vitro previously to transplant into the target organ or tissue. This manufacturing process requires extensive control and safety tests during all steps to fulfill the health agencies' commitment (Hanatani and Takasu 2021; Piao et al. 2021). Due to the cost and complexity of scaling out manufacturing systems, most of the current clinical trials are observational studies to assess the efficacy and safety of ESCs and iPSCs, and far from their approval to be commercialized as advanced therapy medicinal products (ATMPs) (Madrid et al. 2021).

Taking into account these concerns, some authors have focused their attention on the use of adult SCs, especially MSCs. Although they do not retain the pluripotency of ESCs, MSCs do not have ethical implications, they have a lower risk of producing abnormal proliferation and better control under culture conditions. MSCs have the potential to differentiate into mesodermal lineage tissue like bone, cartilage, tendons, skeletal muscle, and adipose tissue under specific cell culture conditions (Gimble et al. 2007; Poloni et al. 2013; Han et al. 2015). Recent studies showed that MSCs

have the ability to differentiate into neurons, pancreatic endocrine cells, hepatocytes, endothelial cells, and cardiomyocytes (Muñoz et al. 2018, 2019; Lo Furno et al. 2018; Refaie et al. 2021; Schäffler and Büchler 2007). For clinical application, different tissues have been used as sources of MSCs. However, the two most used have been bone marrow stem cells (BMSCs) and adipose-derived stem cells (ADSCs) due to the availability and ease with which they are obtained. Specially ADSCs, where the amount of MSC is 500 times higher than in bone marrow (Fraser et al. 2006). The higher frequency of stem cells, easy access, and abundance of adipose tissue facilitate cell culture conditions to reach faster growth rates for expansion protocols (Schäffler and Büchler 2007; Fraser et al. 2006). This contributes to the possibility of obtaining a large number of cells without the need to expand them excessively, reducing the risk of induced senescence or chromosomal abnormalities, and preserving their pluripotency in vitro (Tarte et al. 2010).

In fact, these cells or the biological materials enriched with SCs are widely used in plastic and reconstructive surgery as filler material, for tissue reconstruction, and facial rejuvenation due to their ability to differentiate into endothelial and epithelial cells, as well as secrete cytokines and growth factors that promote angiogenesis through paracrine mechanisms, and cell-cell interactions thus improving neovascularization and accelerating wound healing (Hassanshahi et al. 2019).

Other highly demanded applications are to solve bone problems and regenerate cartilage using SCs directly or in combination with different types of scaffolds (Al-Ghadban et al. 2021).

They have been used for human spinal cord injuries, by intrathecal autologous transplantation of adult SCs with a follow-up of 8 months. None of the patients developed serious adverse effects after the transplant procedure and several of them showed an improved ASIA motor score, ten patients showed an improved ASIA sensory score, and two patients who had no control over the sphincters recovered it after 1 and 4 months after treatment (Hur et al. 2016).

Autologous and allogenic ADSC transplantation has also been used successfully in patients with complex perianal fistulas (Garcia-Olmo et al. 2008; Panés et al. 2016, 2018); in patients with community-acquired bacterial pneumonia (Laterre et al. 2020), knee osteoarthritis (Freitag et al. 2019; Lee et al. 2019), diabetes complications as retinopathies (Gaddam et al. 2019), foot ulcers (Cao et al. 2017) and critical limb ischemia (Soria-Juan et al. 2021), wound repair (Shingyochi et al. 2015), and myocardial infarction (Li et al. 2021; Ma et al. 2017). In this case, percutaneous transendocardial injection with a catheter or by injection of 100 million percutaneous femoral cells guided to the aorta-left ventricle has been used. MSCs were preferentially attracted to and retained in ischemic tissue but not in the remote or intact myocardium. This suggests that injured tissue might express specific receptors or ligands to facilitate trafficking, adhesion, and infiltration of MSCs to the site of injury, but these may be downregulated a fairly short time after injury occurs.

Even its possible use is currently being investigated in patients with a poor prognosis in COVID-19 because it can ameliorate acute respiratory distress syndrome (Sánchez-Guijo et al. 2020; Wang et al. 2021). Other clinical applications of

ADSCs have been related to their use as cell basement to fill calvaria bone defects (Lendeckel et al. 2004), breast reconstruction (Fang et al. 2021), erectile dysfunction following radical prostatectomy (Haahr et al. 2016), and stress-induced urinary incontinence (Trounson et al. 2011).

5.7 Molecular Mechanisms of the Regenerative Effect of SCs

Different mechanisms have been postulated by which MSCs can repair and regenerate tissues apart from differentiation. In fact, it is believed that its regenerative effect is more due to the release of cell factors (paracrine effect) (Al-Ghadban et al. 2021).

MSCs transplanted into damaged or diseased tissue can secrete cytokines and growth factors that stimulate recovery in a paracrine manner (Cai et al. 2020; Mazini et al. 2020; Ooi et al. 2015). MSCs could modulate the host's "stem cell niche" by stimulating endogenous stem cell recruitment and promoting their differentiation into the required cell lineage (Song et al. 2013; Yagi et al. 2010). ADSCs are more resistant to oxidative stress than standard cells (Muñoz et al. 2020; Chen et al. 2011; Kim et al. 2009). This is crucial for the survival of engrafted cells where an oxidative environment is predominant, such as injured, ischemic, or inflamed tissue. ADSCs also play an important role as an immunomodulator (English and Mahon 2011). Therefore, ADSCs can activate or suppress the immune response. A plausible explanation of the dual behavior offered by MSCs against inflammatory stimuli is the possibility that there are heterogeneous populations of MSCs in vivo with different responses to TLR activation, creating divergent answers. Some MSCs would regenerate the damaged tissue while others would remain quiescent, to later activate self-renewal processes, maintaining reserves of MSCs in the tissues (Levin et al. 2014). This mechanism implies that chronic inflammation could lead to stem cell exhaustion as the hallmark of aging (Muñoz et al. 2020; López-Otín et al. 2013).

This immunomodulatory ability has been used in many clinical applications related to uncontrolled inflammatory process. In multiple sclerosis and acute graft versus host disease, ADSCs showed immunosuppressive effects (Fernández et al. 2018; le Blanc et al. 2008; le Blanc and Ringdén 2007). Thus, for example, in a rodent model it was shown that ADSCs injected intravenously act by suppressing the autoimmune response in the early stages of the disease and inducing local neuroregeneration in animals with multiple sclerosis (Constantin et al. 2009).

5.8 Routes of Administration of the SCs

As with any therapeutic alternative, local or systemic application has been considered for the use of MSCs (Parekkadan and Milwid 2010). SC can be applied directly to damaged sites. In principle, this local application would be indicated to repair a specific problem in a specific location. These local applications are used, for example, in traumatology, plastic surgery, for wounds that are difficult to heal, and for myocardial infarction.

An important question is whether, after the local injection, SCs survive at the engraftment site for a long time and whether they differentiate into specialized cells. Using several antibodies bound to fluorescent dyes to detect specific types of cells in brain slices, it can be seen that after local injection into the substantia nigra the SCs are still there after 6 months (Muñoz et al. 2020). In addition, after this period, SCs begin to differentiate into neurons and glial cells at the injection site. Maybe 6 months seems like a long period of time, but it is necessary to keep in mind that what is expected from the SCs is that a cell originally located in a tissue (adipose tissue) behaves differently from how it behaves normally and also that it behaves as we want. Therefore, these cells need time to adapt to their new environment.

In relation to the systemic administration of SC, it would be indicated when the anatomical area to be repaired is extensive or when there are several tissues that are affected, as occurs in aging. There is less information on the in vivo behavior of MSCs. In these cases, it is assumed that the therapeutic effects of SCs are associated with the ability to migrate to the damaged site, differentiate into functional titular cells, and the production of different compounds. Therefore, a plausible hypothesis is that SCs, when injected systemically, are able to recognize tissue damage at early stages, remain in this tissue, and contribute to its repair. In this way, they can be used for the control of aging since, supposedly, SCs injected into the circulation would be able to travel through the body and repair those damages in organs that have not yet been given clinical manifestation. Because of these properties of being able to detect damaged tissues, it is why their use as drug delivery particles is being considered.

It has been described that only a small percentage of the original systemically administered cell mass is able to graft even under the best conditions, and of those that are grafted, only a small percentage has been shown to differentiate into functional replacement tissue. However, again, we must keep in mind that it is not only its capacity for differentiation that justifies its therapeutic effect. Also, SCs are subject to distribution and clearance similar to other intravenous therapies.

One of the tools that is often used to track the SCs after the injection is the transfection of the cells with a reporter gene, for example, the luciferase gene. This gene is integrated into the cell genome and produces the enzyme luciferase, which in contact with luciferin added to the culture medium or injected in vivo produces a photon emission that allows to track the cells in vivo and in vitro. The problem with this type of tracking technique is that the bioluminescent signal is lost in a few days since the cells are diluted and distributed among all the organs and probably many will die.

Using this system, it has been seen that the vast majority of cells that survive after intravenous application accumulate in the lungs. This would be the first capillary bed they would find after passing through the right chambers of the heart; followed by other heavily irrigated organs such as the liver, heart, and spleen, and tissues with active inflammatory processes (Parekkadan and Milwid 2010), most likely explained because expanded MSCs are relatively large and activated and express adhesion molecules. Intraperitoneal application has also been reported to favor the survival and anchoring of MSCs in the colon, which leads to a better therapeutic result for the management of colitis (Liu et al. 2022).

An important issue in systemic administration is the number of cells to be injected (dose) and the number of times it should be done (treatment schedule). Some reports have shown safe and well-tolerated therapeutic doses such as 1×10^6 cells/kg of weight. However, there is no evidence to support this value (Pers et al. 2016; Ra et al. 2011; Lalu et al. 2012). Even more vague is the question about the convenience of the use of repeated doses, which often depends on the disease and particularities of the treated patient (Squillaro et al. 2016).

5.9 Stem Cells in the Control of Aging

Considering the use of SCs for the control of aging, they can be used well both to prevent diseases and for the treatment of any of their clinical stages. For this, we rely on the ability of stem cells to recognize and migrate to sites of damage from the circulation in response to signals that are activated in damaged tissues. Although the mechanisms by which MSCs are recruited into tissues and cross the endothelial cell layer are not yet fully understood, it is probable that chemokines and their receptors are involved, as they are important factors known to control cell migration.

Idealistically, systemic injections could be the best option to use the SCs for the control of aging. But systemic injection has a number of risks and rise up a number of questions. The first question is whether they can produce tumors, which has been described by several authors. In principle, this could be controlled by injecting a limited number of cells and simply to ensure that there is no ongoing tumor process (Parekkadan and Milwid 2010).

In addition, maldifferentiation is very important, because CMs are multipotent and can be differentiated in all cell types that are in the transplanted tissue (Parekkadan and Milwid 2010). For instance, if they are transplanted into the substantia nigra s of Parkinson's patients, the SCs can be differentiated into glial cells, and this could worsen the situation in the case that differentiated glial cells could increase the pathogenesis of disease if they are activated.

Also, SCs undergo a series of biochemical changes with aging that must be considered to determine whether the age of the donor determines the clinical outcome.

Therefore, an important question is whether cell therapies should be limited to a certain age since donor age may have altered its biochemical and regenerative properties. Also, the body mass index and health conditions (underlying disease or comorbidities), might result in a reduced regenerative/immunomodulatory abilities of the SCs (Hassanshahi et al. 2019). Therefore, it is suggested that SCs should be fully characterized and thoroughly screened for in vitro aging and inflammatory markers that might affect their regenerative abilities and detain their usage in clinical application.

Since they are going to be injected into an injured tissue with probably a high index of oxidative stress, it is important to know that they have a good biochemical robustness that allows them to face this oxidation situation. Consequently, parallel to its use in vivo, it would be necessary to conduct an in vitro study of this sensitivity to

oxidative stress to assess whether or not a pretreatment of patients with different antioxidants would be convenient as an adjuvant to cell therapy to facilitate their contribution to tissue regeneration. Similarly, treatment with metformin and resveratrol-based AMPK activators and sirtuins would be indicated to increase the biochemical robustness of the injected SCs.

Other adjuvant treatments may also be indicated. For example, dietary supplementation with NAD precursors can improve their function by increasing mitophagy (Zhang et al. 2016). Vitamin D and E and caloric restriction may reduce mTOR signaling (Meacham et al. 2022). And of course, in the event that they are frozen for possible later use, it is also important to check their viability because after freezing the SCs also behave differently and proliferate or not in in vitro cultures, with which it is likely that the same thing will happen in vivo.

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Novel Strategies for Metformin as an Anti-aging Drug in Skin Aging

6

Gonul Kanigur-Sultuybek and Tugba Soydas

Abstract

The skin aging process is caused by an interaction of epigenetic, genetic, and environmental factors. Thus, skin suffers from extrinsic and intrinsic factors enhancing this process. Both factors contribute to ROS formation in skin aging. ROS is necessary for intrinsic aging; it is produced mainly in mitochondria due to aerobic metabolic reactions. As for extrinsic aging, smoking, UV, and hyperglycemia lead to ROS generation. In skin aging process, generated excessive ROS activates MAPKs and thus induces NF-κB transcription factors. This activation inhibits TGF-β signaling pathway and increases MMPs expression, leading to decreased collagen synthesis and breakdown. Excess ROS related to aging-associated diseases also accelerates skin aging.

On the other hand, metformin, an antidiabetic agent, via inactivating NF-kB, inhibits oxidative stress, ameliorates inflammatory reactions, improves mitochondrial function, and regulates cell death. Up-to-date evidence suggests that NF-kB signaling dysfunction and dysregulation are related to skin aging. There are current mechanisms and novel strategies for the therapeutic manipulation of NF-kB in treating skin aging. This chapter summarizes the current role of NF-kB in dermal aging, underlines the molecular mechanism of metformin in skin aging, attributes its effects to the modulation of NF-kB, and presents novel therapeutic approaches to skin aging through this modulation.

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NF-κB signaling pathway · Metformin · Skin aging · Collagen · MMP

6.1 Introduction

The most prominent features of dermal aging are changes such as wrinkles and skin sagging (Lee et al. 2021). Human skin is generally divided into three main layers: the outer layer (epidermis) contains skin cells, proteins, and pigment, and the middle layer (dermis) contains skin cells, nerves, blood vessels, and sebaceous glands, hair follicles. Dermis makes up 90% of skin thickness. The layer below the dermis (the subcutaneous layer) contains sweat glands, hair follicles, blood vessels, and fatty tissue (Farage et al. 2008). The number of cell layers does not change, but the epidermis layer becomes thinner with aging. In aged skin, the number of melanocytes decreases while their size increases. Aging skin appears paler and more transparent, and thinner. Consequently, alterations in the connective tissue reduce skin strength and elasticity (Kruglikov and Scherer 2018).

Aging skin repairs itself more slowly than younger skin, and wound healing can be slowed up to four times (Gosain and DiPietro 2004). The skin becomes more susceptible to pressure ulcers and infections. Diabetes, blood vessel changes, decreased immunity, and other factors also affect recovery (Spampinato et al. 2020). In line with this information, dermal aging is a very complex process, influenced by more intrinsic and extrinsic factors than other organs. It is divided into two groups intrinsic and extrinsic aging (Farage et al. 2008). The first intrinsic group is our biological clock. One of the theories explaining intrinsic aging is the free radical theory (Poljšak et al. 2012). According to this theory, increases in free radicals, reactive oxygen species, lipid peroxidation in the skin's connective tissue, and inactivation of some antioxidant enzymes cause the release of matrix metalloproteinases (MMPs) and accelerate dermal aging (Shin et al. 2019). Collagen is the most critical component of the extracellular matrix, which makes up about 80% of the dry weight of the skin and forms dermal tension. Elastic fibers that provide elasticity to the skin account for 2–4% of the extracellular matrix. Glycosaminoglycans (GAGs) account for 0.1–0.3% of the dry weight of the skin. These three components have some changes during intrinsic aging (Farage et al. 2008).

In extrinsic aging, extrinsic factors, including exposure to environmental pollutants, UV radiation, tobacco smoking, infrared radiation, and electromagnetic field, lead to premature skin aging (Tsatsou et al. 2012; Krutmann et al. 2021). These factors' combined biological effects in the skin aging process also affect the dermal matrix changes. The essential clinical hallmarks of dermal aging include irregular pigmentation and wrinkling, both affected by the interactions of extrinsic and intrinsic factors. During the dermal aging process, the decrease and disruption of collagen fibers in the dermis leads to wrinkle formation via loss of elasticity in the skin (Shin et al. 2019). These factors cause reactive oxygen species (ROS) formation

and deleterious effects on skin cells' protein, lipid, and DNA structure. The cell aging process is also defined as the gradual failure of maintenance and repair systems that occur because of the accumulation of cellular damages, such as DNA damage, reflecting environmental and internal factors. It causes a decrease in the skin cell's ability to repair genetic damage and increases genetic damage (mutation) (Liguori et al. 2018; Luo et al. 2020).

On the other hand, nuclear factor-κB (NF-κB) and NF-κB signaling pathways are one of the primary mediators of skin aging, and this pathway is activated by genotoxic and oxidative stress and regulates the expression of growth factors, cytokines, and genes that dominate cell cycle progression, cell survival, apoptosis, autophagy, and inflammation (Wang et al. 2019). Therefore, NF-κB activity increases in various tissues with aging, such as skin aging. In this way, inhibition of NF-κB activity and expression causes the delayed onset of skin aging (Adler et al. 2007). Novel anti-aging strategies that emerged from recent experimental studies are NF-kB inhibitors targeting the NF-kB signaling pathway. Recent studies on the molecular mechanisms of skin aging provide clinicians with an expanding range of therapeutic targets. NF-kB inhibitors include topical antioxidants, metformin, resveratrol, curcumin, magnesium lithospermate B (MLB), and MHY384 (Austenaa et al. 2004; Kafi et al. 2007; Bowie and O'Neill 2000; Tanaka et al. 2007; Lee et al. 2015; Moiseeva et al. 2013).

One of the therapeutic targets of skin aging may be metformin. Many studies reported that metformin possessed various bioactivities, including anti-aging and antioxidant effects. The anti-aging mechanisms of metformin were mainly inhibiting oxidative stress, relieving inflammatory reaction, ameliorating mitochondrial function, and regulating apoptosis and proliferation via inhibition of nuclear factor NF-kB (Kanigur-Sultuybek et al. 2019; Podhorecka et al. 2017) (Fig. 6.1).

NF-κB activation is associated with these conditions that can disrupt the activity of genes that cause skin aging. This activation plays a vital role in the overexpression of genes, including interleukins, anti-apoptotic genes, and MMP in skin aging. Induction of ECM component degradation accelerates dermal aging. The accumulation of collagen fibrils inhibits new collagen synthesis. This positive feedback loop causes further degradation of the ECM (Wang et al. 2019). Our previous studies show that metformin significantly decreases NF-kB (RELA/p65) expression and increases collagen expression (*COL1A1*, *COL3A1*) thus having a possible anti-aging effect on aged dermal fibroblasts (Soydas et al. 2021). The results have demonstrated that abnormal NF-κB activation is connected to skin aging (Wang et al. 2019; García-García et al. 2021). This chapter underlines the molecular mechanism of metformin in skin aging and attributes its effects to the NF-kB signaling pathway modulation.

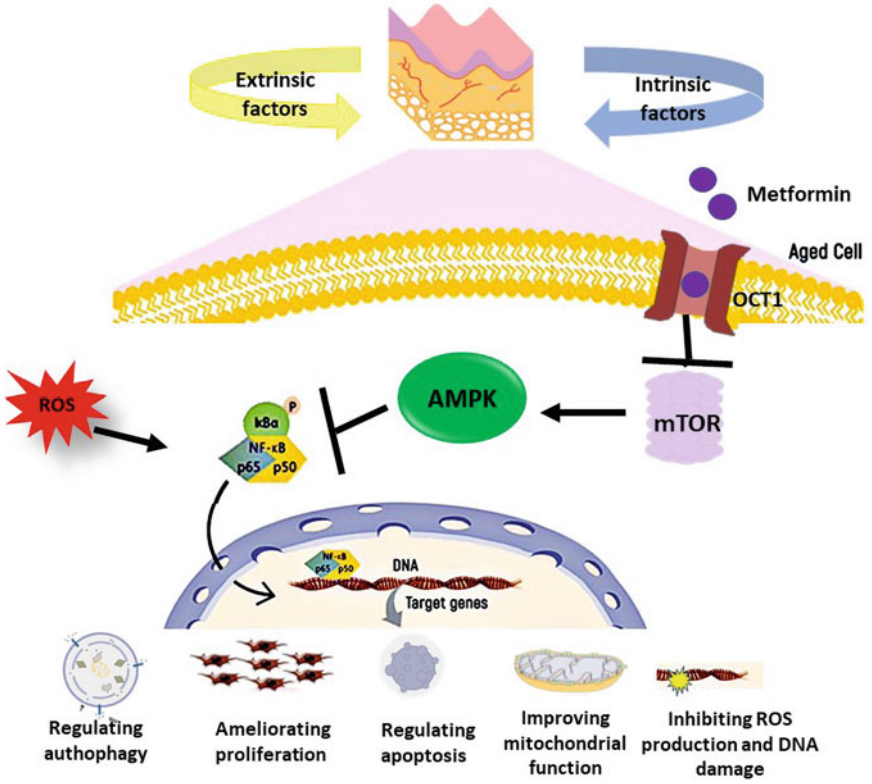

Fig. 6.1 The mechanisms of metformin against skin aging. In the skin aging process, excess ROS produced by extrinsic and intrinsic factors activates mitogen-activated protein kinases (MAPKs), activating the nuclear factor-kB (NF-kB) transcription factor. This activation accelerates matrix metalloproteinase (MMP) expression, causing a decrease in collagen synthesis and degradation. On the other hand, metformin inhibits oxidative stress by inactivating NF-kB, ameliorates proliferation, improves mitochondrial function, regulates cell death such as apoptosis and autophagy, and inhibits ROS production and DNA damage

6.2 Fundamental and Molecular Mechanisms of Dermal Aging

Dermal aging is an effective biological process on the layers of skin; however, the essential changes characterized by facial wrinkles and irregular epidermal thinning are observed in the ECM. The causes of these critical changes are morphological alterations in the connective tissue. ECM of the dermis mostly does not contain cellular components, but glycosaminoglycans are interwoven with fibrous matrix proteins produced by the fibroblasts, such as fibronectin, elastin, and collagen, which comprise its content (Shin et al. 2019). Collagen fibers constitute the essential components of the ECM, they provide 80% of the skin's dry weight, and they

function for elasticity and tensile strength. Almost 80–90% is comprised of Type I collagen, 8–12% is comprised of type III, and <5% is comprised of type V (Talwar et al. 1995). Structural and quantitative changes in collagen fibers become more prominent in the aged skin (Fisher et al. 1997; El-Domyati et al. 2002). Unlike aged skin, which has fragmented and roughly dispersed collagen fibrils, young skin contains many intact collagen fibrils that are tightly bound and well-organized (Quan and Fisher 2015; Yasui et al. 2013). Previous experimental studies have proved that a collagen deficiency occurs due to reduced collagen synthesis and increased collagen breakdown leading to abnormal collagen homeostasis (Fisher et al. 2008; Quan et al. 2010). This process causes clinical modifications, such as loss of elasticity and skin wrinkling, which are observed naturally and in aged skin tissue (Varani et al. 2000).

Another fibrous element of the ECM dermis is elastic fibers. These fibers provide the elasticity of the skin after being deformed or stretched. The proteoglycans and GAGs of the ECM are amorphous elements that surround and embed the fibrous and cellular matrix elements in the skin (Li et al. 2013). They consist of a minimal amount (0.2%) of the dermal dry weight and function as a delicate water-absorbent by regulating the dermis's water-binding and compressibility up to 1000 times their volume (Naylor et al. 2011).

Fibroblasts, differentiated from mesenchymal cells, are dermal-resident cells. They have a role in the biosynthesis and degradation of amorphous and fibrous ECM components, e.g., collagen and fibronectin. Their role and interaction with the microenvironment are mainstays in understanding the molecular and cellular cascades in skin aging (Shin et al. 2019). According to the emerging hypothesis of skin aging fibroblasts, senescence drives dermal aging due to irreversible cell cycle arrest and induced release of a senescence-associated secretory phenotype (SASP). SASP, via pro-inflammatory factors and chemokines, enhances inflammation, decreases proliferation by the impaired release of GFs, and induces the degradation of the ECM by enhanced activation of enzymes such as MMPs (Muñoz-Espín and Serrano 2014).

Moreover, fibroblasts synthesize and secrete MMPs, degrading nearly all ECM proteins involving collagen (Qin et al. 2017). The degradation and synthesis of collagen and MMPs are balanced in healthy skin. This homeostasis may be disrupted by skin aging, radiation, electromagnetic fields, and other processes such as oxidative stress (Tsatsou et al. 2012; Krutmann et al. 2021). Oxidative stress is one of the leading causes of skin aging due to ROS products. High amounts of ROS products cause dermal aging by activating the NF-kB/MAPK signaling pathway and thus the transcription factors activator protein 1 (AP-1) and NF-kB. Therefore, they increase the level of TNF-α and the expression of MMPs, facilitating the degradation of ECM components and accelerating dermal aging (Quan and Fisher 2015).

6.2.1 NF-κB Signaling Pathway: A Master Regulator of Skin Aging

The NF-κB system is an evolutionarily conserved signaling pathway that various external and internal signals can trigger, such as genotoxic and oxidative stress related to aging, involving the dermal aging process (Bassères and Baldwin 2006; Wang et al. 2019). Excessive ROS activates the NF-κB/MAPK signal transduction pathways, responsible for activating NF-κB and AP-1 in the dermal aging process. Subsequently, activating these transcription factors increases the expression of MMPs and levels of TNF-α, which induces ECM degradation and accelerates skin aging. Therefore, the NF-kB pathway system plays an essential regulatory role in dermal aging (Wang et al. 2019; Soydas et al. 2021) (Fig. 6.2).

NF-κB, a vital dimeric regulator protein, are the Rel protein family members. The general characteristic of Rel family proteins is conserved of about 300 amino acids at the N-terminal domain. This conserved domain is significant for binding Rel family members to DNA and other proteins. The binding domains of two dimers of NF-κB protein and the region combined with IκB protein are in this conserved domain (Hayden and Ghosh 2008). Common Rel family protein members are p50, p52, Rel A (p65), Rel B, and c-Rel. These Rel proteins can be divided into two classes, the first includes p50 and p52, and the other contains p105 (NF-κB1) and p100 (NF-κB2). The C-terminal domain having ankyrin repeats (AnkR) is posttranslationally cleaved from p50 and p52. The second class includes p65, Rel B, and c-Rel with transcription transactivation domains (Smale 2012). Eukaryotic cells have two paradigmatic dimers (p50: p65 and p52: Rel B) that play the most

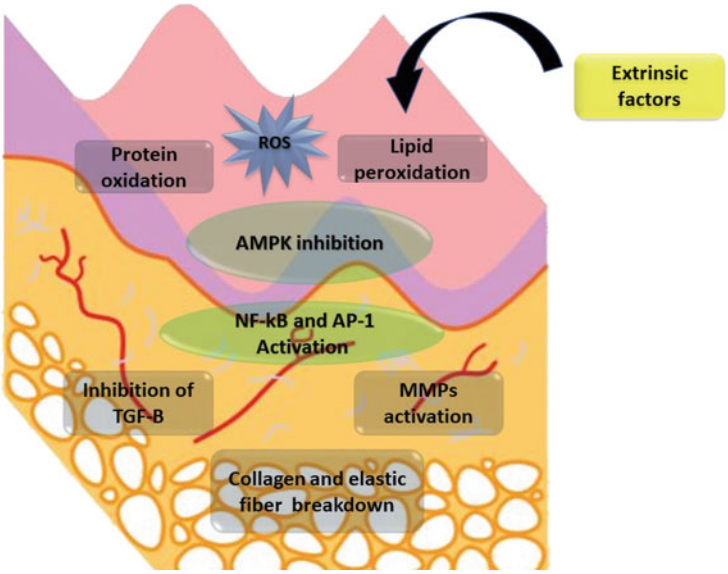

Fig. 6.2 Effects of extrinsic factors on human skin. Schematic representation of oxidative stress-induced skin aging process

critical regulatory role. Although other combinations with homodimers p50: p50 and p52: p52 or heterodimers p52: Rel A and p50: Rel B exist in these cells, they have different functions (Adler et al. 2008; Smale 2012).

In this process, the NF-κB signal transduction pathways are the conventional activation pathway and the nonconventional signal transduction. *The conventional pathway* controls, and many factors, including TNF-α and interleukins such as IL-6 and IL-8, activate it. In the resting state of the cell, NF-kB is inactive in the cytoplasm due to IκB. IkB proteins bind to the RHD region of NF-kB, blocking the nuclear localization signal, and thus preventing translocation of NF-KB to the nucleus. IκB protein variants include IκBa, IκBβ, and IκBγ (Christian et al. 2016). The traditional pathway involves releasing the p65 subunit of NF-κB by cleaving the IκB kinase complex. The most potent form of the complex is a trimer composed of the catalytic subunit IκBα, IκBβ, and the regulatory subunit IκBγ (NEMO, nuclear factor NF-κB key modulator). Inflammatory factors and free oxygen radicals activate the IKK kinase complex. This complex catalyzes the phosphorylation of serine residues in parts of Ser32 and Ser36 in IκB. Phosphorylated IκB, which binds to several ubiquitin molecules, is degraded by the ATP-dependent 26S proteasome system. Active NF-κB (p50/p65 dimer) is translocated into the nucleus through a nuclear pore complex and bound to specific DNA regions. It regulates the transcription of approximately 400 genes, leading to the formation and release of inflammatory cytokines such as IL-2, IL-6, TNF-α, and INF-γ, which can reactivate NF-κB and mediate the inflammatory tissue damage via cascade reaction (Giridharan and Srinivasan 2018; Oeckinghaus and Ghosh 2009).

The *nonconventional activation pathway* is mediated by the 100/Rel B transcription factor, which is essentially active in immune cells. The activation of the nonconventional pathway primarily depends on IκBα instead of IκBβ and IκBγ (Oeckinghaus and Ghosh 2009). The N-terminal region of p100 displays self-inhibition, blocking the transcriptional activity of p100/Rel B in the resting state. Stimulation of ligand can eliminate the K48 ubiquitination of the key protein NF-κB inducing kinase (NIK) and terminate NIK degradation by the proteasome. The stable form of NIK activates p100, resulting in phosphorylation and ubiquitination of p100, which is recognized by the proteasome and partially degraded into p52. Consequently, the nuclear translocation of p52 and Rel B activity-specific gene expressions (Vallabhapurapu and Karin 2009). Both NF-kB signaling pathways are highly effective in skin aging. According to recent evidence, excess free radicals (ROS) in skin aging activate the NF-κB signaling pathway, resulting in increased expression of matrix metalloproteinases involved in collagen metabolism (Wang et al. 2019; Soydas et al. 2021; Kanigur-Sultuybek et al. 2019). MMPs are a family of ubiquitous endopeptidases that can degrade ECM proteins such as collagen, elastin, fibronectin, and the production of these enzymes is regulated by NF-κB and AP-1 transcription factors (Chen et al. 2016). MMPs can be divided into five groups: (1) Collagenases (MMP-1, MMP-8, and MMP-13); (2) gelatinases (MMP-2 and MMP-9); (3) stromelysins (MMP-3, MMP-10, and MMP-11); (4) matrilysins (MMP-7 and MMP-26); and (5) membrane-type (MT) MMPs (MMP-14, MMP-15, and MMP-16). Previous studies have demonstrated that in aged human skin the high

level of MMP-1, MMP-2, MMP-3, MMP-9, MMP-10, MMP-11, MMP-13, MMP-17, MMP-26, and MMP-27 are demonstrated (Quan et al. 2013; Qin et al. 2017; Tewari et al. 2014; Parkinson et al. 2015). Also, MMP-1 is the essential protease that initiates the breakdown of collagen fibers, predominantly type I and III, in human skin tissue. After cleavage by MMP-1, collagen may be further degraded through MMP-3 and MMP-9 (Fisher et al. 1996; Brennan et al. 2003).

Specific tissue inhibitors control MMPs: TIMP-1, TIMP-2, TIMP-3, and TIMP-4. The TIMP-1 is reduced in intrinsically and photoaged aged skin (Nagase et al. 2006). TIMPs and MMPs are regulated together by excessive MMP activity. However, the increase in MMP levels is not accompanied by an increase in TIMP levels in aged skin (Quan et al. 2013; Qin et al. 2017). Therefore, this imbalance accelerates progressive collagen degradation and thus skin aging.

ROS induces skin inflammation by activating the NF-κB signal pathway and thus the various inflammatory cytokines expression in skin tissue (Bell et al. 2003). Increased levels of chronic inflammation trigger destructive processes in cells that rapidly lead to even greater activation of NF-kB and an accelerating inflammatory cycle. In light of all this information, the role of inflammation in skin aging is clear. In addition, inflammation has even been shown to shorten telomeres. When telomeres shorten, this directly contributes to a decreased cell life span (Jose et al. 2017). It leads to cell death, tissue loss, DNA damage, and other deleterious changes associated with skin aging. While NF-kB activation promotes inflammatory processes, blocking NF-kB inhibits all-natural skin aging processes (Zhang et al. 2016; Donato et al. 2015).

6.3 Novel Therapeutic Strategies for Skin Aging

Anti-aging approaches have been developed to achieve healthy and youthful skin. These approaches are currently used to demonstrate dermal rejuvenation, and the related molecular mechanisms will be summarized here. Today, well-known anti-aging strategies include preventive, cosmetic, topical antioxidants, systemic therapeutic agents, and invasive procedures (Ganceviciene et al. 2012). One novel anti-aging strategy that has emerged due to recent in vivo and in vitro studies is NF-kB inhibitors that target NF-kB activation (Balistreri et al. 2013). Many NF-κB inhibitors for skin aging are currently under clinical trials. NF-kB inhibitors include topical antioxidant, metformin, resveratrol, curcumin, magnesium lithospermate B (MLB), and MHY384 (Austenaa et al. 2004; Kafi et al. 2007; Bowie and O'Neill 2000; Tanaka et al. 2007; Lee et al. 2015; Moiseeva et al. 2013) (Table 6.1).

NF-kB is an essential factor for skin aging and plays a role in many skin diseases such as skin cancer, allergic dermatitis, and psoriasis vulgaris (Kim and Pasparakis 2014; Dajee et al. 2006; Goldminz et al. 2013). Thus, although NF-kB has a role in maintaining skin homeostasis, excessive activation of this factor appears pathogenic (Wullaert et al. 2011). The inhibition of NF-kB is considered to prevent the destructive changes in the skin induced by extrinsic factors such as UV, high glucose, and electromagnetic fields (Kagan et al. 2002). Therefore, NF-kB and NF-κB signal

Table 6.1 Skin anti-aging approaches

Inhibitors of NF-kB	Mechanisms of action
Retinoids acid (RA)	Acts through RARs and RXRs. Increases type I, III, and VII collagens. Decreases MMPs.Reorganizes elastic fiber.Normalizes GAG deposition.
Ascorbic acid	Reduces ROS. Acts as a cofactor in the biosynthesis of procollagen and elastin. Induces collagen synthesis in human skin fibroblasts and increase dermal thickness.
Glycolic acid	Stimulates the production of GAGs and collagen in the dermis. Improves histologic quality of elastic fibers.
Peptides	Regulate fibroblasts and control the production of ECM.
Metformin	Suppresses ROS-induced oxidative, regulates apoptosis, inhibits age-related low-grade inflammation, and improves mitochondrial function via inhibiting NF-kB signaling pathway.
Resveratrol	Reduce the expression of AP-1 and NF-kB factors and it slows down the process of photoaging of the skin.
Curcumin	Improvement of cell viability. Reducing the apoptosis and oxidative stress. Decreasing the expression of apoptosis-related proteins and OS biomarkers.
Magnesium lithospermate B (MLB)	Direct removes of reactive oxygen species (ROS). Inhibits NF-κB-dependent inflammation genes.
MHY384	Reduces UVA-induced oxidative stress. UVA-mediated decrease in type 1 procollagen protein level recovers by MHY384 in the fibroblasts.
Parthenolide and magnolol	NF-kappaB inhibitors could block such UVB-mediated skin changes.

pathways are essential targets for inhibiting the formation of wrinkles on the skin induced by factors such as oxidants (Balistreri et al. 2013). In line with this information, it can be said that NF-kB inhibitors affect skin aging.

Topical retinoids are one of the NF-kB inhibitors (Austenaa et al. 2004). They consist of vitamin A, its derivatives, and synthetic molecules acting through the same pathway (Hubbard et al. 2014). Retinaldehyde, retinol, and various retinyl esters are also termed "retinoids." They act through retinoic acid and retinoid X receptors, causing an increase in type I procollagens and a decrease in the level of MMPs (Kang 2005; Kim et al. 1992). Retinoic acid increases the level of type I, III, and VII collagen in the dermis and can reorganize the dermal collagen into newly formed bundles (Woodley et al. 1990). Additionally, retinoic acid stimulates the normalization of the elastic tissue organization and GAG deposition in the dermis (Berardesca et al. 1990). Topical retinoids effectively treat skin aging, involving roughness, wrinkles, and laxity in clinical trials (Olsen et al. 1992; Sorg et al. 2006; Darlenski et al. 2010). Retinoids are still considered topical antioxidants and anti-aging products (Kang et al. 1995; Kafi et al. 2007).

Another NF-kB inhibitor, ascorbic acid (vitamin C), has been used as an anti-aging and hyperpigmentation agent (Bowie and O'Neill 2000). Ascorbic acid, a topical antioxidant, also eliminates ROS production and maintains the normal

physiological state of human skin (Masaki 2010). Furthermore, ascorbic acid is a cofactor for elastin and procollagen synthesis (Myllylä et al. 1984). In vivo and ex vivo studies suggest that ascorbic acid increases dermal thickness and induces collagen synthesis in human skin fibroblasts (Yamamoto et al. 1992; Ohshima et al. 2009). Topical formulations having ascorbic acid have clinical efficacy in skincare treatment (Humbert et al. 2003; Colven and Pinnell 1996; Alster and West 1998).

Another anti-aging product, topical α-hydroxy acids such as glycolic acid, stimulate GAG, and collagen production in the dermis and improve the histological quality of elastic fibers (Ditre et al. 1996; McCook 2016). Topical peptides regulate fibroblasts and control the production of ECM components. Therefore, peptides have recently attracted great interest in the cosmetic industry and showed clinical efficacy in several trials (Katayama et al. 1991; Langholz et al. 1995). Magnesium lithospermate B (MLB) has an antioxidant effect by directly removing ROS and inhibitory effects on inflammation via the NF-kB pathway (Jung et al. 2014).

A recent study showed that the NF-kB inhibitors, parthenolide, and magnolol, inhibit UVB-induced skin photoaging thus raising the possibility that topically applied NF-kB inhibitors may be more effective in preventing skin aging (Tanaka et al. 2005, 2007).

The NF-kB inhibitor MHY384 plays an antioxidant role and reduces UVA-induced oxidative stress. Also, MHY384 dose-dependently reversed the increased level of MMP-1, MMP-12, MMP-13, and type 1 procollagen in UVA-induced fibroblast damage to prevent wrinkles. As a result, MHY 384, a new ingredient for anti-aging cosmetics, can be used to prevent wrinkles by reducing ROS formation (Lee et al. 2015; Natarajan et al. 2014).

Resveratrol, an antioxidant, activates its SIRT, inhibiting the NF-kB signaling pathway. Thus, it reduces UV-induced skin damage (Liu et al. 2011). Curcumin, a bioactive polyphenol, inhibits the degradation of IkBa, and thus NF-κB inhibits translocation to the nucleus. This inhibition activates MMPs and subsequently degrades collagen (Thangapazham et al. 2007).

Metformin is a well-known oral antidiabetic drug and also an NF-kB inhibitor. It has aging-related effects, such as skin aging at the intracellular and extracellular levels (Moiseeva et al. 2013; Kanigur-Sultuybek et al. 2019).

6.3.1 The Therapeutic Effect of Metformin as an NF-kB Inhibitor in Skin Aging

Metformin has been in clinical use for over half a century and is widely studied, with a high safety profile. It intervenes at unique positions in several crucial pathways of aging (Campisi et al. 2019; Kulkarni et al. 2020). Metformin acts on mechanisms attenuating the hallmarks of senescence and their interconnectivity by enhancing nutrient sensing, autophagy, and communication between the cells, protecting against macromolecular damage, and delaying stem cell modulating mitochondrial function, regulating transcription, and reducing telomere shortening and senescence.

These features of metformin make it a promising gerotherapeutic to use in human trials (Hsu et al. 2021; Barzilai et al. 2016).

Metformin, a biguanide known as an NF-kB inhibitor that combats aging and improves healthspan, is the first drug to be tested for its age-targeting effects in the large clinical trial-TAME (targeting aging by metformin) (Barzilai 2017; Kulkarni et al. 2020). NF-kB is also crucial for skin aging and is implicated in many skin diseases such as skin cancer, allergic dermatitis, and psoriasis vulgaris (Kim and Pasparakis 2014; Dajee et al. 2006; Goldminz et al. 2013). Hence, although NF-kB maintains skin homeostasis, excessive activation appears pathogenic. Therefore, the inhibition of NF-kB is considered to prevent the destructive changes in the skin induced by extrinsic factors such as UV, high glucose, and electromagnetic fields (Kagan et al. 2002). Further, NF-kB and NF-κB signals pathways are essential targets for inhibiting the formation of wrinkles on the skin induced by factors such as oxidants (Balistreri et al. 2013). All this information is in line with it; it can be said that NF-kB inhibitors affect skin aging.

One of the NF-kB inhibitors, metformin, is transported into the cell via the organic cation transporters, OCT1, 2, 3, and the plasma membrane monoamine transporter (PMAT) and generally acts on the AMP-activated protein kinase (AMPK) signal pathway (Shikata et al. 2007; Graham et al. 2011). The activation of the AMPK declines in cell aging, and its decline intervenes with autophagy and starts cellular stress and inflammation, which further triggers aging and skin aging (Salminen and Kaarniranta 2012; Stancu 2015). Consequently, the AMPK signaling pathway plays a vital role in aging and skin aging. The increased activation of the AMPK pathway has been shown to extend lifespan in animal models responding to drugs like metformin. Metformin reduces oxidative and ER stress, which is often caused by nutrition overload, ROS formation, and skin aging (Burgos-Morón et al. 2019). Furthermore, metformin downregulates the mTOR pathway in an AMPK-dependent and AMPK-independent manner (Rena et al. 2017). Besides, metformin suppresses the consumption of ATP by inhibiting energy-needing processes, such as protein synthesis over the mTOR pathway (Piskovatska et al. 2019; Towler and Hardie 2007). In addition, metformin influences cell growth, proliferation, and autophagy by downregulating mTOR signaling and insulin-like growth factor 1 (IGF-1) (Vancura et al. 2018).

Clinical studies further suggest that metformin may also alter inflammation as determined by decreased inflammatory markers in cell aging. Metformin also facilitates AMPK activation, inhibiting mTOR, a primary target for cell aging modulation, and limiting the inflammatory pathway. Inflammation, cell survival, autophagy, apoptosis, and protein synthesis are all affected by these mechanisms and are associated with accelerated cell aging (Mohammed et al. 2021). The protective role of metformin against hyperglycemia and inflammation, which are factors that accelerate cell aging, has been reported in various reports (Cai et al. 2020; Soydas et al. 2018). Apart from that, metformin might reduce endogenous ROS production by acting at the mitochondrial level by blocking the reverse electron flow in the respiratory chain complex, which plays a crucial role in aging and skin aging (Algire et al. 2012; Batandier et al. 2006).

Several mechanisms of action of metformin concerning anti-aging have been demonstrated, involving inhibition of inflammation, enhancing autophagy pathways with AMPK activation and inhibition of mTOR, increased antioxidant effect, inhibition of ROS, and improvement of mitochondrial function (Mohammed et al. 2021). The exact cellular and molecular mechanisms of metformin on genotoxic and oxidative stress (ROS production) induced harmful effects in skin aging and its relationship with the expression of NF-kB (p65) remain elusive. As we know, the proliferation of fibroblast is the crucial element affecting skin health; however, the mechanisms of how intrinsic and extrinsic factor disturbs the proliferation and how metformin corrects it could be very complicated and have not yet been elucidated completely. Although past studies have reported that a high glucose concentration is responsible for diminished cell growth and increased apoptosis, we have observed that metformin exposure leads to decreased apoptosis levels and increased proliferation of aged fibroblast in high glucose media (Soydas et al. 2018, 2021). In these studies, we determined the deleterious effects of metformin at high glucose on cell proliferation, apoptosis, and gene expression of *RELA/p65*, *COL1A1*, and *COL3A1* in human primary dermal fibroblasts (HDFs). We also showed that our findings of reduced expression of the *RELA/p65* gene in metformin-treated HDFs suggest that NF-κB may be the regulatory pathway that metformin exerts its anti-aging effect on the skin (Soydas et al. 2021). In several studies, the overexpression of two NF-κB subunits, c-rel, and RelA/p65, induced a senescent phenotype in cultured cells (Seitz et al. 2000). According to Wang et al. loss of p65 gives mouse embryonic fibroblasts can escape senescence, partly due to its role in DNA repair (Wang et al. 2009). Moreover, autophagy, which plays a vital role in regulating cell aging, is necessary for removing damaged proteins and organelles (Levine and Kroemer 2008; Rubinsztein et al. 2011). Additionally, embryonic fibroblasts derived from Atg5 transgenic mice are more resistant to oxidative stress-induced cell death, a tolerance reversible by an autophagy inhibitor, metformin (Pyo et al. 2013). Contemporary studies point to a link between metformin, apoptosis, autophagy, and lifespan extension (Nafisa et al. 2018; Triggle et al. 2020; Kalyani 2021). Song et al. have described a relation between SIRT1, AMPK, and metformin-induced autophagy thus supporting a synergistic alteration between the deacetylase, sirtuin-1, and metformin-mediated effects on skin aging (Song et al. 2015).

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Anti-inflammatory-Dependent Anti-aging Strategies

7

Seyma Dumur and Hafize Uzun

Abstract

Aging and death remain a great mystery of biological science. Many processes associated with aging have been described. In general, the aging process is associated with inflammation. Inflammation is the cellular and vascular response of tissues to infection and tissue damage. Under normal conditions, it provides tissue healing with a controlled humoral and cellular response and prevents the development of infection. The presence of chronic, low-level inflammation without significant infection was termed "inflammaging." The use of methods aimed at regulating or preventing inflammaging will prevent, at least reduce or delay the effects of both the prevention of symptoms that can occur with aging and the emergence of diseases that can be seen. The use of treatments and methods to regulate inflammation in the early period when signs of aging begin to appear will have a positive effect on aging by activating the body's compensatory mechanism. Aging is strongly affected by metabolism. Research on drugs such as polyphenolic compounds, statins, and aspirin will increasingly continue, as they can delay the aging process, prolong lifespan, and reduce age-related degeneration and associated morbidity and mortality by targeting mTOR, NF-κβ, inflammatory cytokines, and related signal transduction pathways. Research on polyphenolic compounds, anti-aging pharmacologic agents will increasingly continue, as they can delay the aging process, prolong life, and reduce age-related degeneration and associated morbidity and mortality by targeting mTOR, NF-κb, inflammatory cytokines, and related signal transduction pathways.

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Keywords

New update · Anti-aging strategies · Low-grade inflammation

7.1 Introduction

7.1.1 Aging

While aging is the reduction and decline in repair with age defined as physiological definition; aging is the increase in mortality rate with age defined as actuarial definition. As for evolutionary definition, aging is the decline of adaptive abilities with advancing age (Péron et al. 2019; Mitina et al. 2020). The concepts of "aging," "old age," and "senility" are concepts that are frequently used and often confused with each other in the fields of gerontology and geriatrics. Since the distinction between these concepts is not clear, they are often used incorrectly interchangeably. Living things age in time due to ongoing mechanisms such as destruction and repair in general. As the lifespan of individuals increases, age-related health problems also increase. Nowadays, it is emphasized that having a chance to live a longer life compared to the past will not make sense without increasing the quality of life and health expectancy is much more important than life expectancy. Therefore, while the goal of medicine in the young population is treatment, the main goal in the geriatric population is to "protect the quality of life" of patients.

7.1.2 Inflammation

Inflammation is the immune system's response to attacks from outside or inside the body. Inflammation is not a disease but a system that must be in balance for a healthy life. Inflammation is actually important to the body because it is a response of the body to protect itself from infection, illness, or injury. Inflammation is divided into acute (short term) and chronic (long term). The classic signs of short-term inflammation are reactions such as redness, pain, warmth, and swelling. However, chronic (long term) inflammation is usually silent and may progress without any visible symptoms such as redness, pain, and edema. As with everything in the body, too much inflammation is harmful and long-term inflammation can cause many diseases such as diabetes, heart disease, fatty liver disease, and cancer (Germolec et al. 1803).

7.1.3 New Mechanisms of Inflammation in Aging

One of the major changes that occur during the aging process is the dysregulation of the immune response leading to a chronic systemic inflammatory state. Among the dysregulated proinflammatory mediators, cytokines and chemokines are the major culprits in the development of chronic inflammation and in the immunoaging

process (Chung et al. 2019). Intestinal microbiota, intestinal-brain axis disorders, inflammatory condition, and low physical capacity pave the way for chronic diseases. With aging, immunodeficiency states increase the susceptibility to infection and cancer (Calder et al. 2017).

Inflammation is increasingly linked to aging and chronic diseases. The acute inflammatory response in conditions such as infection and injury is critical for recovery and health. However, the observed increase in basal inflammatory response with age causes a low level of sustained inflammation that promotes aging. Therefore, there is a need for a good definition of inflammatory pathways that are adaptive (acute inflammatory response) and detrimental (chronic inflammatory response). When this is done, an important step will be taken towards the development of treatments that can interfere with the chronic inflammatory response. Senescent cells release many cytokines that initiate inflammation. In fact, in advanced ages, there are few old cells in organs and tissues, but the factors that initiate the inflammation they secrete affect the functioning of autocrine, paracrine, and endocrine systems, also known as cellular signal transmission mechanisms (Kennedy et al. 2014).

Accumulation of misfolded proteins causes an effect that triggers the aging process or accelerates its progression. Telomeres are repetitive sequences that play an important role in maintaining the stable structure of chromosomes. It is known that telomeres, which are also associated with stress responses, are affected and damaged by DNA damage that increases with aging (Zhu et al. 2019). Structural and functional disorders in telomeres also accelerate aging. Cellular senescence, which means a kind of cellular arrest that prevents the proliferation of cells, occurs due to different reasons such as DNA damage and stress (Di Micco et al. 2021). The number of cells undergoing senescence is also increasing due to the increase in DNA damage with aging. Epigenetics is defined as inherited traits without a change in DNA sequence (Yang and Sen 2018). Along with the aging process, damage also occurs in mitochondrial DNA, and the damage negatively affects the respiratory chain.

Cells with this negativity are known to be prone to aging. At the same time, it has been observed that interventions in pathways such as insulin and insulin-like growth factor-1 (IGF-1) pathway delay aging by showing the effect of calorie restriction (Narasimhan et al. 2009). Other pathways that increase lifespan with genetic interventions and that have been studied extensively are mTOR, AMPK, and sirtuin pathways. Ketone bodies, which play a role in providing energy during long-term fasting and after exercise, are molecules derived from fatty acids. The most active of the ketone bodies, which exist in three different forms, and the most common in the bloodstream, is ß-hydroxybutyrate (ß-HB). It usually occurs in the liver (Newman and Verdin 2017). The activity of enzymes involved in ketogenesis is regulated by complexes associated with aging, such as FOXO1-3 and mTOR. ß-HB is involved in energy metabolism as well as cellular signal transmission. It inhibits class 1 histone deacetylases (HDAC), which are involved in the regulation of ß-HB gene expression (Kolb et al. 2021).

Somatic mitochondrial DNA (mtDNA) mutations accumulate with aging, and these mutations cause dysfunction in the respiratory chain. The observation that cells

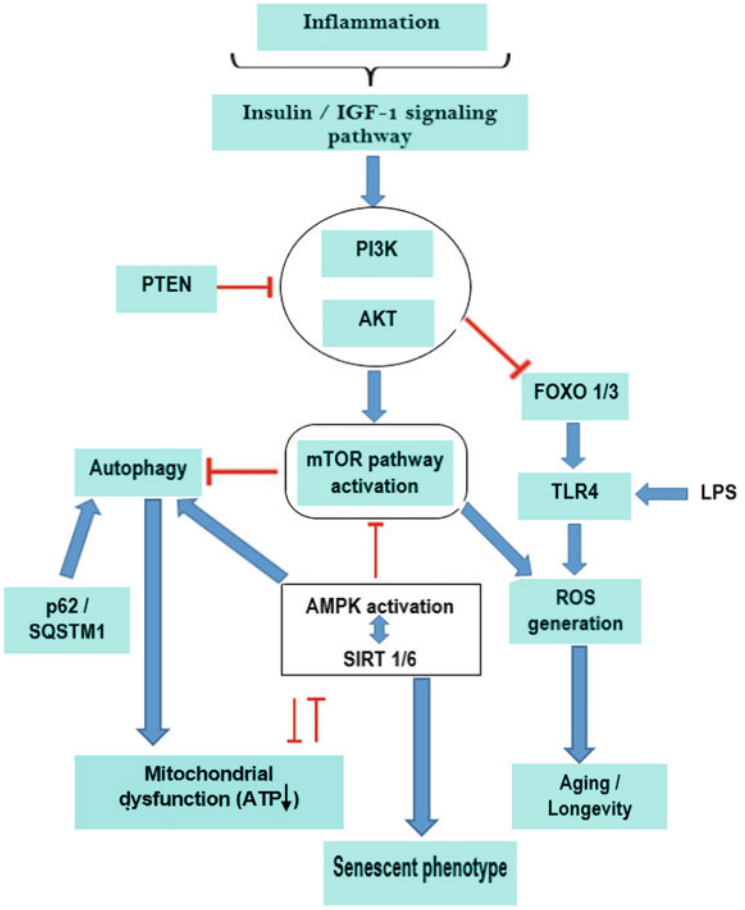

Fig. 7.1 The relationship between inflammation and longevity. *Abbreviations*: Insulin-like growth factor 1 (IGF-1); phosphatidylinositol-3 kinase (PI3K)/AKT (Protein Kinase B); Forkhead box O (FOXO); adenosine 5′-monophosphate (AMP)-activated protein kinase/Sirtuin 1/6 (AMPK/SIRT1/6); sequestosome 1 (p62/SQSTM1); phosphatase and the tensin homolog deleted on chromosome ten (PTEN)

deprived of the respiratory chain are prone to aging proves that dysfunctions in mitochondria are one of the aging markers (Lee et al. 2010). Finding the effects of these mutations on mtDNA suggests that any disorder in mitochondria other than ROTs may also accelerate aging. The fact that the main region where ROS occur is the respiratory chain in the mitochondria, showing that mitochondria are exposed to oxidative damage quite a lot, and it has been suggested that mitochondria itself may be the cause of aging, as well as the free radical theory (Ospelt and Gay 2005; Harman 1972). Although studies conducted in the early years showed that ROS formation and oxidative stress accelerate aging, the opposite has been observed in some studies conducted in recent years (Fig. 7.1).

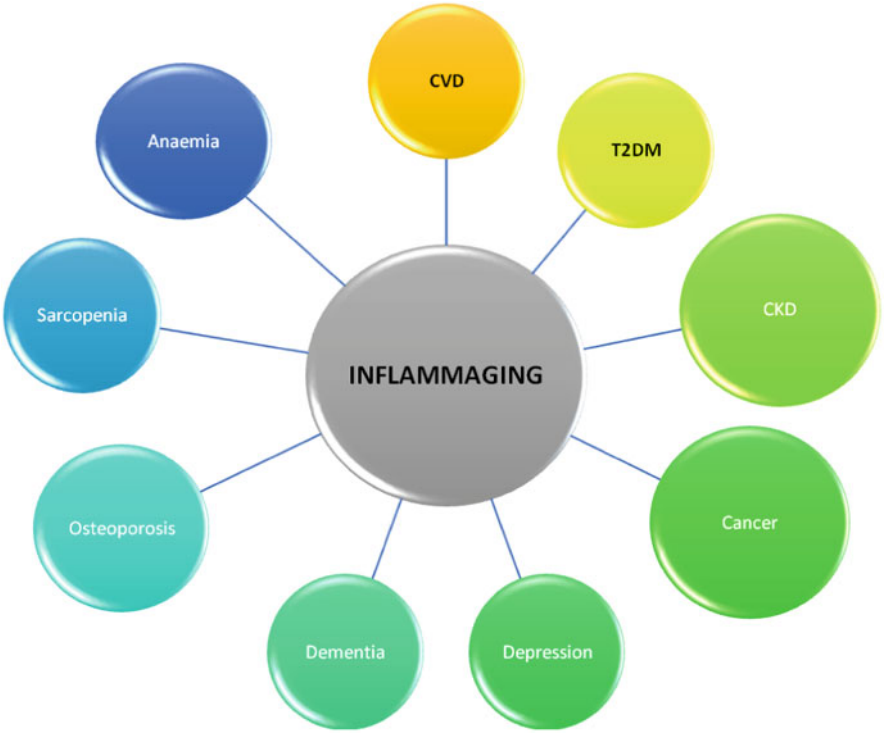

Fig. 7.2 Inflammaging and associated pathologies. *Abbreviations*: *CKD* chronic kidney disease, *CVD* cardiovascular disease, *T2DM* type 2 diabetes mellitus

Aging causes changes in the immune system. Especially with the changes in the immune system in advanced ages, pathogen recognition, warning signal generation, and clearing functions are impaired. Another consequence of persistent inflammation is "inflammaging" and "immunosenescence," which is similar to the picture seen in older people. Both are increased in inflammation and hence ineffective stimulation. When stimulation is ineffective, an effective and effective response to inflammation cannot occur (Mueller et al. 2020). Low-grade persistent inflammation is observed as an impaired immune response in the elderly, without host damage such as trauma or infection. This is actually an immunosuppressive picture in which the immune response is impaired. Similar to persistent inflammation, immunosuppression, and catabolism syndrome (PICS), the circulating level of myeloid-derived suppressor cells (MDSCs) was found to be high in this patient group (Horiguchi et al. 2018). Inflammageing is a risk factor for multiple chronic diseases. Pathologies associated with inflammaging are given in Fig. 7.2.

7.2 Health Benefits of Anti-inflammatory-Dependent Anti-aging Strategies

Even if the aging process cannot be stopped, at least many studies for better quality and longer life are promising for the future. Although preventive health practices, education, lifestyle changes, exercise, prevention, and correction of environmental physical and chemical negative factors, that is, active and healthy aging, play a fundamental role in delaying or preventing aging. The use of products, drugs, and remedies of both modern medicine and alternative or complementary medicine is an approach that is increasingly popular today as an "anti-aging strategies" (Liu 2022).

7.2.1 Anti-inflammatory Immune Modulators Contributing to Anti-aging Strategies

Various studies have shown that immune sufficiency in elderly people decreases and this causes increased morbidity and mortality against infections. The immune system weakens slightly with age, but it varies from person to person. The fever response to infections is usually low in the elderly. It is thought that this may be related to IL-1 reduction or hypothalamic receptors (Cunha et al. 2020).

It has been shown that IL-1 synthesis is decreased in aged mice.

- The thymus gland atrophies with aging (important in the activation of T cells).
- Total lymphocyte count and natural killer (NK) cell functions decrease.
- The speed at which leukocytes reach the area of inflammation decreases (migration).
- Decreased in leukocytosis response to inflammation.
- Cytokines; while IL-1 and IL-2 levels decrease, IL-6, IL-8, IL-10, IL-12, and TNF-α levels increase.

Antibody production is normally slightly decreased and autoimmune diseases increase. RF, ANA, and false positive VDRL are seen. As a result, infections progress more seriously and recovery is prolonged and adequate immunity may not develop. Wound healing is delayed (van der Geest et al. 2016).

Intestinal microbiota, intestinal-brain axis disorders, inflammatory condition, and low physical capacity pave the way for chronic diseases. With aging, immunodeficiency states increase the susceptibility to infection and cancer. Mediterranean type diet and vegetarian diet have been found to be beneficial. Polyphenolic components, antioxidants, fibers, omega 3, prebiotics and probiotics, vitamins D, and E stand as an important nutritional strategy against low-grade inflammation (Calder et al. 2017).

Assuming that aging is a remodeling process caused by chronic exposure to and responses to various stressors, responses to these stressors have been the focus of research on the underlying mechanisms of aging. In fact, strategies aimed at reducing inflammation by acting on the immune response (systemic reduction of stress,

treatment with vaccines, and anti-inflammatory anti-aging drugs) have not yet provided conclusive evidence of their capacity to delay the onset of age-related diseases as well as aging. As a variant, dietary interventions and physical activity fit much better into the conceptual framework of hormesis (Santoro et al. 2020; Kennedy et al. 2014).

In the light of current knowledge that we have, we can say that vitamins (A, D, E, B6, B12, folate, and C) and elements (selenium, zinc, copper, and iron) are necessary to maintain normal immune functions, but there is not enough data to show that they increase immune functions (Maggini et al. 2007; Chandra 2004). However, it is known that the basal metabolic rate decreases significantly with aging, resulting in a decrease in the daily targeted calorie intake (Institute of Medicine (US) Food Forum 2010). In this case, the ratios of micronutrients and vitamins taken by the elderly who consume less calories decrease. The net effect on the elderly is not only immune weakening but also causes negative progression of many other physiological processes. For example, considering that magnesium is effective in the function of more than 300 enzymes in the body, the importance of an adequate and balanced intake of vitamins and elements in foods for the health of the elderly is better understood (Jahnen-Dechent and Ketteler 2012).

Probiotics exert significant anti-inflammatory "tolerogenic" effects that may reduce the burden of infection to a harmless level (Walsh 2019).

7.2.2 Anti-inflammatory Pharmacological Agents Contributing to Anti-aging Strategies

Anti-inflammatory drugs are pharmacological agents that interfere at various points in the highly complex biochemical processes of inflammation. They inhibit the formation of the E-type prostaglandin transporter and are therefore also called "prostaglandin synthesis inhibitors." Anti-inflammatory drugs belong to the class of nonsteroidal anti-inflammatory drugs. They are called NSAIDs in short. For the treatment of inflammation and inflammatory pain, doctors often prescribe chemically produced anti-inflammatory drugs.

7.2.2.1 Anti-inflammatory Drugs with Anti-aging Effects

Impaired glucose regulation accelerates the aging process (Chen 2009). This suggests that maintaining the integrity of glucose metabolism will delay aging. Acarbose delays carbohydrate absorption by inhibiting alpha-glucosidase enzymes in the small intestine (Harrison et al. 2014). Acarbose at 1% concentration has been reported to significantly increase mean and maximum lifespan in male mice. Acarbose decreased key markers (Iba1+ microglia, tumor necrosis factor-α, and glial fibrillary acidic protein) of hypothalamic inflammation, while long-term treatment of acarbose (from 3 to 9 months, 20 mg/kg/day) enhanced memory during aging in SAMP8 mice (Sagagurski et al. 2017; Yan et al. 2015).

Resveratrol, rapamycin, metformin, and aspirin affect autophagy, inflammation, and oxidative stress by targeting key regulators of aging, mainly mTOR, FOXO, and

PGC1α, which have efficacy in life- and healthspan extension of the model organism. Similar mechanisms have been demonstrated for aspirin, rapamycin, metformin, and resveratrol in the modulation of lifespan in nematodes and mammals (Piskovatska et al. 2019). Aspirin, statins, rapalogs, metformin, as well as lisinopril, propranolol, calorie restriction, and exercise target compounds contribute to the same age-related cellular dysfunction known as the senescence-associated secretory phenotype (SASP) seems to be a hallmark of age-related diseases, being a cause of the functional decline. Both statins and aspirin decrease inflammation (Blagosklonny 2017).

Age-related inhibition of p38MAPK pathways and acute phase responses is induced by rapamycin, acarbose, and 17α-estradiol in both sexes. This suggests new approaches for preventing or reversing age-related inflammatory changes in a clinical setting independent of lifespan effects (Wink et al. 2022).

7.2.3 Anti-inflammatory Compounds Contributing to Anti-aging Strategies

There are approximately 5611 both research and review articles published in pubmed under the terms "anti-aging" to date. These published studies reported a large number of anti-aging compounds or molecules. Compounds or extracts from natural products that have significant anti-aging activity are shown in Fig. 7.3. Some have received popular attention, and under intense research, anti-aging activities available in multiple aging models such as resveratrol (Liu 2022), α-lipoic acid (Kim et al. 2021a), astaxanthin (Liu 2022), catechin (Liu et al. 2021), fucoxanthin (Shaposhnikov et al. 2022), spermidine (Liu 2022), acacetin (Okoro et al. 2021), and curcumin (Brinkmann et al. 2022) all show anti-aging activity in both *Drosophila melanogaster* (*D. melanogaster*) and *Caenorhabditis elegans* (*C. elegans*) models.

In mice given resveratrol, a 4.7% increase in life expectancy increased insulin sensitivity, decreased IGF-1 levels, increased AMP-activated protein kinase and peroxisome proliferator-activated receptor-gamma coactivator 1alpha activity, increased mitochondria count, and improved motor function. In *D. melanogaster* treated with resveratrol, it modulates genetic pathways that may reduce cellular damage by prolonging the mean lifespan of females fed a low sugar-high protein diet by 15.0%, by 10.0% for females fed a high-fat diet. It also modulates 18.0% increase in life expectancy in *C. elegans* and autophagy and proteasomal degradation in AMPK and SIR-2.1. In addition, increased NAD(+) and AMPK and Sirt1 activity in the cell inhibit PDE4, JAK2/STAT3 (Ding et al. 2017; Baur et al. 2006; Tung et al. 2015; Toth et al. 2015; Wang et al. 2013; Regitz et al. 2016; Park et al. 2012; Liu et al. 2015; Chen et al. 2015; Madrigal-Perez et al. 2015).

α-Lipoic acid improves memory and oxidative stress but shortens lifespan in old SAMP8 mice. It has been reported that there is a 12.0% increase in life expectancy and antioxidant in melanogaster. In *C. elegans*, 24.0% increase in life expectancy and antioxidant increase the chemotaxis index (Brown et al. 2006; Farr et al. 2012).

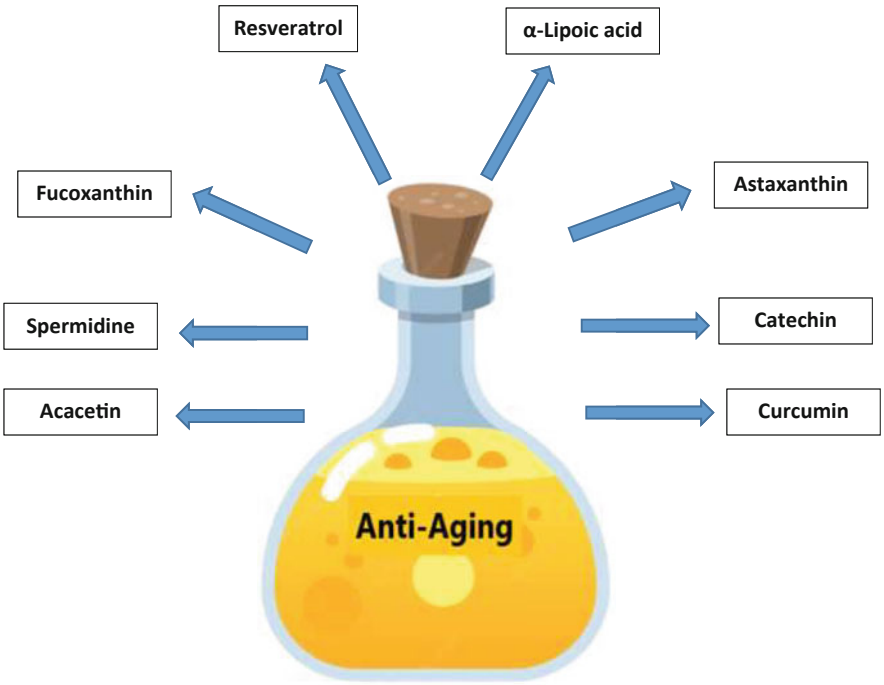

Fig. 7.3 Some natural product compounds with anti-aging activity

Astaxanthin enforced an antioxidant effect in D-galactose-induced brain aging in rats by upregulating BDNF expression. In addition, antioxidant effects have been reported in melanogaster. A 29.0% increase in average lifespan has been reported with *C. elegans* (Mueller et al. 2020; Horiguchi et al. 2018; Liu 2022; Cunha et al. 2020; van der Geest et al. 2016; Calder et al. 2017; Santoro et al. 2020; Kennedy et al. 2014; Maggini et al. 2007; Chandra 2004; Institute of Medicine (US) Food Forum 2010; Jahnen-Dechent and Ketteler 2012; Walsh 2019; Chen 2009; Harrison et al. 2014; Sagagurski et al. 2017; Yan et al. 2015; Piskovatska et al. 2019; Blagosklonny 2017; Wink et al. 2022; Kim et al. 2021a; Liu et al. 2021).

As for catechin, it has been reported to prevent memory decline and DNA oxidative damage in the accelerated aging mouse model (Shaposhnikov et al. 2022). In *D. melanogaster*, an increase of 16.0% (Okoro et al. 2021; Brinkmann et al. 2022) was observed in the mean lifespan and antioxidant capacity (Ding et al. 2017).

It has been reported that UVB-induced epidermal hypertrophy, decreased expression of VEGF and MMP-13 in hairless mice given fucoxanthin (Baur et al. 2006). In addition, *D. melanogaster* had a 33.0% increase in life expectancy and an antioxidant effect. In *C. elegans*, it increased 14.0% in average life expectancy and antioxidant as well (Tung et al. 2015).

Spermidine had a 30.0% increase in mean survival and autophagy effect in *D. melanogaster*, and a 15.0% increase in median survival and autophagy in *C. elegans* (Toth et al. 2015).

Acacetin *D. melanogaster* showed decreased Acacetin rescued amyloid precursor protein (APP) expression, BACE-1 activity and Ab production effect (Wang et al. 2013). In *C. elegans*, it provided a 27.3% increase in life expectancy and upregulation of SOD-3 and GST-4 (Regitz et al. 2016).

When administered to C57BL6/N mice, curcumin caused antioxidant and increases in collagen and AGEs (Park et al. 2012). In *D. melanogaster*, it showed an increase of 25.8% in the average lifespan and antioxidant effect, while in *C. elegans*, it showed an increase of 25.0% in the average lifespan and antioxidant effect (Liu et al. 2015; Chen et al. 2015).

7.2.4 Anti-inflammatory Effect of Some Molecules That Are Related to Genes on Skin Contributing to Anti-aging Strategies

The largest organ in the body, the skin functions as a necessary interface to the external environment. Thus, it constantly protects the body from harmful stimuli, such as ultraviolet (UVB) radiation from sunlight, microorganisms, allergens, and irritants. Anti-inflammatory effect of some molecules associated with genes on the skin contributes to anti-aging strategies. There are numerous bioactive compounds (including antioxidants and polyphenols) that provide anti-aging benefits in plants. Polyphenols function as exogenous antioxidants; They have a donor hydroxyl (–OH) group attached to the aromatic ring that donates electrons and hydrogen to free radicals and other reactive species. Topical application of antioxidants can reduce the induction of matrix metalloproteinases (MMPs). It offers reduced transepidermal water loss (TEWL), increased skin elasticity, increased collagen formation, and reduced facial pigmentation (Campa and Baron 2018; Solano 2020).

The extracellular matrix (ECM) disruption in aged skin is associated with a low level of glycosaminoglycans, hyaluronan, dermatin sulfate, elastin, and collagen fibers. Various environmental factors such as pollution, chemicals, reagents, and UVB radiation cause an increase in dermal enzymes (hyaluronidase, elastase, MMP), which results in ECM degradation (Solano 2020).

Therefore, there is ample molecule derived from a variety of natural sources that have inhibitory potential on these enzymes and can be used as an anti-wrinkle in skin aging. Some of them are listed in Table 7.1.

7.2.5 Anti-inflammatory Effect of Some Molecules on Bone Loss That Contribute to Anti-aging Strategies

Control of bone metabolism is an important factor to reduce the resorption of bone tissue in various diseases where bone healing is reduced. Inflammation causes loss of bone healing ability in the elderly. An increase in chronic inflammation is the main

Table 7.1 Some natural molecules with anti-aging effects on the skin

Marigold (Tagetes erecta): It has anti-inflammatory properties which are important for phytotherapeutic, dermatological, and cosmetic applications (Kang et al. 2018).
Aloe vera (Aloe barbadensis Miller): It has the effect of reducing ROS formation by increasing the activities of mitochondrial reductase and complex II even under the influence of UVB (Lee et al. 2021).
Tomato extracts (Solanum lycopersicum L.): It has the ability to protect the skin from erythema and prevent skin damage caused by UVB rays (Lee et al. 2021).
Cactus extracts (Opuntia humifusa): It has anti-inflammatory and anti-aging effects by effectively inhibiting microphthalmia, reduced matrix metalloproteinase-1 (MMP-1), and transcription factor associated with JNK phosphorylation (Ha et al. 2016).
Green tea (Camellia sinensis): The polyphenols in its structure are antioxidants and they play a role in preventing oxidative damage caused by ROS. It has biological activities against skin aging, including inhibition of melanogenesis and TRP-2 activities and inhibition of MMP-2 through the suppression of tyrosinase and various phenolic acids present in it (Chaikul et al. 2020).
Pomegranate (Punica granatum): Pomegranate contains many compounds, including anthocyanins, catechins, and gallic acids, which have significant antioxidant activity. It has been reported in studies that it supports skin tissue repair and has an anti-aging effect as an important free radical scavenger (Pengkumsri et al. 2019; Kaur et al. 2006; Eghbali et al. 2021; Ko et al. 2021).
Cucumber (Cucumis sativus): Its antioxidant activity prevents and suppresses various diseases and aging by eliminating free radicals (Song et al. 2021; Jo et al. 2022).

reason why injured bones fail to heal with increasing age, according to a study in mice and humans. Bone health is very important in old age and therefore it should be well protected during youth. With the increasing awareness in society, the use of antioxidant compounds and products prepared from plants has increased. Among these compounds, resveratrol, which has anti-aging and anti-inflammatory effects, has become important in the light of various clinical and pharmacological studies. While resveratrol treatment stimulates osteoblastogenesis in vitro, it also inhibits osteoclastogenesis (Tseng et al. 2011). Resveratrol selectively binds to estrogen receptors on bone and cartilage cells in vitro to increase the expression of genes with osteo-protective and chondroprotective effects (Kim et al. 2014).

Cinnamic acid is a natural antioxidant compound found in the essential oil of the cinnamon plant. It has been reported that cinnamic acid treatment prevents postmenopausal bone loss through induction of osteoblast differentiation mediated by enhancement of BMP/TGFβ/Smad signaling by altering the gut microbiome (Hong et al. 2022).

7.2.6 Anti-inflammatory Effect of Some Molecules on Endocrine System That Contribute to Anti-aging Strategies

In modern era, long life expectancies are increasing and studies on the biology of aging are trying to elucidate the biochemical and genetic processes that lead to aging over time and to find new strategies to counter this process. In fact, aging is primarily

caused by a disrupted endocrine circadian rhythm, which has been linked to numerous health complications and pathologies in aging populations (Campisi et al. 2019; Trubitsyn 2020).

The endocrine system changes in many ways as we age. Some endocrine organs and axes become hypoactive due to diseases or physiological downregulation; some have minimal or no changes, and rarely become hyperactive (Campbell and Jialal 2022). Changes in the production and secretion of hormones, changes in their metabolic clearance, changes in hormone receptors or postreceptor mechanisms, and changes in the response or sensitivity of the tissue to the hormone lead to these different and sometimes striking results. Changes in serum hormone levels occur as a result of all these interactions. While the levels of some hormones change in direct relation to these mechanisms, the levels of some hormones also reflect the effects of compensatory mechanisms. Endocrine changes are reported to be involved in the pathogenesis of decreased muscle mass (sarcopenia), age-related cognitive dysfunction, atherogenesis, osteoporosis, and frailty. Endocrine and metabolic control systems also offer important opportunities for the prevention of disability related to aging. Thyroid disorders are quite common and the diagnosis is delayed. Early diagnosis and treatment can prevent disability (Kim et al. 2021b; Strous et al. 2020; Crafa et al. 2021).

7.2.7 Anti-inflammatory Effect of Some Molecules for COVID-19 That Contribute to Anti-aging Strategies

The New Coronavirus Disease (COVID-19) is a pandemic that affects the world. The elderly are much more likely to die from COVID-19 than the young. Perhaps the most important reason for this is the inability of the elderly immune systems to cope with infections and to get rid of them. The pandemic process has made it difficult to implement the steps to be healthy in the old age group. However, the principles have not changed, they have been adapted to the conditions of the pandemic. Important changes brought by pandemic conditions; wearing masks in accordance with the rules, paying attention to physical distance, applying hygiene rules, and complying with the recommendations to stay at home. In order to prevent the disease, individuals have tended to consume foods containing molecules with high antioxidant capacity and anti-inflammatory effect. These nutrients can be used to mitigate the pathological effects caused by COVID-19 infection. Consequently, the use of natural nutrients can provide prophylactic, therapeutic support, and anti-inflammatory effect as well as COVID-19 treatment (Mrityunjaya et al. 2020; Ghidoli et al. 2021; Wong et al. 2022; Ali and Kunugi 2021).

Many people have turned to try drugs that rejuvenate human bodies to boost their immune systems. Worldwide clinical trials are being carried out testing drugs that reverse the effects of age on the body, rejuvenate the immune system, and clean up aged, worn cells (Domi et al. 2022; Alfikri et al. 2020).

Some of these nutrients are:

Resveratrol which represents a broad class of polyphenols, which are anti-aging compounds, is known to be potentially useful in the treatment of disease (Liu 2022; Ghidoli et al. 2021). It regulates the immune system response and proinflammatory cytokines and prevents the onset of thrombotic events that usually occur in COVID-19 patients. Resveratrol, an all-natural agent, can be used as an adjuvant in the treatment of anti-COVID-19 through different mechanisms of action (Hwang et al. 2018).

***Syzygium aromaticum* L.**, rich in bioactive components, has many properties such as anti-inflammatory, antifungal, antiangiogenic, anti-microbial, and antiviral as well as high antioxidant capacity and anti-aging effect. In this context, *Syzygium aromaticum* L. extract, which is one of the important medicinal plants, is thought to reduce oxidative stress by eliminating free radicals (Esmaeili et al. 2022; Batiha et al. 2020; Vicidomini et al. 2021).

Allium sativum, which has a sharp and characteristic odor, has been used for years as both a food and herbal medicine due to its beneficial effects on health (Najman et al. 2020). It has been reported that *Allium sativum* and its compounds have many positive contributions to health, especially the healing effect on immune system functions, anticancer effect, anti-microbial activity, anti-stress, anti-diabetes, antioxidant properties, and protective effects on COVID-19 (Najman et al. 2020; Khubber et al. 2020; Donma and Donma 2020; Mobasheri and Shakibaei 2013; Tesfaye 2021).

The biological aging mechanism is quite complex. One of the most important changes in aging is increased autoimmunity, chronic low-grade inflammation, increased cancer incidences, decreased response to vaccines, decreased ability to fight infections, etc. These are both structural and functional changes in the immune system (Bajaj et al. 2021; Bartleson et al. 2021).

7.3 Conclusion

Inflammation is increasingly linked to aging and chronic diseases. It is important to know what kind of changes occur in the profile of the metabolome (all chemical molecules present in a biological sample) with aging. This issue is also closely related to inflammation. Because the cytokines that initiate inflammation are largely secreted from the adipose tissue. This close relationship between metabolic changes and inflammation also offers opportunities for treatment. There are many ongoing studies to find out whether this condition is caused by the accumulation of genetic mutations or, for example, by food. We will surely grow old and die, but we can develop strategies and slow the process down. Once we discover that aging is reversible, many possibilities open up before us. With non-drug approaches, healthy and long-term aging is aimed at reducing chronic and low-grade inflammation with the support of dietary supplements, mindfulness (attitude, awareness, meditation), complementary medicine practices such as acupuncture, homeopathy, herbal products, and hypnosis. Nutritional intake is a physiological condition that plays an important role in the development of the human body from birth to old age,

causing diseases and treatment of diseases. The nutritional elements in the right dose, in the right indication, and with the right duration will be beneficial in the elderly who develop immune dysfunction. Although the importance of inflammation in the aging process is known, the details of the mechanism that triggers the most important stimulus of inflammation are not yet fully known. Anti-aging medicine appears to be an extremely promising and challenging field that faces many limitations from different perspectives.

Conflict of Interest The authors declare no potential conflict of interest.

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Spermidine, an Autophagy Inducer, as a Therapeutic Antiaging Strategy

8

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Abstract

Aging is an ineluctable physiological process that occurs in humans and is often known to progress towards death. It is associated with organ failures, lowered metabolic rate, and a decline in adaptive capabilities of the body. As aging advances, it exerts its effects on the brain, affecting its size, vasculature, structure, and cognitive functions. Multiple factors like oxidative stress, mutation, protein aggregation, reactive oxygen species, and so on have unfolded themselves as contributors to the aging phenomenon. Majorly autophagy is downregulated in the conditions of aging. Therefore, the autophagic processes have marked their prime importance where their modulation could be instrumental in declining the aging pathologies. Spermidine is an autophagy inducer synthesized from diamine putrescine in our body. The age-linked alterations in the spermidine level link autophagy with cellular senescence. Further, recent research highlight the potential of spermidine to delay the aging process. Still, the antiaging mechanisms of spermidine remain unknown. This chapter aims to explore the role of spermidine in the brain and to summarize the epigenetic effects of spermidine linked with aging. We also expose the effects of spermidine on mitochondrial metabolism, lipid metabolism, circadian rhythm, cardioprotection, and anti-inflammatory effects.

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Spermidine · Aging · Autophagy · Polyamine · Epigenetics · Neurological disorder

8.1 Introduction

Aging is a biological multifaceted process affecting various molecular, cellular, and organ-level functions. It is characterized by a progressive decline of cellular function and therefore reducing the quality of life. With the increasing life expectancy, the aged population is growing at a rapid pace and so are the geriatric health issues and particularly the burden of neurological conditions. Interventions that promote healthy aging are examples of novel powerful disease management treatments that can have a major socioeconomic impact.

Various factors such as neuroinflammation, oxidative stress, epigenetic modifications, defective mitochondrial function, genetic anomaly, and defective autophagy accelerate the process of aging and neurodegeneration (Basu Mallik et al. 2022; Balaji et al. 2020; Chowdhury et al. 2021). The levels of natural polyamine spermidine progressively decrease with aging and its restoration in aging cells prolonged the lifespan in yeast, flies, worms, human cells, and mice (Eisenberg et al. 2009, 2016). Additionally, aged mice fed with a diet rich in spermidine reduced mortality (Soda et al. 2009). Spermidine has immense potential in antiaging and reducing age-related neurological, cardiovascular diseases, and increasing life span. It exerts its action primarily by enhancing autophagy and additionally by anti-oxidant, anti-inflammatory, improving proteostasis, reducing telomere attrition, etc. (Wirth et al. 2021). The antiaging mechanisms will be discussed in detail in the following sections.

8.2 Spermidine: Structure, Sources, and Biosynthesis

A eukaryotic and prokaryotic cell contains broadly distributed polyamines, including putrescine, spermine, spermidine, and cadaverine (Potter and Paton 2014). Polyamines are characterized by two or more amino groups with flexible hydrocarbon chain structures (Potter and Paton 2014). The structural characteristic of polyamines is important in providing stability, the ability to resist at different pH and form a hydrogen bond with water and alcohol (hydroxyl solvents) (Agostinelli et al. 2010; Handa et al. 2018; Muñoz-Esparza et al. 2019). These polyamines differ in hydrocarbon backbone chain structure and amine groups leading to changes in their physicochemical properties (Potter and Paton 2014). In mammalian cells, polyamines such as putrescine, spermidine, and spermine are present in millimolar concentration (Igarashi and Kashiwagi 2000; Pegg and Casero 2011). At physiological pH, polyamines including spermidine get completely protonated (Muñoz-Esparza et al. 2019). The polycation nature of polyamines leads to interaction with

negatively charged polyanionic macromolecules including lipids, proteins, RNA, and DNA. These intracellular interactions of polyamines are crucial for the stability of DNA, cell growth, differentiation, proliferation and death, tissue regeneration, and enzymatic modulation (Potter and Paton 2014; Igarashi and Kashiwagi 2000; Minois 2014). Spermidine is an aliphatic amine [*N*-(3-aminopropyl)-1,4-diaminobutane] and is synthesized from diamine putrescine. Spermidine possesses a low molecular weight, i.e., 145.25 g/mol (Ghosh et al. 2020).

In the human body, the basic polyamine pool is regulated by de novo biosynthesis, exogenous sources such as diet and intestinal microorganisms (Buyukuslu et al. 2014). The amount of endogenous polyamine biosynthesis is lesser than external dietary polyamine (Atiya et al. 2011; Bardócz 1995). Spermine and spermidine are described as only naturally occurring biogenic amines in food. Whereas other biogenic amines accumulate in presence of microorganisms (Wójcik et al. 2021). Dietary polyamines are crucial for maintaining normal metabolism and intracellular polyamine synthesis. Spermidine is one of the prominent polyamines found in all plant-derived food. Soy derivatives, cereal, and legumes are rich in spermin and spermidine (Muñoz-Esparza et al. 2019; Okamoto et al. 1997; Nishimura et al. 2006). Peas, pistachios, almonds, chestnut, hazelnut, mushroom, spinach, cauliflower, green bean, red kidney beans, white bread, rice bran, green pepper, green tea, and wheat germ are enriched with spermidine. Fruits such as tangerines, pears, oranges, and cherries contain lower levels of polyamines. Animal-derived food such as lamb, rabbit, veal, turkey, beef, chicken, pork, duck meat, egg, milk, and fish are also containing a significant amount of spermidine. Fermented food products such as sausages, sauerkraut, and cheese contain high polyamine concentrations (Muñoz-Esparza et al. 2019; Minois 2014). In the human body, intestinal microbiota also takes part in spermidine biosynthesis (Matsumoto and Benno 2007). Systemic spermine and spermidine concentration are also upregulated with oral consumption of prebiotics producing polyamines. In the intestine, the polyamine precursor arginine also contributes to the stimulation of spermidine synthesis (Kibe et al. 2014). With age the levels of polyamines are decreasing, due to reduced uptake, altered intestinal microbes, decrease intracellular biosynthesis, loss of transporters, and increased polyamine degradation (Madeo et al. 2019). It has been also suggested that in the elderly, levels of polyamines can be maintained by ingesting a spermidine-rich diet, spermidine-rich extracts, synthetic spermidine, and supplementation of pre and probiotics (Madeo et al. 2019). However, levels of polyamines have been negatively correlated with cancer, by promoting cancer cell growth (Gerner and Meyskens 2004).

In the body, polyamine levels are maintained in the optimal range by the import/export system and highly regulated metabolic pathways (Casero et al. 2018). Through the urea cycle, arginase 1 (ARG1) converts amino acid arginine into ornithine. Ornithine decarboxylase (ODC) is a rate-limiting step in the biosynthesis of polyamine, which produces putrescine from ornithine (Casero et al. 2018). Putrescine is the first mammalian polyamine formed in the body. In the biosynthesis of higher polyamines, such as spermidine and spermine, decarboxylated *S*-adenosylmethionine (AdoMetDc) plays an important role as an aminopropyl

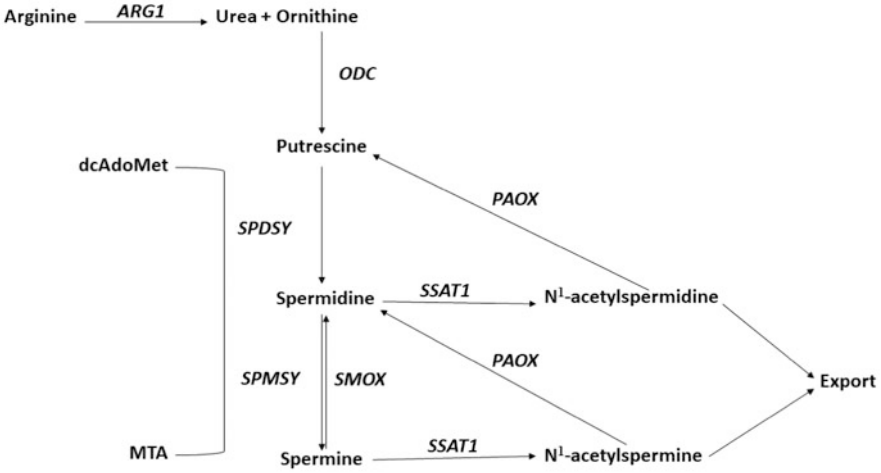

Fig. 8.1 Biosynthesis of spermidine

donor, for spermidine synthase (SPDS) and spermine synthase (SPMS) (Pegg and Casero 2011) (Fig. 8.1). Polyamine putrescine is then converted into spermidine by SPDS. Further, spermine is formed by spermidine through SPMS. The SPDS, SPMS, and AdoMetDc are encoded by SRM, SMS, and AMD genes, respectively (Pegg and Casero 2011; Casero et al. 2018). To maintain the relative amount and free concentration of spermine and spermidine, the action of AdoMetDc and ODC are highly regulated at different levels such as transcription, translation, and degradation (Pegg 2006; Wallace and Pegg 2009).

This biosynthesis pathway of polyamine is effectively irreversible but due to the action of Spermine oxidase (SMOX) and polyamine oxidase (PAOX) it can be interconvertible (Casero and Pegg 2009). Spermidine/spermine *N*1-acetyltransferase 1 (SSAT1) is essential for the excretion of spermine and spermidine. SSAT1 is encoded by the SAT1 gene. It forms *N*1-acetylspermine and *N*1-acetylspermidine which can be excreted by the body or converted into 3-acetylaminopropanal (3-APP) (Casero et al. 2018). The spermine and spermidine are also served as substrate/product PAOX. Peroxisomal enzyme PAOX catalyzes *N*1-acetylated polyamine. 3-APP is a serve oxidation product of PAOX-mediated acetylated polyamine oxidation. SSAT1 transfers the acetyl group to either spermidine or spermine from acetyl-CoA (Casero et al. 2018). SMOX also known as nuclear and cytosolic amine oxidase, plays important role in the oxidation of spermine and spermidine. SMOX-mediated oxidation of spermine leads to oxidation product 3-Aminopropanal (3-AP). SMOX (present in cytoplasm and nucleus) is often more active than PAOX (present in peroxisome) due to its cellular localization (Casero et al. 2018). In a cell, endocytosis and exocytosis processes regulate polyamine uptake and secretion across the plasma membrane and, it is characterized in an energy-dependent manner (Madeo et al. 2019; Poulin et al. 2012; Soulet et al. 2004). Studies of intestinal perfusion highlighted the rapid absorption of polyamine

in the intestinal brush border via the action of polyamine transporters and distributed to other tissues and organs. Similarly, after administration, within 1 min spermidine levels were also observed in the portal vein (Milovic 2001; Uda et al. 2003). In Mammals, pancreas contains the highest spermidine levels (Minois et al. 2011). In rats, 83 mg/kg/day dose of spermidine did not show adverse effects (Til et al. 1997). Earlier studies reported that a high dose of spermidine (250 nmol, i.c.v.) is responsible for hypothermia, focal encephalomalacia lesions sedation, adipsia, and seizures in rats (Anderson et al. 1975; Guerra et al. 2016).

8.3 Spermidine and Brain

Various studies interlinked spermidine intake with beneficial effects on the brain. The brain also consists of a high spermidine concentration. Nutritional spermidine supplementation correlated with improved brain functions, cardioprotective, anti-inflammatory properties, and improved mitochondrial function (Xu et al. 2020; Soda 2010; Wirth et al. 2019). Astrocytes and synaptic vesicles store brain polyamine. Astrocytic glutamatergic transmission and astrocytic Toll-like receptor 4-mediated dopaminergic signaling have been implicated in neurodegeneration and cognitive disorders (Satarker et al. 2022; Gurram et al. 2022). Thus, the distribution of polyamine in astrocytes and synaptic vesicles might be important for various polyamine regulatory activities with receptors located on the glial and neuronal cell surface (Masuko et al. 2003; Takano et al. 2005). In synaptic vesicles, accumulated polyamines are released under depolarized state (Masuko et al. 2003). Through polyamine transporters surrounding glial cells take up released polyamines. Intracellular and extracellular polyamines are having their distinct role. Intracellular polyamines are responsible for rectification and blockage of the AMP-activate protein kinase/kinate receptor and kir channel (Masuko et al. 2003). On the other hand, extracellular polyamines play important role in the modulation of *N*-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor transmission (Masuko et al. 2003; Chen et al. 2010; Kelly et al. 2009). For NMDA receptor, spermidine act as a positive allosteric modulator, and it has variable action on the NMDA receptor (Girardi et al. 2020). At low concentrations, spermidine facilitates channel opening and at higher physiological concentrations antagonistic effects have been seen on NMDA receptor (Jamwal et al. 2015). Gamma amino-butyric acid (GABA) receptor functions are also modulated by polyamines (Gilad et al. 1992). Changes in polyamine level results in alteration of polyamine interactions with different neuronal receptors and transmitters leading to various psychiatric and pathological conditions such as schizophrenia, mood disorders, anxiety, suicidal behavior, and stress (Chen et al. 2010; Genedani et al. 2001; Guipponi et al. 2009; Gilad and Gilad 2002; Ramchand et al. 1994). In psychiatric disorders, along with traditional monoaminergic transmission attention should also be given to the polyamine system (Fiori and Turecki 2008; Sequeira et al. 2006; Chen et al. 2010).

The age-related memory loss can be attenuated by an external supplement of spermidine. Indeed, in Drosophila dietary spermidine supplementation showed restoration of polyamine levels including spermine and putrescine in aged flies' head. An age-induced decline in intermediate and short-term olfactory memory was effectively protected in the fly as compared to juveniles (Gupta et al. 2013). Similarly, in human basal ganglia, spermidine and spermine levels are declining with age which further contributes to age-related changes in white matter (Vivó et al. 2001). In flies, spermidine intake can overcome the age-related dysfunction of autophagic machinery and hence prevent poly-ubiquitinated protein accumulation (Gupta et al. 2013; Sigrist et al. 2014). Spermidine is a natural autophagy inducer that prolongs lifespan in an autophagy-dependent manner across various species (Eisenberg et al. 2009, 2016; Yue et al. 2017). Spermidine shows its action through autophagy to maintain synaptic plasticity/transmission and clear out cellular waste such as pathogenic aggregates of protein (Maglione et al. 2019). In aged mice, dietary spermidine crosses the blood-brain barrier and thereby improves temporal and spatial memory. Spermidine increases eukaryotic translation initiation factor 5A hypusination in the hippocampus and mitochondrial function. In Drosophila, spermidine improves mitochondrial function by autophagy and mitophagy-related manner (Schroeder et al. 2021). Spermidine-mediated regulation of synaptic activity and aging helps in improving neuronal dysfunction (Eisenberg et al. 2016; Filfan et al. 2020). The restoration of striatal neurochemistry and alleviation of neuroinflammation and oxidative stress were also reported with spermidine treatment in the striatal region of the brain (Jamwal et al. 2015). The release of systemic and neuronal proinflammatory cytokines and NLRP3 inflammasome activation has been correlated with cognitive impairment, fatigue, and decreased locomotion (Mudgal et al. 2020; Kinra et al. 2021).

The intracerebral and intraperitoneal spermidine infusion causes an increase in cognitive performance, and it is correlated with the impact of spermidine on NMDA receptors (Guerra et al. 2016). Similarly, bilateral infusion of spermidine into the hippocampus showed memory improvement through protein kinase A/cAMP response element-binding protein signaling pathway (Guerra et al. 2011). In the mice model, intraperitoneal administration of spermidine in mild cognitive impairment showed restoration of memory (De Risi et al. 2020). The brain tissues from suicide completers with and without major depressive disorders exhibited downregulated expressions of SAT1 (Chen et al. 2010). To treat age-associated Alzheimer's disease spermidine is considered a potential therapeutic approach (Joaquim et al. 2019). Researchers also tried to establish an interlink between behavioral modification with polyamine levels. The mice subjected to social isolation showed an increase in brain spermidine but not spermine levels and aggressive behavior. However, reintroduction of experimental animals to their colony showed a decrease in both spermidine levels and aggressive behavior (Tadano et al. 1974; Tadano 1974; Guerra et al. 2016).

8.4 Spermidine as an Autophagy Inducer in Aging

Autophagy is a cellular quality control process that degrades components such as defective cellular organelles and misfolded proteins through lysosomes. Impaired autophagy is strongly associated with aging (Aman et al. 2021). It is also instrumental in maintaining homeostasis at a cellular level, preventing stress-induced damage, along with promoting the growth and pathogenic defense mechanism (Arachiche and Gozuacik 2015). It can be divided into three categories namely macroautophagy, where the contents to be degraded are packed into vesicles named autophagosomes; microautophagy, where the lysosomes undergo invagination to incorporate the contents of the cytoplasm followed by its degradation; and chaperone-mediated autophagy, where degradation of specific proteins occur upon their transportation to lysosomes (González-Polo et al. 2016). Spermidine promotes autophagy through several molecular mechanisms. At an epigenetic level, it inhibits histone acetyltransferases (HATs) and modulates the increased expression of several autophagy-related genes to induce autophagy (Eisenberg et al. 2009, 2016; Morselli et al. 2011; Madeo et al. 2014).

Besides HATs, spermidine stimulates autophagy by inhibiting several acetyltransferases and through promoting deacetylation of various cellular proteins similar to caloric restriction mimetics (CRMs). Of note, the competitive inhibition of E1A-associated protein p300 (EP300) acetyltransferases is sufficient to induce autophagy by spermidine. EP300 functions as a sensor of nutrient-starved acetyl-CoA (AcCoA) levels and increases the acetylation of several autophagy-related proteins. It also stimulates the deacetylation of tubulin by inhibiting α-tubulin acetyltransferase1. (Eisenberg et al. 2009; Lee and Finkel 2009; Morselli et al. 2011; Mackeh et al. 2014; Mariño et al. 2014; Pietrocola et al. 2015). Nutritional starvation causes rapid depletion of AcCoA, leading to quick reduction of acetylated cytosolic proteins and induction of autophagy. Reduced levels of AcCoA favor the inhibition of EP300, which in turn promotes autophagy flux. AcCoA and EP300 promote autophagy-inhibitory mechanistic targets of rapamycin complex1 (mTORC1) (Mariño et al. 2014). Catabolism of spermidine requires AcCoA, thus spermidine may lower the activity of acetyltransferase by depleting the AcCoA levels (Madeo et al. 2018a, b). Therefore, spermidine, through regulating AcCoA levels and EP300 activity inhibits the mTORC1 to induce autophagy (Madeo et al. 2014; Mariño et al. 2014; Pietrocola et al. 2015).

Of the several targets, spermidine-mediated acetylation of microtubule-associated protein 1S (MAP1S) plays an important role in life span expansion. Spermidine enhances the stability of MAP1S and stimulates autophagy flux by depleting cytosolic histone deacetylase 4 (HDAC4). Spermidine-specific acetylated site lysine residue 520 of MAP1S is important for autophagosome-lysosome fusion (Yue et al. 2017). Resveratrol, a SIRT1 activator is a potent inducer of autophagy. Both resveratrol and spermidine promote autophagy synergistically through different mechanisms and share convergent effects on the modification of acetylproteome. Both these compounds induce changes in the acetylation and deacetylation of nuclear, cytosol, and mitochondrial proteins (Morselli et al. 2011).

8.5 Spermidine as an Epigenetic Regulator of Autophagy and Connection with Aging

The autophagy proteins are encoded by autophagy-related genes (ATG) like ULK1 serine-threonine complex, Beclin 1/class III phosphatidylinositol 3-kinase (PI3KC3), ATG9A, WIPI (WD repeat domain phosphoinositide-interacting) proteins, ATG2A/ATG2B, ATG12, ATG5 and ATL16L1, and so on (Levine and Kroemer 2019). Therefore, modulation of ATG especially by natural polyamines like spermidine and spermine is essential in maintaining autophagy and regulating the phenomenon of aging (Minois 2014).

As a result, the inhibition of EP300 by spermidine (which competes with the acetyl group donor acetyl coenzyme A) stimulates autophagy (Minois 2014; Soriano-Tárraga et al. 2019). Autophagy is required for the antiaging effect of spermidine as indicated by the fact that genetic inhibition of autophagy (by knockout or knockdown of essential autophagy-relevant genes) abolishes the longevity-extending effects of spermidine on yeast, worms, and flies (Pietrocola et al. 2015).

Epigenetic modifications are associated with the worsening of cellular mechanisms seen in aging conditions (Soriano-Tárraga et al. 2019). Spermidine is instrumental in the epigenetic regulation of autophagy via modulating various components. It regulates Atg genes and inhibits the enzyme E1A-associated protein p300 (EP300) (Pietrocola et al. 2015). The EP300 knockdown is known to enhance autophagic conditions (Lee and Finkel 2009). As it endogenously inhibits autophagy, it acetylates lysine residues of proteins involved in autophagy mechanisms. Therefore, spermidine treatment causes competitive inhibition of the EP300 to stimulate autophagy (Madeo et al. 2018a, b). Peitrocola et al. showed that aspirin-mediated inhibition of EP300 acetyltransferase promoted autophagic conditions in mice and nematodes (Pietrocola et al. 2018). Chromatin modifying enzyme-mediated histone acetylation plays a role in the epigenetic regulation of transcription factors. HDACs are associated with the inactivation of chromatin structures that represses transcription ultimately affecting autophagy (Gray and Ekström 2001). Spermidine inhibits Histone deacetylase (HDAC) and eukaryotic translation initiation factor 5A (elf5A) thus facilitating autophagic processes (Ni and Liu 2021). Hypusinated elf5A levels express mitochondrial proteins and maintain the tricarboxylic acid (TCA) cycle and oxidative phosphorylation thus modulating autophagy in macrophages (Puleston et al. 2019). Higher hypusinated elf5A levels are necessary for the TFEB expression that is ultimately important in the hematopoiesis and B cell Activation. Spermidine promotes higher expression of hypusinated elf5A and TFEB with an associated improvement in the functioning of aged B cells, especially in aged mice (Zhang et al. 2019). The decline in hypusinated elf5A levels in the aged Drosophila brain has been ameliorated by spermidine treatment possibly via translation of autophagic components and mitochondrial proteins (Liang et al. 2021). Spermidine also can delay aging through specific signaling pathways, such as SIRT1/PGC-1α, insulin/IGF, AMPK-FOXO3a, and CK2/MAPK signaling

pathways (Park and Kim 2012). Its action on transcription factor TFEB is also instrumental in autophagic processes (Alsaleh et al. 2020).

Spermidine and spermine promoted anti-aging effects in the senescence-accelerated mouse-8 (SAMP-8) model by enhancing autophagic process and mitochondrial functioning. It was shown to prevent cognitive decline and oxidative stress, improve synaptic plasticity, recover mitochondrial dysfunction, and initiate autophagy in the SAMP-8 model (Eisenberg et al. 2009). Similarly, Eisenberg et al. reported that spermidine treatment prevented aging in human-based immune cells and mice along with other organisms like yeast and flies and induced autophagy (Eisenberg et al. 2009). It also lowered histone H3 acetylation in human immune cells and mice hepatocytes and inhibited histone acetyltransferase (HAT) activity (Sacitharan et al. 2018).

Surprisingly, in the aged and osteoarthritis conditions, chondrocyte autophagy was induced by the activation of EP 300 by spermidine (Sacitharan et al. 2018). This was in contradiction to the findings that spermidine is a competitive inhibitor of EP 300 (Morselli et al. 2011; Pietrocola et al. 2015). This concern was aptly raised by Borzi et al. highlighting the findings of Sacitharan et al. to be contradicting in the interest of spermidine and EP 300 research (Borzì et al. 2019). Interestingly, addressing the comments of Borzi et al., Sacitharan et al. put forth that their research was specific to the human chondrocytes, focusing on bone physiology in comparison to other findings which were based on cell-free systems (Sacitharan et al. 2019). The deletion of Atg5 in podocytes enhanced aging and led to glomerular disease progression (Liang et al. 2020). Spermidine altered the uptake of glucose in aged mice and promoted cardioprotective effects thus signifying its role in aged conditions (Wirth et al. 2021). It was also shown to favor DNA synthesis in rat hepatocytes induced by hepatocyte growth factor (Higaki et al. 1994). An interesting study by Gupta et al. reported a decline in spermidine levels in aging fruit flies with an associated decline in cognition. A randomized clinical trial (NCT03094546) was conducted to assess the effect of spermidine supplements on the cognition of older adults (Gupta et al. 2013). The administration of spermidine promoted autophagy and ameliorated memory deficits (Madeo et al. 2018a, b). It also stabilized microtubule-associated protein 1S (MAP1S) in aging mice that was associated with life span longevity and autophagy induction via HDAC4 depletion (Madeo et al. 2018a, b). Similarly, an interesting study by Yue et al. demonstrated that spermidine treatment reduced liver fibrosis and liver carcinoma in mice and showed greater levels of MAP1S that promoted autophagy and improved their life spans (Yue et al. 2017). In a study performed in nematodes and yeast cells that lacked SIRT1, the administration of spermidine increased their life span and promoted autophagy via AMP-dependent kinase/mTOR-independent pathways (Morselli et al. 2011). Therefore, the polyamines like spermidine play an important role in the epigenetic regulation of autophagic processes in conditions of aging.

8.6 Crosstalk Between Mitochondrial Metabolism and Autophagy

Mitochondrial metabolism and autophagy are the two most common active cellular processes crucial for regulating organism longevity. Perturbation in either of these processes, influences each other's activity, impairs cellular homeostasis and promotes inflammation (Levine et al. 2011; Green et al. 2011; Soto-Heredero et al. 2017; Gabandé-Rodríguez et al. 2019). Dysfunctional mitochondria are the major sources of reactive oxygen species (ROS) generation, and thus it is important to clear them. The selective autophagy process involved in mitochondrial clearance is known as mitophagy (Wade Harper et al. 2018). Accumulation of mutations in mitochondrial DNA (mtDNA) promotes mitochondrial degeneration and compromises its function, which eventually leads to vicious cellular aging. Impaired crosstalk between mitochondria and autophagy, can also stimulate severe inflammatory pathways which commonly occur during aging (Gabandé-Rodríguez et al. 2019; Jordan et al. 2019).

The calorie restriction (CR) approach was one of the first classical antiaging strategies employed. CR treatment improves autophagy and mitochondrial health by promoting autophagosome formation and mitophagy. CR approach also exhibits potential anti-inflammatory activity. A class of new compounds, known as CR mimetics, mimics the effect of CR (Gabandé-Rodríguez et al. 2019; Jordan et al. 2019). Spermidine is one such molecule that promotes mitochondrial fitness through mitophagy and exhibits anti-inflammatory properties (Eisenberg et al. 2016). Spermidine slows cardiac aging by improving mitochondrial respiratory function and titin phosphorylation by inducing cardiomyocyte autophagy and mitophagy (Abdellatif et al. 2018).

8.7 Anti-inflammatory Effects

Aging is associated with chronic progressive inflammation, termed inflammaging (Franceschi and Campisi 2014). Spermidine showed direct anti-inflammatory effects and reduced the levels of inflammaging-associated cytokines such as TNF, IL-6, IL-1β, and IFNγ in mice (Eisenberg et al. 2016). It also maintains tolerance by elevating the anti-inflammatory cytokine, IL-10 (Hasko et al. 2000). It inhibits microglial activation observed with LPS by inhibiting NF-κβ under in vitro conditions. Importantly, spermidine inhibited the increased leukocyte migration during aging by attenuating the adhesion molecule expression (Choi and Park 2012). It hypermethylates and reduces the expression of *Itgal* gene encoding the adhesion molecule, LFA-1 in lymphocytes (Kano et al. 2013; Gabandé-Rodríguez et al. 2019).

8.8 Lipid Metabolism

Lipid metabolism plays a crucial role in health and life span. Disruption of lipids can have a severe impact on health and aging. Spermidine-fed flies showed elevated levels of triglycerides and altered levels of phospholipid and fatty acid profiles, which are associated with an increased life span in flies. Spermidine is essential for adipogenesis. In 3T3-L1 cells, spermidine interacts with acidic nuclear phosphoproteins 32 (ANP32) and blocks the interaction between human antigen R (HUR) and protein phosphatase 2Ac (PP2Ac). Decreased PP2Ac activity promotes the nuclear import of HUR, which in turn interacts with CCAAT/enhancer-binding protein β (C/EBP-β) to promote the expression of transcription factors such as peroxisome proliferator-activated receptor γ2 (PPAR-γ2) and sterol-regulatory element-binding protein (SREBP-1c). Elevated levels of these transcription factors promote differentiation of preadipocyte to mature adipocyte. (Minois 2014; Minois et al. 2014).

8.9 Cardioprotection in Aging and Hypertensive Heart Failure

In the aging population incidence and prevalence of heart failure are increasing at an alarming speed, which necessitates the use of cardioprotective agents. Spermidine is one such molecule whose treatment enhances cardiac function. Spermidine improves mitochondrial volume and function and also inhibits cardiac remodeling by promoting cardiac autophagy and mitophagy in cardiomyocytes. Spermidine improves cardiomyocyte structure and function by inhibiting circulating inflammatory TNF-α levels. This ameliorates titin phosphorylation and reduces titin-based myocardial passive stiffness. Spermidine enhances arginine bioavailability, which improves the production of vasodilator nitric oxide (NO). NO exhibits cardioprotection in aging through improving titin phosphorylation and reducing blood pressure (Eisenberg et al. 2016).

8.10 Circadian Rhythm and Aging

Mice with an innate circadian period of close to 24 h showed enhanced longevity than their littermates with deviations from the circadian period (Libert et al. 2012). Levels of polyamines decline with age and are associated with various pathologies. Clock-dependent mechanisms regulate polyamine biosynthesis through BMAL1 and CLOCK transcription factors that drive the transcription of the period (PER2) and cryptochrome (CRY1). Polyamines in turn control the circadian period by regulating the interaction between core clock protein PER2 and CRY1 in cultured cells and mice. Nutritional interventions such as polyamine supplementation can work against the disruption of circadian rhythmicity with age (Zwighaft et al. 2015).

Various epidemiological and clinical studies suggested an inverse correlation of overall mortality with the intake of polyamines such as spermidine (Kiechl et al.

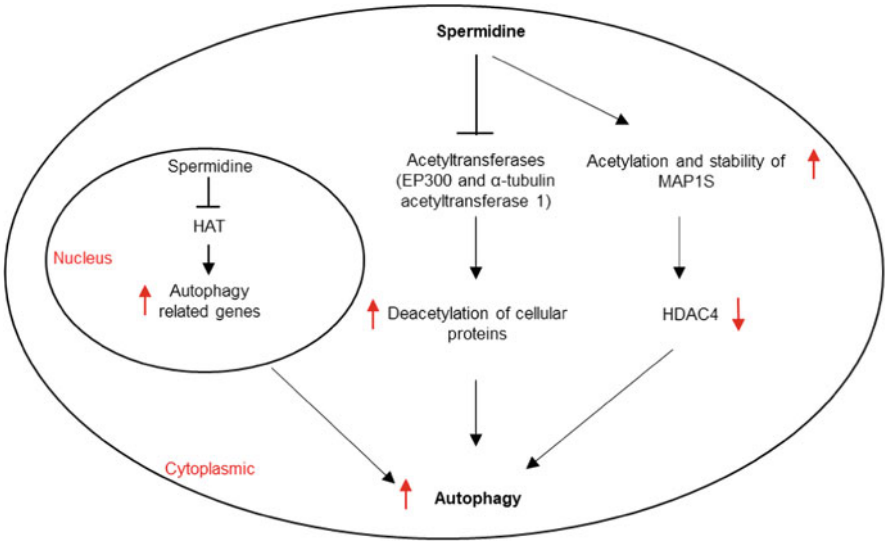

Fig. 8.2 Induction of autophagy by spermidine. Spermidine through HAT inhibition regulates the transcription of autophagy-related genes to induce autophagy. Spermidine by inhibition of acetyltransferase EP300 promotes deacetylation of autophagic-related cellular proteins to stimulate autophagy. It also induces autophagy by promoting acetylation and stability of MAP1S target. Spermidine shares convergent effects to drive induction of autophagy to promote health and longevity

2018; Hofer et al. 2021). Mice fed with chow containing probiotic-*bifidobacterium* LKM52 inhibited senescence and enhanced longevity by enhancing gut bacterial polyamines (putrescine, spermidine, and spermine) production (Matsumoto et al. 2011). Supplementation of probiotic LKM52 in combination with arginine led to upregulation of blood concentration of spermidine and spermine which showed reduced inflammation and enhanced longevity in mice (Kibe et al. 2014; Madeo et al. 2018a, b). In aging skin, *Streptococococcus* secreted spermidine promoted skin structure rejuvenation through upregulation of collagen and lipid synthesis (Kim et al. 2021). A schematic diagram of the autophagy pathway of spermidine is depicted (Fig. 8.2) and Table 8.1 describes the antiaging mechanisms of spermidine.

8.11 Conclusion

Spermidine by inducing autophagy modulates the activity of targets involved in aging. Since autophagy is involved in the neural mechanisms linked with aging, probably the therapeutic efficiency of spermidine in the aging process may be attributed to autophagy. Electrophysiological studies and optogenetics may open vistas in elucidating the mechanism of action of spermidine in aging. Studying the

Table 8.1 Antiaging mechanisms of spermidine

Mechanism of action	Target	Function	References
Inhibition of histone acetyltransferases (HATs)	Deacetylation of histone H3	Suppression of oxidative stress and necrosis and promotes longevity	Eisenberg et al. (2009)
Deacetylation of cellular proteins	Inhibition of mTORC1 complex	Induction of autophagy	Morselli et al. (2011), Pietrocola et al. (2015)
Inhibition of EP300 acetyltransferase	Deacetylation of autophagy-related (ATG) proteins, and increases acetylation of α-tubulin	Induction of autophagy	Pietrocola et al. (2015)
Acetylation of MAP1S	Depleting cytosolic HDAC4	Stimulation of autophagy flux	Yue et al. (2017)
Controls period (PER2) and Cryptochrome (CRY1) transcription factors	BMAL1 and CLOCK transcription factors	Normalizes circadian rhythm	Zwighaft et al. (2015)
Reduced levels of AcCoA	EP300	Induction of autophagy	Mariño et al. (2014)
Inhibition of NF-kβ	Inhibits microglial activation	Anti-inflammatory	Choi and Park (2012)
Hypermethylation of *Itgal* gene	Lower levels of LFA-1 in lymphocytes	Anti-inflammatory	Kano et al. (2013), Gabandé-Rodríguez et al. (2019)
Inhibition of circulatory TNF-α levels	Ameliorates titin phosphorylation	Cardiac protection	Eisenberg et al. (2016)
Increases arginine bioavailability	Stimulates production of nitric oxide-titin phosphorylation	Cardiac protection	Eisenberg et al. (2016)
Interacts with ANP32	Blocks HUR and PP2Ac interaction-promotes expression of transcription factors PPAR-γ2 and SREBP-1c	Maturation of adipocytes	Minois (2014), Minois et al. (2014)

target and developing spermidine-based ligands with actions on epigenetics of aging could be a future method for the therapy of age-associated neurological dysfunction.

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Melatonin in Aging and Aging-Related Disorders

9

Sibel Suzen

Abstract

Mammalian cells have multifaceted systems and structures to avoid accumulation of unfolded or misfolded proteins, the accumulation of which is a feature of aging and aging-related conditions. With a gradually increasing elderly population, aging-related pathologies such as neurodegenerative disorders, cancer, and cardiovascular diseases are becoming a growing economic, social, and personal problem. Few or no effective treatments are presented for aging-associated neurodegenerative conditions, which progress in an irreversible way.

Melatonin (MLT), a pineal hormone and an endogenous antioxidant, has numerous physiological functions in the brain, including regulating circadian rhythms, scavenging free radicals, preventing oxidation, and suppressing neuroinflammation. Clinical studies revealed that MLT levels are significantly reduced in patients with neurodegenerative diseases. Research proved that MLT prevents activity on neurodegeneration. As a chronobiotic, MLT can modify *circadian* rhythm. As a cytoprotective molecule, it prevents inflammatory injury in neurodegenerative diseases and aging.

This chapter discusses neurodegenerative diseases, which are the main risk elements of aging, antioxidant properties, and action of MLT, as well as MLT achievement in the pathogenesis of neurodegenerative diseases and its associations with the nine biological hallmarks of aging. The main physiological mechanisms of aging and their potential as targets of novel treatments for neurodegenerative diseases are also discussed.

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Keywords

Melatonin · Aging · Oxidative stress · Neurodegenerative diseases · Cancer

Abbreviations

3-NPA	3-Nitropropionic acid
5-HIAA	5-Hydroxyindoleacetic acid
5-HT	Hydroxytryptamine
Aβ	Aggregated β-amyloid
AD	Alzheimer's disease
AFMK	*N*1-acetyl-*N*2-formyl-5-methoxykynuramine
ALS	Amyotrophic lateral sclerosis
AMD	Age-associated macular degeneration
AMK	*N*1-acetyl-5-methoxykynuramine
AMPK	AMP-activated protein kinase
βAPP	Amyloid-β precursor protein
BBB	Blood-brain barrier
CAT	Catalase
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
COX-2	Cyclooxygenase-2
fALS	Familial ALS
GIT	Gastrointestinal tract
GPCR	G-Protein coupled receptors
GSH	Glutathione
HAT	Acetyltransferase
HATs	Histone acetyltransferases
HD	Huntington's disease
HDACs	Histone deacetylases
IGF-1	Insulin-like growth factor 1
IL	Interleukin
LRRK2	*Leucine-rich repeat kinase*
MCI	Mild cognitive impairment
MDA	Malondialdehyde
MLT	Melatonin
MS	Multiple sclerosis
MSCs	Mesenchymal stromal cells
MtDNA	Mitochondrial DNA
Mtor	Mammalian target of rapamycin
NAS	*N*-Acetylserotonin
NFTs	Neurofibrillary tangles
NF-κB	Nuclear factor kappa B
NSCs	Neural stem cells

OS	Oxidative stress
PD	Parkinson's disease
PINK1	PTEN-induced kinase 1
POCD	Postoperative cognitive dysfunction
ROS	Reactive oxygen species
sALS	Sporadic ALS
SASP	Senescence-Associated Secretory Phenotype
SCN	Suprachiasmatic nucleus
SIRT	Sirtuin
SOD2	Superoxide dismutase 2

9.1 Introduction

The average lifespan of human has enlarged significantly due to the control of infectious diseases by the discovery of antibiotics and vaccines. Furthermore, healthy nutrition, improved sanitary conditions, and good developments in the management of diseases such as cancer or diabetes (Finch 2010). Aging is described by consequences of loss of physiological activity, causing impaired function and increased weakness. This weakening is the main risk factor for many diseases including cancer, diabetes, cardiovascular disorders, and neurodegenerative disorders.

Oxidative stress (OS) or free radical theory of aging is associated with age-related capacity losses that caused the accumulation of oxidative damage to vital macromolecules such as lipids, DNA, and proteins (Liguori et al. 2018; Suzen et al. 2017). This theory describes that aging is an outcome of the failure of defensive processes to battle the reactive oxygen species (ROS)-related injury, predominantly at the mitochondria (Islam 2017). Elderly people are vulnerable to OS because of a weakening in the effectiveness of their endogenous antioxidant systems. Organs, particularly brain with high rates of oxygen consumption and restricted respiration levels, are most defenceless to this occurrence which partly describes the high prevalence of neurological conditions in the elderly (Corb et al. 2008; Tan et al. 2018).

Melatonin (MLT), a neuroregulatory hormone synthesized by pineal gland, is a powerful endogenous antioxidant, which protects the body from the effects of numerous harmful agents. Thus, it was assumed that the decline of MLT levels with age correlated to the aging progression. Agreeing to all of the positive data gained from investigational and clinical trials, MLT may have a prophylactic and therapeutic action on neurodegenerative diseases (Li et al. 2021). Since MLT is known as a neuroregulator hormone that has free radical scavenger, anti-inflammatory, and immunosuppressive actions as well as preventive effects against autophagy, it could have a significant part in the pathophysiological processes of neurodegenerative diseases. It can protect neurodegeneration maintaining the

homeostasis and survival of neurons (Luo et al. 2020b). This data proves that MLT is a nontoxic and essential protecting molecule and displays favorable effects on numerous neurodegenerative conditions. Administration of MLT in patients suffering from neurological disorders could be one of the approaches researchers have been looking for (Gunata et al. 2020).

9.2 Hallmarks of Aging and Melatonin

Mechanisms of aging and aging-related diseases still need to be clarified. Nine hallmarks of aging have been defined, with signs that these are also conserved in humans The cellular and molecular markers for aging are under investigation (Van der Rijt et al. 2020). There are many factors related when determining the aging phenotype (Lopez-Otín et al. 2013). Theories related to aging point the main importance of damaging changes to DNA that accumulate throughout life (Hoeijmakers 2009).

Multiple protective actions of MLT and its metabolites are present related to the decrease of inflammatory reactions and development of inflammaging of the nerves, prevention of free radical production, avoidance of NADPH oxidase stimulation and inhibition of nitric oxide synthase, as well as modulation of proinflammatory cytokines which are closely related to the hallmarks of aging (Hardeland et al. 2015).

9.2.1 Genomic Instability

One of the important hallmarks of aging is genome instability, which is connected to a variety of DNA alterations such as point mutations, chromosomal rearrangements, and whole chromosome numerical changes. Genomes are unstable due to the constant induction of damage to DNA, either from endogenous or exogenous foundations. DNA damage usually is effectively repaired while repair enzymes do not know DNA mutations. Therefore, the build-up of mutations with age is not reliant on DNA repair capability (Vijg and Montagna 2017; Santra et al. 2019). Although genomes are tolerated some quantity of mutation, it is possible to have a linear increase of somatic mutations with age. Animal studies confirmed that MLT extended the lifespan. Comparative to genomic instability, MLT was found active in decreasing DNA damage produced by aging. MLT displays an effective antioxidant performance assisting in modulating genetic and physiological modifications due to aging (Damiani et al. 2020).

9.2.2 Telomere Attrition

Telomere length is a marker of biological age and has significant role in multiple cellular processes since they protect chromosome fusions and chromosomal instability (Aksenova and Mirkin 2019). Two important elements of human telomerase

are known as telomerase reverse transcriptase and telomerase RNA component aiding the telomere action (Rubtsova and Dontsova 2020). Due to many human somatic cells having very low or no telomerase action, it consequences in age-linked pathological conditions. Telomere shortening can be one of the reasons for mammalian aging, while telomerase can oppose this occurrence. Stimulation of telomerase is considered as an encouraging therapeutic approach in the management of neurodegenerative aging-related diseases (Prieto 2020). Conversely, telomerase undeniably has potential as an antiaging therapeutic however it was found that it is overexpressed in almost 90% of human cancers increasing uncertainties about the use of telomerase activator compounds for treatments (Smith 2020). MLT was found as a perfect inhibitor of the vascular endothelial damage which is described by the over formation of inflammatory cytokines, raised ROS, MDA, and SOD quantities, and more apoptotic endothelial cells (Xie et al. 2021).

OS has also been shown to stimulate telomere attrition. Furthermore, injury by free radicals is associated with reduced circadian rhythmicity. MLT, being an exceedingly pleiotropic regulator neurohormone, cooperates directly or indirectly with all the antioxidant processes. Administration of MLT produced a healthy aging. This has been detected in animal experiments and is highly likely in humans (Hardeland 2013).

9.2.3 Epigenetic Alterations

Recent research has pointed to epigenetic mechanisms in the regulation of the gene expression alters modifying many aging-linked diseases such as cancer and neurodegenerative conditions. Epigenetics includes all the mechanisms regulating gene expression independent of the DNA sequence (Pagiatakis et al. 2021). There are three main types of epigenetic alteration namely DNA methylation, histone modification, and noncoding RNA. The level, type, and distribution of epigenetic alteration changes throughout an organism's life thereby changing the expression of genes in ways that are deleterious to the organism. Neurodegenerative disorders are strongly associated with the procedure of aging. It was proved that enhanced epigenetic age was related to the progress of neurodegenerative conditions (Dolinar et al. 2018). Alzheimer's disease (AD) and Parkinson's disease (PD) are neurodegenerative diseases that have been found connected with epigenetic modifications (Tecalco et al. 2020). It was found that specifically three types of the sirtuin (SIRT) namely SIRT1, SIRT3, and SIRT6 are related to aging process. Overexpression of SIRT1 advances health features during aging regardless of not increasing lifespan (Herranz et al. 2010). Dropping the activity of SIRT6 decreases the lifespan (Kanfi et al. 2012). MLT and SIRTs are geroprotective compounds that effect on the aging progress (Carbone et al. 2020). Many studies describe an increase in action, particularly on SIRT1, in a variety of cells after MLT administration (Mayo et al. 2017).

Histone acetylation is one of the most common epigenetic modifications. Two enzymes namely histone acetyltransferases (HATs) and histone deacetylases (HDACs) regulate histone acetylation and deacetylation (Tain et al. 2014a). It was

observed that MLT maintained the expression of HDAC-2, HDAC-3, and HDAC-8 after the administration (Sharma et al. 2008). Results display the probability that MLT can perform as an HDAC inhibitor and effects on HDACs to control gene expression alteration (Tain et al. 2014b).

9.2.4 Loss of Proteostasis

Proteostasis deterioration is a well-known hallmark of aging (Klaips et al. 2018). Aging and some aging-associated disorders are related to impaired protein homeostasis, similarly known as proteostasis (Powers et al. 2009). In healthy cells, a multifaceted proteostasis system, including molecular chaperones and proteolytic mechanisms and their regulators, controls the maintenance of proteostasis. These influences organize protein synthesis with polypeptide folding, the protection of protein structure, and protein degradation (Hipp et al. 2019). While cells age, environmental stresses add up and mechanisms responsible for controlling proper protein composition begin to drop. Proteins lose their stability, autophagic progressions start to fail, and misfolded proteins build-up. The subsequent build-up of misfolded and aggregated proteins disturbs especially postmitotic cells, such as neurons, exhibiting in disease (Ottens et al. 2021). It seems that maintenance of proteostasis is crucial for cell health and longevity.

Regulation of proteostasis and SIRT1 which are related to aging and neuroprotection is getting more interest since they are closely related to the development of specifically neurodegenerative diseases. MLT prompts the overexpression of SIRT1 and downregulates the p53-MDM2 pathway. Animal studies showed that in β-amyloid peptide injected rats, MLT upregulated SIRT1 and the mitochondrial transcription factor A which are crucial features for accurate mtDNA replication and mitochondrial biogenesis. This means MLT regulates hippocampal neuronal homeostasis (Ramos et al. 2019).

9.2.5 Cellular Senescence

Cellular senescence is a stable state of cell cycle arrest that happens in proliferating cells exposed to different stresses. Senescence is hence a cellular defense instrument that stops or avoid the cells to gain harm. Senescent cells are able to build-up with age and at locations of age-associated pathologies, like osteoarthritis (Pignolo et al. 2021) and atherosclerosis (Wu et al. 2020). Among all of the aging hallmarks, cellular senescence processes are the main aspect of the complexity of aging (Mchugh and Gil 2018).

Administration of SIRT1 inhibitor such as sirtinol or MLT receptor antagonists such as luzindole and 4-P-PDOT abolished MLT defensive activity therefore representing its role in defending stem cell senescence via SIRT1 stimulation via MLT membrane receptors. Results revealed that MLT protected against estrogen deficiency-associated bone loss. Thus, MLT-intermediated antisenescence activity

on stem cells delivers crucial evidence to the progress of an approach for handling postmenopausal bone-related diseases (Chen et al. 2022). The association between OS and cellular senescence is a significant investigation topic. MLT is linked with aging and has an important part in redox homeostasis, but its part in sustaining physiological stability in the brain is still not clear (Sumsuzzman et al. 2021b).

9.2.6 Mitochondrial Dysfunction

The best recognized hypothesis to describe aging is the free radical concept, which has a crucial part for the mitochondrion as the main foundation of ROS causing mitochondrial DNA (mtDNA) mutations (Payne and Chinnery 2015). Aging and neurodegeneration are associated with increased mtDNA mutations (Kraytsberg et al. 2006; Bender et al. 2006; Lin et al. 2002). Generally, mitochondrial function is reduced with age, noticeable by changed mitochondrial respiration, lessened energy production, and extensive alterations in metabolites related to mitochondrial activity (Houtkooper et al. 2011). In aged cells, enhanced mitochondria-mediated apoptosis pays to a rise in the percentage of apoptotic cells. With aging, the mitochondrial density in skeletal muscle was found to reduce gradually and may suggest a decrease in mitochondrial biogenesis (Crane et al. 2010; Chistiakov et al. 2014).

The inhibition of pineal MLT as a consequence of diverse actions stimulates aerobic glycolysis in immune cells through an inflammatory condition, and thus cytokine storm and mitochondrial dysfunction characteristics of these disorders (Reiter et al. 2019). MLT is a potent anti-inflammatory molecule, and its decrease at the mitochondrial level would definitely overstress the inflammatory reaction (Martin Giménez et al. 2021).

Age-associated macular degeneration (AMD), a retinal neurodegenerative disorder, is the most important origin of blindness among the elderly. The activity of MLT on mitochondrial function consequences in the decrease of OS, inflammation, and apoptosis in the retina; these results prove that MLT has the effect to stop and cure AMD degeneration (Mehrzadi et al. 2020).

9.2.7 Deregulated Nutrient Sensing

Four pathways of nutrient sensing regulate metabolism and effect aging. The four related key protein groups are insulin-like growth factor 1 (IGF-1), mammalian target of rapamycin (mTOR), SIRTs, and AMP-activated protein kinase (AMPK). These proteins are known as nutrient sensing since their nutrient levels influence their activity. Reduction of the IGF-1/GH pathway (IIS) seems to expand lifespan in many organisms. Reduced activity of mTOR increases lifespan in animal tests.

Upregulating some SIRTs produces antiaging or health supporting special effects. Similar to SIRTs, advanced activity of AMPK has prolonged lifespan effects. Turning down the pathways of IGF-1 and mTOR increased longevity. Contrariwise,

turning up the action of SIRTs and AMPK improves longevity. They work to promote catabolic metabolism and increase with nutrient scarcity (Milman et al. 2016).

It is known that metabolic processes and its by-products can put stress on cells. Too much activity and changes in nutrient availability and composition cause cells to age faster. Nutrient signaling via insulin/IGF-1 was the primary pathway established to control aging and age-related conditions. Caloric control, the most extensively studied longevity promoting intervention, acts through multiple nutrient signaling pathways, whereas inhibition of mTOR reproducibly delays aging-related conditions (Johnson 2018). On the other hand, it is important to know that the inhibition of mTOR may be a reason to slow wound healing and insulin resistance (Zaza et al. 2018). According to research, caloric restriction may prolong the lifespan of many organisms by modifying the SIRT family (Kapahi et al. 2017). Hence, the synthesis and decomposition procedures of nutrient metabolism could be significant for targeted antiaging therapies.

Metabolic processes are described by modifications in nutrient uptake and some crucial molecular pathways associated with malignant transformation. Regarding malignant metabolisms, MLT presents substantial anticancer activities through the maintaining of the main element of aerobic glycolysis, gluconeogenesis, the pentose phosphate pathway, and lipid metabolism. MLT usage affects some metabolic contributors such as glucose transporter expression, glucose-6-phosphate dehydrogenase activity, and lactate production (Samec et al. 2021). Studies show that MLT's benefits are accredited to the stimulation of nutrient-sensing signals (Qi and Wang 2020). Similarly, MLT's defense against hypertension is related to the stimulation of the AMPK/SIRT1/PGC-1α pathway (Tain et al. 2018).

9.2.8 Stem Cell Exhaustion

Stem cells achieve varied functions, including useful signaling that develops tissue function, regulation and health, replacement of injured or lost red blood cells, white blood cells, and solid tissues (Hattangadi et al. 2011; Madrigal et al. 2014). Changes in cell configuration are almost universal in aging. The lifelong persistence of stem cells in the body makes them predominantly vulnerable to the increase of cellular damage, which eventually may cause cell death. Stem cells in numerous tissues have been observed to experience profound changes with age. Higher quantities of damaged DNA in aged stem cells could consequence in a build-up of damage over time (Oh et al. 2014). Studies showed that even though mature tissue stem cells may be the key cell type in the aging procedure, they could be able to decrease their differentiation rates, rather than becoming shattered. According to this idea, when stem cells divide but do not differentiate, they can make many daughter stem cells (Bodmer and Crouch 2020). Numerous molecular procedures concerned with stem cell aging are conserved such as the accumulation of damaged macromolecules, DNA damage, ROS production, TOR, and WNT signaling (Jones and Rando 2011). Understanding the mechanisms-related stem cell aging with the promising

approaches will allow us to create substantial progress toward providing preventive and personalized medicine for aging (Bae et al. 2017).

Mesenchymal stromal cells (MSCs) are essential for their capability to change into different tissue. Studies revealed that MSCs, via paracrine pathways, can recognize receptor-independent reactions to MLT and then stimulate a sequence of downstream pathways, which display numerous activities, containing anti-tumor and anti-inflammatory (Feng et al. 2021).

Stem cell treatment for tissue regeneration has some restrictions in the fact that transplanted cells could not live for a long time. Several studies are performed on the antioxidants to escalate survival ratio of neural stem cells (NSCs). MLT prevents NO formation and defends NSCs against inflammatory stress. It was revealed that MLT might be a vital element for the survival and proliferation of NSCs in neuroinflammatory disorders (Song et al. 2015).

9.2.9 Altered Intercellular Communication

Like the decline in stem cell renewal, the age-related alterations in intercellular media are integrated effects of the other hallmarks of aging. Specifically, senescent cells activate chronic inflammation that can further damage aging tissues. Lacking intercellular interactions, tissues would not be able to coordinate the necessary number, distribution, and localization of different cell types. Intercellular communication results in the accumulation of inflammatory tissue damage. The causes of this damage are mainly, the accumulation of proinflammatory tissue damage, dysfunctional immune system, and dysfunctional host cells (Malaquin et al. 2016; Axel et al. 2014). It was revealed that senescent cells excrete a specific set of molecules called the SASP (Senescence-Associated Secretory Phenotype) which prompt senescence in neighboring cells. On the other hand, lifespan-extending operations aiming at one tissue may slow the aging progression also in other tissues (Nelson et al. 2012; Lavasani et al. 2012).

In mammalian ovaries, oocytes are actually coupled to somatic granulosa cells, and this is essential for the development of oocytes. Oocyte feature is effected by aging-mediated dysregulation in communication. MLT has a part in preventing age-linked germline-soma communication defects, assisting the relay of antioxidant metabolic agents for the preservation of oocyte quality from cumulus cells, which have significant activity for improving deficient phenotypes of aged oocytes and the handling of woman infertility (Zhang et al. 2022a).

9.3 Aging-Related Disorders and Oxidative Stress

Age-related disorders that arise more often in people as they get older, meaning age is a significant risk factor (Luo et al. 2020a). Some of the more common age-related diseases are cardiovascular disease, cerebrovascular disease, high blood pressure, many types of cancer, type 2 diabetes, neurological disorders (such as PD and AD),

dementia, chronic obstructive pulmonary disease (COPD), osteoarthritis, osteoporosis, cataracts, AMD, and hearing loss.

Participation of free radicals in aging process has raised increasingly and become one of the more probable cause of the aging process. There are many ways in which free radicals can be formed, but the most important foundation is the mitochondria. It has been found that enzymatic and nonenzymatic free radical sources have the ability to cause oxidative damage to vital biological components (Wickens 2001).

The biggest alteration that happens in the cardiovascular system with aging is a lessening in the elasticity of the vasculature and therefore augmented arterial stiffness. This consequence of elevated elastolytic activity in the vascular wall leads to elastin degradation and higher smooth muscle tone. This situation is especially vital in large vessels such as the aorta, which establishes reduced compliance with aging (Cheitlin 2003). Emerging suggestion shows that mitochondria within cardiomyocytes contribute to age-linked augmented ROS generation that acts a crucial role in aging-correlated cardiac diseases. Aging alerts mitochondrial and extramitochondrial processes of ROS accumulation within the heart (Rizvi et al. 2021). Mitochondrial activity is lessened with age, noticeable by changed mitochondrial respiration, reduced energy making, and extensive alterations in metabolites related to mitochondrial activity (Houtkooper et al. 2011). Many stages are still necessary to fully understand all the processes connected to aging and to OS-related modifications in cardiovascular diseases in order to avoid or delay cardiovascular decay (Paneni et al. 2017).

Numerous studies have evidenced that aging or senescence is a risk factor that aggravates stroke. The reduction of energy in ischemic stroke can be the reason for a sequence of damage to stimulate the progress of stroke, and OS takes place in all steps of ischemic stroke proses (Shao et al. 2020). ROS has effects on cerebral blood flow. ROS triggers vasoconstriction and escalates platelet aggregation and endothelial cell permeability, in that way affecting blood circulation (Anwar and Eid 2016).

Aging and cancer are multifaceted progressions that are controlled by multiple mechanisms. The significant role of free radicals and ROS in cancer and aging is widely studied for many years. Extreme conditions of OS always enhance cell senescence and aging, also able to trigger both inhibition and activation of tumor development (Afanas'ev 2011). ROS are recognized not only to attack DNA but also other vital components, such as proteins and lipids, producing reactive species that can attack DNA bases leading to DNA injury that works as a biomarker of carcinogenesis (Jomova et al. 2020). Between other significant regulators of aging and cancer are SIRTs, which were revealed to lessen with age. Studies prove that the action of SIRTs is also involved in cancer development, distressing the activity of p53,38,39 as well as that of other regulators (Taylor et al. 2008).

Research deliver encouraging data, signifying the efficiency of dietary antioxidants in aging and cancer mechanism. However, considering the significant role that free radicals show in cellular signaling, more clinical studies and research is essential before natural antioxidants can be clinically used.

Brain aging is primarily expressed by proinflammatory factors, altered signaling, and the build-up of senescent glia (Harry 2013). For example, the hallmarks of AD

include extracellular amyloid plaques, intracellular neurofibrillary tangles (NFTs), and hyperphosphorylation of Tau protein (Janczura et al. 2018). Generally, microglia act as a "scavenger," which is the chief phagocytic cell in the brain, and show an essential role in the clearance of Aβ. Nevertheless, the efficacy of this clearance drops with the occurrence of AD (Olah et al. 2018).

OS has a significant part in the degeneration of dopaminergic neurons in Parkinson's disease (PD). Disorders in the physiologic balance of the redox potential in neurons may affect numerous biological procedures, eventually causing to cell death. PD affecting gene products including DJ-1, PTEN-induced kinase 1 (PINK1), parkin, alpha-synuclein and *Leucine-rich repeat kinase* (LRRK2) also influence in complex ways mitochondrial action leading to ROS generation and OS (Dias et al. 2013). Early indication for the presence of OS in PD came from studies of brain tissue from patients with PD that confirmed higher levels of oxidized proteins, lipids, and nucleic acids (Kumar et al. 2012).

Among numerous processes, pathways and mechanisms, OS has been suggested to have a crucial part in the rate of ageing. Oxidative damage resources to the hallmarks of ageing and important elements in pathological pathways which are assumed to drive multiple age-related diseases (Luo et al. 2020a).

9.4 Is Melatonin a Miraculous Indolamine

MLT was discovered more than 60 years ago. It is found extensively in different types of food such as meat, eggs, nuts, and medical herbs (Reiter et al. 2001). It is a hormone synthesized in the brain by the pineal gland, from the amino acid tryptophan. Besides the pineal gland, MLT is also most likely created in gastrointestinal tract, airway epithelium, pancreas, adrenal glands, thyroid gland, thymus, urogenital tract, placenta, and other organs (Kvetnoy 1999; Suzen 2013). MLT is recognized to effect sleep and mood patterns. It is a free radical scavenger molecule and has antioxidant properties. Furthermore, MLT has been recognized to regulate immune mechanisms and carcinogenic processes and control reproductive functions (Karaaslan and Suzen 2015).

The biological activities of MLT are enormously widespread. MLT has antioxidant and free radical scavenger properties (Tan et al. 2015), effects on inflammatory pathways (Kim et al. 2012), immune system supporting activities (Claustrat and Leston 2015), apoptosis (Ligen et al. 2018), organization of light/dark cycle and season after birth (Cipolla and do Amaral 2018), role on diabetes-related metabolic disorders (Espino et al. 2011), obesity (Guan et al. 2021), kidney disease and renal protection (Russcher et al. 2012), cardiovascular benefits (Jiki et al. 2018), cancers (Reiter et al. 2017; Talib et al. 2021; Gurunathan et al. 2021), neurological disorders (Sanchez-Barcelo et al. 2017), and aging-related conditions (Hardeland 2018). Due to its amphiphilic, pleiotropic structure, MLT has both direct actions and receptor-mediated effects. These effects explain the MLT antioxidant and free radical scavenger activities both in vitro and in animal models. MLT decreases malondialdehyde

(MDA), a marker of OS, when the patient was treated with MLT within the first 2 h post-trauma (Melhuish et al. 2021).

Besides its antioxidant properties, MLT stimulates the actions of antioxidant enzymes and decreases pro-oxidant enzymes. Synthesis of antioxidant enzyme glutathione (GSH) is activated by MLT (Reiter et al. 2020). Regulation of superoxide dismutase 2 (SOD2) is realized by MLT via the elevation of the action of SIRT3 (Reiter et al. 2018).

The chronobiotic behavior of MLT is controlled by the MLT receptors, which have been recognized in the central nervous system (CNS) and in the periphery (Dubocovich et al. 2010). MT_1 and MT_2 receptors are members of the G-protein coupled receptors (GPCR). Recently GPR50 was described as another MLT receptor subfamily. MLT stimulates MT_1 and MT_2, to employ favorable activities in sleep and circadian abnormality, mood disorders, learning and memory, neuroprotection, and cancer (Liu et al. 2016; Cecon et al. 2018).

Some studies have proposed that MLT is more appropriate than benzodiazepines in controlling circadian rhythm and sleep disorders (Ghaeli et al. 2018). The beneficial effect of MLT has been established in sleep disorders and cardiovascular disease. The link between MLT and β-blockers has shown a good influence on sleep disorders. Anti-inflammatory properties of MLT was observed by regulating amounts of proinflammatory cytokines, including interleukin (IL)-6, IL-1β, and tumor necrosis factor-α. MLT administration has been confirmed to reduce IL-6 and IL-10 expression levels (Anghel et al. 2022).

MLT can decrease mitochondrial dysfunction by lessening the formation of mitochondrial ROS, supporting mitochondrial membrane potential, and the performance of mitochondrial ATP (Yuan et al. 2019). MLT and its metabolites have potent antioxidant activity. They support in the protection of cell membrane by scavenging extremely toxic hydroxyl radicals which prompts lipid peroxidation. They can fight back OS and cell apoptosis by directly scavenging free radicals, indirectly motivating antioxidant enzymes to stop oxidase activity (Reiter et al. 2016).

Usual concentrations of the MLT defend the cells from the harmful effects of many chemicals and processes including carcinogenesis and neurodegenerative diseases. Medicinally, MLT's most remarkable activity is its anticancer property. The oncostatic activity of MLT is multidimensional, related to the advancement of apoptosis, the arrest of the cell cycle, inhibition of metastasis, and antioxidant activity (Samanta 2020).

Studies have continuously exposed an ever-increasing number of procedures in which MLT is recognized to have significant acts in mammals. Among these acts is its involvement in cell multiplication, differentiation, and survival in the brain. Research demonstrates that MLT may successfully accomplish these functions by inducing transcription factors which regulate neuronal and glial gene expression (Onaolapo et al. 2020).

9.5 Melatonin in Aging

The antioxidant activity of MLT includes scavenging free radicals and trigger the action and expression of antioxidant and pro-oxidant enzymes. Because of these properties, numerous MLT-related molecules like MLT metabolites and synthetic MLT-based derivatives are studied to define which display the maximum activity with the lowest side effects (Suzen 2006; Gurer-Orhan and Suzen 2015).

MLT has been associated with the formation of several degenerative disorders owing to its potent biological functions. For example, MLT is related to the improvement of oxidative damage by restoring the osteoarthritis-impaired intracellular antioxidant protection system in articular cartilage (Zhang et al. 2022b). The capacity of MLT to scavenge free radicals was first revealed in 1993 (Tan et al. 1993). MLT, at the physiological concentrations, has the ability of rising either mRNA levels or the actions of the main antioxidative enzymes. The activity of MLT to scavenge the free radicals is receptor-independent (Reiter 2000).

MLT can react with free radicals which it is metabolized into metabolites counting *N*1-acetyl-*N*2-formyl-5-methoxykynuramine (AFMK), *N*1-acetyl-5-methoxykynuramine (AMK), and cyclic-3-hydroxymelatonin. These metabolites also are active free radical scavengers (Tan et al. 2001; Mor et al. 2004). The research showed that the indole moiety of the MLT is the active part of contact with free radicals due to its high resonance stability and very low activation energy barrier (Tan et al. 2002; Suzen et al. 2013). Numerous pharmacological activities of MLT have been defined causing much attention to the discovery of new compounds (Suzen et al. 2011; Yilmaz et al. 2012; Shirinzadeh et al. 2010; Gurkok et al. 2009).

Indeed, no single aspect has been defined, which suitably describes the aging process. The concepts involving the pineal gland and its secretory neurohormone MLT to aging have been suggested, but the part of this molecule in aging development is still uncertain. MLT levels significantly drop progressively over the lifespan and could be associated with reduced sleep efficacy, progressing age, and worsening of various circadian rhythms (Karasek 2004). Reiter also (1993) proposes MLT's decline in the elderly decreases the antioxidant defense against the toxic oxidants that accumulate with age.

Interestingly there is a link between caloric intake and MLT levels in gastrointestinal tract (GIT). Animal research reveals that fasting significantly improved the production of GIT MLT. It is concluded that being constantly hungry, a prolongation of human life could be succeeded by a replacement MLT administration. Intake of MLT may rise body MLT levels, which will defend people from the effects of aging and age-related diseases like PD and AD (Bubenik and Konturek 2011). In addition, Cardinali (2021) suggests that MLT has activity as a chronobiotic and a cytoprotective to support healthy aging. MLT levels are constantly declining in metabolic syndrome, ischemic and non-ischemic cardiovascular diseases, and neurodegenerative disorders. Clinical studies using MLT in the 2–10 mg/day range have proposed the possible beneficial value of MLT.

MLT is able to mend and develop mitochondrial dysfunction linked with the aging progression by eliminating active oxygen, as well as stopping lipid

peroxidation to fight free radical attack (Jiang et al. 2022). The suggested antiaging and neuroprotective possessions of MLT are determined by mitochondrial function (Hardeland 2013). MLT is also able to stimulate the PI3K/Akt signaling pathway and triggers the NAD^+-dependent protein deacetylase SIRT1 (Mayo et al. 2017). SIRT1 prompts neuroprotective activities against AD brain pathology by maintaining the acetylation homeostasis of key proteins (Corpas et al. 2017, 2018).

MLT receptors activity could include the stimulation of antioxidant genes, such as SOD and catalase (CAT), via receptor-mediated transcriptional signaling. Therefore, MLT receptors could be possible targets for new molecules against OS and neuroinflammatory progressions. Triggering of MT_2 receptors infect has been associated with the defense of MLT against neuronal injury that follows ischemic strokes (Mozaffarian et al. 2015). Furthermore, MLT supports neurogenesis and cell proliferation via an MT_2 receptor-dependent mechanism (Chern et al. 2012).

In a study effect of MLT decline on important physiological, metabolic, and biochemical markers associated to aging was revealed. Pinealectomy caused an age-related weakening of muscle power, motor activities, food consumption, and impaired lipid profile in rats (Tchekalarova et al. 2022). MLT treatment in aged animals responds to a significant number of senescence-related alterations. It employs an important cytoprotective act by scavenging free radicals and reversing inflammation via downregulation of proinflammatory cytokines, prevention of low-grade inflammation, and avoidance of insulin resistance.

Decreases in circulating MLT are detected in numerous age-related disorders. Postoperative cognitive dysfunction (POCD) is a known problem of the CNS in aged patients after operation. It will extend the stay at the hospital, lessen the independence of the patient, and escalate the possibility of death. Latest research has revealed that MT is able to stop and treat POCD by regulating circadian rhythm, reestablishing cholinergic system function, and neuroprotection (Wei et al. 2022). Social isolation that harmfully effect diverse social considerations, such as mental and intellectual function, anxiety, and social communication, is liable on the age of isolation. The potential positive effects of MLT and exercise in improving social isolation-associated communicative alterations in aged rats were observed (Alghamdi 2022).

It has been proved that MLT lessens the formation of proinflammatory cytokines by inhibiting nuclear factor kappa B (NF-κB) (Rosales-Corral et al. 2003). Furthermore, it stops the establishment of cyclooxygenase-2 (COX-2), which is an important proinflammatory factor, in neurodegenerative disorders (Yokota et al. 2003; Chen et al. 2020b). There is an appreciated act of MLT in many neurodegenerative disorders, which essentially happen by inhibiting OS and reducing the unusual protein accumulation that causes cell death (Miller et al. 2015). Due to MLT's relatively small size and amphiphilic property, it can pass through BBB and holds antioxidant activity in CNS (Tarocco et al. 2019) as well as accumulate in subcellular organelles, particularly the mitochondria (Menendez-Pelaez and Reiter 1993). There is evidence that MLT decreases OS and protects against neurodegeneration in animal experiments (Liu et al. 2009). The only problem is the low bioavailability and first-pass effect for oral administration could lessen the amount of MLT in the brain. Oral

MLT tablets (2 and 4 mg) displayed very low bioavailability, this could be due to poor oral absorption, large first-pass metabolism, or a combination of both (DeMuro et al. 2000). In a study, elimination half-lives after oral and IV MLT administration was 54 min and 39 min, respectively. The bioavailability of oral MLT was found about 3% (Andersen et al. 2016).

9.5.1 Melatonin in Alzheimer's Disease

MLT is suggested as one of the effective treatment methods for neurodegenerative disorders (Govitrapong and Shukla 2017). AD is an age-associated neurodegenerative disease that is described by the formation of extracellular senile plaques of Aβ and intracellular neurofibrillary tangles, mostly enclosing the hyperphosphorylated microtubule-associated protein tau (Zhao et al. 2018). MLT powerfully defends neuronal cells from Aβ-mediated injury via anti-amyloid activity. It can also stop the development of amyloid fibrils by dealing with Aβ (Lin et al. 2013; Gurer-Orhan et al. 2016). Prevention of the development of these harmful peptides, which activate neurodegenerative diseases, could only be succeeded by inhibition of β- or γ-secretase or activating the nonamyloidogenic α-secretase. Shukla et al. (2017) have shown a possible mechanism by which MLT activates the nonamyloidogenic action and deactivates the amyloidogenic action of amyloid-β precursor protein (βAPP) by triggering α-secretase and subsequently preventing regulation of β- and γ-secretases. Moreover, MLT improves Aβ-induced neurotoxicity and possibly supports Aβ clearance via glymphatic-lymphatic drainage, blood-brain barrier (BBB) transportation, and degradation pathways (Li et al. 2020). It prevents and eliminates Tau protein hyperphosphorylation in AD. Furthermore, it is able to lessen proinflammatory cytokines expression and factors IL-8, IL-6, and TNF (Asefy et al. 2021). There are some evidence signifying an interface between MLT and Aβ (Masilamoni et al. 2008). There has been a direct contact between the 5-methoxy group of MLT and His-13 of Aβ detected (Carter and Weaver 2003) and amounts of Aβ aggregates in the brain were found decreased by MLT administration in animal experiments (Lahiri et al. 2004).

As a potent antioxidant (Galano et al. 2013), MLT presents anti-amyloidogenic activity, which supports it to be an encouraging and beneficial drug for AD (Rosales-Corral et al. 2012). Studies have proposed that patients with AD also have reduced MLT and pineal gland activity (Song 2019).

Stimulation of MLT receptors in the suprachiasmatic nucleus (SCN) shows a crucial role in synchronizing the phase and amplitude of circadian rhythms (Dubocovich 2007). It was found that MLT receptors in dementia are powerfully supported by postmortem histological founding in hippocampus from AD patients presenting higher MT_1 and reduced MT_2 receptor immunoreactivity (Savaskan et al. 2005). MLT employs several mechanisms of action against AD in animal studies. It presumably displays neuroprotection via triggering of G-protein-coupled MLT receptors. Furthermore, MLT may show activity intracellularly to defend mitochondria and neurons by scavenging free radical (O'Neal-Moffitt et al. 2015).

MLT receptors have been stated to be associated with aging and AD, and their expression is reduced with the development of AD. Overexpression of MLT receptors can cause MLT-like actions to repair the signs of AD. Activation of MT_1 in vivo brain using a Cas9 activator could be a new AD treatment approach (Park and Kim 2022). Ramelteon, piromelatin, and Agomelatine which are receptor agonists of MT_1 and MT_2 were investigated regarding their effect on AD. Interestingly ramelton presented no pharmacological effects in a B6C3 transgenic mouse model of AD (Otalora et al. 2012). The capacity of piromelatin to decrease neuronal apoptosis was more effective than MLT (He et al. 2013). Studies showed that agomelatine has effect on Aβ, p-tau, oxidative stress, apoptosis, and neuroinflammation, causing positive developments in AD (Ilieva et al. 2019). In vitro studies established that MLT could stop the advanced development of β-sheets and amyloid fibrils (Poeggeler et al. 2001).

MLT administration was revealed to reduce Aβ aggregation, tau hyperphosphorylation, OS, neuroinflammation, and neuronal apoptosis. Preclinical research exhibited that MLT is able to improve AD pathologies and repair cognitive damage. Hypothetically, inhibition of the pathological development of AD by MLT administration should also repair the weakened neurotransmission (Roy et al. 2021). On the other hand, it was observed that MLT levels in the peripheral blood were decreased in amnestic mild cognitive impairment (MCI) but increased with the severity of AD. The discovery shows that the results represent that MLT presented efficacy only in MCI but not in AD. Whether the amount of MLT has potential to be a treatment response biomarker for AD, specifically its early stage requests advance studies (Lin et al. 2021). Nevertheless, the efficiency of MLT treatment in animal studies was reliant on the disease stage and treatment durations (Roy et al. 2022). MLT is effective in neuroblastoma cells and not only reduced the hyperphosphorylation of neurofilaments but also disallowed tau hyperphosphorylation (Chen et al. 2020a). Furthermore, animal studies revealed that MLT had antioxidant activity and may considerably decrease the amount of lipid peroxidation, ROS (Gunasingh et al. 2008), and nitric oxide synthase (Luengo et al. 2019).

MLT has anti-inflammatory, antioxidant, anti-fibrillogenic, anti-hyperphosphorylating, and anti-amyloidogenic activities (Vincent 2018), and cerebrospinal fluid MLT concentrations have been revealed to negatively associate with Braak stages in AD (Zhou et al. 2003). This finding shows that studying MLT levels in patients could be beneficial for understanding the effects of AD and more studies with blood MLT could give ideas as possible biomarker to track disease development. Nous et al. (2021) showed that MLT levels in cerebrospinal fluid, blood, saliva, and urine are different in older people and more decreased in AD patients. The daytime MLT rhythm vanished in cerebrospinal fluid of AD patients.

Insomnia seems to be more disturbing and severe in the elderly, and is related with a higher risk of AD (Winsky-Sommerer et al. 2019). The reasons underlying the consequence of insomnia in increasing the severity of AD have been proposed to include augmented Aβ formation and reduced Aβ clearance by poor sleep patterns (Cordone et al. 2019). Some research have been accomplished concerning the use of

MLT to increase cognition in dementia (Wang et al. 2017); however, results did not show how the administration of MLT effects dementia of severity classes of AD. It was established that MLT developed spatial memory in sporadic AD mice. MLT enhanced *Creb1* gene expression and expressively improved *Bdnf* gene expression in the hippocampus of AD model mice compared with the AD group (Labban et al. 2021).

Animal research has revealed that MLT may protects against AD-type disorders, even though clinical studies have not been entirely certain. The management of circadian rhythm and better quality of sleep can prove the benefit of MLT administration to AD patients (Zisapel 2018). Sumsuzzman et al. (2021a) presented that MLT treatment for over 12 weeks could be beneficial for mental functioning in AD. They recommend that MLT can be an alternative to known hypnotics in the management of insomnia.

According to Reiter et al. (2022), MLT capacity reduces evidently throughout aging, parallel with the development of numerous neurodegenerative disorders and the increase of the related neurotoxins. Research points out that MLT eliminates pathogenic toxins, such as Aβ, from the brain to defend against neurocognitive degeneration.

9.5.2 Melatonin in Parkinson's Disease

PD is the second most common neurodegenerative disease (Tysnes and Storstein 2017). The damage of dopaminergic neurons has been associated with low-degree inflammation and oxidative damage (Abramov et al. 2020). As chief components of inflammatory reactions, the microglia stimulated and produced ROS and proinflammatory cytokines in PD (Kam et al. 2020). Therefore, many researchers (Davies et al. 2017; Perez-Lloret and Cardinali 2021) have suggested application of antioxidants may be beneficial for neurodegeneration protection.

The etiologies of PD continue to be indefinable. One of the facts is that the excess formation of ROS in the brain escalates OS in PD patients. Dopamine demonstrates oxidation to form dopamine quinones and free radicals, which could contribute to neurodegeneration in PD (Monzani et al. 2019). At present, the most common treatment approach is done with L-dopa to reestablish dopamine content. MLT has been established in numerous in vitro and in vivo models of PD and results consistently display that it is extremely efficient (Mayo et al. 2005). Kataoka et al. (2020) compared endogenous MLT quantities between patients with PD and non-PD senior adults. They proposed that MLT amount in PD patients getting regular levodopa doses are comparable with those in senior adults, even after seeing confounding issues. This suggestion was modulated by daily levodopa dose in PD patients. Infect treatment with 2 mg MLT was found to be effective for clinical use and was connected with major progress in night-time frequency and nocturnal voided volumes in PD patients (Batla et al. 2021). Randomized and placebo-controlled experiments to demonstrate the effectiveness and safety of prolonged-release MLT in PD patients with poor sleep quality were tested. MLT treatment was

found as an option for sleep quality in PD patients (Ahn et al. 2020). Another double-blind, placebo-controlled clinical study was done on patients who received either 25 mg of MLT or placebo at lunchtime and 30 min before sleeping at night for 3 months. Results revealed that in patients treated with MLT, a major decrease in lipoperoxides, nitric oxide metabolites, and carbonyl groups in plasma samples from PD patients compared with the placebo group (Jimenez-Delgado et al. 2021).

The earliest indication of a close association between MLT and PD was obtained from results of a lessened pineal action and a following decrease in circulating MLT concentrations in patients (Sandyk 1990). Instabilities of biological rhythms have been observed in PD patients. Sleep disturbance also affect PD patients often and effect destructively on quality of life (Porter et al. 2008).

Immunomodulation by MLT involves proinflammatory and anti-inflammatory activity (Hardeland 2018). The GABAergic system is included in MLT-mediated neuroprotection. MLT employs anti-excitatory and sedative properties and there is evidence that MLT defends neurons from Aβ peptide toxicity via the stimulation of GABAergic receptors (Louzada et al. 2004). Treatment with MLT causes inhibition of some pathways connected to apoptosis, autophagy, oxidative stress, inflammation, α-synuclein aggregation, and dopamine loss in PD. Furthermore, MLT recovers some nonmotor symptoms of PD patients (Tamtaji et al. 2020). In a systematic review and meta-analysis, MLT was found considerably increase the subjective and objective sleep quality of patients with PD with good safety and tolerability (Ma et al. 2022).

MLT modified the viscosity enhancement of the mitochondrial inner membrane (Reiter et al. 2014). The protection of the mitochondrial membrane reveals a part of MLT in moderating the mitochondria-dependent apoptotic pathway. The defensive action of MLT against apoptosis is well recognized, particularly in neurodegeneration (Wang 2009). MLT is synthesized by neuronal mitochondria. Reduced MLT levels damage mitochondrial homeostasis, resulting in mtDNA release and stimulation of cytosolic DNA-mediated inflammatory reaction in neurons. Cytosolic mtDNA stimulates the inflammatory reaction in aging and neurodegeneration, a process lessened by MLT (Jauhari et al. 2020).

9.5.3 Melatonin in Huntington's Disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease (Ross and Tabrizi 2011) and is initiated by an active mutation of the HTT gene, placed on the short arm of the fourth chromosome. A continuing build-up of deposits of misfolded huntingtin and the loss of neurons in different areas of the brain are detected during the progression of the disease (Herzog-Krzywoszanska and Krzywoszanski 2019). Although HD varies from more common illnesses like AD and PD regarding predominant clinical sleep pathology, there are significant data related to the timing and nature of sleep and circadian disorders (Voysey et al. 2021). MLT circadian rhythms are disturbed in HD and associated with disease severity giving a description of disrupted sleep (Kalliolia et al. 2012).

Research proved the neuroprotective activity of MLT, primarily due to its antioxidant properties, against neurotoxicity in HD (Wongprayoon and Govitrapong 2017). Disturbances of circadian rhythm in HD patients, as supported by cortisol and MLT blood concentrations. These patterns of circadian changes were found alike to the changes that happen with aging (Adamczak-Ratajczak et al. 2017).

Analyses of the blood of patients with HD or brain injury for levels of 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and MLT showed that 5-HIAA decreased in HD. MLT concentration augmented on tryptophan administration. It was observed that patients displayed irregularities in tryptophan metabolism, which could be associated with augmented inflammatory conditions and OS (Christofides et al. 2006). Furthermore, MLT proposed favorable effects in alteration of learning linked to fine motor regulations through the repair of striatal and cortical spines (Chakraborty et al. 2014). The brain weight in HD patients is almost 30% lesser than in healthy people. The striatal and cerebral cortex projection neurons are more subject to the disease than interneurons. Damage to energy metabolism in HD is associated with neuronal death.

MLT effect on the oxidative modifications is created by 3-nitropropionic acid (3-NPA) in animal studies. Examination of striatal and cortical synaptosomes showed that 3-NPA initiated an increase in lipid peroxidation and an augmentation in SOD activity. These modifications could prohibit by previous treatment of MLT. It can be explained by the capacity of MLT to adjust the neural response to 3-NPA with the defensive mechanism connecting the antioxidative properties of MLT (Tunez et al. 2004).

MLT levels are decreased in HD. Altered MLT patterns may give us a reason for sleep disturbance in HD, and characterize a biomarker for the disease. MLT has a significant role in the maintenance of sleep and its synthesis is controlled by the hypothalamic SCN. Aziz et al. (2009) proposed that disordered SCN action may increase abnormal MLT secretion in HD patients. Results propose a circadian rhythm irregularity in early stage HD and MLT concentrations may gradually drop with progressing disease (Bonilla 2000).

MLT delays disease onset and mortality in animal studies of HD. It was observed that injury in cells is linked with damage and absence of the MT_1 receptor. MLT is able to stop mutant htt-induced caspase activation and conserves MT_1 receptor expression (Caballero et al. 2008). The association of MT_1 receptor in HD is explained by the modulating brain function and mood. Thus, MT_1 subtype needs to be further inspected as a novel approach for neuropsychopharmacological drug discovery (Comai et al. 2019).

MLT stops mitochondrial cell death in experimental models of HD. Furthermore, this activity is possibly mediated by the MT_1 receptor. Studies of HD reveal that MLT reduces the speed of disease development and stops the mitochondrial cell death pathway. These results suggest that the synthesis of selective MT_1 receptor agonist compounds may be a new approach to treating patients with HD (Wang et al. 2011).

At present, no treatment exists to battle hypothalamic alterations, nor circadian rhythm and characteristic sleep disturbances in HD (Bartlett et al. 2020). MLT

therapy may help the sleep disorders seen in HD (Kalliolia et al. 2014). Therefore, well-organized, detailed clinical trials are desirable to advance evaluation of the potential and effectiveness of MLT for the prevention and treatment of the symptoms of neurodegenerative diseases (Van Wamelen et al. 2015).

9.5.4 Melatonin in Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal motoneuron disorder of diverse etiologies, basically with oxidative stress. ALS symptoms comprise of advanced muscle spasticity, hyperreflexia, fasciculations, atrophy, and paralysis. Unfortunately, the disease could be fatal within a few years due to respiratory failure. There are two types of the disease acknowledged as familial ALS (fALS) and the more common sporadic ALS (sALS) (Paula et al. 2015).

Research in ALS patients existing solid indications of mitochondrial dysfunction (Tan et al. 2014). Furthermore, mutant SOD1 animal study for ALS displays morphological changes in motor neurons and skeletal muscle tissue and parallel anomalies are observed in sporadic ALS patients (Sasaki and Iwata 2007). In ALS, the most favorite activity of MLT are stopping apoptotic pathways and decreasing oxidative injury (Leon et al. 2005). It has been stated that MLT not only successfully suspensions the development and reduced mortality but also considerably prevents motor neuron death by incapacitating the receptor interacting protein-2 (Rip2)/caspase-1 pathway and caspase-3 in animal studies of ALS (Zhang et al. 2013).

Effects of MLT as a neuroprotective and antioxidant compound in sporadic ALS patients showed that MLT normalized circulating serum protein carbonyls, which provide a surrogate marker for oxidative stress, in ALS patients. This data confirmed protection in humans and recommends that high-dose MLT could be appropriate for clinical treatments intended at neuroprotection in ALS (Weishaupt et al. 2006).

Treatment of high-dose MLT over 1 year ALS patients was performed in a study in Göttingen to discover probable activity and side-effects showed that MLT has a powerful effect in the treatment of ALS (Jacob et al. 2002).

9.5.5 Melatonin in Multiple Sclerosis

Multiple sclerosis (MS) is a prolonged inflammatory disease described by CNS lesions that can cause severe physical or cognitive incapacity as well as neurological effects. Conventional therapies for MS are established on the use of anti-inflammatory and immunomodulatory treatments, but these are not capable to stop or prevent the damage of nerve tissue (Ghasemi et al. 2017).

MLT has been associated with the pathophysiology of MS. The treatment of MLT confirmed a development in clinical status, a reduction in OS and inflammation (Escribano et al. 2022). Several mechanisms of the neuroprotective properties of MLT including mitochondrial defense and antioxidant, anti-inflammatory, anti-apoptotic activities, and anti-demyelinating function might be related to MS

pathology. MLT effectively controls the immune system, demyelination, free radical generation, and inflammatory responses in neural tissue (Yeganeh et al. 2019). Throughout the demyelination stage, MLT exhibits a neuroprotective activity in animal studies with mice. It is supported by enhanced locomotor action, elevated antioxidant levels, and reduction of MDA and inflammatory elements. Interestingly male mice had protective activity after MLT treatment, while no effect was detected in female mice. This data proposes a multifaceted interaction including exogenous MLT, remyelination, and endogenous female sex hormones (Abo and Alghamdi 2020).

MLT ameliorates MS by regulating the balance between effector and regulatory cells, proposing that MLT-stimulated signaling pathways are possible targets for drug developments (Farez et al. 2016). Recent data shows that MLT secretion is dysregulated in MS patients, signifying that MLT might be a potential target for a therapeutic approach (Skarlis and Anagnostouli 2020). The luck of mitochondria MLT together with augmented *N*-acetylserotonin (NAS) has allegations for changed mitochondrial activity in many cell types that are related to MS pathophysiology. NAS is elevated in progressive MS, representing activity for alterations in the mitochondria melatonergic pathway in the development of MS (Anderson et al. 2019). In MS, decreased MLT levels were linked with acute attacks as well as period and clinical features (Kern et al. 2019).

Investigation of the effects of MLT administration on memory weaknesses in animal studies of MS showed that MLT considerably developed memory weaknesses by modifying *cAMP-response element-binding protein* and by elevating postsynaptic density protein 95 genes in the prefrontal cortex. This data specifies that MLT might have protective effects against memory weakening linked with MS (Alghamdi and AboTaleb 2020).

The association between MS and MLT has been recognized in patients where a decline has been detected as well as the correlation with the stages of the disease and symptoms such as fatigue, insomnia, or depression (Alvarez-Sánchez et al. 2015). It is recommended that a combination therapy might be more beneficial, particularly using drugs that target neuroinflammation and neurodegeneration (Martinez and Peplow 2020).

9.6 Conclusions and Future Perspectives

Many studies have suggested the possible therapeutic application of antioxidants for aging-related disorders. Numerous clinical trials have evaluated antioxidants' effects on aging events but the results so far have been controversial (Kris-Etherton et al. 2004; Gonzalez et al. 2011). Between antioxidants, vitamins have revealed some activity in short-term avoidance while long-term activity was non-encouraging. Unluckily, it is not possible to use endogenous antioxidant enzymes, SOD, CAT, or glutathione peroxidase, or any synthetic drug. Some efforts have been done to produce such molecules, mimetics, and scavenging enzymes, but until now, no

compounds have been successful, and new compounds are still in trial (Izzo et al. 2021; Patel et al. 2011).

At the physiological level, aging results from the impact of the accumulation of a wide variety of molecular and cellular damage over time. Some important aging mechanisms have been described, mainly pointing to genomic instability, telomere shortening, and cellular senescence. It is well known that aging is closely related to numerous diseases, including neurodegenerative diseases, cardiovascular diseases, cancer (Chobotova 2009), immune system disorders, and musculoskeletal disorders. These age-associated conditions and disorders produce a heavy economic and psychological problem for patients and society (De Magalhães et al. 2017). Research to develop drugs that improve the health span by directing the pathogenesis of aging is still a hot topic in this arena. The increase in the aging population shows the requirement to discover new and better active molecules and approaches in order to avoid, treat, and better overall outcome of age-associated diseases.

Clinical research proposes that MLT is a powerful antioxidant that could be active in the cure of neurodegenerative diseases. Depending on the investigation, MLT performs to have a good safety profile. It is clear that MLT is able to prevent curative effects on many neurodegenerative diseases. MLT shows nearly no side effects, according to cumulative evidence even with high-dose and long-term treatment, some adverse reactions such as drowsiness, slight fever, headache, vomiting, thrombosis, drowsiness, hyperkinesia, or restless leg syndrome were observed (Hardeland 2010).

MLT treatment can decrease brain injury during neurodegeneration by modifying mainly OS, neuroinflammation, autophagy, and apoptotic cell death. The precise mechanisms of the neuroprotective action of MLT are still not clear, additional research should be dedicated to defining the neuroprotective activities of MLT.

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Intermittent Fasting as an Anti-Aging Strategy

10

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Abstract

Aging is a multifactorial process that encompasses a wide range of physiological implications including the onset of age-associated diseases and eventually death. Although recent years have seen a surge in the number of anti-aging interventions after the elucidation of the hallmarks of aging, a special attraction for gerontologists is the dietary restriction interventions which comprise caloric restriction (CR) and intermittent fasting (IF) strategies. CR is the reduction in calorie intake by 30–40% without causing malnutrition and improves health and increases lifespan in many model organisms. An alternative to CR is Intermittent Fasting, another DR intervention widely popular since ancient times in the form of religious fasting that is now being scientifically explored for its ability to impact metabolism in a way that is beneficial in reducing age-associated ailments and overall health and physiology. IF exerts its action through the activation of bioenergetics sensors and genes associated with longevity like AMPK and sirtuins. Some of the health benefits of fasting include reduction in body weight and obesity, and reduced incidence of diseases like cancer, cardiovascular disorders, neurodegeneration, inflammation, and metabolic syndrome. The ketogenic diet and Mediterranean diet are among the variants that are widely popular as IF regimen today.

Keywords

Aging · Dietary restriction · Calorie restriction · Intermittent fasting · Ketogenic diet

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10.1 Introduction

Aging is a complex and gradual physiological change occurring in every organism eventually leading to death. The main characteristics of aging include steady accumulation of damage in macromolecules, and disruption in physiology and metabolism according to various observational and descriptive studies conducted on a wide range of aging model organisms (Rattan 2006). As a result, aging has become a substantial problem, serving as a key risk factor for a wide range of human diseases, including diabetes, cardiovascular disease, neurological disorder, and cancer (Niccoli and Partridge 2012). Although scientific breakthroughs in recent decades have resulted in effective treatment approaches that have drastically increased human life expectancy.

In recent years, aging research has progressed at an unforeseen rate, and nine hallmarks of aging have been identified at the molecular and cellular levels that are stated as follows: genomic instability, epigenetic alterations, loss of proteostasis, telomere attrition, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and impaired intercellular communication (López-Otín et al. 2013). Researchers have been captivated by possible treatments that could slow the aging process since the dawn of time, and recent improvements in our understanding of the mechanism(s) of aging have resulted in a surge in anti-aging methods. The identification of the gene (daf-2) that influences longevity in Caenorhabditis elegans (Klass 1983) urged scientists to discover a number of pathways (such as mTOR, AMPK, sirtuins, and insulin/IGF-1) have been found that regulate the aging phenotype. These discoveries have almost always resulted in the identification of novel potential therapeutic molecules that target various signaling pathways by activating or inhibiting specific intermediary proteins (Johnson et al. 2013; Salminen and Kaarniranta 2012).

Several proposed interventions have been used to delay the onset of aging in various organisms over the years, including caloric restriction (CR), antioxidant supplementation, autophagy induction, hormonal therapies, epigenetic regulation, and telomerase activation (Saraswat and Rizvi 2017). This chapter discusses the DR interventions specifically caloric restriction and more broadly intermittent fasting, both of which are promising strategies to achieve healthy aging in near future.

10.1.1 Dietary Restriction as an Anti-Aging Intervention

The dietary restriction (DR) intervention is presently widely popular worldwide due to its efficiency in inducing weight loss in obese individuals and scientifically is a well-established non-genetic, non-pharmacological method that has been shown to extend active and healthy lifespan in a variety of organisms (Katewa and Kapahi 2010). CR, genetic modifications, and pharmacological delivery have been the most common therapies of DR (Liang et al. 2018). DR is defined as a reduction in nutrient intake, either specific or total, that does not result in malnutrition. CR, in which total calorie intake is lowered, as well as research including the limitation of main dietary

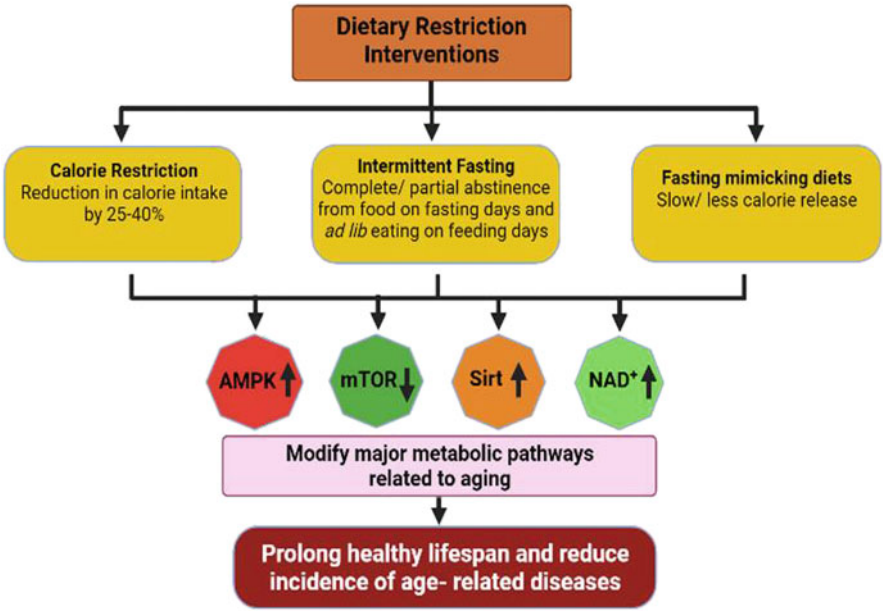

Fig. 10.1 Types of dietary restriction interventions and their common targets for metabolic action and effect on health and metabolism

components (protein, fat, or carbohydrates) or temporal fluctuations of food consumption are all examples of dietary restriction. In recent years, research into the molecular pathways by which DR slows aging and age-related disorders has accelerated (Fig. 10.1) (Bartke et al. 2001). This is largely due to the use of sophisticated genetic tools in simple and short-lived model organisms such as *Saccharomyces cerevisiae, Caenorhabditis elegans*, and *Drosophila melanogaster* to figure out the core mechanisms of DR's protective effects. These researches set the groundwork for studying mammals' conserved basic systems. Insightful research in mammalian systems is bringing closer the aim of using knowledge of DR to delay aging and age-related pathologies in humans (Fontana 2007).

10.1.2 Types of Dietary Restriction

10.1.2.1 Caloric Restriction

CR is the reduction in total calorie consumption (by 10–40%) without causing malnutrition. CR, in combination with intermittent fasting (a type of CR in which bouts of ad libitum food are alternated with periods of up to no calorie consumption), is the only known approach for improving health and lifespan in most, of the living creatures (Madeo et al. 2019). Remarkable findings were observed from CR studies that revealed its influence on health span, which in turn, was associated with a considerable reduction in age-related disorders such as cardiovascular diseases,

diabetes, neurodegenerative disorders, and malignancies (Balasubramanian et al. 2017). The positive effects of CR are mediated by a diverse set of biological mechanisms, many of which overlap with the prevention of the hallmarks of aging (Gensous et al. 2019).

Several factors, including dietary composition, the duration of the imposed dietary regimen, and the timing of diet commencement, appear to influence the overall efficiency of CR. Several scientific findings on the effects of CR on the first and second halves of life have also been examined, with the conclusion that CR administered in the first or second half of life is more effective than CR applied in both halves. An increase in the length of CR to more than the median age of the animal enhances the lifespan by reducing lifetime reproduction, according to a study utilizing rodents. As a result, CR has been advocated as a promising method that should be researched further in order to promote healthy aging (Erbaba et al. 2021; Weithoff 2007).

Caloric Restriction Mimetics (CRMs)

The term "caloric restriction mimetics" was coined recently to characterize pharmacologically active drugs that replicate some of the effects of caloric restriction. Many studies claim that prospective CR-mimicking chemicals could be a prime factor in achieving a healthy lifespan and improve age-related disorders in model organisms. CRMs ought to be able to induce autophagy, a complex degradative process that maintains cellular homeostasis by destroying damaged or unnecessary proteins or cellular structures by reducing protein acetylation via depletion of acetyl-CoA, activation of deacetylases, or inhibition of acetylases (Yin et al. 2016; Madeo et al. 2019).

CRMs also have the ability to imitate more general metabolic, physiological, and hormonal changes caused by CR, as well as the activation of stress response pathways and greater stress resistance. Several chemically varied CRM candidates have been found, with potential sources and work as either inhibitors of lipid and carbohydrate metabolism, mammalian target of rapamycin (mTOR), glycolysis, or act as activators of AMP-activated protein kinase (AMPK), sirtuin, and polyphenols (Ingram et al. 2006).

Rapamycin, a molecule originally discovered from Streptomyces bacteria and used to reduce organ transplant rejection due to its immunosuppressive effects, is probably the most well-known CR mimic. Rapamycin stops mTOR from controlling protein synthesis and cell proliferation in response to nutrition and growth hormones. Autophagy, a cellular recycling process that is thought to renew cells and underlie the anti-aging benefits of rapamycin, is activated when mTOR is inhibited (Johnson et al. 2013).

Metformin, an anti-diabetic agent, is also considered a CRM. Metformin was discovered to improve insulin sensitivity through a variety of mechanisms, including inhibition of complex I of the electron transport chain, activation of AMPK, and modulation of the gut microbiota (Rena et al. 2017). Resveratrol, a polyphenol present in low amounts in red wine, blueberries, raspberries, and peanuts, is another CR mimetic substance that can disrupt the electron transport chain and activate

AMPK (Martel et al. 2021). Human cartilage contains glucosamine, an amino monosaccharide that inhibits glycolysis, activates AMPK, and stimulates mitochondrial biogenesis to exert its action (Weimer et al. 2014). Spermidine (found in soybean and mushroom) extends lifespan of model rats by inhibition of acetyltransferase and by the activation of autophagy (Hofer et al. 2021; Madeo et al. 2018).

10.2 Intermittent Fasting

IF is a potential non-genetic, non-pharmacological strategy known for granting several health benefits which significantly improves overall health and confers increased lifespan. IF is defined as the umbrella term for the different eating patterns comprising cycles of feeding and fasting. IF serves as an anti-aging strategy and can be categorized under the broad spectrum of dietary restriction interventions. IF relies on the rhythmic or arrhythmic pattern of eating and abstaining from food maintained in regular intervals or on a specific routine based on the type of fasting practiced. The feeding phase during IF may comprise ad libitum intake of food or restricting food intake to a mere 25–30% with regular fasting intervals. There are several types of IF that can be followed based on one's need and ability to fast.

IF is a commonly practiced intervention mainly by individuals who choose to fast for religious reasons such as the Ramadan fasting among Muslims during the holy month of Ramadan, and among Christians, Buddhists, Orthodox, Jews, and Hindus to name a few. The religious fasts rely on the timing and duration of the fast according to their calendar year and the type of food consumed during this period. These are the widely discussed religious fasts whose detailed effects and benefits have been elucidated (Persynaki et al. 2017; Trepanowski and Bloomer 2010).

The various types and patterns of IF are as follows:

(i) **Alternate day fasting (ADF):** The ADF method consists of alternate days of feeding and fasting, i.e., feeding ad libitum (no energy restrictions) on a particular day and fasting (no caloric intake, total energy restriction) the subsequent day following repeated cycles. Some individuals may acquire some food (20–30% of one's energy needs or more) on the eating day as per one's preferences and needs. ADF confers several health benefits aside from weight loss like glucose and insulin resistance, cardio-protection, and improvement in metabolism and mental well-being.

(ii) **Periodic fasting (PF): 5:2 or other** Periodic fasting refers to an arrhythmic pattern of feeding and fasting where 2 days of complete fasting or fasting with the intake of fewer calories is followed by the remaining 5 days of normal feeding in a week. The days of fasting may be any day of the week or 2 consecutive days followed by 5 days of ad libitum eating.

(iii) **Time-restricted feeding (TRF):** While IF relies on the cycles of feeding and fasting, time-restricted eating crucially entails the time period of fasting and feeding. TRF generally implies 8:12 h of feeding and fasting, respectively. TRF

is based on the ability of the suprachiasmatic nucleus (SCN) to regulate the synchronization of the peripheral clocks and the circadian cycle of the organism. TRF presses on the intake of food during the active phase of the organism since this confers various metabolic and physiological benefits to the organism.

(iv) **Fasting-mimicking diets (FMD):** FMDs tend to mimic fasting-like effects and comprise diets such as ketogenic diets (KD's), very low carbohydrate diet (VLCD), low carbohydrate diet (LCD), or high-fat plant-based diets. This is a hypocaloric diet plan which intends to cause no micronutrient deficiency and observe fasting-like benefits.

(v) **Long-term fasting (LF):** LF refers to prolonged durations of fasting lasting from a few days to a few weeks (21 days or more). It is followed by taking very low/reduced amounts of calorie intake (<1000 calories in a day) which is given in appropriate amounts for a specified duration of the day (Wilhelmi de Toledo et al. 2020).

10.2.1 Metabolic Switch During Fasting and Its Mechanism

The basic difference between IF and CR is that during CR organism relies on eating at regular periods but with a reduced amount of calories (restriction in calories of about 25–30% in food) whereas during IF, the fasting period requires complete abstinence from food in the majority of the types of fasting. Therefore, the initial response of the body to both of these methods of DR is a slightly different approach, but gradually these strategies almost share the same mechanism of action and functions (Hofer et al. 2022). When an organism initiates a fast, the body responds to the starting phase by breaking down the stored glycogen in the liver by glycogenolysis. The hepatic glycogen store serves as the glucose reservoir during the first 12–36 h of the fasting cycle. Post all the glycogen depletion, the organism undergoes a process generally referred to as "flipping the metabolic switch" or "flipping the switch" which refers to the breakdown of reserved fat to free fatty acids (FFAs) and utilization of ketone bodies (β-hydroxybutyrate, acetoacetate, and acetic acid). Most of the β-hydroxybutyrate generated is utilized by the brain whereas the ketone bodies also serve as an energy source in other organs requiring energy for functioning like muscles. Therefore, flipping the switch mechanism transfers the functioning of metabolic machinery from glycogen to ketone bodies and FFAs from adipocytes which supply the required energy to neurons and other organs for maintenance.

10.2.2 Molecular Mechanisms of Intermittent Fasting

IF is a widely demonstrated DR strategy with various evidence-based studies reporting its role in retarding aging and associated diseases. The basic mechanism of IF involves a network of genes, transcription factors (TFs), hormones, and biochemical markers in an organism. During IF, the fasting period erupts a wave of changes in the functioning of the metabolic pathways which helps the body to

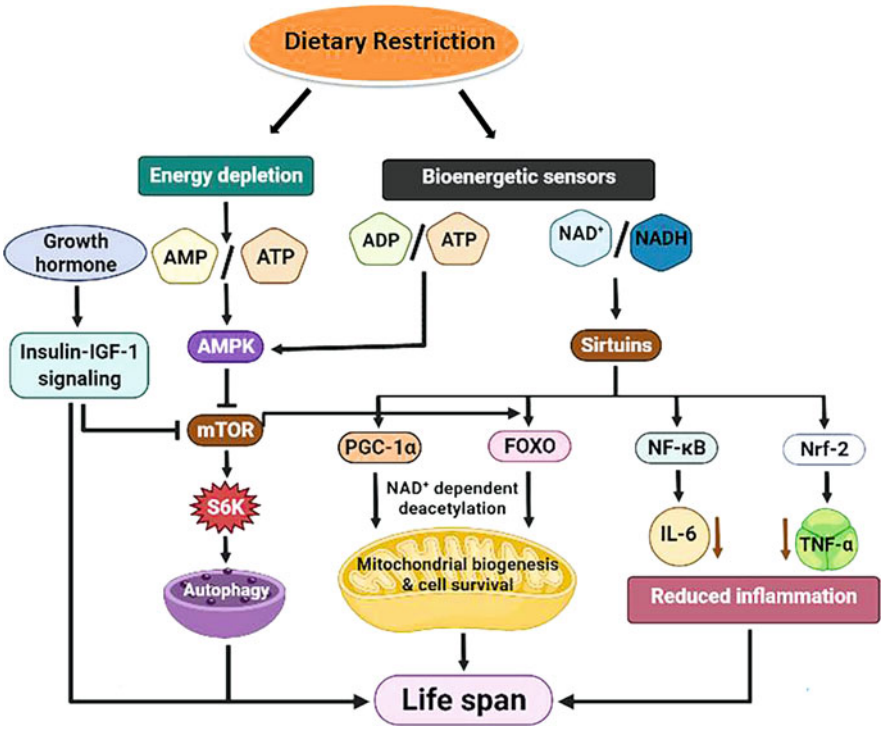

Fig. 10.2 Role of dietary restriction in cellular metabolism. Intermittent fasting activates bioenergetic sensors and replenishes energy depletion pathways which inhibit autophagy, increases mitochondrial biogenesis, and reduces inflammation all of which benefits in delaying aging

cope with the starvation induced in the body. IF basically affects the following factors: (1) reduces oxidative damage caused by ROS and inflammation, (2) induces autophagy, (3) reduces blood glucose and insulin levels and initiates body weight loss, (4) reduces the production of advanced glycation end-products (AGEs), (5) releases adiponectin and ghrelin and reduces leptin, and (6) reduces inflammation and generation of lipid and protein oxidized products.

IF activates a few energy sensors which help in stress resistance and impacts organismal health span. The activity of the mTOR pathway is downregulated in response to lowered amino acids and the availability of growth factors. The inhibition of mTOR also reduces ribosomal biogenesis and induces autophagy which is a major factor for IF-induced protection against cancer and tumor formation (Fig. 10.2). The oscillations arising due to changes in the ratio of ATP/AMP, NAD^+/NADH, and acetyl-CoA/CoA is the cause of the activation of the AMPK pathway and Sirtuins (SIRTs). The SIRTs activate a network of transcription factors (TFs) FOXO, PGC-1α, NRF-2, and FGF-21, all involved in coordinating stress resistance, mitochondrial biogenesis, cell survival, proteostasis, and glucose and lipid metabolism (de Cabo and Mattson 2019).

1. The peroxisome proliferator-activated receptor-α (PPAR-α) is a transcription factor which binds to fatty acid derivatives and regulates genes related to ketogenesis, fatty acid oxidation, gluconeogenesis, and amino acid utilization (Goldstein and Hager 2015).
2. The Forkhead box factor (FOXO) is activated during fasting as a metabolic adaptation to regulate energy homeostasis and glucose metabolism. The FOXO plays a crucial role in upregulating several longevity controlling factors most likely conferring stress resistance, cellular proliferation, autophagy, and antioxidant activity (Fontana and Partridge 2015).
3. The PPAR Ɣ coactivator (PGC-1α) is activated via the upstream energy sensing pathways (AMPK and Sirtuins) and mainly regulates fatty acid oxidation, mitochondrial biogenesis, and antioxidant defenses.
4. The nuclear factor-erythroid factor 2-related factor 2 (Nrf-2) pathway plays a major role in resistance against stress by upregulating NRF 2-ARE (antioxidant response element) activation and promoting antioxidant defenses against oxidative stress.
5. Collectively, major changes occurring in an organism during intermittent fasting are the inhibition of anabolic pathways involving growth and reproduction and activation of catabolic processes like DNA repair, cell survival and recycling of damaged organelles, autophagy, maintenance of antioxidant activity, and stress resistance.

10.2.3 Fasting and Circadian Rhythm

The coordinated regulation of the metabolic pathways, biological activities, and behavioral processes occurring in an organism is possible due to the presence of the circadian clock machinery. The circadian clock is the rhythmic oscillations taking place within a 24 h cycle and is primarily regulated by the suprachiasmatic nucleus located in the hypothalamus. The circadian rhythms in an organism arise due to the sleep-wake cycle, exposure to light and darkness, fluctuations in body temperature, hormones, and changes in pattern of feeding and fasting. The circadian cycle influences the rhythms of the sleep-wake cycle and the physiological functioning of the digestive system, the endocrine, reproductive, and cardiovascular systems, the rhythmicity of the brain and immune systems, the renal system, and hepatic metabolism (Froy and Miskin 2010; Manoogian and Panda 2017). The SCN controls the biological functioning of the various peripheral secondary clocks located in various organs like the liver, adipose tissue, heart, retina, intestine, pancreas, skeletal muscle, and parts of the brain (Queiroz et al. 2021).

10.2.4 Time-Restricted Feeding and Circadian Rhythm

The circadian cycle is regulated by a systematic network of transcription and translational feedback loops which involves the circadian clock genes and their

transcription factors CLOCK (Circadian Locomotor Output Cycles Kaput) and BMAL1 (Brain and muscle ARNT-like protein 1). The downstream effects of these clock-controlled genes synchronize the interplay of the circadian and peripheral clocks with metabolism which influences changes in glucose tolerance, redox state, memory, and lipid functions (Challet 2019). Erratic schedules of eating and fasting in modern lifestyle hampers the coordination of the circadian cycle with the metabolic clock giving rise to diseases which can well be an indicator of aging. Therefore, the eating window in TRF must be scheduled in such a way that maximizes the beneficial effects imposed on organismal physiology and slows aging. TRF is the provision of eating within a <12 h timespan and the rest of the time period is the fasting timespan. The beneficial effects of TRF can mitigate the negative impact associated with chronodisruption. TRF during the active phase of an organism helps in initiating body weight loss, lowering glucose and insulin in the morning, and also peak other hormones related to aging like cortisol and growth hormone. Therefore, TRF following a robust feeding/fasting regimen in rodents helps in reprogramming the smooth synchronization of the circadian cycle which enables the harmonious functioning of the metabolic pathways which leads to extended longevity.

10.2.5 Beneficial Effects of Intermittent Fasting

10.2.5.1 Hormonal Changes During Intermittent Fasting

Intermittent energy restriction (IER), commonly practiced as ADF or PF reduces levels of blood glucose and insulin. It also lowers insulin resistance which corresponds to improved insulin sensitivity (Hofer et al. 2022). Nutrient deprivation during fasting causes the reduction in plasma insulin and glucose which leads to decline in Insulin-like growth factor (IGF-1) and IGFBP-1. This occurs due to the G-to- K switch which activates the gluconeogenesis cycle in the liver. The adiponectin levels rise during this metabolic switch and this plays a crucial role in fasting-induced improved longevity and lowered stress (Golbidi et al. 2017). The dramatic increase in adiponectin also mediates the cardioprotective effects during fasting.

The adipose tissue largely affects the body's metabolism because it secretes hormonal adipokines and uncoupling proteins. ADF exerts beneficial effects on adipose tissue by increasing the secretion of serum adiponectin and ghrelin hormones. Adiponectin and ghrelin are protein-derived hormones indicator of hunger and satiety which peak during the fasting state, adiponectin in response to hunger and ghrelin enhances feeding and promotes energy conservation after a brief duration of fasting (Challet 2019). Whereas leptin secretion from the fat cells is reduced in response to the metabolic switch, signaling the brain of reduction in appetite. Hunger levels remain elevated during fasting due to reduced leptin, a hormone which signals the body and brain about condition related to appetite.

10.2.5.2 Obesity and Body Weight

IF is an immensely popular dietary protocol for reducing body weight and, in turn, obesity and related causes. Intermittent calorie restriction (ICR) is an efficient phenomenon in rats and humans to decrease visceral fat from adipocytes and initiate weight loss in overweight individuals along with reduction in waist circumference, fat mass, fat-free mass, and body structure (Seimon et al. 2015). A number of studies have reported ADF to be a standard method for weight loss studies. Intermittent fasting accompanying restricted meal intake (or ad libitum) is effective in promoting weight loss and deduction in body fat (Tinsley and La Bounty 2015). IER-led organisms show some adaptive responses in return of energy restriction most likely which include a decline in physical activity, increases appetite, conservation of body energy, and hormonal stimulations which reserve fat tissues and dispense loss of lean mass. TRF with a feeding window of <10 h a day during the active phase has a significant role in weight loss and fat mass reduction in humans (Hoddy et al. 2020).

10.2.5.3 Fasting and Cardiovascular Diseases

IF immensely affects the functioning of the heart and exerts cardioprotective effects. The increase in BDNF in brain during fasting influences neurons that respond to heart and stabilizes the heart rate (Mattson et al. 2017). ADF reduces the rate of myocardial infarction in humans and rats. ADF reduces the risk of blood pressure associated with increasing age. ADF protects the heart against age-associated risk factors like inflammation, oxidative stress, and hyperlipidemia which are the main cause of heart attacks and stroke. Rise in adiponectin and reduced inflammation help in reducing the risk of heart diseases.

10.2.5.4 Fasting and Cancer

Fasting is helpful in reducing tumor incidence since 2 days or more of periodic fasting initiates autophagy, a cellular conservation process which helps eradicate dead and dysfunctional proteins and organelles. Fasting promotes glycolytic inhibition and decline in IGF-1 level which induces protective effects by inhibiting carcinogenesis and DNA damage. IF-induced protection against cancer is also attributed to the sirtuin genes. Sirtuins activate downstream transcription factors FOXO and Nrf2 which are concerned with inducing apoptosis during fasting and block the inflammasome pathway (Lee et al. 2020).

10.2.5.5 Fasting and Neurodegeneration

Aging increases manifold the vulnerability of the brain to neurodegenerative diseases and oxidative stress-associated damages. An aging brain is highly susceptible to neurodegenerative disorders like Alzheimer's disease (AD) and Parkinson's disease. Accumulation of oxidatively damaged molecules (lipid peroxidation products and carbonyl-modified proteins) damages portions of the brain and accelerates aging.

Fasting elicits major beneficial changes in brain and enhances cognitive power and neuronal functions. IF increases mitochondrial biogenesis in neurons, increases stress resistance, reduces markers of inflammation Interleukin-6 (IL-6) and Tumor

necrosis factor (TNF-α), improves synaptic plasticity, and stimulates neurogenesis. The rise in BDNF (Brain-derived neurotropic factors) during fasting presses its protective role in enhancing overall cognitive functioning. BDNF signaling during IF activates the antioxidant enzymes superoxide dismutase (SOD) and catalase in the brain which protects it from various neurodegenerative diseases. IF elicits these protective effects due to decreased oxidative damage and an overall improvement in cellular bioenergetics (Longo and Mattson 2014). BDNF stimulates neuron regeneration and synaptic plasticity and regulates cognitive functions like learning and memory. The mitochondrial bioenergetics is improved and an overall reduction in oxidative damage is seen (Kalsi 2015; Longo and Mattson 2014).

10.2.5.6 Fasting and Metabolic Syndrome

Metabolic syndrome is a combination of various metabolic complications associated with aging health, i.e., abdominal obesity, insulin resistance, elevated levels of triglycerides, and hypertension. The cumulative effect of these parameters is the cause of cardiovascular diseases, stroke, and diabetes (Anton et al. 2018). IF reverses the negative features which cause metabolic syndrome like inflammation, elevated blood pressure, and reduced adipocyte fat deposits. IF increases insulin sensitivity, reduces total cholesterol, and visceral fat mass nullifying the risk factors associated with heart disorders and diabetes (Longo and Mattson 2014).

The hormonal changes induced by fasting which include elevation in adiponectin and ghrelin levels and reduction in leptin are correlated to lowered inflammation and increased sensitivity to insulin. Overall fasting reduces all conditions corresponding to metabolic syndrome in rodents and humans like cardiovascular diseases (CVDs), AD, blood pressure, and diabetes.

10.2.5.7 Fasting and Inflammation

Lifestyle changes in the present era have given rise to a number of metabolic disorders like diabetes, metabolic syndrome, cancer, cardiovascular disorders, and neurodegenerative diseases. The disturbance caused by these disorders also gives rise to chronic inflammation due to the activation of certain harmful metabolic pathways in an organism. The molecular pathways which cause an increase in inflammatory biomarkers in blood and other organs are the STAT3 (signal inducer and activator of transcription 3), COX2 (cyclooxygenase 2), NF-κβ (Nuclear factor kappa light chain enhancer of activated B cells), MMP9 (matrix metallopeptidase 9), and MAPK (mitogen-activated protein kinase) (Margină et al. 2020). Whereas, IF induces activation of the NRF2, AMPK, mTOR pathways, and sirtuin genes that coordinate in a systematic way during fasting to reduce the level of inflammatory cytokines and molecules in body.

The rise in inflammation is known by a significant increase in cytokines including TNF-α, IL-6, CRP (C-reactive protein) in blood, liver, and adipocytes. In addition to these, the adipose tissue also produces other adipokines such as resistin, angiotensinogen, and plasminogen activator inhibitor-1 (PAI-1).

10.3 Intermittent Fasting in Model Organisms

The laboratory-based study of CR and IF requires the use of certain model organisms which basically are treated as models for trying and testing the efficacy of the DR routine upon them. Animal models are widely used since they are reliable to study and be experimented upon for longer periods than humans. Laboratory rats and mice are widely used model animals for longevity studies. However, several other model organisms are also extensively used to help determine the molecular pathways of action and its metabolic regulation. The most extensive laboratory-used model organisms are yeast, flies, worms, rodents, non-human primates (monkeys), and mammals too. In bacteria ***Escherichia coli*** the transfer of *E. coli* culture from a normal nutrient medium to a deficient one may cause an increase in lifespan asserting the belief that nutrient/dietary restriction is a strong determinant of lifespan extension even in lower organisms like bacteria (Lushchak et al. 2018).

In budding yeast ***Saccharomyces cerevisiae,*** nematode ***Caenorhabditis elegans,*** and fruit fly ***Drosophila melanogaster*** restriction of calories either by transfer to a low nutrient medium or alternate day fasting has proven to be effective in inducing healthy lifespan. *C. elegans* lifespan increase can be attributed to a reduction in levels of Daf-2, 16 transcription factor, whose similarity corresponds to the IGF-1 pathway in rodents and humans (López-Lluch and Navas 2016). The sensory abilities in *Drosophila* and *C. elegans* including gustatory and olfactory sensing neurons tend to increase lifespan. Any sensation of alterations in the nutrient-sensing pathways specifically IGF-1 network in *C. elegans* and of steroidal and growth hormonal signaling pathways in mice plays a stimulatory role in the longevity process (Fontana and Partridge 2015). Fasting in *Drosophila* has shown a positive effect on health with an increase in autophagy and higher stress resistance. Moreover, lowered IIS pathway activity and dietary restriction promote longevity rate and survival in *Drosophila* (Fontana et al. 2010).

The preliminary evidences of IF being a successful DR anti-aging approach was found in **rodents**. Rats were kept on 20–50% of calorie restriction for CR studies or were maintained on 1–3 days of fasting on alternative days or periodically for IF studies. The genetic makeup of rodents is most closely similar to humans and therefore rodents are widely used model organisms for dietary restriction studies. Studies have also been conducted on **non-human primates** like rhesus monkeys and primates including humans (Most et al. 2017). Overall, it is the reduction in the incidence of age-related diseases and hence longer health span that defines the purpose of dietary restriction interventions in longevity studies because these anti-aging interventions have produced successful results in few cases of human studies as well (Green et al. 2022; Longo et al. 2015).

10.3.1 Mediterranean Diet

The Mediterranean diet is popular because of its cardiovascular benefits offering properties. Mediterranean diet comprises a high intake of fruits and vegetables along

with fish and unsaturated fats with restricted consumption of saturated fat and processed food (Voglhuber et al. 2021). Mediterranean diet has mainly effects on markers of cardiovascular risk like lowering of HDL and LDL cholesterol and atheroprotective effect. The Mediterranean diet has shown to lower the risk of CVDs by about 30% and also includes reduced incidence of heart diseases, metabolic syndrome, and diabetes.

10.3.2 Ketogenic Diet

Intermittent fasting causes a metabolic transition in the body which induces conversion of the fatty acids to ketone bodies. This change in metabolic state is now adapted in a dietary form referred to as the ketogenic diet (KD). KD involves the complete reduction in carbohydrate intake and excess consumption of fats (>70%) and scarce protein (<20%) content which forcefully generates ketone bodies. The generation of a ketogenic state is being adapted as it is useful in the treatment of a number of maladies including diabetes, cancer, CVDs, obesity, multiple sclerosis, neurological effects, and others (Green et al. 2022). KD produces several benefits as it imitates some molecular effects of IF such as downregulation of IGF-1, inhibition of mTOR, and stimulation of autophagy. KD lowers the generation of ROS and oxidative stress by causing fatty acid oxidation in the liver and cessation of fatty acid synthesis (Bhoumik and Rizvi 2021).

10.4 Conclusion

Aging as a biological problem has attracted attention from gerontologists to find a possible way to improve the duration of healthy lifespan. The cumulative effort of the worldwide aging research has therefore given us diverse approaches to target aging which are broadly classified under “anti-aging strategies.” Dietary restriction interventions have been a fruitful approach to delay aging of which intermittent fasting is a modern dietary intervention that provides metabolic and physiological benefits to organisms. IF is further classified into its types based on time period and duration of fast. IF studies conducted on model organisms have revealed interesting results in improving longevity. Moreover, modifications of diet based on its constituents are a trending dietary pattern that induces each of its own beneficial impact on human health and metabolism. In conclusion, Intermittent Fasting may prove to be beneficial in reducing the age-associated implications and improving health span.

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The Role of Curcumin as an Anti-Aging Compound

11

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Abstract

It is claimed that delaying aging is more effective and less expensive than treating specific age-related disorders. Compounds that may postpone the onset of age-related symptoms, particularly natural compounds included in the average diet, are being thoroughly researched and Curcumin (CUR) is one among them. It alleviates age-related symptoms, increases the lifetime of model organisms, and delays the course of age-related disorders in which cellular senescence is directly implicated. It has been established that removing senescent cells enhances mice's quality of life greatly. The quest for molecules known as senolytic medicines,

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which selectively destroy senescent cells from organisms, is ongoing. In this study, we attempt to evaluate the existing understanding of CUR's anti-aging impact and highlight its senolytic potential.

Keywords

CUR · Aging · Anticancer · Autophagy · Analytics · Microbiota · Senescence

11.1 Introduction

According to demographic data, the number of old and very elderly persons is growing (Roberts et al. 2018). The population over the age of 65 accounts for 8.7% of the total. The ratio ranges from 15% to 16% in Europe, North America, and Central Asia to less than 5% in the Middle East, North Africa, and South Asia. Longevity is useless unless it is accompanied by improved health. We want to live longer and healthier lives so that we may fully appreciate the beauty of our home planet. An increasing amount of research suggests that the rate and quality of aging can be influenced (Amron 2018). Understanding the mechanics of aging is critical for postponing the onset of the illness. Cellular senescence complicates organismal aging and age-related illnesses. Senescent cells have been seen in the tissues and organs of elderly animals and humans, and cell proliferative capacity diminishes with age (Song et al. 2020). Even though the proportion of senescent cells appears to be small, ranging from a few to a dozen %, changes in the extracellular milieu caused by senescent cells' increased cytokine production, as well as senescence-related impairment of regenerative processes, can cause dramatic organismal dysfunctions. Senescent cells may have a role in the beginning and progression of illnesses that are becoming more frequent as individuals get older (Streit et al. 2020). Almost all age-related disorders have been connected to the formation of senescent cells. In animal studies, the role of cell senescence in the development of aging and age-related diseases has been convincingly proven. It has been demonstrated that removing senescent cells improves the quality of life of genetically modified animals while decreasing indications of aging and age-related illnesses (Bielak-Zmijewska et al. 2019). It is trendier these days to prevent individuals from getting old than to treat specific diseases, and to go even farther, to abolish cell senescence to lessen age-related dysfunctions. Despite tremendous advances in slowing the aging process in animal models, many therapies are not suitable for humans due to the possible negative effects of long-term anti-aging medication usage (not to mention genetic alterations). The ideal anti-aging strategy should be easy to get, devoid of side effects, and readily incorporated into our daily lives through food or physical exercise (Lokhande and Pathak 2021; Bhattacharya et al. 2021; Rahman et al. 2021). Natural chemicals are currently receiving a lot of attention, and some promising results have already been reported. Curcumin (CUR), a polyphenol, is

one of these chemicals (Akter et al. 2021b; Sindhu et al. 2021b). CUR's capacity to prevent aging in animal models has been demonstrated, although long-term human research is required to corroborate certain findings (Lefcheck et al. 2018; Tagde et al. 2021b; Rahman et al. 2021).

11.2 Cellular Senescence

Leonard Hayflick and Paul Moorhead defined cellular senescence for the first time around 60 years ago. Since then, there has been a concentrated effort to understand more about the role and processes of this critical biological activity. Senescence serves a range of functions depending on the age of the organism. Cell senescence is required for the development of a young organism (von Kobbe 2018). It is required for embryonic development, tissue regeneration (as part of body sculpting, immune cells eliminate senescent cells), and as a cancer barrier (senescent cells cannot multiply). Senescent cells multiply as an organism age, resulting in reduced chronic inflammation, increased reactive oxygen species (ROS), and microenvironmental alterations that favor cancer development via the senescence-associated secretory phenotype (SASP) (Fane and Weeraratna 2020). Cell senescence is typically harmful due to the function of senescent cells in aging and age-related diseases. On the other hand, the function of cell senescence in organismal homeostasis should not be overlooked (Zhang et al. 2021). Senescence is a complicated process governed by genetic and epigenetic modifications. An organism is subjected to both extrinsic (chemical and physical genotoxic events) and intrinsic (metabolic processes such as ROS production and DNA replication) stressors throughout its existence. Cells repair damaged DNA to limit the risk of mutations that might lead to malignant growth (Di Micco et al. 2021). Cells must repair their DNA precisely to entirely heal harmed cells; otherwise, cells with permanent damage may undergo apoptosis or senescence (Ogrodnik 2021).

Senescence is distinguished by a decrease in proliferation (it affects proliferation-competent cells), an increase in cell cycle inhibitors (p21, p16), activation of the DNA damage response (DDR) pathway, increased activity of a lysosomal enzyme known as senescence-associated -galactosidase (SA-β-gal), an increase in the number of DNA double-strand breaks (DSBs), and changes in gene expression (Volman et al. 2022). The formation of a senescence-associated secretory phenotype is one of the most essential characteristics of senescent cells (SASP). SASP is caused by an increase in protein synthesis and secretion, which is connected to the beginning of low-grade inflammation (Salminen et al. 2020). This has a bystander effect, which means it causes senescence in nearby cells. Senescence can be induced by external (chemical and physical) and internal (oncogene overexpression, DNA damage, increased ROS generation, ER-stress, and chromatin structural dysfunction) stressors, as well as telomere lengthening (replicative senescence) (Koloko Ngassie et al. 2021). Stress-induced senescence is the later kind of senescence (SIPS, stress-induced premature senescence). Non-senescent cells may be distinguished from senescent cells since they have a separate identity (Gasek et al. 2021).

Cell senescence, on the other hand, is less evident than it once was. An increasing amount of research suggests that the key properties of cell senescence, as well as the processes that cause it, may differ depending on the cell and the type of senescence. This includes alterations in cell sensitivity to stress, metabolic discrepancies between stress-induced and replicative senescence, and changes in SASP components (Zhu et al. 2020). The question of whether post-mitotic cells may undergo cell senescence and if cancer cell senescence can be reversed, as well as whether SA-β-gal activity in these cells is suggestive of senescence or just unspecific, is intriguing (Liu et al. 2020).

11.3 Age-Related Diseases and Senescence

Senescent cells have been linked to neurodegenerative diseases (Alzheimer's and Parkinson's disease), glaucoma, cataract, cardiovascular diseases (atherosclerosis, CVD, and hypertension), idiopathic pulmonary fibrosis (IPF), sarcopenia, chronic obstructive pulmonary disease (COPD), osteoporosis, diabetes type II, osteoarthritis, and certain types of cancer (Khaltourina et al. 2020; Akter et al. 2021a; Rahman et al. 2021).

11.4 Intervention Against Aging

Human life has been prolonged because of scientific and medical achievements. Ailments associated with old age, on the other hand, are becoming more frequent as individuals live longer lives (Partridge et al. 2018). As a result, developing a plan to minimize or postpone the start of age-related illnesses is crucial. Animal studies have provided us with a wealth of knowledge and insights, and several ways for prolonging the lifespan of model animals, the bulk of which entail genetic manipulation, have been developed. Dietary/calorie restriction (DR/CR) is the only non-genetic strategy that has yet to be proven to increase longevity (Bielak-Zmijewska et al. 2019). This strategy involves lowering calories by 20–40% without deprivation, and it has been found to work in several species, including yeast, monkeys, fruit flies, nematodes, rats, primates, dogs, and humans. Furthermore, epidemiological research back up the favorable impacts of CR on people (Blesso and Fernandez 2018). The number of centenarians (those above the age of 100) on the Japanese island of Okinawa, for example, is five times greater than anywhere else on the earth. According to one research, adult Okinawans consume 17% fewer calories than the average Japanese adult and 40% fewer calories than the average adult American citizen (Imada and Furumitsu 2020). Calorie restriction has been found to lower the risk of cancer, improve cardiovascular and metabolic health, and postpone the onset of neurodegeneration and sarcopenia. Because sticking to a CR program might be difficult, researchers are seeking medications, supplements, or less severe nutritional therapy that can be utilized in the same way as CR. Intermittent fasting (IF) is one such strategy that has lately gained popularity (Keenan et al.

2020). According to Good rick's results, rats on an alternate day fasting diet lived 30% to 100% longer than that fed ad libitum, depending on the age at which the diet was begun. Catterson et al. observed that IF (2 days fed, 5 days fasting) can lengthen a fruit fly's life by 10%. It has been proposed that CR works through increasing autophagy because blocking this process reduces the anti-aging effects of the diet. Another study found that sirtuins, whose expression increases in response to CR, regulate the effects of CR (Zhu et al. 2021). Sirtuins have a role in the cell's response to many stimuli, including oxidative and genotoxic stress. A reduction in the level of activity of these enzymes reduces the longevity of several model species, whereas an increase in activity/level increases both lifespan and health span. Several natural and synthetic substances (quercetin, CUR, lutein, kaempferol, fisetin, and catechins) have been demonstrated to increase sirtuin expression hence extending the life and lowering the risk of age-related disorders (Mayack et al. 2020). Mild physical exercise, along with a nutritious diet, has been found to increase health and longevity. Low-intensity exercise is intended to act as a minor stressor, inducing the stress response and preparing the body to respond to a larger threat. This causes lowering oxidative stress, antioxidant enzymes to be activated, and perhaps activating sirtuins (Kitada et al. 2019). Finally, practical and promising anti-aging therapies in humans are associated with a reduction in food consumption and/or the use of certain chemicals or physical activity similar to diet restriction (Rock et al. 2020).

11.5 Curcumin

CUR is a potential anti-aging substance that is readily available and simple to incorporate into the diet, as well as safe and inexpensive (Karthika et al. 2021; Kabir et al. 2021). CUR is a polyphenolic nutraceutical derived from the rhizome of the plant Curcuma longa (turmeric), a member of the ginger family. Because turmeric has 12 active ingredients, the ratio of CUR (chemically known as diferuloylmethane) per dry weight of turmeric powder is no more than 3.14%. CUR is widely ingested since it is used as a spice (turmeric) and a yellow food color (E100) (Rahman et al. 2021; Bhattacharya et al. 2021). CUR, on the other hand, is poorly absorbed by intestinal cells (because of its low water solubility and stability), rapidly metabolized by the liver (resulting in the formation of less active CUR glucuronides), and promptly removed from the body. Following 3 months of oral administration of 8 g of CUR per day for 3 months, the peak blood CUR level was around 1.77 μM 1 h after therapy, or even 3.6 μM if such a dosage was maintained for 3 months. Daily, Hindus take the most CUR (an active component found in turmeric spice), up to 100 mg (Shaito et al. 2020). CUR's therapeutic usefulness may be called into doubt due to its low bioavailability. Several research however has been conducted to address these critical difficulties. Furthermore, several attempts have been made to improve CUR absorption, such as coadministration of CUR with piperine, the active ingredient in pepper, which can boost CUR levels in the blood by up to 30 times (Tabanelli et al. 2021). To improve cell targeting, self-assembling peptide nanofiber carriers, CUR-alginate conjugates,

CUR-phospholipid complexes, liposomes, microemulsions, polymeric micelles, and CUR nanoparticles are all alternatives. Furthermore, studies have demonstrated that even extremely high daily CUR dosages (12 g/day) are completely safe for patients (Mantzorou et al. 2018). CUR, like other polyphenols, has pleiotropic effects (CUR is a PAINS or pan-assay interference chemical), which is seen as a significant defect in natural goods. Indeed, due to its capacity to interact with a diverse set of receptors (e.g., EGFR, CXCR4), enzymes (e.g., DNA pol, COX2), kinases (e.g., MAPK, FAK), growth factors (e.g., EGF, TGF), transcription factors (e.g., NF-, STAT1-5), and adhesion molecules (e.g., ICAM-1, VCAM-1) (Bielak-Zmijewska et al. 2019).

However, we believe that the ability of a single compound to affect a variety of biological processes, such as inflammation, apoptosis, redox state, migration, proliferation, and wound healing, and thus positively affect memory, aging, and age-related diseases, such as atherosclerosis, is an advantage rather than a disadvantage (Martel et al. 2020). CUR-based therapy looks to be a potential therapeutic option for complex, chronic, multigenic, civilization- and age-related illnesses caused by aberrant signaling pathways. CUR has been utilized in a significant number of clinical studies as a medication or adjuvant in the treatment of a variety of illnesses due to all of its qualities (Maleki Dizaj et al. 2022; Akter et al. 2021c). The therapeutic or detrimental effects of CUR are also dependent on its concentration. This behavior is known as a hormetic effect because it functions as a stimulant at low levels and an inhibitor at high concentrations (Calabrese and Kozumbo 2021; Sindhu et al. 2021a). CUR has a similar function: at low quantities, it may work as a protective agent, while at large concentrations, it may act as a cytotoxic, cytostatic, and genotoxic agent (Bebianno et al. 2020).

11.6 Curcumin and Its Anti-Aging Role

Because low-grade inflammation is a characteristic of aging, polyphenol-rich foods with anti-inflammatory and antioxidant capabilities can help to alleviate age-related symptoms. The anti-aging benefits of CUR have been established in a variety of ways. CUR extended the lives of fruit flies, nematodes, and mice (Zia et al. 2021). CUR has also been shown in clinical research to aid persons with age-related disorders such as atherosclerosis, diabetes, and cancer. It may have neuroprotective effects as well. CUR has also been proven to protect breast cancer patients against the adverse effects of chemotherapy, such as doxorubicin cardiotoxicity, and radiation-induced dermatitis. CUR protected HUVEC against peroxide-induced senescence, but a CUR analog, bis-dimethoxy CUR, protected WI38 fibroblasts from oxidative stress-induced senescence (Malavolta et al. 2018). CUR also improved the capacity of human epidermal keratinocytes to differentiate during replicative senescence. Some believe that CUR's anti-aging properties stem from its ability to prevent cellular senescence (Benameur et al. 2021). CUR did not affect VSMC or EC replicative or doxorubicin-induced senescence. At low dosages (0.1–1.0 μM), CUR did not delay replicative senescence or protect cells against doxorubicin-induced senescence, but it did enhance sirtuins and AMPK levels in

VSMC experiencing replicative senescence. As a result, rather than inhibiting cellular senescence, the beneficial effects of CUR supplementation on the organism are more likely to be linked to sirtuin and AMPK activation (Hou et al. 2019). In mice and rats, CUR supplementation was associated with higher levels/activity of AMPK and sirtuin 1 in muscles, which decreased the impact of exercise, delayed exhaustion, and avoided weariness (Snyder and Cinelli 2022).

Sirt2 has been revealed to be required for the lifetime extension mediated by CUR in Caenorhabditis elegans. In summary, CUR affects nutrient-sensing signaling pathways (effect on sirtuins, AMPK) and can thus mimic caloric/diet restriction while simultaneously boosting the benefits of moderate physical exercise (Bielak-Zmijewska et al. 2019). CUR concentrations are comparable to those seen in the blood after meal supplementation were also investigated (5–7.5 μM for VSMC and 2.5–5 μM for EC). Surprisingly, both the VSMC and the EC died because of this therapy. CUR-induced senescence was produced through its impact on many signaling pathways, rather than by DNA damage (Grabowska et al. 2019). Early modifications showed lower levels of sirtuins and AMPK, indicating that these proteins may play a role in the onset of senescence. Overall, these findings imply that, while CUR cannot prevent or postpone aging, it may have an anti-aging impact by changing the amounts of proteins implicated in the aging process (sirtuins, AMPK). Furthermore, the likelihood of positive effects from CUR-induced cell senescence cannot be ruled out (Sikora et al. 2019). CUR-induced senescence in cancer-associated fibroblasts (CAF) lowered cancer aggressiveness, while CUR-induced senescence in hepatic stellate cells (HSC) protected against liver fibrosis (Rahman et al. 2021).

CUR also has an anticancer impact, which is a significant, albeit indirect, anti-aging benefit. Age is one of the most important risk factors for a wide variety of cancer types. Women and men over the age of 65 are four to seven times more likely to develop cancer than their younger counterparts (Melucci et al. 2022). Prostate, lung, and colorectal cancers account for about half of all malignancies identified in older males. Breast, lung, colon, and stomach cancers are the most frequent among older women, accounting for 48% of all cancer. CUR can prevent carcinogenesis (for example, by shielding against the toxicity of certain environmental or therapeutic stimuli), decrease cancer cell population (through cancer cell death activation), and restrict metastasis (anti-angiogenic properties) (Baillie et al. 2019).

This can be explained by several things. The first is concerned with the pace of cell proliferation. CUR suppresses mitosis, causing cancer cells to divide more quickly than normal cells. This has been connected to, among other things, mitotic spindle malfunction, cdk1 kinase inhibition, and sirtuin 7 suppression. The second avenue is CUR's capacity to suppress the NF-kB transcription factor, which is found in cancer cells. This is related to the inhibition of IB-IKK and the activation of sirtuins 1 and 6, which deacetylate (sirtuin 1) or indirectly engage RelA/p65, an NF-kB component (sirtuin 6). Another cause is that β-glucuronidase (a glucuronide conjugation enzyme) activity is raised in cancer tissue while UDP-glucuronosyltransferases (UGT) (a glucuronide synthesis enzyme) activity is lowered. This might result in a higher local concentration of free, previously

glucuronide molecules, improving apoptotic efficiency. CUR also possesses this feature, which may boost its anticancer potency. Inducing cell senescence is one potential tumor-eradication strategy. CUR can induce cellular senescence in cancer cells. This may be harmful to normal cells but beneficial to cancer cells (Moyer and Reid 2022). However, in the long run, such a strategy looks to be troublesome, as some research shows that cancer cell senescence can be reversed and cancer can reappear (Wang et al. 2018).

11.7 Curcumin and SASP

CUR's ability to decrease inflammation is one of its anti-aging properties. Senescent cells are alive, metabolically active, and can impact their surroundings even though they are not reproducing. Senescent cells can emit interleukins, chemokines, and other pro-inflammatory cytokines, as well as proteases, metalloproteinases, and growth factors (Larouche et al. 2018). SASP has been associated with inflammation, angiogenesis, extracellular matrix remodeling, proliferation activation, and immune system regulation. This might be advantageous or detrimental depending on the biological setting. SASP is favorable because several secreted cytokines, including interleukin-6 (IL-6) and interleukin-8 (IL-8), play an essential role in the autocrine process of senescence development, maintenance, and reinforcement (Xenaki et al. 2021). SASP is also capable of communicating with immune cells. Certain subsets of senescent cells may release chemokines that attract NK cells, monocytes/macrophages, neutrophils, B cells, and T cells (e.g., RANTES, GRO α, MCP-1). As a result, these immune cells detect and eliminate damaged, senescent, and malfunctioning cells. This is a critical stage in tissue regeneration and fibrosis management. Senescent cell proteins, on the other hand, have been shown to promote cancer growth by boosting cancer cell motility and hence metastasis formation, as well as promoting senescence in adjacent normal cells (He et al. 2018). Furthermore, cytokines affect the tissue environment, which may lead to malfunction. According to cocultivation of senescent and normal cells, the senescent phenotype is transmitted to neighboring cells via soluble SASP proteins. The secretory phenotype is influenced by both senescence-inducing events and cell type. ATM and CHK2 are two proteins that can influence the effectiveness of the DNA damage response system (DDR pathway) (Huang et al. 2021).

Furthermore, the transcription factor NF-kB regulates the synthesis of many SASP components, including the pro-inflammatory cytokines IL-8 and IL-6. When one of the NF-kB components, p65, was downregulated, there were less secreted IL-8 and mRNAs encoding IL-8, RANTES, and GRO α (Strzeszewska et al. 2018). Chronic inflammation has been associated with a variety of disorders, including Alzheimer's disease, cardiovascular disease, cancer, diabetes, and other age-related problems. CUR's anti-inflammatory qualities allow it to reduce TNF α, the most potent NF-kB activator, while also decreasing NF-kB activity. CUR has been shown to benefit normal young cells by decreasing the amount of pro-inflammatory cytokines generated over time (Fernández-Lázaro et al. 2020).

After just one application, this therapy reduced IL-8 and VEGF levels. This impact is not detected when cells are permanently treated during replicative senescence. CUR did not affect IL-6, IL-8, or VEGF levels. CUR, on the other hand, boosted IL-6 and IL-8 levels in response to aging. Sirtuin 1 inhibits NF-kB signaling, whereas CUR increases sirtuin synthesis, reducing inflammation (i.e., NAD-dependent deacetylases) (Zendedel et al. 2018). CUR can reduce or increase the levels/activities of proteins implicated in age-related secretory phenotypes (Fakhri et al. 2021).

11.8 Curcumin's Role in Autophagy

The function of autophagy in aging has been highlighted in a variety of studies spanning from yeast to mice. The synthesis of autophagy-related proteins is required for life extension (especially those encoded by the ATG gene family). Furthermore, overexpression of certain autophagy proteins is enough to extend life. The primary autophagy regulators are the mammalian target of rapamycin (mTOR) kinase and AMP-activated kinase (AMPK) (Tamaddoni et al. 2020). The AMPK system was boosted while the mTOR pathway was suppressed in several model animals, resulting in increased longevity and health span. Like the insulin/IGF1 system, which has been shown to reduce aging in animal models, these signaling pathways are critical for food sensing and autophagy control. A variety of dietary supplements and drugs that inhibit mTOR or activate AMPK pathways, such as resveratrol and spermidine or rapamycin and metformin, can induce autophagy (Yang et al. 2021). Long-term metformin use, on the other hand, has been linked to immunosuppression. CUR regulates the quantity and activity of AMPK, and it has been demonstrated in several studies to diminish the level and activity of mTOR, indicating that it may impact autophagy. Autophagy can be a double-edged sword during cancer growth (Zhang et al. 2021; Tagde et al. 2021a; Sharma et al. 2022). Functional autophagy works as a cancer suppressor in the early stages of cancer by removing damaged cells and organelles, reducing cell proliferation, and maintaining genomic integrity. Autophagy provides much-needed energy and building materials to metastasizing, rapidly reproducing cancer cells, allowing them to complete their cell cycle uninterrupted. Cancer cells can also tolerate the negative effects of the cancer microenvironment, such as hypoxia, inflammation, and energy depletion, thanks to active autophagy (Janji et al. 2018). As a result, if induction or autophagy flux is lowered in later stages of the illness, cancer cells may be injured (Shu et al. 2019).

CUR has both activating and inhibiting characteristics in terms of autophagy. The efficiency of CUR is strongly reliant on the kind of cancer cells. CUR can influence a number of molecular targets, including Beclin-1 and p53 (Shakeri et al. 2019). CUR can also hasten autophagy-associated cell death (type II PCD), which is a non-apoptotic, caspase-independent kind of programmed cell death that does not cause an inflammatory response. Finally, CUR's ability to impact autophagy contributes to its potential to influence cancer cell senescence and cancer

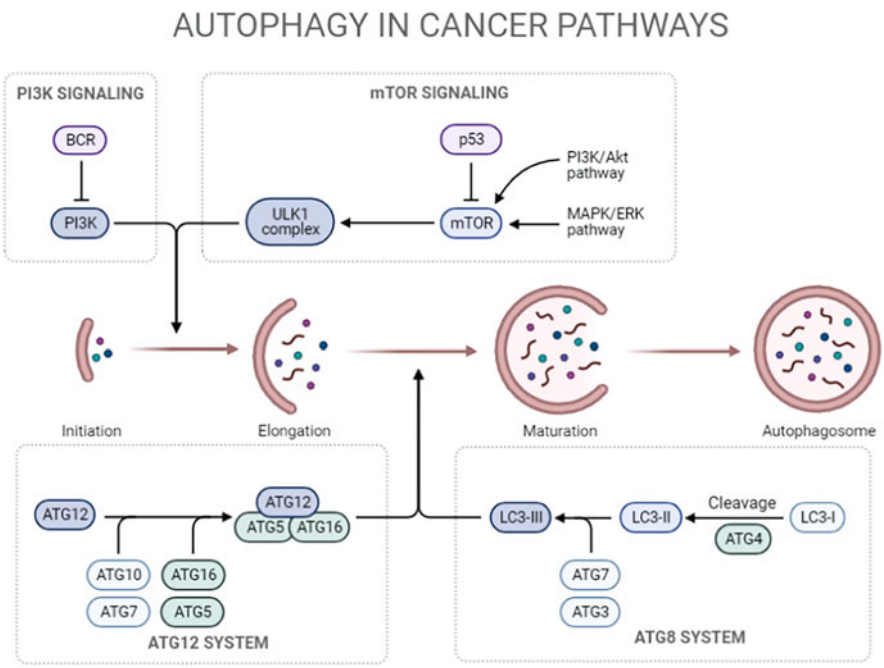

Fig. 11.1 Autophagy in cancer pathway

development (Wang and Wu 2020; Fatima et al. 2021) The autophagy in the cancer pathway is given in Fig. 11.1.

Autophagy dysregulation has a function in neurodegenerative disorders. The build-up of mutant huntingtin or misfolded amyloid-beta proteins (A) in neurons is responsible for the genesis of Huntington's and Alzheimer's diseases (Karthika 2022). CUR can cause misfolded proteins or damaged organelles to disintegrate in a variety of ways. It begins by activating the transcription factor TFEB, which is involved in the formation of lysosomes (Pastore et al. 2019). Second, CUR restores normal HSP70 levels, allowing cargo to enter lysosomes properly. CUR also enhances mitophagy, which reduces oxidative stress and increases neuronal lifespan (Yang et al. 2022; Walia et al. 2021).

11.9 Cancer and Curcumin

One element that contributes to the development of cancer is age. CUR's anticancer qualities are only one of the numerous age-related health benefits it offers. CUR has been proven to have a role in cancer formation at several stages, including cancer genesis, growth, and metastasis (Shakeri et al. 2019). CUR has a diverse set of molecular targets and signaling pathways with which it interacts. Transcription factors such as NF-kB and AP1 regulate the expression of inflammatory cytokines,

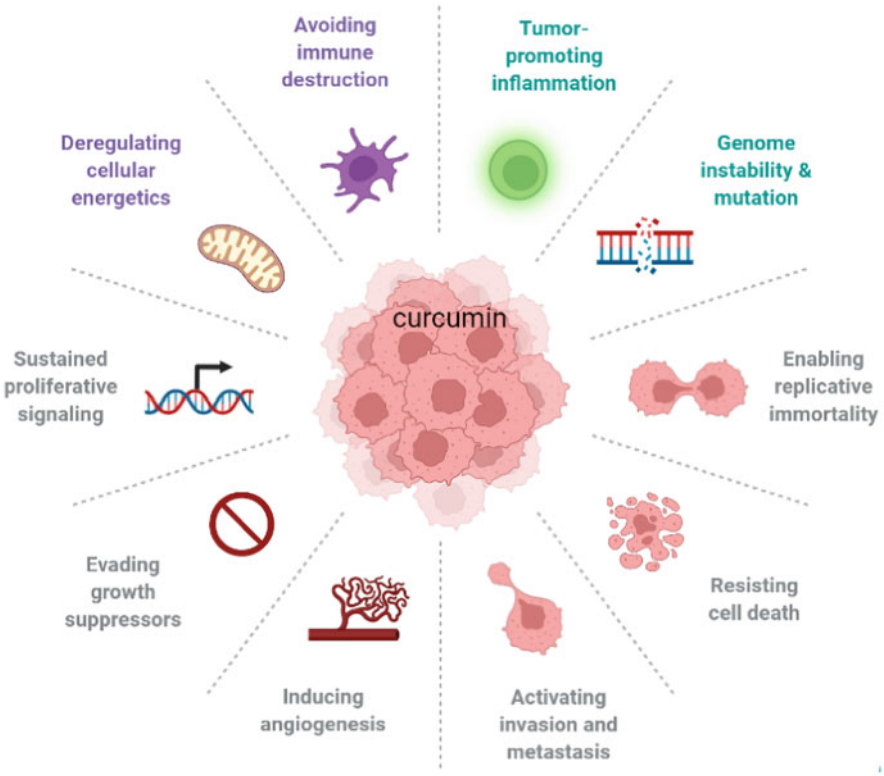

Fig. 11.2 Curcumin and cancer

receptors, kinases, growth factors, and many other genes. CUR has been shown in vitro to trigger cell death in a variety of cancer cells (Cinar et al. 2022). According to our results, CUR can also kill cancer cells that are resistant to regularly used chemotherapeutic medicines by triggering apoptosis or mitotic catastrophe. CUR has been shown to induce atypical apoptosis in cells that do not exhibit DNA fragmentation or activation of the caspase-3 and -7 enzymes. DNA fragmentation factor 40 (DFF40), a caspase-activated DNA endonuclease, was inhibited in dying cancer cells, leading to a lack of fragmentation (Kulbay et al. 2021). However, it should be noted that CUR was administered at a somewhat high dosage (50 M) in these investigations. Increased genomic instability has been related to both cancer and aging. The role of CUR in cancer is given in Fig. 11.2. As we age, these cancer-causing DNA mutations accumulate in our bodies. During the aging process, both increased hypomethylation and faster ROS generation are found. Mutations in protooncogenes and cancer suppressor genes have been associated with these factors (Kontomanolis et al. 2020). Cancer growth is generated by the growing activation or inactivation of certain genes, according to a well-defined description of cancer development. CUR has been shown to reduce the chance of mutation. For starters,

CUR has been shown to have chemopreventive effects. Several studies have shown that just reducing the mutagenesis impact of certain carcinogens prevents cancer development (Eslami et al. 2019).

CUR is a powerful antioxidant that can help protect DNA against mutation. Finally, CUR influences the epigenetic landscape via histone acetylation, DNA methylation, and miRNA expression. As a result of this action, we can infer that CUR possesses anti-aging and anticancer properties; nevertheless, additional study is required. EGFR is an example of an oncogene. The EGFR gene is overexpressed and/or mutated in a variety of malignancies (Eng et al. 2022). CUR's antiproliferative action in cancer cells is based, among other things, on the breakdown of the EGFR/EGF/TGF autocrine loop. It prevents the receptor's phosphorylation as well as the production of its ligands. CUR also suppresses downstream EGFR signaling pathways such as the PI3K/Akt/mTOR and ERK/MAPK (Jantan et al. 2021). Recently, anticancer medication has been related to cellular senescence as a major side effect. Chemotherapeutic drug therapy causes DNA damage and activation of the DNA damage response system, which results in cell senescence. As a result, we observed that CUR triggered senescence in human colon HCT116 cancer cells, MCF-7 human breast cancer cells, and U2OS human osteosarcoma cell line at low, non-cytotoxic doses. The capacity of CUR to cause DNA damage, which is the primary cause of senescence, is controversial (Bielak-Zmijewska et al. 2019). Despite previous research suggesting that CUR might cause DNA damage, we discovered no indication of DNA damage in cells after treatment with extremely high, lethal dosages of CUR. Due to disrupted mitosis progression caused by incorrect mitotic spindle formation, double-strand DNA breaks (DSBs) in mitotic chromosomes have been seen in CUR-treated cancer cells. CUR, through suppressing the DNA damage response system, delayed senescence and increased the number of growing cells in cancer cells. CUR's ability to inhibit cancer cell development and kill cancer cells through the senescence pathway implies that it may have anticancer properties (Lestari et al. 2019). The subject of whether cancer cell senescence may be reversed is being debated right now. According to our findings, cancer cell senescence can be accompanied by polyploidization. Polyploid cells may regain their proliferative capacity because of defective cell division. Cancer cells, too, have been shown to acquire the stemness characteristic as they develop (Pasani et al. 2020). As a result, cells that avoid senescence because of mutations have a far higher tumor-initiation potential than cells that have never been forced to senesce. As a result, a combination of prosenescent anticancer treatment with senolytics looks to be the best and safest therapeutic choice (Basu 2021).

11.10 Curcumin's Senolytic Activity

Senolytic medications are chemicals that can exclusively eliminate senescent cells. The concept of removing senescent cells originates from research that shows they have a role in aging and age-related disorders. Senescent cells increase as people age, and they can be seen in areas where age-related illnesses are prevalent (Sikora et al.

2019). As previously indicated, removing senescent cells enhanced life expectancy considerably. According to one study, analytics induce senescent cells to die in vitro. Chemotherapy is a novel field of biogerontology that use analytics to increase immune cells' ability to eliminate senescent cells or to lessen the low-grade inflammatory state caused by senescent cells. Analytics are tiny molecules such as dasatinib, A155463, A1351852, navitoclax, and FOXO4-related peptides, as well as natural substances such as quercetin, piperlinguine, and fisetin. Although CUR has been proven in several tests to have anti-aging properties, just a few studies have proved that it can influence cell senescence (Zia et al. 2021). CUR was therefore investigated in a mouse model of chemically induced diabetic Mellitus, a condition characterized by endothelial progenitor cell failure (EPCs). CUR administration to type I diabetic mice enhanced blood circulation and increased capillary density in ischemic hind limbs, according to the findings. According to an in vitro investigation, CUR treatment also restored EPCs' migratory, angiogenesis, and proliferative capabilities, as well as the number of senescent EPCs, as measured by the number of SA-β-gal-positive cells. The earliest definition of cell senescence was the stop of cell proliferation in previously growing cells. In nonproliferating neurons in vitro and in the brain, cell senescence signs such as increased SA-β-gal activity can be detected. Lipofuscin is collected by senescent cells, which include both previously proliferating and non-dividing post-mitotic cells such as neurons (Sikora et al. 2019).

When combined with piperine, CUR decreased lipofuscin clumps in the CA1 area of the rat hippocampus when cells were forced to senesce by d-galactose treatment. Another study utilizing the same rat aging model found that CUR treatment reduced p16 mRNA levels in premature ovarian failure (Yang and Zhang 2020). CUR did not induce apoptosis in p16-positive cells or increase apoptotic markers in the ovaries. CUR reduced SA-β-gal activity in the aorta and the amount of an inflammatory marker, MCP-1, in the blood in atherosclerotic rats. CUR extended the longevity of rat mesenchymal stem cells while indirectly decreasing population-doubling time, indicating that it can impact replicative senescence (Panahi et al. 2020). CUR decreased cell quantity somewhat less than fisetin, a potent senolytic, in the culture of mice embryonic fibroblasts derived from prematurely aged animals. CUR did not affect replicative vascular smooth muscle cells (VSMCs), but it did affect stress-induced senescent VSMCs. Although the word "senolytic" was originally used for medications that caused apoptosis in senescent cells, we believe it should be broadened to include senescent cancer cells (Lin et al. 2019). We believe that inducing senescence in cancer cells is dangerous because it can result in cancer recurrence owing to polyploidization/depolyploidization and senescence-associated cell regrowth. As a result, finding medications that can destroy senescent cancer cells is critical. To the best of our knowledge, there is no evidence that CUR can specifically destroy senescent cancer cells. In both senescent cancer cells and normal senescent cells, SASP promotes a pro-inflammatory microenvironment and cell senescence. The transcription factor NF-kB is the primary activator of SASP (Lopes-Paciencia et al. 2019). Large Reed–Sternberg cells in Hodgkin's lymphoma were recently reported to have significant NF-kB activity and to exhibit a variety of cellular senescence features. CUR works as an NF-kB inhibitor to prevent senescent

cells from producing IL-6. According to our findings, CUR cytotoxicity and SASP activity did not differ between non-senescent HCT116 cells and cells forced to senesce, or between normally replicating and prematurely senescent VSMC (Sikora et al. 2019). Overall, our findings, as well as those of previous research, do not support CUR's effectiveness as a senolytic agent. It should be noted however that no one has demonstrated that senolytic medicines are frequently used. Because they are cell type specific, it is not impossible that CUR's potential as a senolytic medication is currently being investigated (Beltzig et al. 2021).

11.11 The Microbiome and Bioavailability

CUR's limited bioavailability has caused a storm of controversy. CUR's limited bioavailability, on the other hand, does not rule it out of use because it has been shown to have beneficial effects at modest dosages. As previously stated, CUR's low bioavailability is owing to poor absorption and rapid removal from the system as a result of the high rate of metabolism (Dei Cas and Ghidoni 2019). In addition, a greater CUR level may be detrimental. CUR is degraded by bacteria as it goes through the digestive system (mostly the small intestine) and the liver. The most prevalent adverse effect of CUR metabolism is glucuronides. Because these conjugates (mono- and diglucuronides) are less active in cell culture, the in vitro findings may differ. CUR glucuronide is a substrate for glucuronidase, a lysosomal enzyme in living organisms that deconjugates glucuronides (Chen and Liu 2018). This protein is present in all cell types, with macrophages being the most abundant, and its activity is highest in the liver. Inflammation increases glucuronidase activity, and a low-grade inflammatory state has been linked to aging and age-related illnesses. The amounts of CUR and other polyphenols in cancer tissue are expected to differ from those in serum. It is impossible to rule out the possibility of greater non-metabolized CUR concentrations in sick tissue/organs due to pro-inflammatory circumstances associated with aging and age-related disorders. Depending on the type of condition, this might have negative consequences (e.g., senescence induction in neighboring non-senescent cells) (Wyld et al. 2020). A variety of variables, including CUR glucuronidation, impact CUR bioavailability. Low blood and tissue concentrations are determined by two factors: low food concentration (curcuminoids account for around 4% of turmeric, whereas CUR accounts for 70% of curcuminoids) and interaction with other dietary components (the most recognized example is the already mentioned piperine). CUR can pass the blood-brain barrier (BBB); however, its permeability is restricted. Despite having a lower quantity in brain tissue than in serum, CUR reduces neuroinflammation (Askarizadeh et al. 2020). Most polyphenols, including CUR, are thought to be mediated by microbiota rather than performing a direct role in the organism. Furthermore, research shows that gut bacteria create a lot of glucuronidase, which might contribute to an increase in free chemicals. This shows that bacteria may have a role in medication metabolism and bioavailability. The microbiome evolves through time, and aging is related to a decrease in microbial diversity in terms of composition, quality, and number

(Dzidic et al. 2018). It has been proposed that microbiome diversity is associated with good aging. The microbiome regulates innate immunity, sarcopenia, and cognitive impairment, all of which lead to frailty. CUR has been demonstrated to influence the gut microbial makeup (i.e., biodiversity). CUR, through changing the microbiota, may be able to lessen some of the negative effects of aging, particularly those associated with frailty. Finally, the effects of CUR on the microbiota may enhance a variety of organismal processes, and CUR bioavailability may be regulated by the microbiota's capacity to metabolize it (Nakov and Velikova 2020).

11.12 Conclusion

Cellular senescence, according to accumulating evidence, is one of the reasons for organismal aging. SASP identifies senescent cells, which, in addition to losing their capacity to reproduce, promote low-grade chronic inflammation, cancer growth, and hampers regeneration. As people age, the number of senescent cells grows, as does their effect on neighboring cells and the microenvironment. Senescent cells have also been discovered in locations where individuals are suffering from age-related disorders. Researchers discovered that removing senescent cells (p16 +) from genetically engineered mice avoided cancer, improved tissue function, and extended the animals' lives, but not to their full capacity. The mice appeared to be younger and healthier. These findings show that reducing the number of senescent cells may be enough to prevent age-related disorders, prompting researchers to explore senolytic medications and supplements that may destroy such cells. Researchers studying CUR identified a host of benefits for this molecule. Although research on CUR's involvement in the control of aging has grown rapidly in recent years, and the issue has been passionately debated, most of the studies have focused on its anticancer properties. Some studies show a positive influence, while others raise serious concerns owing to unexpected, and sometimes negative, consequences (e.g., the induction of cell senescence).

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The Role of Telomerase Activators in Antiaging Strategies and their Clinical Potential

12

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Abstract

Recent studies have focused on telomere shortening for the prevention of cellular aging and diseases related to aging. Re-extension of shortened telomeres is achieved by various naturally or synthetically produced telomerase activators. Naturally, every cell division can induce telomere shortening, as well as factors such as food, lifestyle, stress, and past diseases that affect the shortening of telomeres. Age-related diseases such as cardiovascular, cognitive, neurodegenerative diseases, and cancer are seen as a result of aging of the cell due to telomere shortening and thus aging of the organism. Telomerase activators play a very important role in the prevention of age-related conditions and diseases. This review focuses on telomeric aging, the molecular mechanism of action of telomerase activators, and the clinical importance of these activators by compiling studies in the context of increasing human lifespan and healthy aging. In this perspective, this study examined the telomerase activators produced both naturally and synthetically, revealing the signaling pathways used by these activators and their clinically relevant concentrations. It will also guide future research that can be conducted to determine the function of telomerase activation in the treatment of human aging-related diseases.

Keywords

Antiaging · Telomere · Telomeric aging · Telomerase · Telomerase activators

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12.1 Introduction

Aging encompasses the physiological changes that occur in all living cells over time. These physiological changes that occur during the aging process reduce the adaptation to daily life and cause an increase in the incidence of chronic diseases. With aging, the frequency of diseases such as cancer, diabetes, cardiovascular diseases, chronic lung diseases, dementia, depression, and anxiety increases (Jin 2010). One of the most critical changes affecting aging is the deterioration of DNA stabilization in genetic accumulation. Many factors, endogenous and exogenous, play a role in the degradation of DNA integrity. Among the exogenous factors, UV and ionizing radiation, chemotherapy drugs, and heavy metals can be counted, while endogenous factors can be listed as unrepaired errors in replication, reactive oxygen species, lipid peroxidation products, and telomere shortening (Iachine et al. 2006). All of these factors are indisputably critical in the aging process, but in this section, we will discuss aging and telomeres in detail. Telomeres are located at the ends of eukaryotic chromosomes and consist of specialized DNA repeat sequences that do not encode any genes. Telomeres, located at the ends of the DNA helix, act as protective regions on chromosomes. In the absence of telomeres, DNA may be damaged over time and dysfunction may occur in the cell (Greider 1991). It has been determined in research that chromosomes shorten in each cell division cycle, which is necessary for the growth and regeneration of cells. Because of the telomere regions, these base losses in the chromosomes do not result in DNA damage (Canela et al. 2017). If a cell undergoes DNA replication without a telomere, a significant portion of the chromosome is lost. As a result, DNA integrity, which must be preserved for life, is impaired due to the decrease in the number of division cycles in cells in telomere deficiency (de Lange 2005). The absence of telomere regions at the ends of chromosomes can result in these non-telomere-free ends merging with each other. These mismatches, on the other hand, can cause cell loss or uncontrolled division as a result of causing a lot of damage to DNA (Maciejowski and de Lange 2017).

With the increasing number of studies revealing the relationship of telomere shortening with aging and aging-related diseases, solutions for preventing telomere shortening have been focused on. At this point, the target of studies in recent years has been the discovery of telomerase activators, which prevent telomere shortening or enable the activation of telomere regions. This review has covered the genetic and cellular mechanisms of telomerase activators in telomere activation, and the clinical importance of telomerase activators in aging and senescence-related diseases associated with telomerase activity.

12.2 Telomere, Telomerase Activity, and Telomeric Aging

Telomeres are complex nucleoprotein structures consisting of repetitive sequences of 5–15 kb base pairs, TTAGGG, located at the ends of linear chromosomes and protecting the ends of chromosomes. The presence of telomeres in linear chromosomes protects the chromosomes from DNA degradation, end-to-end

fusions, rearrangements, and chromosome loss thus preserving the nuclear structure. Due to the DNA replication-end problem that occurs in every DNA replication and hence cell division, a DNA sequence called telomere, located at the ends of the chromosomes, is lost (de Lange 2009).

Mammalian telomeres contain tandemly repeating sequences (5′-TTAGGG-3′) that terminate at 30–400 nucleotides rich in G base at the 3′ end of DNA. Mammalian telomeres are bound by a protein complex called the shelterin complex, rather than just these tandem repeats. This shelterin complex of the telomere consists of six proteins called telomeric rebinding factors 1 and 2 (TRF1 and TRF2), protection of telomere protein 1 (POT1), TRF1 interacting protein 2 (TIN2), TIN2 and POT1 interacting protein (TPP1), and suppressor/activator protein 1(RAP1) (de Lange 2002, 2005). The telomere is recognized by this protein complex, which binds to it to physically protect it. This complex plays a critical role in suppressing fusion and recombination processes and preventing DNA damage response in addition to telomere protection. The shelterin complex is also thought to be functional in binding the telomerase enzyme, which is involved in the resynthesis of the lost telomere, to be able to function. The telomerase enzyme ensures that telomeric DNA is added to the broken ends that lack the shelter complex (de Lange 2009). The proteins that constitute the Shelterin complex each have a specific function. TRF1 promotes telomere DNA replication and negatively controls telomere length. TRF2 protein of the complex shields double-stranded DNA breaks from detection. The RAP1 protein prevents homologous recombination by collaborating with TRF2 (Chen 2019). The TPP1/POT1 heterodimer complex, two other proteins that constitute the shelterin complex, carries out its protective function in telomeres by blocking ATR kinase, one of the DNA repair machinery, and promoting telomerase activity (Kibe et al. 2017). The main protein of the shelterin complex, TIN2, both binds with TRF1 and TRF2 to stabilize them and interacts with the TPP1/POT1 heterodimer complex to play a key role in suppressing ATM and ATR signaling pathways, which act as DNA repair machinery (Rice et al. 2017). Deficiency of these proteins causes various problems with telomeres. Cells lacking TRF2 in the Shelterin complex are arrested in the cell cycle due to upregulation of p53. Diotti and Loayza (2011) found that the absence of POT1 protein causes a telomere damage response.

Telomeres are involved in many cellular events, such as maintenance of chromosome stability, nuclear remodeling, gene expression, chromosomal replication, tumorigenesis, senescence, and cell division (Liu 1999). Telomeres become dysfunctional when they shorten below a threshold length, which triggers a DNA damage response at the ends of the chromosomes. This results in the activation of apoptotic and/or cellular senescence programs and the subsequent dysregulation of numerous functional processes (O'Sullivan and Karlseder 2010). In order to delay aging, it is crucial to maintain the stability of telomere length. This is provided by a complex found in the telomere called the telomerase holoenzyme, which is in charge of elongating chromosomal ends (Greider and Blackburn 1987). Telomerase, also called telomere terminal transferase, was first discovered in 1985 in *Tetrahymena thermophila* (Greider and Blackburn 1985). Telomerase is a ribonucleoprotein that

primarily consists of two main components: a functional RNA component (TR) that serves as a template for telomeric DNA synthesis and a telomerase reverse transcriptase (TERT) protein with reverse transcriptase activity. Additional proteins (DKC1, NHP2, GAR1, and NOP10) that ensure the stability of this complex are also present (Cohen et al. 2007). Telomerase attaches to the terminal end of the telomere region, which remains as a protrusion at the 3′ end of the DNA, adding telomere repeats to the opposite strand, allowing the completion and elongation of the end of the chromosome. The cycle of the telomerase reaction occurs in three steps: substrate recognition and binding; elongation with the addition of a single TTAGGG sequence; and translocation to repeat the process or dissociation from the telomere (Nicholls et al. 2011). The TERT subunit of the telomerase enzyme was first found by genetic screening in yeast and was identified by biochemical purification from *Euplotes aediculatus*. TERT has three main components: a long N-terminal end containing DNA and RNA binding sites; a central reverse transcriptase domain; and a short C-terminal extension (Wyatt et al. 2010). It is known that the N-terminal extension of TERT plays an important role in DNA substrate recognition and elongation (Zaug et al. 2008). The catalytic domain of the TERT unit in the telomerase enzyme consists of a central reverse transcriptase domain, which contains seven highly conserved reverse transcriptase motifs (Harrington et al. 1997). Another important subunit of the telomerase holoenzyme is the presence of an RNA subunit (TR) for DNA synthesis. The TR subunit of telomerase cooperates to mediate TR-TERT interactions, nucleotide addition and re-addition, and enzyme compatibility (Theimer and Feigon 2006).

It is known that telomerase enzyme activity is infinite in stem cells, cancer cells, and in vitro immortalized cells, while it is limited in somatic cells. This causes the cell to have a certain division limit since telomeres shorten with each division and cannot be replaced. This division limit is called the Hayflick limit and indicates that the cell has reached its maximum division capacity and can no longer divide (Hayflick 1965). Cell senescence is prevented by telomerase activity, which delays the time it takes for cells to reach the Hayflick limit (Harley et al. 1990). If telomerase activity is absent or decreased after the cells reach the Hayflick limit, aging occurs in the cell or organism as the length of telomeres will shorten. This shortening of telomeres with aging is called telomeric aging (Epel 2012). Along with defining telomeric aging, researchers also focused on the conditions in which it occurs. By identifying these conditions and altering and avoiding them, stopping or reversing telomeric aging and, by extension, the aging of cells and the organism, has been a priority. The question of whether the telomerase enzyme, which may lengthen short telomeres, can halt or stop the signs of cellular aging has been the subject of numerous investigations (Kipling and Faragher 1999). According to studies, having a chronic illness, particularly when young (between the ages of 1 and 12), shortens telomeres, reduces telomerase activity, and increases oxidative stress (Sapolsky 2004). When human somatic cells are cultured in vitro, a condition known as telomeric senescence is seen. An essential first step in the therapy of age-related disorders will be to create telomerase immortalized cells utilizing tissue engineering methods using human somatic cells in vitro. These cells will be used to treat

atherosclerosis, liver cirrhosis, skin, muscular, and neurological diseases (Klapper et al. 2001).

12.3 Telomerase Activity and Antiaging

Telomere shortening plays a major role in cell aging. As a result of base losses experienced in each cell division, the shortening of the chromosome length and reaching critical levels leads to the arrest of cell division. Telomere length, which plays a role in the aging process and brings about apoptotic death, is directly related to cancer and increased mortality (Blasco 2007). Since every organ and tissue in the body consists of cells, telomeres have a critical role in the vitality and health of the organism. The enzyme called telomerase, found in young cells, adds TTAGGG repetitive sequences to the ends of chromosomes that shorten as a result of cell divisions thus preventing the loss of a large number of bases in the telomere (Zhu et al. 2019). However, since the enzyme telomerase cannot keep up with the bases that need to be added in each cell division, the length of the telomeres becomes shorter as the cell ages through cell divisions. The amount of telomerase, which is involved in the protection of the length of the telomeres at the ends of the chromosomes and therefore the DNA, is higher in cells that have the ability to divide very often, such as stem cells and germ cells, compared to other cells (Blasco 2007). This prevents germ cells and stem cells from aging and losing their essential functions. Due to the lower amount of telomerase in somatic cells, the cells age and can perform their functions less than before. In addition, the higher presence of telomerase in cancer cells causes the survival of these pathological cells to increase and their proliferation continues. The telomerase enzyme, which adds repetitive sequences to the telomere region at the end of the chromosome, consists of two subunits, TERT and TERC, which are responsible for preventing telomere shortening (Shay 2018). Human TERC (hTERC) is expressed especially in stem cells and cells containing telomerase, and its expression is suppressed in somatic cells at birth. A mutation in the expression of the hTERC gene can lead to autosomal dominant dyskeratosis congenita. Abnormal skin pigmentation and related skin cancer, nail dystrophy, and oral mucosal leukoplakia can be seen in individuals with this disease.

By lengthening telomeres, the Astragalus membranaceus-derived telomerase activator TA-65 molecule can decrease the proportion of dangerously short telomeres and DNA damage foci (Bernardes de Jesus et al. 2011). Rats receiving TA-65 as a dietary supplement see improvements in a number of health indicators, including osteoporosis, glucose tolerance, and skin firmness and vitality, without a discernible rise in cancer incidence. The cardiovascular system, bone mineral density, metabolism, and immune system of healthy senior volunteers who received TA-65 supplements showed considerable improvement in the research (Yao and Fernandez 2017).

In a study using the antiaging protein Klotho (KL), which is primarily produced in the kidney and secreted into the systemic circulation, it was demonstrated that KL

protein synthesis and secretion decrease during the aging process and that this causes an increase in the incidence of age-related diseases (Kuro-o 2009). This study showed that interactions between KL proteins and telomeres, as well as telomerase subunits TERF1, POT1, and TERT utilizing TGF-ß, insulin, and Wnt signaling, regulate the stem cell aging process (Ullah and Sun 2019). Therefore, it is possible to imagine that the KL protein actively contributes to longevity, which is controlled by the telomerase enzyme's activity.

According to the claim suggested by Denham Harman in 1956 on the relationship of free radicals with the aging process, free radicals that emerge as by-products during aerobic respiration react with important structures and components of the cell due to their highly reactive properties, and the changes occurring in the cell as a result of these reactions lead to cell aging. This theory, suggested by Harman, is now accepted as the mitochondria-related oxidative stress theory (Harman 1992). Haendeler et al. (2004) investigated whether ROS levels, whose production increases with aging, induce nuclear TERT transport and endothelial cell senescence. Haendeler et al. found that, in direct proportion to the rising ROS levels, the nuclear TERT protein was transported from the nucleus to the cytoplasm and activated the Src kinase (Haendeler et al. 2004). Recent research on the telomerase enzyme's antiaging abilities has revealed that the telomerase enzyme may lengthen shorter telomeres and safeguard long telomeres for stability. Telomerase activation, which might be a means to turn back the biological clock, can increase life expectancy by minimizing age-related diseases.

12.4 Telomerase Activators: Molecular Mechanism of Action

Telomerase, a reverse transcriptase responsible for the replication of telomeres, is critical for cells (Fragkiadaki et al. 2022). Unavoidable telomere shortening occurs in cells lacking telomerase, resulting in cellular senescence. While excessive telomerase activity is associated with cancer, telomerase deficiency plays an active role in the incidence of age-related diseases (Bodnar et al. 1998). Studies have shown that individuals with lower-than-average telomere length have diseases such as stroke, coronary artery diseases, diabetes, and arteriosclerosis, as well as a decrease in the healing rate of infections. Therefore, telomerase activators are critical molecules for delaying aging and preventing telomere-related diseases (Boccardi and Paolisso 2014).

In recent years, the discovery of telomerase activators or the successful use of their synthetic products in the treatment of diseases related to age or telomere shortening and dysfunction has increased the interest in these activators. A telomerase activator named GRN510 was identified in an ex vivo and in vivo study using the mTERT heterozygous mouse model. This activator not only suppressed the development of lung fibrosis induced by bleomycin but also prevented the accumulation of senescent cells. Thus, the study found that GNR510 is a telomerase activator that stimulates telomerase activation in the treatment and prevention of pulmonary fibrosis (Le Saux et al. 2013).

In a study examining whether leptin can function as a telomerase activator, the mechanism of leptin-induced hTERT transcription was evaluated on breast cancer cells, and it was seen that it significantly reduced the rate of cancer cell division. In the study, the role of leptin in the hTERT mechanism was examined through STAT3 inhibition, and it was shown that leptin directly stimulates telomerase and hTERT expression levels by increasing the binding of STAT3 to the hTERT promoter (Ren et al. 2010).

The function of CDC5L as a telomerase activator in colorectal cancer, which ranks third among cancer types worldwide, has been studied. The link between CDC5L and hTERT was examined in colorectal cancer cells, and it was determined that this pathway plays an important role in the proliferation and migration of cancer cells, in which CDC5L functions as the hTERT promoter. It has been proven in colorectal cancer patients that the degradation of CDC5L inhibits tumor formation by suppressing hTERT expression and can be a therapeutic agent (Li et al. 2017).

A study evaluated the telomerase activity of various fatty acids in human cancer cells. In this study, it was shown that saturated and trans fatty acids did not affect telomerase activity in DLD-1 human colorectal adenocarcinoma cells, but cis-unsaturated fatty acids such as eicosapentaenoic and docosahexaenoic acids decreased telomerase activity and gene expression levels. The mechanism of action of these polyunsaturated fatty acids on telomerase has been revealed by suppressing the expression of hTERT and c-myc through inhibition of protein kinase C by these fatty acids (Eitsuka et al. 2005).

One of the most frequently studied natural telomerase activators in the literature is cycloastragenol (CAG, GRN665, or TA65). Studies have determined that it shows CAG activity through many signaling pathways. In a study evaluating CAG activity in osteoblastic damage, it was found that CAG stimulated the activation of JAK2 and STAT5, resulting in an increase in TERT expression. The increase in TERT expression, leading to increased phosphorylation of STAT5, has proven the effectiveness of the JAK/STAT signaling pathway in telomerase activation (Yu et al. 2018a, b). Another pathway used by CAG is the ERK signaling pathway. It has been determined that CAG induces ERK phosphorylation in studies conducted in many cells originating from lung, brain, and breast tissue. The presence of CAG activated the Src/MEK/ERK pathway through phosphorylation of the extracellular signal-regulated kinase, resulting in telomerase activation (Yung et al. 2012).

Studies have found that neurodegenerative disorders are among the most common age-related diseases, with neuronal and cognitive losses as the causes (Calina et al. 2020). When these neurodegenerative diseases such as Parkinson's, Alzheimer's, and Huntington's are examined, they are associated with telomere shortening, and it is aimed to search for agents that target the increase of telomerase activity in treatment processes. A supplement containing D3, vitamin C, and zinc, prepared as a natural telomerase activator, was applied to the brain tissues of rats in a study. According to the results of the study, it was found that TERT expression and telomerase activity were increased not only in the embryonic and postnatal periods but also in the brain tissue of middle-aged rats (Tsoukalas et al. 2021). These

findings showed that telomerase activators could be promising in nervous system diseases.

According to studies, the number of telomerase activators that target telomere activity in aging and aging-related diseases is increasing day by day. While the most commonly used of these activators are tried to be examined under the title, telomerase activators, whose effectiveness and reliability have been proven by studies, are listed in Table 12.1 together with their molecular mechanisms.

12.5 Telomerase Activators in Antiaging Clinical Aspects

Telomerase activators are very important in the prevention of aging-related diseases and in antiaging interventions. It is known that shortening telomeres play a role in the emergence of aging-related diseases (Zhu et al. 2011). Restructuring or increasing the activity of telomerase enzyme as one of the antiaging treatments has been an important part of regenerative medicine as a therapeutic approach in minimizing the effects of aging and preventing aging-related diseases. There are various approaches to increasing the activity of telomerase, such as classical gene therapy by transfection of telomerase sequences, reexpression of silenced telomerase, reactivation of enzymatic activity, and modulation of the intracellular location of telomerase (Jäger and Walter 2016). Telomerase activators have gained attention, and new telomerase activators are found every day while these methods are being researched therapeutically (Table 12.2). One of the most well-known telomerase activators is cycloastragenol (Bernardes de Jesus et al. 2011). TA-65, with the trade name cycloastragenol, was first shown to activate telomerase in T lymphocytes (Fauce et al. 2008). A study conducted in 2011 found that it increased telomere length and reduced the number of extremely short telomeres in mouse embryonic fibroblasts. In the same study, TA-65 was shown to increase telomerase levels, increase telomere length, and improve aging-related deteriorations such as glucose tolerance, bone health, and skin quality when taken as a dietary supplement (Bernardes de Jesus et al. 2011). In a human study published in the same year, they gave TA-65 (10–50 mg) as a vitamin supplement to 114 people aged 51–74 for 1 year, and the subjects participating in this study showed that T cells in the immune system were renewed and a decrease in natural killer cells at the end of 1 year. It has been determined that the immune systems become younger with this disease (Harley et al. 2011). Since 2013, TA-65, which first became available in 2007 in limited numbers, has been offered as a food supplement. As a result of numerous clinical investigations, it improves metabolic, cardiovascular, and bone markers associated with aging (Harley et al. 2013), improves age-related macular degeneration in humans (Dow and Harley 2016), and lengthens telomeres (Salvador et al. 2016). Patients with cardiovascular risk can dramatically lower their risk ratio by taking a daily dose of 16 mg TA-65 (Fernandez et al. 2018). By administering TA-65, it has been shown to be a new therapeutic approach for the recovery of hindlimb ischemia in mice (Saitoh et al. 2019). In a study with CD8+ T cells from HIV patients, another telomerase activator and cycloastragenol derivative, TAT-2, was found to delay telomere

Table 12.1 Mode of action of telomerase activators

Molecule	Mode of action	Reference
TA-65	Controls telomerase at the transcriptional level through regulation of the MAPK pathway	Bernardes de Jesus et al. (2011)
TAT2	Leads to enhancement in human telomerase reverse transcriptase mRNA levels; increases the expression of telomerase via the MAPK/ERK pathway	Fauce et al. (2008)
GRN510	Regulates the Wnt/β-catenin pathway involved in induction of hTERT transcription and telomerase activity	Fragkiadaki et al. (2022)
Resveratrol	Activates telomerase and delays cell senescence by inducing the direct phosphorylation of TERT and the serine-threonine enzyme Akt	Xia et al. (2008)
Selenium	Induces the upregulation of TERT expression	Yu et al. (2009)
Cynomorium species (Maltese mushroom)	Increased the level of hTERT expression by delaying telomere shortening	Cui et al. (2013)
Hydrogen sulfide	Delays the initiation of replicative senescence by preserving hTERT expression depending on NAMPT mRNA expression and SIRT1 activation	Sanokawa-Akakura et al. (2016)
ZEB1	The complex formed by ZEB1 with the coactivator YAP binds to the hTERT promoter and acts as a transcriptional activator	Yu et al. (2018a, b)
SPT5	The tumor-specific TERT promoter-binding protein SPT5 induces upregulation of TERT and activates colon cancer	Chen et al. (2015)
EPO	Enhances hTERT gene expression through induction of the JAK2/STAT5/c-Myc axis and TERT phosphorylation via the PI3K/Akt pathway	Kawauchi et al. (2013)
Leptin	Regulates expression of hTERT through recruitment of STAT3 and Myc/max/mad network proteins in binding sites on hTERT promoter	Stefanou et al. (2010)
RFPL3 and CBP	CBP co-localized with RFPL3 to the hTERT promoter region activates transcriptional activity of hTERT and telomerase activity	Li et al. (2022)
PROX1	Induces TERT transcription due to its high affinity for the mutant hTERT promoter	Kim et al. (2018)
Vitamin D	Promotes telomerase activity, improves telomere protection in peripheral blood mononuclear cell; but its mechanism remains unclear	Zhu et al. (2012)
Ginkgo biloba extract	Activates telomerase and delays the onset of cell senescence by interacting with the PI3k/Akt signaling pathway	Dong et al. (2007)

shortening by regulating telomerase (Fauce et al. 2008). Another small molecule called GNR510, another cycloastragenol derivative and synthetic telomerase activator, has been shown to have telomerase-enhancing properties. In this study, it was found that daily supplementation of 10 mg/kg GNR510 increased telomerase

Table 12.2 Clinical effects of telomerase activators

Telomerase activator	Source	Study model Dose	Clinical effects	Reference
TA-65	*Astragalus membranaceous*	Human trials 8–50 mg Haploinsufficient mouse embryonic fibroblasts (MEFs) 25 mg/kg/body weight per day	• Increased average telomere length, • Decreased the percentage of critically short telomeres and DNA damage, • Improvement of glucose tolerance, osteoporosis, and skin fitness, • Improved markers of metabolic, bone, and cardiovascular health, • Improved key markers of cardiovascular disease risk, • A therapeutic option to rescue ischemic tissue.	Bernardes de Jesus et al. (2011) Harley et al. (2011)
TAT2 or GRN665	*Astragalus membranaceous*	Human $CD8^+$ T lymphocytes 0.01–10 μM	• Enhances cytokine/chemokine production and antiviral activity.	Fauce et al. (2008)
AGS-499 and AGS-500	Synthesized triaryl compounds	Human bone marrow mesenchymal stem cells (hMSC) 1.5, 3, 6 and 12 mg/kg Human adipose arterioles10 nM Hippocampal neurons of ICR mouse 20, 50, 100, and 200 nM	• Increases expression and activity of telomerase in brain and spinal cord of mice, • Improves coronary artery disease, • Changed the dilatation associated with coronary arterial disease, • Enhance the expression of neurotrophic factors and neuronal plasticity genes	Eitan et al. (2012) Hughes et al. (2019) Baruch-Eliyahu et al. (2019)
SPT5	Suppressor of ty homolog-5	Colon cancer cells	• Potential tumor biomarker and/or cancer therapeutic target	Chen et al. (2015)
RFPL3 and CBP	Ret Finger Protein-Like	Human lung cancer tissues and human	• Affect hTERT expression and	Qin et al. (2015)

(continued)

Table 12.2 (continued)

Telomerase activator	Source	Study model Dose	Clinical effects	Reference
	3 (RFPL) and CREB binding protein	lung cancer cell lines (H1299, H460, H322, A549)	telomerase activity, • Possible targets for lung cancer treatment	
Leptin	Adipose cells and enterocytes	MCF-7 breast cancer cells10, 80, 160 ng/mL	• Critical player of oncogenesis	Ren et al. (2010)
Resveratrol	Red wine	Human mammary and endothelial progenitor cells10^{-8} M Primary human aortic smooth muscle cells (ASM cells) and A549 cells 200, 100, 50, or 25 μM	• Activates the telomerase gene, • Anti-oxidative and anti-inflammatory effects on age-related diseases, • Induces telomere maintenance by activating WRN helicase and telomerase, • Beneficial effects on human cardiovascular diseases	Wang et al. (2011) Huang et al. (2015)

activity up to four times, reducing the number of senescent cells in murine lungs (Le Saux et al. 2013).

Chemical compounds and telomerase activators, AGS-499 and AGS-500, have been shown in various studies to increase telomerase activity and TERT levels in many tissues and cells (Eitan et al. 2012; Tracy et al. 2020; Hughes et al. 2019; Tichon et al. 2013; Baruch-Eliyahu et al. 2019). Studies have suggested that it may be a possible new therapeutic strategy for the treatment of neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS) by increasing telomerase in motor neurons thanks to compounds such as AGS-499. In a study by Eitan et al. (2012) with the mouse model of ALS, the compound AGS-499 was administered to mice at various doses (1.5, 3, 6, and 12 mg/kg). According to the results of this study, it was found that telomerase enzyme activity increased in the brain tissue. In another study, it was observed that the administration of AGS-499 to old female mice improved coronary artery disease (Tracy et al. 2020). In a study on human arteoli isolated from adipose tissue, they found that AGS-499 (10 nM) changed the dilatation associated with coronary arteriole disease (Hughes et al. 2019). The use of AGS-499 in the treatment of Alzheimer's disease, which occurs mostly with aging, has shown some beneficial effects. In a study conducted for this purpose, AGS-499 (20, 50, 100, and 200 nM) was applied to the hippocampal neurons of mice, and telomerase enzyme activity was found to increase as a result of the study. The increase in TERT activity caused an increase in the amount of β-catenin in the

Wnt signaling pathway, which resulted in an increase in the amount of neurotrophin, resulting in neurogenesis. Finally found to stop neuronal death (Baruch-Eliyahu et al. 2019).

Various genetic products in the cell, especially transcription factors, have been the focus of attention of scientists in recent years, acting as telomerase activators as potential therapeutics in many aging-related diseases. Uncovering how these transcription factors function and developing approaches to increase the concentration of these factors is an important step in the prevention of age-related diseases. One of these transcription factors, Prospero homeobox protein 1 (PROX1), is a transcription factor that is particularly necessary for the maintenance of the lymphatic lineage (Shresta et al. 2020). It has been found that PROX1 is involved in the recovery of pulmonary fibrosis after lung injury (Baluk et al. 2020), preventing age-related senescence of lymphatic endothelial cells in the lung (Shrestha et al. 2020), and increasing the lifespan of human lymphatic cell lines by activating telomerase (Nisato et al. 2004). The cell division cycle 5-like (CDC5L) protein is a core protein in a transcription complex and is known to be involved in prolonging the lifespan of human endothelial cells (Voglauer et al. 2006), repairing DNA damage in cells (Lepperdinger et al. 2008), and maintaining homeostasis in the skin of elderly individuals (Yeh et al. 2021).

Resveratrol, a natural polyphenol and telomerase activator used in the treatment of Werner's Syndrome, known as a premature aging disease, has been revealed by studies in recent years to have an important place in reversing many aging-related conditions and in antiaging interventions (Wang et al. 2011; Xia et al. 2008; Huang et al. 2015; Uchiumi et al. 2011; Liu et al. 2013; Li et al. 2017). In a study on the senescence of endothelial progenitor cells, it was determined that resveratrol delayed senescence by increasing telomerase activity when applied to cells at concentrations of 1, 10, and 50 μmol/L (Wang et al. 2011). In another study, scientists suggest that the effects of aging can be minimized and cardiovascular diseases can be prevented by increasing human TERT and telomerase activity in aortic smooth muscle cells that cause aging-related cardiovascular diseases by resveratrol (25, 50, 100 μM) (Huang et al. 2015). Resveratrol has been found in numerous studies on mice and rats to delay kidney aging and reverse aging symptoms (Uddin et al. 2021). The ovarian aging was delayed, telomerase activity, telomere length, and age-related gene activity profile in the ovary were similar to those in young people after resveratrol at a level of 30 mg/L was given to mice with drinking water for a year (Liu et al. 2013).

12.6 Conclusion

Telomerase activity can be self-regulated by the body in many genetic ways, as well as by induced polyphenols found in nature, anticancer drugs, and synthetically produced components. The reason why telomerase activity has gained such importance today is the aim of preventing aging-related diseases, especially aging and cancer. Because the aging process is a highly complex process at the molecular level,

it is extremely difficult to unravel the mechanisms underlying its reversal. Studies with telomerase activators have been very promising for this process. If the molecular mechanisms of telomerase activators are further clarified and their clinical use is increased despite the difficulties that may be encountered in individual differences, the aging process may pass later and more prosperously. As a result of these approaches, the fact that cells respond differently to the discovered telomerase activators and use different signaling pathways shows the difficulty of studying telomere biology, but the increasing results of these studies in the future are of critical importance in improving the aging process and protecting human health.

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Clinical Laboratories and Their Role in Anti-Aging Strategies

13

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Abstract

The contribution of medical biochemistry to anti-aging strategies can be suggested under two main headings: invention of new measurement parameters and making measurements with existing parameters. New marker invention is a topic that can excite the world of gerontology at any moment. For this reason, there is a constant struggle applied by researchers. Biomarkers related to nutrition, cardio-metabolic state, and immune system stand out in this issue. Cardio-metabolic markers are important for cardiovascular health, immune-related markers are important for inflammaging and susceptibility to infection, and those related to nutrition are important for general well-being and deficiencies. The priority should be to focus on well-being rather than diseases while dealing with those biomarkers and anti-aging strategies. Data from centenarian individuals and countries with high life expectancies are very important sources of information in this issue. This information should be evaluated holistically and presented in a comparative way to see the elderly healthier.

Keywords

Aging · Dyslipidaemia · Immune system · Frailty · Vitamins

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Abbreviations

HDL High-density lipoprotein
LDL Low-density lipoprotein
TC Total cholesterol
TG Triglyceride
WHO World Health Organization

13.1 Introduction

For an individual who was born in any part of the earth, "life expectancy" is the average total number of years of life expected for that person. According to the official report of the World Health Organization (WHO), the average life expectancy had increased to 73.4 worldwide just before the Covid-19 pandemic. According to similar data published regularly by WHO, this crucial number of calendar years can vary according to gender, and the general development status of the country based on financial and health-related issues. When the aforementioned statistics are examined in detail, it is easily seen that these numbers have been increasing rapidly in the last two centuries, almost all over the world, at a rate that has never been seen in human history. The main reasons for rising general welfare and increasing health service quality are rapid increase in state of art technology, easier communication and transportation tools, hygiene, and also increased access to vaccines and antibiotics. While the 40s were the highest life expectancy ages in the previous century, it has approached the 90s in some societies today (Aburto et al. 2020). People are getting healthier and living longer. As a consequence, while the world progresses at these similar rates, the population over 60 will soon outnumber the population under 5 years old (Fuster 2017) and when we come to the half of the century we live in, the number of people over the age of 60 will exceed two billion (Caruso et al. 2012). On the other hand, "life span" is the maximum number of years a person can live. While life expectancy was increasing rapidly, lifespan remained rigidly unchanged for decades (Dong et al. 2016). The gap between these two values was and is still gradually closing even though one side remains the same. We give different names to the closure of this scissor gap. Some scientists call it "healthy aging," some call it "healthy life span," some call it "improving of public health," and finally some scientists call it "anti-aging." While anti-aging is a difficult concept to define for gerontologists, it is also a very popular and exciting topic culturally. Anti-aging is not only physical but also psychiatric and social well-being continuation in a similar way to the rate in the reproductive period, regardless of the progression of the calendar ages.

Ever since the human beings on earth started to breathe for the first time, they have constantly struggled with the problem of aging, which they saw as the beginning of the process that leads to death. At this point, just like the rest of the clinical

sciences medical biochemistry, which is also one of the most important branches of life sciences, was involved as an important factor. The contribution of medical biochemistry to anti-aging can be suggested under two main headings: presenting new biomarkers and changing biological matrixes into mathematical results by robust measurements. Medical biochemistry, which is primarily involved in the diagnosis, treatment, and follow-up of diseases, also makes serious contributions to anti-aging phenomenon. In this chapter of the book, the subject of routine medical biochemistry biomarkers and anti-aging will be started, followed by advanced examinations that require high technology and experience, and finally future expectations will be discussed. Those biomarkers will be discussed under three titles cardio-metabolic, immune, and nutrition-related.

13.2 Cardio-metabolic Biomarkers

Anti-aging strategies to protect cardiovascular health, which is the primary cause of mortality, should always be a priority. While elderly populations are boosting in many countries, the highest mortality and morbidity rates due to cardiovascular problems are not expected to change in foreseeable future (Turakhia and Tseng 2007). Diabetes and dyslipidemia, which are modifiable and measurable risk factors, will be highlighted as the main topics under this title, apart from non-modifiable risks such as chronological age, genetic risk, and male gender.

According to health statistics in the world, it is estimated that the number of diabetes patients will double in 25–30 years in geriatric populations. At the same time, of course, the number of elderly people will be already increasing. These diabetes-related rates include not only countries with large populations such as China and India but also industrialized and developed North American and European countries (East and Africa 2017). It is known that every geriatric individual has an increased risk of Type 2 Diabetes, as well as other clinical conditions such as Alzheimer's disease, Parkinson's disease, cataract, malignancies, frailty, fractures, osteoporosis, sarcopenia, osteoarthritis, depression, cardiovascular diseases, and renal insufficiency (Ford et al. 2002). In this case, it is logical that the concepts of anti-aging and controlling geriatric diabetes and dyslipidemia will overlap. But which biomarkers are involved in this overlapping issue?

According to different population data, approximately 1/5–1/3 of all people in the geriatric time period, already suffer or will suffer from Type 2 Diabetes Mellitus (Chentli et al. 2015). These patients may have been diagnosed with diabetes at a younger age, or they may have had diabetes in their 60s. Genetic background and environmental factors basically determine this newly diagnosed or ongoing malady of the disease ratio. Robust biomarkers are always needed to monitor fasting and/or postprandial blood glucose levels.

There are markers for long-term glucose control indicators instead of short-term. Glucose binding can occur without the aid of enzyme activity to the N-terminal region of the valine amino acid located in the beta chains of hemoglobin proteins (Little and Sacks 2009). The clinical threshold for glycated hemoglobin (HbA1c),

Table 13.1 The most commonly used and routinely measured cardio-metabolic lipid and carbohydrate biomarkers in clinical laboratories, their general population reference ranges for healthy adults, and desired values in the geriatric population on the aspect of anti-aging

Biomarker	Reference ranges for general adult population	Optimal levels or clinical threshold/cut-off values for the elderly	Reference
Fasting plasma glucose	70–100 mg/dL	90–130 mg/dL	Kalra and Sharma (2018)
Glycated hemoglobin (HbA1C)	<6%	<6.6%	Masuch et al. (2019)
Low-density lipoprotein cholesterol (LDL-C)	<130 mg/dL	<189 mg/dL	O'Keefe et al. (2004), Adeli et al. (2015)
Triglyceride (TG)	<150 mg/dL	<301 mg/dL for males, <212 mg/dL for females	Adeli et al. (2015)
Total cholesterol (TC)	<200 mg/dL	<240 mg/dL	Félix-Redondo et al. (2013)
Apolipoprotein-B	<130 mg/dL	<150 mg/dL	Motta et al. (2009)

which reveals the diagnosis for prediabetes status is 5.7%, and for diabetes it is 6.5% (Table 13.1) (Petersmann et al. 2019). For follow-up patients who have the disease, the target value is below 7–7.5% for HbA1c, directed by associations such as American Diabetes Association and American Geriatrics Association (Longo et al. 2019). This limit can be increased up to 8.5% for more fragile patients. It should be kept in mind that in elderly patients, HbA1c may lose its measurement value specificity due to the combination of different pathologies occurring at the same time that cause early/premature destruction of erythrocytes and interlaboratory measurement technique differences. Postprandial glucose monitoring can be considered as a more effective follow-up method in this case. Also, other very important questions here are whether the disease follow-up should be carried out very strictly and what the consequences will be for the senior citizens. Type 2 DM may not be seen as a typical semiology in the geriatric population. But its microvascular and macrovascular complications like neuropathy, retinopathy, nephropathy, and cardiovascular diseases can be prevented with disciplined follow-up aforementioned biomarkers. These risks can be completely controlled and as a result, anti-aging strategies can become successful.

According to the data from the World Health Organization, acute coronary syndrome, which is the most important cause of cardiovascular death, has been associated with dyslipidemia at a very high rate (Shanmugasundaram et al. 2010). Dyslipidemia is accepted as one of the most important modifiable risk factor for this issue and it comprises;

i. Elevated levels of triglyceride (TG) (>150 mg/dL) and/or
ii. Elevated levels of total cholesterol (TC) (>200 mg/dL) and/or
iii. Elevated levels of low-density lipoprotein cholesterol (LDL-C)(>130 mg/dL) and/or
iv. Decreased level of high-density lipoprotein cholesterol(HDL-C) (for women <50 mg/dL, for men <45 mg/dL) (Table 13.1).

In some studies, dyslipidemia rates were found to be 64% in elderly populations (Lin et al. 2019). Dyslipidaemia can become more evident with advancing chronological age even after childhood. However, very interestingly, a plateau trend begins to be seen in both men and women in their 50s and after 60 years of age (Liu and Li 2015). On the contrary, for individuals over 70 years of age, dyslipidemia may have a reverse momentum based on the principle of survival. Some studies report that the risk of insulin resistance, which is a diabetogenic condition, increases with high triglyceride levels (Lamarche et al. 1997; Kiliç et al. 2021; Yeh et al. 2019).

The relationship between "adequate but restricted calorie diet," which is one of the proven anti-aging strategies, and dyslipidemia and hyperglycemia is another interesting situation that needs to be addressed here. Calorie restriction without malnutrition is a protective tool against aging-related cardiovascular changes (Weiss and Fontana 2011). There are many studies in current medical literature and alive proofs in Japan the island "Okinawa," showing that both dyslipidemia and inflammatory markers improve for humans with caloric restriction applications together with exercise (Weiss and Fontana 2011; Fontana et al. 2007; Walford et al. 2002). Beyond protection, even some studies claim to reverse even preexisting vascular pathologies. Attenuation of oxidative stress and inflammation, reduction of myocardial hypertrophy, and fibrosis are beneficial anti-aging events demonstrated by calorie restriction (Weiss and Fontana 2011). For these reasons, it can be said that individuals who apply calorie restriction without disturbing the nutritional balance are more advantageous in terms of anti-aging strategy.

13.3 Immune-Related Biomarkers and Anti-aging

The whole world experienced a very big and deep health problem from the end of 2019 until the beginning of the summer of 2022. The problem, whose effects still continue, was COVID-19. When the factors that increase the risk of morbidity and mortality during the fight against the COVID-19 pandemic are listed, "old age" was taking its place in the first line. Presence of comorbid diseases, longer hospital stay, high intensive care unit admission, and higher death rates were observed in elderly individuals (Prendki et al. 2022). The main reason for this situation, which especially threatens the experienced senior individuals of our society, is undoubtedly the changing organization of our immune system over the years. The presence of continuous low-grade inflammation, higher susceptibility to malignancies and infections, more frequent autoimmunity-related reactions, and a relative decrease

in the response to vaccines and regular antigens are the results of the changes in the immune system caused by aging (Sadighi Akha 2018; Goodwin et al. 2006).

In order to examine the changes of the immune system during aging (also called immunosenescence), this extraordinary, multipartite, defensive system must be divided into two interweaving compartments, innate and adaptive (Müller et al. 2019). For innate immunity; (a) different levels of dysfunctions in phagocytosis, chemotaxis, and respiratory burst functions for neutrophils while their numbers are preserved (Shaw et al. 2010; Montgomery and Shaw 2015; Bulut et al. 2020), (b) lower release of pro-inflammatory cytokines, impaired functionality during the production of reactive oxygen and nitrogen radicals, transmission of cytokines, apoptosis-cellularity balance, and MHC-II antigen for macrophages that are very similar to neutrophils (Müller et al. 2019; Bulut et al. 2020; Fernández-Morera et al. 2010; Gonzalo 2010; Aprahamian et al. 2008), (c) decline in numbers and maturation, relocation, antigen processing, and cytokine release deterioration for dendritic cells (Müller et al. 2019; Desai et al. 2010; Della Bella et al. 2007), (d) increased number of NK cells but disturbance in their signaling routes (Müller et al. 2019; Dewan et al. 2012) can be counted as critical changes. For adaptive immunity, (i) altered T cell surface glycoprotein performance and structure (most striking one is loss of CD 28 surface protein) (Weyand and Goronzy 2016; Sadighi Akha and Miller 2005) (ii) decline in both naive T cell number and T cell receptor variety but increase in memory lymphocyte counts (Qi et al. 2014; Kogut et al. 2012), (iii) increase in regulatory T lymphocytes (Moro-García et al. 2013) (iv) impaired antibody development (Bulut et al. 2020) can be listed as important changes. These are age-related changes that are commonly observed and can be measured in clinical laboratories.

In order to combine immune system and anti-aging strategies, which is the main topic of our chapter, it is necessary to approach two patient groups more carefully. First, during the pandemic we observed that some of the elderly people have remained extremely strong even aging of the immune system normally leads to having lower capacity to recognize new antigens, decreasing neutralizing capacity of antibodies against viral pathogens and carrying big risks to secrete huge levels of pro-inflammatory cytokines (Witkowski 2022). Diminished type-I Interferon response (Molony et al. 2017) and generation of elevated levels of interferon-γ induced protein-10, monocyte chemotactic protein-3, IL-6, IL-12, and IL-1β release (McGonagle et al. 2020; Lagunas-Rangel and Chávez-Valencia 2020; Yang et al. 2020) were associated with severe Covid-19 infections for the elderly (Brodin 2021). Neutrophil-lymphocyte ratios were measured to be high in elderly and diabetic patients who had severe Covid-19, but on the other hand high or restored lymphocyte counts also indicated good prognosis (Brodin 2021; Zhou et al. 2020; Chen et al. 2020). Secondly, it is informative to detail the immune status of people over the age of 100, also called centenarians. Centenarians not only live longer but also live a life with reduced frailty and morbidity (Andersen et al. 2012). In their T cell subset analyses, T4/T8 ratio was 0.72 (Thompson et al. 1984), number of standard T cell counts were decreasing very deeply, while the number of NK cells and extra-thymic T cell clones were increasing (Miyaji et al. 2000). Centenarians were also having elevated counts of neutrophils and those polymorphonuclear leukocytes had

powerful resistance to dysfunctions that comes with aging (Miyaji et al. 2000; Miyaji et al. 1997; Alonso-Fernández et al. 2008). As a reminder statement, it should be kept in mind that those tests may have been done at different stages of the disease or centenarians, there may have been asymptomatic disease courses, and epigenetic modifications are possible for centenarians.

13.4 Nutrition-Related Biomarkers

There is a very important saying whose validity has been accepted by researchers: "we are what we eat." There is a constant need for a balanced diet for growth, development, continuation of cellular turnover, and metabolic reactions (Picó et al. 2019). Nutrition has different dimensions in terms of anti-aging: healthy aging with continuous high-quality nutrition, being healthier with a balanced diet, and development and measuring biomarkers of nutrition. In this section, we will approach the subject from the point of view of medical biochemistry, that is, biomarkers. Nutrition of people in sickness or in healthy periods can be evaluated with nonobjective questionnaires (Thompson et al. 2010). Biomarkers that are objectively measured and give quantitative results are of great benefit for anti-aging interventions. The most commonly used nutritional biomarkers for senior citizens in medical laboratories are iron, folic acid, vitamin B12, and Vitamin D (Table 13.2).

Iron is like a double-edged sword, deficiency and anaemia on one side, excess and oxidative stress on the other. Iron deficiency, which is the most common problem associated with unbalanced nutrition, is related to the deterioration of cognitive functions, worsening of immunity, presence of frailty, bone mineral density loss, and increased psychiatric problems in the elderly (Fairweather-Tait et al. 2014; Goodnough and Schrier 2014; Manckoundia et al. 2020). There are researches stating that the pro-inflammatory and oxidant roles of iron could cause damage to the central nervous system and this problem increases with aging (Watson et al. 2018). After iron, which is the most abundant trace element in the body, two other vitamins associated with anemia, B12 and B9 (folate), should be mentioned in this chapter and their relationship with old age. Vitamin B12 deficiency is a condition that can be seen between 1 in 20 and 2 in 5 in the elderly according to studies with different threshold limits (Palacios et al. 2013). Vitamin B12 deficiency, that is quite often observed in the general population too, is associated with reversible megaloblastic anemia and a different range of neurologic manifestations (Stabler 2013). While B12 deficiency creates a tendency to depression, B12 is actually protective against depression in older adults (Petridou et al. 2016). Folate deficiency, that is quite common as well, is associated with malabsorption and dietary deterioration, also brings along megaloblastic anemia and dermatological problems in senior citizens (Watson et al. 2018). For anti-aging strategies, the possibility of silent progression of the deficiency of these two vitamins together and without clinical indication should always be considered. In addition, the recommendations in Table 13.2 and the laboratory relevance sections should be carefully examined by the reader.

Table 13.2 The most significant nutrition-related/commonly deficient biomarkers in clinical laboratories, their general population reference ranges, optimal levels or clinical threshold/cut-off values for the elderly for anti-aging aspect

Nutritional biomarker	Reference ranges for general adult population	Optimal levels or clinical threshold/ cut-off value for the elderly	Laboratory relevance	Reference
Iron	80–180 μg/dL for males, 60–160 μg/dL for females	Serum ferritin >50 μg/L and hemoglobin >12 g/dL	Serum ferritin is recommended for confirmation	Busti et al. (2014)
Vitamin B 12	>150 pmol/L	>220 pmol/L	Methylmalonic acid and/or homocysteine measurements are recommended for confirmation	Stabler (2013), Yao et al. (1992), Wolters et al. (2004)
Folic acid	>5 nmol/L	>7 nmol/L	Homocysteine measurement is recommended for confirmation	Palacios et al. (2013), Clarke et al. (2004)
Vitamin D	>25 nmol/L	>50 nmol/L	Parathyroid, calcium, and phosphate measurements are recommended for confirmation of the deficiency	Mosekilde (2005), Kweder and Eidi (2018)

During the last two decades, even though it is not an actual one, vitamin D is the most studied vitamin at all on metabolite selection and measurement methods, determination of different/seasonal reference ranges, list of associated diseases, clinical follow-up recommendations, etc. Vitamin D deficiency is a primary subject in geriatrics due to atrophy of the skin, changes in clothing/work/residency and less solar exposure, unhealthy dietary habits, and decreased renal function (Mosekilde 2005). In some studies, the rate of patients with deficiency was found to be much higher than those without (Annweiler et al. 2010). Its deficiency causes direct dysfunction in the musculoskeletal system and aging-related frailty can increase dramatically. Calcium and vitamin D regulation is very important for anti-aging strategy, especially in specific societies living in high latitudes.

As technology advances, more state of art tests will become available and those tests should be evaluated holistically for anti-aging. In conclusion suggestions, tests focusing on well-being should be prioritized instead of tests based on diseases for anti-aging strategy. Data from centenarians, supercentenarians, and countries with high life expectancy rates are critical in this issue. These specific featured populations can contribute greatly to the upcoming tests. In addition to tests, it may be necessary to record information such as lifestyles, work and home lives, family and dietary habits of these people and convert it into big data. Those data can

be evaluated with different soft wares and effective comparisons can be applied for the rest of the people.

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Antiaging Strategies Based on Sirtuin Activation

14

Geetika Garg and Sandeep Singh

Abstract

Aging is one of the main risk factor for many diseases and conditions that limit lifespan. The key focus of antiaging research is to explore whether the lifespan will be regulated by modulators of signaling pathways that trigger and/or progress age-related disorders. Among various antiaging approaches, the best is to target the proteins belonging to the sirtuin family. Sirtuins proteins are a conserved family of nicotinamide adenine dinucleotide (NAD^+)-dependent protein deacetylase, and are known to control longevity from yeast to mammals. Furthermore, sirtuins are the most promising therapeutic targets for antiaging and age-associated diseases and scientific communities have always been interested to develop natural and synthetic modulators of sirtuins. The sirtuins are always fascinating because it is possible to modulate the activity by small molecules that could be developed into drugs. Therefore, in this chapter, we summarize the potential role of sirtuins in delaying aging and interventions.

Keywords

Aging · Caloric restriction · Mimetics · Sirtuins

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14.1 Introduction

Aging is an intricate biological phenomenon which is generally manifested by progressive and persistent decline of physiological activity as well as an increased probability of morbidity and mortality (Finkel and Holbrook 2000). However, it has been found in different studies and hypothesized that aging can be modulated by biomedical approaches or pharmaceutics. Among various antiaging interventions, one of the most promising strategy is to target the proteins belonging to the sirtuin family (Guarente 2007; Grabowska et al. 2017). Earlier, sirtuins had been primarily discovered as transcription factors in yeast. However, nowadays they are also present in bacteria and eukaryotes as well (including mammals) (Sinclair and Guarente 1997; Wang et al. 2020). Sirtuins are nicotinamide dinucleotide (NAD^+)-dependent deacylases involved in preventing diseases and reversing aging in some aspects (Westphal et al. 2007; Giblin et al. 2014).

Sirtuins control different cellular and metabolic processes related to lifespan-associated diseases such as diabetes and neurodegenerative disorders (Kaeberlein et al. 1999; Lin et al. 2000; Michan and Sinclair 2007). There are Seven SIRT (SIRT1-SIRT7) family members have been identified in mammals. SIRT1 is present in the nucleus and SIRT2 localized in the nucleus and cytoplasm however SIRT3, SIRT4, and SIRT5 are present in mitochondrial, and SIRT6 and SIRT7 are nuclear (Fig. 14.1) (Sauve et al. 2006). These proteins have enzymatic activity with NAD^+(Cofactor)-dependent and controlling the cellular process such as gene expression, DNA repair, oxidative stress response, and mitochondrial function. (Schwer and Verdin 2008; Chang and Guarente 2014). Therefore, on the basis of these function of sirtuins, deregulation of sirtuin activity leads to various degenerative diseases such as cancer, diabetes, and cardiovascular disease (Haigis and Sinclair 2010).

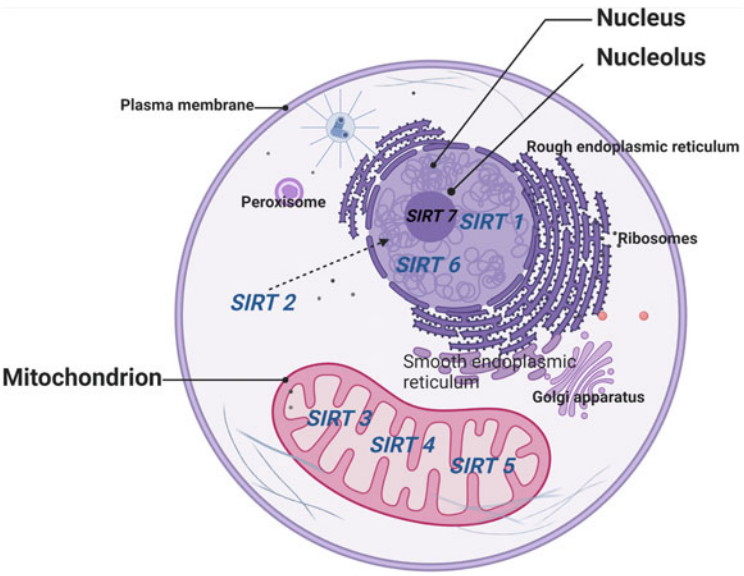

Fig. 14.1 Localization of sirtuins

This chapter will emphasize the potential roles of sirtuin during cellular senescence and improving health span, and will also summarize natural and pharmacological agents able to enhance sirtuin activity that could have clinical potential to develop drug to cure age-associated diseases. This chapter also suggests that interventions to maintain sirtuin activity might be on the new horizon to forestall age-associated diseases

14.2 Role of Sirtuin in Lifespan of Organism

In addition to cellular aging, sirtuins modulate the lifespan in different animal models. It has been found that an increased level of sirtuin in yeast Sir2 and its homologs which extends lifespan of *S. cereviseasae*, *C. elegans*, *D. melanogaster*, and mice (Kaeberlein et al. 1999; Tissenbaum and Guarente 2001; Kanfi et al. 2012). Sirtuins are associated to the signaling pathways of insulin and insulin-like growth factor-I (IGF-I). IGF-I increases SIRT-1 expression via JNK1 (c-Jun N-terminal kinase 1) pathway (Ng and Tang 2013). As a result, SIRT-1 and SIRT-2 restore the IGF-I activity and deacetylate the insulin receptor substrate 2 (IRS-2). SIRT-1 has been also reported to involve in the CR through the influence on FOXO (Lee and Min 2013; Sasaki et al. 2010) (Fig. 14.2). The main role of SIRT-2 during aging

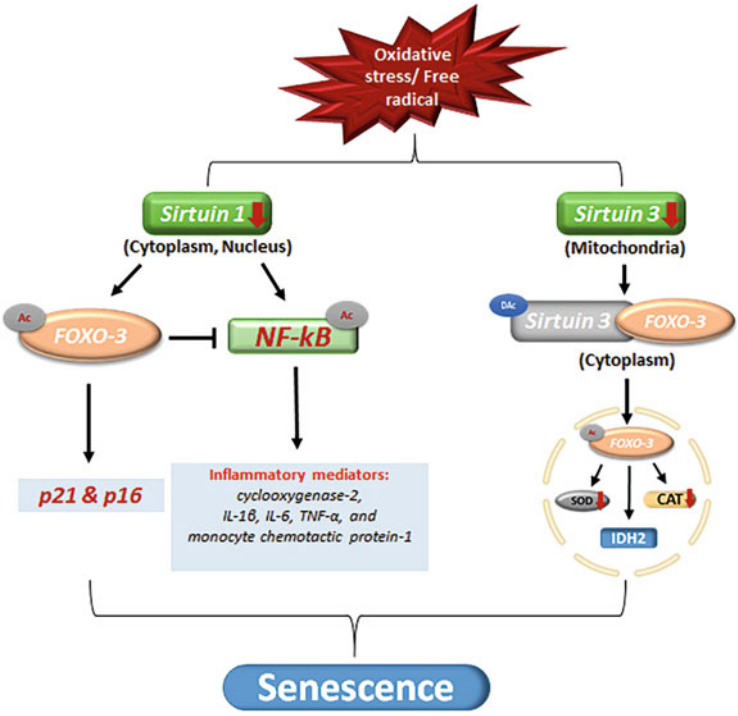

Fig. 14.2 Role of SIRT1 and 3 in cellular senescence under oxidative stress

results from a relation between human longevity and a polymorphism in the regulatory elements of sirtuin gene (Crocco et al. 2016). Furthermore, SIRT-2 level indifferent region of brain has been observed during aging animal models such as mice and rats (Braidy et al. 2015; Maxwell et al. 2011).

Due to these interesting findings during the last couple of decades scientists have been excited and studies have shown that either activation or inhibition of sirtuin activity may be desirable for ameliorating disease state (Imai and Guarente 2010). Accordingly, efforts have been undertaken to identify compounds that can either activate or inhibit specific sirtuins and serve as leads for the development of human therapeutics. Most of these efforts have been focused on developing modulators of major nuclear sirtuin, SIRT-1; however more activators or inhibitors are still being identified which are targeting the sirtuins.

14.3 Antiaging Potential of Sirtuins

Aging is a physiological process that can be successfully modulated by medicinal and pharmaceutical approaches (Ferrari 2004). It is associated with numerous alterations at the cellular, tissue, and organismal level. With age, senescent cells accumulate in various tissues affecting their regular functioning (Ma et al. 2011). The elevation of DNA damage with age is the result of impaired efficiency of DNA repair systems and it is the main cause of cellular senescence resulting from increased damage accumulation, and finally it causes cell senescence (Borodkina et al. 2014). Sirtuins have an essential role during DNA repair, inflammation and anti-oxidative properties which provide them good anti-senescence/antiaging targets (Wang et al. 2020). Among all, SIRT-1 is the main target for drug development because it shows many different and unrelated positive effects that are significant to health and might have a role in lifespan through different metabolic signaling pathways.

14.4 Caloric Restriction and Caloric Restriction Mimetics

Caloric restriction (CR) is a nongenetic intervention reliably extends life and health span, in overall it involves the reduction of total calorie intake without malnutrition for a particular duration. Caloric restriction promotes health and protects against many age-related diseases in mammals such as diabetes, cardiovascular disease, nephropathy, and neurodegenerative disease (Fontana and Klein 2007; Cangemi et al. 2010; Yang et al. 2016).

Despite the uncontestable health-promoting effects of CR, it becomes very difficult to maintain the CR lifestyle due to excessive willpower and subjective discomfort. It has also been reported that CR causes a number of physiological and psychological side effects, such as hypotension, infertility, osteoporosis, cold sensitivity, loss of strength and stamina, delay of wound healing, depression, emotional deadening, and irritability (Dirks and Leeuwenburgh 2006). Due to these reasons,

there has been great interest in the development of such drugs (CR mimetics) that can serve as alternatives to classical CR (Ingram and Roth 2015). Thus, pharmacological approaches that induce health and lifespan without causing malnutrition linked to CR have promoted the emergence of caloric restriction mimetics (CRMs). CRMs have been reported to modulate several cellular signaling pathways and have also been identified and investigated as sirtuin-activating compounds (STACs) which act as mTOR inhibitors, AMPK activators, and enhance autophagy in various experimental models (Kitada and Koya 2013). So far, the most likely candidate CRMs are SIRT-1 activators, especially resveratrol (Chung et al. 2012) and fisetin (Iside et al. 2020), inhibitors of mTOR, especially rapamycin (Blagosklonny 2010, 2013), 2-deoxy-D-glucose (2DG) and other glycolytic inhibitors (Ingram and Roth 2011), insulin pathway and AMPK activators (Cantó and Auwerx 2011; Stenesen et al. 2013), autophagy stimulators (Mariño et al. 2014), and α-lipoic acid (LA) (Merry et al. 2008) dietary antioxidants may also delay specific aspects of aging.

14.5 Sirtuins and Cellular Senescence

Sirtuins are essential factors that delay cellular senescence and extend the organism's lifespan through the regulation of various cellular processes (Wang et al. 2020). The suppression of cellular senescence by sirtuin is directed by delay in the age-associated telomere attrition, sustaining genome integrity and promotion of DNA damage repair (North and Verdin 2004; Toiber et al. 2011). The lifespan of an organism is modulated by sirtuins which interact with different signaling pathways including insulin/IGF-1, AMP-activated protein kinase (AMPK), and forkhead box O (FOX-O) (Bonda et al. 2011). Although cellular senescence is an important process to eliminate the accumulation of unwanted cells caused by stress in the young however the level of senescent cells increases during aging. The level of sirtuins has been found to decrease in senescent cells of embryonic fibroblasts, lung epithelial cells, endothelial cells, and macrophages in presence of oxidants (Sasaki et al. 2006; Anwar et al. 2016; Son et al. 2016). Moreover, the reduction in the level of SIRT1 and SIRT6 in presence of an inhibitor promotes premature senescence (Mostoslavsky et al. 2006; Ota et al. 2007). On the other hand, increased levels of SIRT1 and SIRT6 inhibit cellular senescence in angiotensin II-treated cells (Kim et al. 2012; Chen et al. 2018). The sirtuins present in the nucleus, SIRT 1, 6, and 7, act as transcriptional regulators to suppress the expression of genes by maintaining the chromatin structure (Toiber et al. 2011), which provide protection during cellular senescence. All the research findings suggest that sirtuins have a potential role in cellular senescence and are still being actively investigated.

It has been reported that deactivated SIRT1 in young Mesenchymal Stem Cells (MSCs) recapitulated the cellular senescence in aged MSCs; however, overexpression of SIRT1 mitigates aging which enhanced the MSC proliferation (Chen et al. 2014). Autophagy has been also found to contribute to the downregulation of SIRT1 protein during senescence in different cells of mice

model and in human T cells, even though the deficiency of SIRT1 protein has also been observed in a different model of senescence and aging (Sasaki et al. 2006; Huang et al. 2008; Zu et al. 2010; Chen et al. 2014). The SIRT1 also actively participate in autophagy because it is present in the nucleus, which is recognized by LC3 and further move to cytoplasm autophagosomes for degradation which causes cellular senescence (Xu et al. 2020).

SIRT6 is a chromatin-associated protein that stabilizes genomes and telomeres that prevents cells from premature senescence (Michishita et al. 2008). Sirt6 knock-out mice showed the premature senescence phenotype whereas Sirt6 over-expressing mice had shown a longer lifespan with cardio-protection against stressed condition (Mostoslavsky et al. 2006; Maksin-Matveev et al. 2015).

14.6 Sirtuins as a Powerful Tool in Antiaging Medicine

Among various antiaging strategies, one of the most pivotal targets for antiaging interventions is to modulate the proteins of sirtuin family. In humans, the sirtuins family consists of seven members (SIRT1-7) that have either mono-ADP ribosyl-transferase or deacetylase activity. It is well documented that daily lifestyle, i.e., exercise and diet can improve health span which modulates the sirtuins (Ajami et al. 2017; Radak et al. 2020). Furthermore, recent interest is focusing on to search for the modulator of sirtuins is one of the most extensive and robust topics in the field of gerontology. Natural compound have shown always a great promise for improving health span and longevity and also preventing age-associated diseases (Ferrari 2004).

Resveratrol is a natural phenolic compound found in several plants such as grapes, peanuts, and blueberries. The Sirt-1 activator resveratrol extends the lifespan (Howitz et al. 2003; Baur and Sinclair 2006) and provides protection against insulin-dependent diseases (Baur and Sinclair 2006; Baur et al. 2006). It also ameliorates aging-related metabolic phenotypes by inhibiting cAMP phosphodiesterases, as consequence increases $NAD^{(+)}$ and the activity of Sirt1 (Pirola and Fröjdö 2008; Park et al. 2012). Resveratrol has been shown to be involved in the activation of immune cells such as macrophages and T cells as well as in the suppression of CD^{4+} CD^{25+} regulatory T cells (Yang et al. 2008; Švajger and Jeras 2012). These effects are due to its capability to eliminate Reactive Oxygen Species (ROSs), inhibition of cyclooxygenase, and trigger anti-inflammatory pathways through activation of SIRT 1 (Miceli et al. 2014; Malaguarnera 2019).

Fisetin (3,3,4,7-tetrahydroxyflavone), a flavonoid found in different fruits and vegetables, has antioxidant, anti-inflammatory, antitumor effects, neuroprotective, and other health benefits (Maher et al. 2011; Khan et al. 2013). Multiple mechanisms of action have been proposed to explain diverse health-promoting effects of fisetin such as antioxidants and activation of sirtuins (Yen et al. 2017; Currais et al. 2018). Fisetin has been reported as a caloric restriction mimetic have possible neuroprotection against aging-induced oxidative stress, apoptotic cell death, neuro-inflammation, and neurodegeneration through upregulation of sirtuin-1 and neuronal

markers (NSE and Ngb) and downregulated the expression of inflammatory (IL-1β and TNF-α) and Sirt-2 genes in rat brain (Singh et al. 2018). The fisetin also increases SIRT1 activity by the regulation of FOXO and p53 signaling pathways (Salminen and Kaarniranta 2009).

Curcumin is a natural bioactive polyphenol, isolated from Curcuma longa, a compound mediating a wide spectrum of biological functions that has several health benefits (Sharifi-Rad et al. 2020). Curcumin activates AMPK, which increase SOD and ATP level finally enhance NAD^+ levels and SIRT1 activation in smooth muscle cells (Zendedel et al. 2018). It has been also found that in diabetic mice models, supplementation of Curcumin for 2 months, promotes indirect activation of SIRT1 through AMPK signaling pathways (Jiménez-Flores et al. 2014).

There are several small molecules that have the potential that delay aging and aging-related diseases (Espeland et al. 2017). One such molecule is the biguanide metformin used as a first-line therapy for type 2 diabetes (Bailey 2017). Metformin has an indirect pleiotropic effect during aging through the insulin/IGF-1 and AMPK/mTOR signaling pathways and it has also downstream effects on inhibitory action on mitochondrial respiratory complex I (López-Otín et al. 2016). The phosphorylation and activation of AMPK lead to further inhibition of mTOR, activation of PPAR-gamma coactivator-1 alpha (PGC1-a) and mitochondrial biogenesis, activation of SIRT1 and other nutrient-sensing signaling pathways, further inhibition of AGEs and pro-inflammatory cytokines, activation of Ulk1 and regulation of autophagy, among others (Kim et al. 2011, p. 1; Salminen and Kaarniranta 2012; Barzilai et al. 2016). Metformin direct activates SIRT1 without interaction with AMPK which directly inhibit mTORC1 via Rag-GTPases, suppression of adipogenesis through inhibition of p70S6K pathway, activation of DNA-damage-like response via the activation of ATM-Chk2 pathway, and activation of nuclear factor erythroid 2-related factor 2 (Nrf2), all of which result in downregulation of inflammatory responses (Kalender et al. 2010; Prasad et al. 2017; Cuyàs et al. 2018). These findings revealed that metformin and other biguanides such as phenformin can improve the catalytic efficiency of SIRT1 operating in conditions of low NAD^+ in vitro (Cuyàs et al. 2018).

Nicotinamide adenine dinucleotide NAD^+ is a central metabolic cofactor involved in numerous metabolic transformations. Sirtuins utilize NAD^+ to deacetylate proteins thus making its levels rate-dependent. Increasing levels of NAD^+ activate sirtuins; however, high levels can inhibit sirtuins (Mouchiroud et al. 2013). One strategy is providing nicotinamide. When nicotinamide is fed to Alzheimer's model of mice over a several month period, there are significant reductions in amyloid and improvements in cognition (Green et al. 2008; Gong et al. 2013). Nicotinamide undergoes methylation and generates hydrogen peroxide in mitochondria, which served as a hormetic signal to activate protective pathways to promote longevity.

14.7 Conclusion

The modification of SIRT activity could lead to a new platform opportunity for drug discovery related to aging and age-associated diseases. However, SIRT1 is a novel target and comes out with the challenge of novel molecules that are activators rather than inhibitors and might be developed as therapeutics. More work is still required to explore the potential roles of the sirtuins in age-associated diseases and further identify new related target proteins. Furthermore, this class of mammalian enzymes signifies a naive and possible approach to cure many diseases.

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A Vaccine for the Pandemic of Aging? Conceptual and Ethical Issues

15

Christopher Simon Wareham and Pablo Garcia-Barranquero

Abstract

In this chapter, we develop and extend the above analogy by means of a thought experiment in which a vaccine for the pandemic of aging is developed. We ask first, whether the concept of a vaccine for the pandemic of aging is *conceptually* coherent, and second whether such a vaccine (or similar aging preventive) is ethically desirable. This chapter makes the case that, while there are some clear disanalogies between aging and typical pandemics like the COVID-19 pandemic, there are some striking similarities that advocate for similar degrees of urgency. Moreover, the comparison throws important light on some of the flawed objections to healthy life-extending technologies.

Keywords

Ageing · Anti-ageing · Vaccine · Covid · Life extension · Ethics

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15.1 Introduction

Writing against the backdrop of the COVID-19 pandemic, Aubrey De Grey provocatively claimed that we are living in a "pandemic of aging" drawing an analogy between interventions against aging and treatments for other medical conditions (De Grey 2020, p. 92). The implication is that aging ought to be treated like so many diseases, and if it were, it would become an absolute research priority, leapfrogging even the COVID-19 pandemic in terms of urgency. However, De Grey stops short of proposing what might be referred to as a "vaccine for aging," and considering its value relative to a COVID-19 vaccine.

In this chapter, we develop and extend the above analogy by means of a thought experiment in which a vaccine for the pandemic of aging is developed. [1] We ask first, whether the concept of a vaccine for the pandemic of aging is *conceptually* coherent, and, second, whether such a vaccine (or similar aging preventive) is ethically desirable.

We begin in Sect. 15.2 by outlining a thought experiment in which a "vaccine for the pandemic of aging" is proposed. In Sects 15.3 and 15.4, we consider whether the concept of a vaccine for the aging pandemic is a coherent one. In particular, in Sect. 15.3, we question whether aging is indeed a "pandemic" and, in Sect. 15.4, whether "vaccine" is a concept that could be appropriately applied to a treatment that prevents aging. In Sect. 15.5, we raise three bioethical questions about the "vaccine" for aging. First, is it an intervention that would be good for people? Second, would its development as high a priority as de Grey suggests it ought to be? Third, how should such a preventive be distributed?

The thought experiment we propose is not the first thought experiment regarding an anti-aging treatment. [2] However, part of the contribution of this article is to explicitly compare a vaccine for the pandemic of aging, to a modern-day emergency—the COVID-19 pandemic. This provides an opportunity to revisit problems and objections, and to explore analogies and disanalogies with the aging vaccine. This chapter makes the case that, while there are some clear disanalogies, there are some striking similarities that advocate for similar degrees of urgency. Moreover, the comparison throws important light on some of the flawed objections to healthy life-extending technologies.

15.2 A Revolutionary Proposal

This section proposes a thought experiment. The function of thought experiments is to draw out relevant considerations in evaluating outcomes, to determine whether the outcomes are desirable, and to clarify concepts. at all. A significant purpose is to

[1] Parts of this chapter are reworked from an article which appears in Spanish in the journal *Pasajes.*

[2] See, e.g., Bostrom (2005); De Grey (2007); Gems (2011); Williams (2009). All these thought experiments are different, as are their the philosophical and ethical implications.

cause us to reflect on the *ends* of an activity, in this case, scientific research into aging. In order to do this, thought experiments are typically permitted to depart from what is strictly considered realistic. With this clarification in place, consider the proposal below.

15.2.1 A "Vaccine for the Pandemic of Aging"

Suppose that, in the near future, a renowned scientist, Professor Makropulos, claims that with 18 billion dollars, [3] her team will be able to fast-track a technique allowing the development of "vaccine for the pandemic of aging" within a year. The vaccine, though expensive to develop, is unlikely to be particularly expensive to develop or produce, and probably no more expensive than the COVID-19 vaccine. From this point, she claims anyone who takes the vaccine will be drastically protected from biological aging. She anticipates that most of the people given the vaccine will no longer age at all, and those who do age will age slower, and will experience the symptoms of aging with far less severity. People with complete protection from aging will no longer experience, or die of age-related causes, and their susceptibility to these diseases will not increase with chronological age. Those nonetheless age, will experience significant, though incomplete protection from age-related causes of death. They may age, though it will be far slower, and their (chronological) age-relative risk of dying from age-related causes will be far lower.

Professor Makropulos is careful to point out that her vaccine is *not* an immortality drug (her surname also belonging to the infamous immortal Elina Makropulos is just a curious coincidence (Williams 2009)). It will be necessary to take a "booster" vaccine every year, and if one wishes to age, one can choose not to take the vaccine. People who take the drug will still die of various conditions. Non-age-related cancers and heart disease are still possible, so the vaccine-protected person would still need to be watchful of her health. Moreover, like everyone else, they will need to be careful crossing the street, driving a car, or going to countries with a high crime rate or prevalence of infectious diseases. [4]

On the other hand, as time progresses, they will not have cause to be quite as fearful as a biologically older person would be of, for instance, pandemics like the COVID-19 pandemic. While a person who takes the vaccine can still die of innumerable causes, they are far less likely to die from aging, or diseases that mainly afflict those with advanced age. Their chances of dying of these causes will increase more slowly with chronological age, and in some cases, will not increase at all.

[3] This is the amount invested in Operation Warpspeed, the US government's project to develop a COVID-19 vaccine (Kim et al. 2021). However, other projections place the figure much higher https://www.devex.com/news/interactive-who-s-funding-the-covid-19-response-and-what-are-the-priorities-96833

[4] There is also the possibility of non-aging-related biological causes of death that correlate with chronological age, as in the case of birds that die "catastrophically" (Ricklefs and Scheuerlein 2001). In this case, we would die without aging.

Professor Makropulos is a highly well-respected gerontologist, and her consistently groundbreaking work has for a long time hinted at this possibility. So there are good reasons to think that she is onto something that really could achieve this result. However, she is making some very strong, and, some suggest, outlandish or simply erroneous claims about what we will refer to as the "Makropulosjab." Does her claim about a "vaccine" for "pandemic" of aging make scientific and conceptual sense. Furthermore, do we have strong moral reasons to give her the 18 million dollars required?

15.3 Is Aging a Pandemic?

The scientific and conceptual questions of whether the Makropulosjab could justifiably be called a vaccine for an aging pandemic should be treated in two parts. In this section, drawing on the example of the COVID-19 pandemic, we discuss various criteria for a pandemic and question the extent to which aging can justifiably be seen as a *pandemic*. In Sect. 15.4, we turn to the question of whether the concept of an aging *vaccine* makes conceptual sense.

15.3.1 What Is a Pandemic?

In scientific terms, COVID-19 is an exemplary pandemic. Morens et al. (2009) suggest key characteristics that are definitive of a pandemic (Morens et al. 2009). [5] These characteristics provide additional detail to the three previously mentioned conditions. After briefly discussing these, we will assess whether aging fits the same framework for COVID-19.

1. *The wide geographical extension* of the disease over large geographical areas (interregional, transregional, or global).
2. The *spread and movement of the disease* through transmission that can be traced from one location to another.
3. High disease attack rates and *strong detrimental effects* on people's health, including the possibility of death. The most notorious health crises have tended to exhibit a more explosive character, i.e., multiple cases appearing in a short time).
4. Minimal population immunity is a form of natural resistance against the most severe effects of the disease.
5. The disease must be *novel* or at least be associated with different variants of existing microorganisms.
6. The disease has a robust *infectious character*.

[5] See also, Sampath et al., (2021).

Table 15.1 Comparison between COVID and aging in light of the essential characteristics of a pandemic

	COVID-19	Aging
Wide geographical extension	Yes	Yes
Disease movement	Yes	No
High attack rates and explosiveness	Yes	No
Minimal population immunity	Yes	Yes
Novelty	Yes	No
Infectiousness	Yes	No
Contagiousness	Yes	No

7. The disease *is highly contagious* by one or more means of transmission (either between members of different species or between members of the same species).

Not all of these characteristics are equally important when defining what a pandemic is. For instance, while most pandemics, including the COVID-19 pandemic, do have the characteristic of novelty, we would be justified in attributing the term to less novel diseases if the other conditions were met.

15.3.2 Is Aging a Pandemic?

These clarifications notwithstanding, the criteria discussed present good reasons why aging would not match the ordinary scientific understandings of a pandemic. As shown in Table 15.1, the conceptualization of aging as a pandemic is problematic from this scientific point of view. Of course, it might be suggested that, even more than COVID-19, aging has a "wide geographical extension." Indeed, other things being equal, it is a universally experienced condition. It could also be suggested that there is minimal population immunity to aging—there is no immunity at all, an issue we return to in the next section when we consider whether the Makropulos drug is a vaccine. However, acceptance of Professor Makropulos's idea of an "aging pandemic" on these or other grounds seems to stretch ordinary scientific understandings of the term pandemic.

15.3.3 Is Aging a Disease?

In addition to the above, there is perhaps a deeper reason why many would be disinclined to refer to aging as a pandemic: typically a pandemic is a term referring to *diseases*. The idea that aging is a disease is extremely controversial. Considering whether this classification is possible is one of the most important philosophical questions relevant to geroscience (Lemoine 2020, pp. 2–3). On the face of it, if any other condition caused organisms to suffer progressively greater cognitive and physical deterioration, ultimately resulting in death, we would be strongly inclined to treat them as a disease. Then why not aging? Some approaches argue that it is not a disease (Hayflick 2000; Schramme 2013); others that it is (Caplan 2005; De Winter

2015); still others argue that it is helpful to view it as a disease, and not merely dismissed as a "natural" process (Murphy 1986; Callahan and Topinkova 1998). We cannot do justice to this significant conceptual debate here, but will instead adopt a "pragmatist" approach to the possibility that aging is a disease. Philosophical pragmatism is, roughly, the idea that the words and concepts ought to serve a practical function, and, further, that the meaning of concepts should be guided by, and perhaps exhausted by their practical usefulness. Saborido and García-Barranquero note that

> The history of medicine shows many conceptual changes of this type—conditions that cease to be diseases or begin to be interpreted as such—and presumably more conceptual changes will happen in the future because medicine is in constant evolution. New diseases will arise and old diseases will change their meanings in the cases in which such changes prove useful to achieve the goals of medicine (Saborido and García-Barranquero 2022).

In this kind of view, the definition of the concept of disease is not determined by who has the best scientific or philosophical arguments. Instead, whether aging is a disease will depend upon whether viewing aging as a disease has useful results, for example, in terms of medical practice. One such useful result of employing the "aging as disease" concept might be highlighting the possibility of a treatment for aging.

> Whether adopting the conception of "aging as a disease" is beneficial or not is something that gerontology will decide depending on whether it helps to achieve its epistemic goals. Treating aging as a disease would make it such (Saborido and García-Barranquero 2022).

In this case, we might say that the question of whether aging is a disease is an open question, one which will have a clearer answer when it is obvious whether or not treating aging as a disease has a pragmatic purpose.

15.3.4 A Broader Understanding of "Pandemic"

So far in this section, we have discussed narrower, technical conceptions of a pandemic, some of which incorporate the idea that pandemics must involve disease. Methodologically, these more technical conceptions draw on the history of disease pandemics in defining what a pandemic is in the epidemiological context (Morens et al. 2009). However, this is neither the only context in which the term pandemic is used, nor is this the only methodology that we could use in determining the correct usage of the term pandemic. Below, we suggest that it would be possible to interpret Professor Makrupulos's claim in a broader etymological way, which nonetheless coincides with other common uses of the term.

First, we consider it relevant to approach the word's etymology. The origin of the word pandemic is found in the Greek word *pandēmos*. This is made of two words

pan (all) and *dēmos* (all the people), i.e., "that which affects the whole people." [6] This definition underlies the one that states that a pandemic implies a contagious epidemic disease that spreads to several countries and attacks many individuals simultaneously. We could say that the meanings discussed earlier are narrower and more technical and require more specific conditions than the one that refers only to its broader etymological meaning. From this latter reading, it is logical to point out that aging is a biological process that *all* humans undergo, arguably throughout our lives (Gems 2014; Lemoine 2020). This inherent condition of aging will (in the absence of an anti-aging intervention) accompany us throughout our existence without exception. In this sense, aging seems to fit squarely with the original meaning of the term pandemic, indeed perhaps better than disease pandemics themselves, since the latter spare at least *some* people.

In addition to this etymological approach, there are some "common usage" reasons to regard Makropulos's broader usage of the term as appropriate. Certainly, a less restrictive approach is employed in speaking of the "pandemic of obesity" or the "smoking pandemic"(Moodie et al. 2013; Meldrum et al. 2017). Both problems can lead to other conditions that drastically reduce our quality of life (whether due to disorders, illnesses, or stigma). Thus, even if we do not accept that aging can be classified as a (new) disease, it is unclear that all pandemics should be governed by this criterion. A less rigid disease-based concept allows us to reflect on what is important about pandemics. In all these cases, there are concomitant health problems that cause a decrease in the well-being of many people. Seen in this way, we are motivated to reverse pandemics with medical, public health, and social measures. This theoretical framework shows a practical aim of the term pandemic that is arguably understated by purely epidemiological definitions: to urge the development of measures that can improve human health and generate greater widespread awareness of the problem to be remedied.

From the above considerations it is clear that, when the term pandemic is understood in a narrow, technical, epidemiological sense, *aging* cannot be seen as a pandemic. This is partly because there is doubt over whether it can be classified as a disease, although, as we suggest there may be pragmatic reasons to do so. However, this narrower conception is not the only conception. "Pandemic" can be understood *etymologically* as a condition that affects *everyone*, or in *common usage* as a ubiquitous cause of health problems. In these latter senses, aging ought arguably to be seen as the greatest pandemic of all since absent other causes of death, it affects everyone, without exception.

[6] https://bcmj.org/blog/origin-pandemic-related-words

15.4 A Vaccine for Aging?

The above is not to suggest that the broader conception of the pandemic should have priority. However, it does present a clear sense in which Makropulos is justified in claiming that there is a "pandemic of aging." The next question is whether her use of the term "vaccine" could be justified. In the same way that we have written about the term "pandemic," we will start by considering the typical scientific definition.

15.4.1 What Is a Vaccine?

Vaccination is a preventive approach that consists of administering a substance to a healthy subject to generate its own immunity against a disease. Such diseases are generally mediated by a virus, bacterium, and, exceptionally, parasites, as in the case of malaria. Vaccines are substances that prepare the immune system to fight a disease-causing germ or other pathogens by imitating an infection. They train the immune system into making a "memory"' of that germ, so that when the immune system encounters the real pathogen—whether a virus, bacterium, or other microbes—the immune system is better prepared to fight infection. [7] As a result, the vaccinated person gains a degree of protection from the effects of the infectant. It should be noted that RNA vaccines generated a change in the understanding of a vaccine. Before the RNA vaccines developed for COVID-19, acquired immunity was generated by the use of an antigen—a substance, usually a protein that generates an immune response. By contrast, RNA vaccines do not involve the physical introduction of an antigen. Instead, these novel vaccines use the machinery of our cells to generate the antigen (Kwok et al. 2021). According to some, this modifies the vaccine concept. Although the global definition does not appear to be affected, vaccine denialists have nonetheless used this to cast doubt aspersions on COVID-19 vaccines.

15.4.2 A Vaccine for Aging?

Aging is clearly not transmitted by viruses or other foreign infectious elements. Indeed a major point of disanalogy between the function of vaccines and the function of an anti-aging intervention is that vaccines typically function by discriminating between "self" and "non-self," and eliminating "non-self," while leaving "self" intact (Kwok et al. 2021). This is different in the case of aging, since aging cells are *our own* cells. So it appears conceptually problematic to suggest that our own aging cells are something that can be vaccinated against. Nonetheless, being

[7] WHO "Vaccines and Immunization: What is vaccination" https://www.who.int/news-room/questions-and-answers/item/vaccines-and-immunization-what-is-vaccination

Table 15.2 Comparison between COVID and aging in light of the essential characteristics of a vaccine

	COVID-19 vaccine	Aging vaccine
Uses acquired immune response	Yes	Yes, but partially
Distinguishes between self and a foreign biological agent	Yes	No
Removes a proliferative agent that is harmful to the organism	Yes	No

open-minded, we might consider what approaches to an anti-aging preventive might share similarities with the vaccine paradigm.

Loosely, a vaccine trains the immune system what to detect as foreign, and, therefore, what to attack. This enables the creation of cells prepared to identify a specific enemy (the specific antigen) and eliminate it from the system. If there were a vaccine against aging, a specific marker of aging would have to be identified that would distinguish the aged cell as foreign. The immune system would have to be trained or otherwise altered to recognize and eliminate cells carrying the marker. In this way, when a cell ages, the marker would become identifiable by the immune system and the aged cell would be eliminated by the body, removing it from circulation and preventing it from making other aged cells.

Arguably, a similar approach is employed in immunotherapy, whereby, roughly, T cells are genetically modified to eliminate cancer cells expressing relevant makers, before being reintroduced to the patient to eliminate cancers (Provinciali and Smorlesi 2005). Perhaps even more relevantly, there is an ongoing effort to develop cancer vaccines (Kwok et al. 2021). Such efforts derive their plausibility in part from the idea that cancers routinely form, but are often eliminated by the immune system (Shankaran et al. 2001). However, applying this idea to *aging* is merely notional and faces serious difficulties. For instance, cancer cells are harmful due to their tendency to proliferate, so that eliminating them is beneficial. Normal aging cells are however beneficial generally and contribute to the homeostasis of the organism. Eliminating aging cells from the body appears likely to damage the functioning of the organism (Table 15.2).

Thus, if Makropulos's "vaccine" used these or similar methods that employ the immune system machinery, she would be involved in a hitherto rather unexplored field. The incredulity of gerontologists, vaccinologists, and the scientific community might well be justified. Without further reasons to the contrary (which we are unable to provide) it might be most charitable to treat Makropulos's claim that the intervention is a vaccine as at best metaphorical. [8] She is proposing that her intervention

[8] Perhaps, for instance, Makropulos believes that a hormesis paradigm, which is sometimes implicated in the effects of caloric restriction, underlies both her intervention and the success of some vaccines (Masoro 2005; Calabrese et al. 2013). Hormesis is, roughly, the idea that small doses of something often induces a response that is favorable to health (Calabrese et al. 2013). Again, though pointing to a similar mode of action would not make her intervention a vaccine.

could somehow prevent or slow the progression of, or reduce the effects of aging *preemptively*, just as vaccines prevent or slow the progression, or reduce the effects of some diseases *preemptively*. Without further evidence, the idea that such an intervention might employ the immune system in a way that would justify the use of the term "vaccine" seems, at best, far-fetched.

15.5 Ethical Implications

Returning to the thought experiment, what should we conclude about Dr. Makropulos's claim to be able to develop a vaccine for a pandemic for aging? Though imprecise, metaphorical, and somewhat rhetorical, it is not entirely outlandish or necessarily disingenuous (at least not unusually so for a funding proposal). Considered alone, the conceptual stretches in Makropulos's public claim provide no strong reason to turn the request down. Moreover, per the thought experiment, we should assume the scientific aspects of her proposal are credible and there is a real prospect that her research could slow or halt aging. [9]

The question then moves to the *rationale* for funding the research. If we assume that Makropulos's work could successfully slow or prevent aging, are there reasons (not) to give her the 18 million dollars? From a public health perspective, biogerontologists and biodemographers have argued that there are enormous financial gains to be achieved from anti-aging interventions (Olshansky et al. 2006, 2007; Huber and Sierra 2009; Miller 2009). Assuming this to be the case, what ethical considerations ought to be considered? While the ethical need for a functional covid vaccine is clear, the desirability of intervening in aging has been debated for millennia. Would it ethically desirable to "immunize" (again, metaphorically) against aging?

In what follows, we consider and rely on three objections to this developing and using the Makropulos "vaccine": First, that slowing aging would be harmful to the individual; second that it would be harmful to society, and third that it would compromise the social values of equality and fairness.

15.5.1 Would Extending Lifespan Using Makropulos Vaccines Be Harmful to the Individual?

As we age we tend to become less healthy. Older people are more likely to have diseases like cancer, cardiovascular disease, diabetes, and Alzheimer's disease. As a

[9] We might think of this as science fictional, but one function of ethics thought experiments is to draw out relevant considerations in evaluating outcomes, to see whether the outcomes are desirable at all. In other words, they cause us to reflect on the ends of an activity, in this case, scientific research.

result, some fear that if we take a life-extending intervention like the Makropulos vaccine, we will simply live longer in a very unhealthy state(Partridge et al. 2009).

For example, suppose that, on average, people are fully healthy until 65, after which they tend to become progressively less healthy until they die at 80. A concern is that if people lived longer than 80 they would age normally until that point, with the increased frequency of disease that typically characterizes old age. Thereafter, they would get even less and less healthy until life became unbearable(De Grey 2008). If this possibility holds true, if we take the life-extending Makropulos vaccine we will eventually have more age-related diseases like cancers, and more disabling mental diseases like Alzheimer's disease. Even though we live longer, we might wish we had not taken the life-prolonging vaccine. Reaching 120 years old, for example, will not be a blessing, but a curse. There is no reason to extend lifespan since doing so would be bad for us. [10]

To some extent, the realization of this unpleasant scenario, along with other negative side effects of this vaccine is an empirical question, the answer to which will depend on the nature of the vaccine developed by Makropulos and may only be discovered in the very long term. In advance of this information, though, it would be reasonable to consider evidence from studies of slowed aging in calorically restricted (CR) organisms. Biologists investigating calorie restriction have observed that CR extends lifespan by slowing down the processes of aging (Giacomello and Toniolo 2021). Because aging is decelerated throughout life, healthy lifespan is extended, postponing the onset of age-related diseases until later in life. This means that, depending on when one initiated the vaccine, rather than health beginning to decline at 65, one might live in good health until 80, and live much longer after that.

Nonetheless, if the healthier part of the aging process is slowed down and extended, perhaps it is possible that the unhealthy part would be slowed down and extended. Just as pulling on the ends of a rubber band elongates all the rubber segments, slowing aging will extend both the healthy and unhealthy segments of our lives (Gems 2011). If we take Makropulos vaccines, it could be that the unhealthy part of life at the end of life will also be extended. And if we take it when we are older, we will be unhealthy for a greater proportion of our lives.

Again, it makes sense to consider evidence from studies of slowed aging in calorically restricted organisms. Some biologists suggest that slowing aging will shorten, rather than lengthen, the period of reduced health at the end of life. Health in late life may actually be improved. While it remains possible that the time in which one experiences worse health will be prolonged, studies on CR suggest that the number of diseases suffered will not increase (Gems 2011, p. 110). That is, although the length of time in which a person is more likely to get sick is longer, at any point during extended old age she is less likely to have a particular age-related disease. So despite the fact that one would be more susceptible to age-related diseases for longer,

[10] An immediate thought is that if life reached the state in which it is not worth living, we might simply end it (Harris 2002). However, this option might not be available or simple to some, for example, for religious reasons.

the frequency of diseases appears to be lower. In this sense, prolonged health decline should be seen as an improvement over normal aging.

Importantly, although health is not as good as when they were younger, older people live good lives. Indeed, studies suggest that older people tend to be far happier than middle-aged people (Blanchflower and Oswald 2008). Thus, although later years are not as healthy, one can still benefit from them, even if one benefits less than one would if one was in full health (Wareham 2012). Provided a life-extending intervention would not reduce well-being below a desirable threshold—and there is no reason to think Makropulos's "vaccine" would do this—later years in an extended life can be as good as the last years of a normal elderly person's life. Since these years are worth having, there is reason to think that even extending this period of worse health would be beneficial. Certainly, there is no strong reason to think the Makropulos vaccine would be harmful to the person who uses it.

15.5.2 Would the Makropulos Vaccine Be Harmful to Society?

It might be thought that the Makropulos vaccine would increase the welfare levels of society if it made individuals better off. A society with citizens that have greater well-being is, generally better than a society with citizens that are not as well off. If so, there is an argument that society would be improved with each additional person that took the anti-aging vaccine.

However, this simple argument ignores population-level effects that are extremely important for public health ethics. In the last century, life expectancy has already risen substantially. One extremely important demographic consequence of people living longer is that the proportion of elderly people in most parts of the world has increased substantially (Fukuyama 2002). If the maximum lifespan was further increased as a result of the Makropulos vaccine, it should be expected that this proportion would be even greater. Many think that the effects of an older society will eventually reduce welfare (Fukuyama 2002). Would population aging that would occur due to the use of the Makropulos vaccine be bad for society?

One reason to think so is the possibility of unsustainable dependency. The past century has already seen great increases in life expectancy, with few increases in the average age of retirement. This means that the ratio of working people to retired people is decreasing. If people using the Makropulos vaccine continued to draw a pension from age 65, and could be expected to live until 100, an even greater proportion of people would then be above pensioning age. In this circumstance, perhaps a diminishing proportion of workers would need to support an expanding population of retirees. If so, this could place an unsustainable burden on society, since less money would be available for social programs such as public healthcare (Fukuyama 2002).

It is true that if citizens continued to retire at age 65 and lived longer afterward, this could pose serious difficulties. Is this an argument against the use of the Makropulos vaccine? As an initial consideration, it might be illuminating to consider this objection in light of our attitudes to the COVID-19 vaccines. These were treated

as life-saving and valuable interventions, in major part because of their implications for preventing death in the very elderly. The idea that we should not use these vaccines because the elderly place a burden on health and social systems would rightly be considered monstrous. Why should this argument be acceptable in the context of the Makropulos vaccine? At minimum, we would have to see the likely social cost as extremely high and very likely if we are to reject the Makropulos drug on these social grounds.

With that said, are there reasons to think the Makropulos vaccine would be harmful to society more broadly? If citizens made use of the Makropulos vaccine, they would—as discussed in the previous section on individual welfare—age more slowly and be healthy for longer. Ill health in later life is arguably one of the main reasons for having a retirement age in the first place. Older people who remain healthy tend to work for longer (Clark et al. 2008). Thus, if people remained healthy for longer, citizens could contribute productively for longer. Indeed many nations have policies to gradually increase retirement ages. The successful development of a Makropulos vaccine would require careful thought about how to arrange the distribution of work and social benefits in society. In a super-aged nation, the longer lived population that made use of the intervention would have to be *healthy* to avoid depletion of the proportion of workers. Though not guaranteed, given available knowledge of slowed aging, this condition appears likely to be fulfilled.

15.5.3 Should Life-Extending Vaccines Be Fairly Distributed?

People who are poorer tend, on average, to die younger. This is already a serious ethical problem. A significant concern is that lifespan gaps might widen even further if life-prolonging interventions became available. The wealthy would make greater use of life-extending vaccines because they tend to be better educated and have more disposable income (Pijnenburg and Leget 2007).

One way to respond to this problem is to suggest that Makropulos vaccines should be provided to everyone by the state, much as COVID-19 vaccines have been (Wareham 2016). Given their health effects, in countries with universal public health cover, perhaps public health services should supply Makropulos vaccines in the same way that other health interventions are. Doing so might go some way towards preventing the lifespan gap from expanding.

However, there are important ethical arguments for and against this type of universal provision of healthy life-extending vaccines. One objection to the wider provision of the Makropulos vaccine is that, typically, health services are obligated only to provide treatments for diseases or disabilities(Mackey 2003). They should restore a person to health. Health services do not typically provide enhancements—such as beautifying cosmetic surgery—that improve a person's condition above a normal level. The Makropulos vaccine would raise people above a normal level by allowing them to live much longer than a normal person. They would therefore constitute an enhancement, which health services do not—and perhaps should not—provide(Barazzetti and Reichlin 2011).

Against this, proponents of the public provision of Makropulos vaccines could argue that the interventions do not *aim* to enhance above normal health. Instead, they should be considered as treatments, since they would cure, prevent or postpone acknowledged diseases like cardiovascular disease, diabetes, and cancer. As such, Makropulos vaccines should fall into the category of preventive interventions. Their provision should be regarded as similar to public health interventions to reduce smoking and obesity (Wareham 2016), and indeed COVID. These are regarded as pivotal and valuable aims of public health services. Thus, if Makropulos vaccines are similarly effective, there are reasons to think that their provision should be prioritized alongside these. At least in the developing world, provision of Makropulos vaccines would be fairer, since it would benefit the worse off.

On the other hand, lower income nations often have different health priorities and emphases. In some contexts, the use of public resources to undermine the causes of infant mortality and curing and preventing HIV/AIDS would prevent more premature deaths than slowing aging. In such contexts, anti-aging interventions would not be fair or effective, since people mainly die of non-age-related causes. Nonetheless, even in lower income nations, the burden of age-related disease is extremely high (Christensen et al. 2009). Noncommunicable diseases, which are often related to aging, represent a large fraction of premature deaths in the developing world. Moreover, these populations are aging at a much faster rate than those of the developed world (Abegunde et al. 2007). Thanks to better healthcare, more people are reaching adulthood. This means that more people will die of age-related diseases, rather than communicable diseases. As a result, even in worse-off nations, forward-thinking policy-makers would have an interest in provision of Makropulos vaccines to slow the large, and growing, burden of age-related disease. Doing so will benefit many people who would otherwise die prematurely. Thus, respecting fairness is not an argument against developing the Makropulos vaccine. Instead, it provides strong reasons to develop and distribute it fairly.

15.6 Conclusion

Anti-aging interventions prevent a wide array of conceptual and ethical challenges. To investigate some of these challenges, this article proposed a thought experiment in which a researcher proposes a "vaccine for the pandemic of aging." The above sections made the case that Makropulos's use of the term pandemic of aging is a departure from the ordinary scientific use, but accords with a broader etymological understanding, as well as common usage. The use of the term "vaccine" was even more problematic, since it is difficult to see how an anti-aging intervention would employ the immune system in the required sense.

Nonetheless, allowing for some colorful and metaphorical language, it could be permitted that Makropulos could have developed a preventive anti-aging intervention. This raises ethical questions: should such an intervention be permitted, or, as with COVID-19 vaccines, should states *provide* it? The relative priorities and values of individual states play a role in this. However, for states that value fairness, there

appear to be strong reasons to disseminate the drug, so as to increase the lifespans of those who die of age-related causes.

In conclusion, it is important to note two disturbing disanalogies between our response to the "pandemic of aging" and the COVID-19 pandemic. Firstly, it would be considered monstrous to suggest that one should not develop a COVID-19 vaccine because death by COVID-19 prevents people from experiencing the harms of aging. Yet, as discussed above, this is the objection that is often made against life extension. That is, a common objection to life extension is the fear of ill health in old age. But very few would have thought it acceptable to fail to develop COVID-19 vaccines so that old people die earlier and thereby be spared age-related health decline by death.

Secondly, suggesting that it was not worth developing the COVID-19 vaccine because longer lived old people increase dependency and place a drain on the economy would have been considered immoral in extreme. Again, this is precisely the sort of consideration presented against the Makropulos "vaccine" above. These apparent inconsistencies in our attitudes demand ethical attention. If we think 18 million USD is too high a price to pay for Makropulos's drug, it is important to consider and justify why aging is treated with nothing approaching the urgency of the COVID-19 pandemic.

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Cognitive and Emotional Aging Across the Life Span: Implications for Building the Cognitive Reserve and Resilience

16

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Abstract

Aging is a cognitive-affective-behavioral construction, apart from being a biological reality which makes it a complex phenomenon. Over the last few decades, a major demographic transition at the global level has been witnessed in the aging population, with a robust finding of positivity bias among older adults. It is now stated that an increased capacity for emotional regulation is a key feature of human aging because of which older adults are more adaptive to positive affective experiences. Henceforth, a large amount of work has focused on cognitive and emotional aging, marking the importance of affective prioritization in support of building cognitive reserve. Neuroscientific studies have also shown evidence for a positivity effect in terms of enhanced connectivity between amygdala and medial prefrontal cortex at rest for memory positivity. However, there is less research to investigate whether these effects are gradual across the adult life span or become exaggerated (or only appear) with increasing age across populations. One of the recent studies has explored the adult age differences in the interaction between cognitive and affective aging in the Indian context. This chapter focuses on the life span perspective of cognitive and emotional aging and its implications and strategies for building resilience and cognitive reserve. We also propose the affective reserve hypothesis (ability to adapt to stressful situations and prevent the impact of emotional distress) as a plausible outcome of the positivity effect, which needs to be investigated empirically.

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Cognitive aging · Affective aging · Positivity effect · Cognitive control · Life span perspective · Cognitive reserve · Resilience

16.1 Introduction

Cognitive aging refers to the decline in one's cognitive processing with increasing age. More specifically, it is associated with decline in memory, processing speed, some aspects of language, visuospatial functions, and executive functions. Declines in gray and white matter volume contribute to the observed cognitive changes with aging. These cognitive changes can affect an individual's day-to-day functioning and can help distinguish normal from disease states associated with aging such as mild cognitive impairment and dementia. Cognitive functions play an important role in the overall psychological well-being of an individual. Starting from middle age, they tend to define the quality of life with a recent claim that the determinants of successful aging can be identified from young adulthood (Park 2019).

Aging is primarily associated with a decline in physical and cognitive health; however, few other changes that come with aging are positive. Cumulative knowledge and skill-based experience are well maintained with aging. In addition, compared to cognitive decline, emotional well-being is known to be stable with increasing age (Kunzmann and Wrosch 2015). Emotion regulation motivation shows a positive trajectory with age such that emotional-motivational development across the life span shows shifts in emotional goals across adulthood. Since affect closely interacts with cognition in terms of modulation of attentional mechanisms and memory (Mather and Cartstensen 2005) and it is likely to contribute to cognitive reserve. In this chapter, we focus on the interaction between cognitive and emotional aging from a life span perspective and attempt to bring out the adaptive effects of this interaction on aging.

16.1.1 Cognitive Aging

The dynamic relationship between brain and cognition changes over a life span. Age-related changes are not uniform across brain structures, cognitive domains, or individuals. In particular, attention and memory are the most affected cognitive processes as a result of aging. Tasks demanding selective attention like complex visual search or Stroop show slowness in response among older adults compared to young adults (Mager et al. 2007). Similarly, divided attention tasks demanding simultaneous attention on two or more sources of information reflect age-related decline among older adults when compared with younger adults (McDowd and Craik 1988). These are attributed to declining cognitive resources and task-switching difficulties in older adults. Working memory and long-term memory, which require active maintenance and manipulation of information are equally affected as a

consequence of aging. Slowness in processing speed (Luszcz and Bryan 1999), reduced abilities in ignoring irrelevant information (Darowski et al. 2008), and reduced usage of strategies improving learning and memory (Isingrini and Taconnat 2008) show a trend of natural decline with aging. Both attention and memory pose demands on "executive control" to streamline goal-oriented information while keeping distractors at bay to function optimally.

Executive control broadly is the basic mechanism modulating the operations of many cognitive processes like attention and memory to regulate cognition (Miyake et al. 2000). Aging affects these executive control processes causing a decline in basic cognitive operations like resistance to interference (or inhibition), task shifting, and memory updating (Miyake et al. 2000) and may also result in affective-behavioral changes.

16.1.2 Cognitive-Affective-Behavioral Changes with Aging

From the very early times beginning from the work of Thomas Aquinas (1225–1274), the study of behavior was divided between cognition and affect which were viewed as separate systems rarely interacting with each other. However, the last two decades of neuroscientific research challenged the notion of functional specialization. The research went on to evidence the interaction between cognition and emotion and established the fact that the neural mechanisms integrated into the brain jointly contribute to one's overall behavior (Ochsner and Phelps 2007; Pessoa 2008, 2009). It was later empirically shown how emotions can affect cognition at variant intervals particularly encoding and recollection (Bower and Forgas 2000).

The past decade has witnessed an abundant amount of work on the crucial role that emotion and motivation play in the overall behavior through tasks involving executive control, perception, attention, and decision-making (Clore and Storbeck 2006; Rowe et al. 2007; Isaacowitz and Riediger 2011). Such motivated cognition has been found to have behavioral implications for young and older adults. Kanning and Schlicht's (2010) bio-psychosocial model of successful aging suggests a dynamic interaction between physiological, psychological, cognitive, and sociological factors influencing an individual's subjective well-being. These include a combination of psychosocial and demographic factors such as age, education, occupation, lifestyle, and physical health contributing to the experience of age-related changes in cognition and behavior. Dziechciaż and Filip (2014) suggest that the inevitable decline in cognition and psychosocial aging may be controlled through a positive approach and change in one's perspective towards age-related decline. One of our recent studies in the Indian context, on adult age differences in memory, general cognitive performance, behavioral/affective changes, and use of emotion regulation strategies, examined through a set of neuropsychological and self-report measures, clearly demonstrates the early cognitive changes related to memory, behavioral/emotional issues, and emotion regulation as early as middle age. Moreover, it was interesting to find that cognitive changes may coexist with

affective and behavioral changes during the early stages of aging and have implications for healthy adaptive aging (Nigam and Kar 2020).

16.1.3 Emotional Aging

Emotional aging refers to subjective experience of relatively more positive than negative emotions, which is achieved by pursuing emotionally meaningful goals. A shift in emotional goals from negative to positive also relates to a shift in emotional motivation to regulate emotions and improve one's competence. Emotion regulation is found to be relatively unaffected by aging and even improves with age as compared to the other declining functions such as memory and executive functions (Scheibe and Carstensen 2010). Emotion regulation in aging revolves around the goal of achieving emotional well-being. Although well-being as a protective factor to healthy aging or as one of the various other factors responsible for healthy aging is still debatable and therefore needs greater understanding.

16.1.3.1 Theoretical Accounts of Emotional Aging

Socioemotional Selectivity Theory

The prominent life span theories on emotion processing suggest that adverse challenges associated with aging can be dealt with through continuous engagement in attaining goals related to better affective well-being. Older adults are motivated to prioritize affective information (a mode of emotion regulation), particularly more positive than negative information, as compared to young adults while regulating emotions. This "motivational shift" forms the basis of a major research area in cognitive aging which results from the optimization of emotional experiences due to limited time left in life (Carstensen 1993; Carstensen 2006) (described in greater detail later in the text). A crucial outcome of this motivational shift is its influence on preference for social partners, differences in problem-solving strategies, and executive functions such as attention and memory that optimize the emotional experience and its regulation (Charles and Carstensen 2007).

The Selection, Optimization, and Compensation (SOC) Model

Baltes and Baltes (1990) proposed a metamodel of general developmental processes that are Selection, Optimization, and Compensation (SOC). The SOC Model postulates that successful aging is a result of lifelong usage of SOC processes to maximize gains and minimize losses in order to optimize the available resources at a given age. This resource management becomes specifically important in old age due to depleting resources. The model explains how older adults prioritize goals (selection) with regard to the significance of increasing gains and simultaneously avoid losses (to compensate) for the available resources. It states that the limited resources and time perspective in old age lead to greater losses than gains due to exhausting larger number of resources while replenishing it less. Hence, the resources accumulated at earlier stages in life become increasingly significant and their

management in later life becomes crucial for successful aging. This can cause a shift in the orientation of goals of particular positive functioning (in the form of regulating emotions) while optimizing external and internal resources to promote growth as well as managing losses (compensation) to ensure maintenance of functioning. These are said to influence an individual's sense of autonomy, environmental mastery, personal growth, positive relations, purpose in life, and self-acceptance in old age (Freund and Baltes 2002).

Dynamic Integration Theory

The dynamic integration theory postulates that the declining cognitive abilities due to aging cause a decrease in the ability to process emotional complexity which leads to increased positivity in later life. Some researchers have also proposed that it is easier to process positive information resulting in preferential processing of positive information with age (Labouvie-Vief et al. 2010) compared to negative information. This integrative model aims to explain both gains as well as losses that incur in cognitive-affective functioning over a life span. The theory hence suggests the positivity effect as an automatic gating selection mechanism.

Discrete Emotions Perspective

In addition, a new era of research on emotion aging is exploring multidirectional age differences with respect to specific emotions under the discrete emotions perspective which has long-term implications for affective well-being (Kunzmann et al. 2014). It emphasizes the role of motivation and experience in terms of adaptiveness to negative emotions, such as anger and sadness, which may modify the cognitive and physiological responses across the adult life span yielding age effects. Chipperfield et al. (2003) demonstrated more positivity than negativity in their study while analyzing discrete emotions in very old individuals (72–99 years). They highlighted the presence of optimistic views on emotions in later life even with the presence of sadness and sickness. However, the presence of negative emotions in very old age did not undermine the presence of positive emotions. Therefore, aging is marked by a unique approach towards emotion processing despite the negative emotionality experienced in old age.

16.2 Positivity Effect in Old Age

Spared emotion regulation abilities in old age are linked to positive affect bias. The "positivity effect" reflects a relative preference for positive over negative information in attention and memory as a consequence of aging. The purpose of the positivity effect in old age is to maximize well-being and thus achieve emotionally satisfying goals (Larsen 2000). Mather and Cartstensen (2003) in their study examined attention to and memory for positively and negatively valenced faces. Their study revealed older adults displaying attention away from negative stimulus by faster detection of a dot probe appearing in the location previously occupied by a neutral face as compared to when it appeared at the location previously occupied by

a negative face. They further revealed older adults having greater memory for positive compared to negative facial expressions.

Among the various life span theories, Socioemotional Selectivity Theory (SST) by Carstensen (1992) has been the most successful in explaining the preserved nature of emotional processes with age. Socioemotional Selectivity Theory (SST) highlights the involvement of motivational shift which makes older adults orient more towards positive information and ignore negative information (Carstensen 1992; Carstensen et al. 1999). This was explained in terms of the Future Time Perspective (FTP) which stated that the perception of time plays a fundamental role in one's selection and pursuit of goals, particularly goals related to knowledge acquisition, social contact, and emotional experience (Carstensen et al. 1999). For instance, if the time is perceived as limited, then people focus more on positive affective states and close social partners. The motivational shift benefits older adults in achieving better affective well-being by optimizing their limited time left in life with emotionally and socially meaningful goals. Affective bias among older adults has been an established phenomenon in the West and is likely to vary across different cultures and populations.

16.2.1 Neuroscientific Evidence on Positivity Effect

Neuroscience research has shown a reduction in the subcortical activation in the course of exposure to negative stimuli with age coupled with increased activation in cortical areas which are responsible for executive control (Samanez-Larkin and Carstensen 2011). Moreover, it has been found that when prefrontal regions mediating cognitive control are damaged in Alzheimer's patients, the positivity effect is no longer observed (Wright et al. 2007). These patients show greater (rather than lesser) amygdala activation in response to negative pictures. It is fascinating to assume that in these patients, the cognitive control processes are no longer implicit in inhibiting amygdala activation in response to the negative stimuli. Hence, it supports the idea that emotion regulation requires cognitive control. Ochsner and Gross (2005) suggest that emotion regulation is dependent upon the prefrontal and cingulate cortex which modify the activation in the subcortical systems such as the amygdala and insula associated with the emotional response. Recently, a large interest in research has grown in exploring the cognitive demands associated with emotion regulation, focusing specifically on the positivity effect on attention and memory. Theoretically, the execution of emotion–regulatory goals needs cognitive control abilities like focusing attention, maintaining attention in spite of distraction, and suppressing unwanted thoughts (Schmeichel et al. 2008). The more cognitive resources older adults have, the better they seem to be able to selectively attend to positive stimuli and avoid negative ones.

16.3 Interaction Between Cognitive and Affective Aging

There have been a few studies to investigate the interaction between cognitive and affective aging. For example, Sasse et al. (2014) showed the presence of controlled selectivity in terms of focusing on positive information among older adults as compared to younger adults when voluntary attentional selection in a multiple item display was linked to the general ability of cognitive control. Older adults show preference for emotional goals and there is evidence for preferential allocation of attention for positive over negative stimuli in older adults (Isaacowitz and Choi 2012; Mather et al. 2004). This effect has been observed in the early stages of attention and memory among older adults using an RSVP task across emotion valence (positive negative and neutral distractors) (Kennedy et al. 2020).

In general, positive and negative emotional stimuli are both salient and attract attention more than neutral stimuli (Straub et al. 2020). However, valence along the negative-positive dimension has different neuropsychological functions. While negative emotions narrow the breadth of attention (Fredrickson 2001), positive emotions broaden the individual's scope of visual attention (Johnson et al. 2010). These latter results show that positive emotions may have an opposite effect by either enhancing or hindering performance depending on the type of executive control (i.e., cognitive, emotional). These results also align well with studies that showed positive emotions to increase distractibility (Dreisbach and Goschke 2004) and to reduce the use of informative cues in cognitive functions (Fröber and Dreisbach 2012). These findings need to be tested in the context of the trajectory of cognitive and emotional aging across the life span.

16.4 The Life Span Perspective of Cognitive and Emotional Aging

Individual development is an ongoing, lifelong and dynamic process which is driven by biological (cognitive capacity, physical health, gender), psychological (family environment, coping strategies, knowledge, self-beliefs), and sociocultural (language, healthcare facilities, social relations, financial status, social organization) factors (Baltes et al. 2006). A life span view considers that all life periods are comprised of developmental experiences that are unique to the life period the individual is in and can only be entirely understood when their relations with previous life periods are taken into consideration.

Many studies have evidenced changes in brain structure which trigger biological changes leading to a decline in old age (Lachman 2004; Debette et al. 2011). Although it is worth noticing that while studying variability in middle age, a decline in processing speed, memory, and fluid intelligence was observed thereby suggesting variability in cognition beginning from middle age (Zimprich and Mascherek 2010). Similarly, various neurodegenerative diseases were found to have a beginning from middle age while detecting very subtle cognitive impairments which are usually overshadowed by protective and compensatory mechanisms

(Sperling et al. 2011; Villemagne et al. 2013). As such the study of cognitive aging requires a shift of focus from old age to middle age which possibly marks the beginning of the aging process.

Labouvie-Vief et al. (1989) first investigated cognitive-emotional complexity across the life span while coding people's understanding of emotions and their description of themselves and other individuals. They evidenced that the emotional understanding increased many folds in middle age as compared to adolescence which included increased ability to adjust to positive and negative feelings related to oneself and others. This cross-sectional developmental trajectory of complex emotional processing and integration was further confirmed by other studies (Labouvie-Vief et al. 2007). Further investigations found a tapering effect in this cognitive-emotional complexity with age (Labouvie-Vief and Medler 2002).

There is evidence to suggest that cognitive functioning becomes immersed with affectivity in later adulthood and peak cognitive performance occurs in middle age. Adult cognitive development comprises of the reintegration of subjective information into existing knowledge structures. Conceptually, this increased complexity in cognitive operations is linked with more complex and adaptive emotional responses (Diehl et al. 1996). It is therefore of importance to examine changing emotional trajectory from middle to old age to find how early are the effects of emotional aging.

16.4.1 Examining Emotion Regulation for Positive and Negative Affect Across the Life Span: East Asian and Indian Context

Emotion regulation allows flexibility in one's emotional response based on their momentary as well as long-term goals and motivations (Gross and Thompson 2007). It is found that attention regulates emotional responses in a way to attend to the emotionally salient information over others (Todd et al. 2012). Research based on implicit regulatory processes suggests that one indulges more in implicit emotion regulation strategies like emotion conflict adaptation in situations demanding rapid responses as they involve a more unconscious modulation of emotion control in behavior evoked by the stimulus itself (Williams et al. 2009). Effortful emotion regulation is difficult to indulge in all the time. Rather implicit emotion regulation proves to be more efficient in regulating emotions most of the time in everyday life since it is automatic and hence is critical for well-being.

Explicit emotion regulation is mostly examined in terms of the use of strategies to modulate emotional responses and experience. Typically, it involves an instruction-based task seeking participants to engage in effortful down- or upregulatory process at different points of the emotion generative process. Aging studies mostly compare strategy use over positive and negative valence to examine the effectiveness of each strategy with age (Livingstone and Isaacowitz 2018; Nakagawa et al. 2017). The interplay of implicit and explicit emotion regulation processes is important for efficient affective well-being (Gyurak et al. 2011) to examine the trajectory of emotional aging across young, middle-aged, and older adults in the light of affect-based goals. In the Indian context, we examined adult age differences in implicit

emotion regulation and explicit affective experience using lab-based behavioral experimentation and self-report measures.

16.4.1.1 Shift in Affective Bias Through Cognitive Control: Effect of Aging on Emotion Regulation

There is a shift in affective bias for positive emotions in the context of a higher order control process with increasing age. Cognitive control (detection of conflict and subsequent control of conflict) is the basis of goal-directed behavior (Botvinick et al. 2001). Emotion induced blindness effect shows difficulty in detecting targets after emotional distractors (Kennedy et al. 2020). Older adults show this effect more for positive distractors giving evidence for the positivity effect in early attention. Berger et al. (2019) found no difference between Young and Older adults in deploying cognitive control for positive affect where proactive control was manipulated by varying the proportion of congruent and incongruent trials. In the Indian context we examined age-related differences in affective conflict adaptation (measured in terms of trial congruency sequence effects) as a measure of upregulation of cognitive control in case of positive and negative affect using the Face word Stroop task (Nigam and Kar 2021). Results showed a shift towards positive affect for conflict-driven cognitive control in middle-aged and older adults. Stronger conflict adaptation effect (greater upregulation of cognitive control) was observed in young adults for fear and among middle-aged adults for happy emotion (see Fig. 16.1). Older adults showed a similar trend. These findings can be explained in terms of the SST to suggest a priority shift in motivation across the life span as a consequence of time left in life thus resulting in a preference for positive affect and its influence on the recruitment of proactive control in older adults. The effect of positive affect on

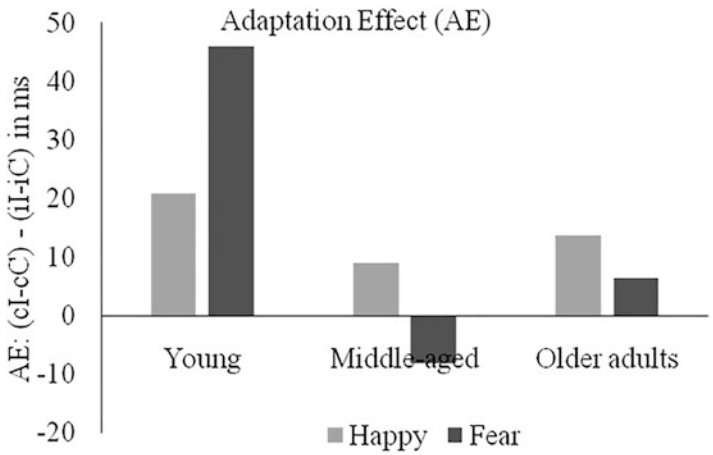

Fig. 16.1 Adaptation Effect across young, middle-aged, and older adults for the emotions Happy and Fear. *Note*: AE: Adaptation effect; [(cI-cC) – (iI-iC)]: cI = previous congruent-current incongruent); cC = previous congruent-current congruent); iI = previous incongruent-current incongruent); and iC = previous incongruent-current congruent)

adaptation effect in older adults could also be understood as a processing advantage related to the broadening of attention and involvement of reactive control mechanisms for positive affect with less cognitive and affective complexity.

16.4.1.2 Effect of Aging on Emotion Regulation, Affect Representation, and Affective Experience

Research shows stability or rather an improvement in emotional well-being (EWB) with an increase in age (Riediger and Rauers 2014). However, the findings on improvement in EWB with aging in the Asian population are largely inconsistent (Fung 2013). Grossmann et al. (2014) showed smaller improvements in emotional experience of Japanese aging individuals. However, Yagi et al. (2020) showed evidence for positive affect bias and experiencing pleasantness in the context of subjective well-being in Japanese individuals. In the Indian context, using self-report measures and experience sampling method, our preliminary results suggest higher magnitudes of positive emotion experience and lower magnitudes of negative emotion experience in middle-aged and older adults compared to young indicate a positive trajectory of EWB compared to young adults (see Fig. 16.2) also supported by previous work (Mather 2012; Carstensen et al. 2011). In addition, greater reports of prohedonic motivation (maintaining positive affect) for positive emotion was observed with an increase in age ($p = 0.009$) (see Fig. 16.3).

The results presented in Fig. 16.3 are consistent with those observed in the European population showing age-related increase in maintaining positive well-being (Riediger et al. 2009) and greater prohedonic motivation in old age (Riediger et al. 2014). Prohedonic motivation is stated as a regulatory process used by middle-aged and older adults to maximize subjective well-being (Carstensen et al. 2006; Larsen 2000). Overall, bias towards positive affect and away from negative affect sustain in middle-aged and older adults (Charles et al. 2001; Riediger and Rauers 2014) in the Indian context as evident in the context of the effect of positive emotion on recruitment of cognitive control as well as explicit self-report measures. The

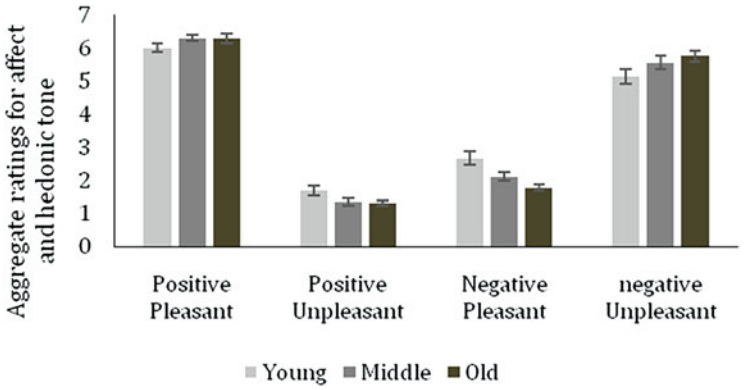

Fig. 16.2 Ratings for feeling-based preferences for positive and negative affect as pleasant or unpleasant across young, middle-aged, and older adults

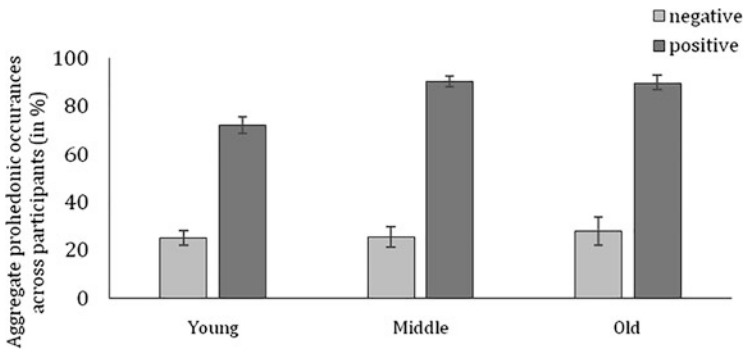

Fig. 16.3 Aggregate occurrences of prohedonic motivation for positive and negative affect among young, middle-aged, and older adults

self-reported preference for pleasant hedonic tone for affect valence was observed among middle-aged and older adults along with significantly increased frequencies for experiencing positive affect suggesting an emerging motivation towards emotionally satisfying goals and maintaining EWB, beginning from mid-life.

16.5 Why Do We Find Positivity Effect with Aging?

Positive affective experience reported among older adults is mostly explained in terms of better or more adaptive emotional regulation with an increase in age. There is robust evidence to suggest that older adults report greater experience of positive affect and less experience of negative attention. These findings have been replicated across cultures including western and eastern populations. Better emotion regulation could be understood in terms of better ability to shift strategies of emotion regulation or implementation of strategies or better ability to adapt or select/match the strategy to the context/situation. These effects have been tested through lab-based experimentation and experience sampling methods. However, both methodologies have limitations and have not consistently found age-related differences in positivity effect. Isaacowitz (2022) in his recent review suggests that it could be a difference in preference for positive affect and selection of strategy that favors positive affective experience on one hand and effectiveness in terms of successful use of the strategy on the other which defines better emotion regulation in older adults. Wang et al. (2020) reported older adults in both the UK and China showed a preference to focus on and remember pleasant pictures, providing evidence of a positivity effect in both cultures. Older adults are known to use attention deployment as the most effective strategy than reappraisal compared to younger adults (Demeyer et al. 2017). Evidence also suggests that affective experience-related age differences are less likely due to differences in emotion regulation. Therefore, although we have convincing evidence in favor of positivity effect yet the explanations for the same are not well routed in the age-related differences in emotion regulation. Our findings

related to the interaction between emotion valence and recruitment of cognitive control suggests the involvement of attentional control mechanisms.

16.6 Implications and Strategies for Building Resilience and Cognitive Reserve as a Function of the Interaction Between Cognitive and Affective Aging

Aging research has shown that although brain plasticity decreases with age, there is growing evidence that old age is indexed by a limited but large amount of biological, cognitive, motivational, and emotional plasticity and reserve capacity. With increasing evidence for accelerated physical and brain aging in old age, there is a growing need to identify other important dimensions, such as resilience, that may be probable intervention targets to enhance one's potential for better outcomes with age and build cognitive reserve across the life span, despite the potential for worse physical functioning.

16.6.1 The Cognitive Reserve Hypothesis

The cognitive reserve hypothesis concerns the ability to improve and maximize cognitive performance. Passive reserve, also known as brain reserve is a term which refers to genetically determined characteristics like brain volume and the number of neurons and synapses present, which allow for more efficient use of preexisting brain networks. In contrast, the concept of cognitive reserve depends on the activation of alternative brain networks holding the capability for plasticity and reorganization in neural processing to compensate for numerous neuropathologic changes. It refers to how flexibly and efficiently one can make use of available brain reserve. The cognitive reserve hypothesis states that some individuals have a greater ability to bear against pathologic changes to the brain, like accumulation of amyloid protein resulting from greater brain reserve (Stern 2002). This hypothesis posits that higher levels of education, participation in certain activities, higher socioeconomic status, and baseline intelligence protect against the clinical reflection of brain disease (Fotenoz et al 2008).

16.6.1.1 Lifetime Experiences and Cognitive Reserve

A combination of lifetime experiences, such as educational and occupational attainment, occupational complexity, literacy attainment, bilingualism, and involvement in cognitive and socially stimulating activities, are thought to increase the effectiveness of cognitive processing in later life. As no single direct cognitive, functional, neuronal, or structural measure of cognitive reserve exists (Stern et al. 2019), a number of psychosocial, clinical, and demographic variables are used as proxies (Leary et al. 2018; Stern et al. 2018). For example, demographic measures such as higher levels of education are associated with a lower incidence of mild cognitive impairment. Education has been associated with the generation of cognitive

strategies (Manly et al. 2004), with synaptic growth (Katzman 1993), and with reduced cognitive decline in the elderly (Yaffe et al. 2011), and should therefore provide a reliable measure of cognitive reserve. Studies have shown that higher NART IQ (National Adult Reading Test, Nelson and Willison 1991), a valid assessment for literacy attainment is associated with greater CR capacity (Stern 2012) as well as with episodic and working memory (Jefferson et al. 2011). Paplikar et al. (2020) validated the Lifetime of Experiences Questionnaire (LEQ; Valenzuela and Sachdev 2007) with the Indian cultural context. It is a comprehensive instrument to assess lifetime cognitive activities (Kartschmit et al. 2019), combining three major aspects of cognitive reserve activities—educational attainment, occupational complexity, and lifetime engagement in cognitive and social activities across age. Studies have found that the elderly who participated more in complex mental activities in middle and late adulthood have better cognition (Karp et al. 2009). The differences in mid-life scores in LEQ among healthy elderly and dementia groups suggest that there is a protective effect against dementia coming from cognitively stimulating activities undertaken in mid-life (Chan et al. 2018). The negative correlation between clinical dementia rating scores and young adulthood, mid-life, and total scores on LEQ also suggests that a more active lifestyle predicts less severity of dementia and thus lifetime experiences have a protective role against cognitive decline in late life.

Combining evidence from all these studies, they show that CR proxy variables reduce cognitive decline in case of healthy aging as well as in case of cognitive disorders like dementia. Primarily, cognitive reserve continues to evolve across the life span and is not fixed. In the last decade or more, certain other factors such as bilingualism have also been found to contribute to cognitive reserve.

16.6.1.2 Bilingualism and Cognitive Reserve

Large amount of evidence shows that the use of second language promotes the maintenance of cognitive and neural efficiency during old age (Del Maschio et al. 2018). The first indication that supports the role of bilingualism for cognitive reserve comes from studies linking multiple language use to enhancements in executive control (EC) across the life span. Initial evidence indicated that bilinguals outperform monolinguals in EC tasks across different age groups (Bialystok 2017). Apart from EC tasks, now plenty of evidence reports that older bilinguals do maintain better cognitive efficiency, outperforming their monolingual allies on different cognitive measures (Rosselli et al. 2019; Zunini et al. 2019), and also extending to executive-related memory recall tasks (Rosselli et al. 2019), semantic memory (Arce Rentería et al. 2019), and general intelligence (Bak et al. 2014). This evidence posits an explanation as to why bilingualism could act as a cognitive reserve (Bialystok 2017). Older individuals, particularly those in retirement, are not exposed to so many cognitively challenging activities anymore; therefore, the effect of bilingualism on their cognitive performance is stronger (Valian 2015), if the speaker is still exposed to her second language and actively uses it (Del Maschio et al. 2018).

Bilingualism indeed promotes reserve in healthy aging peers, as its effects are visible when the elderly have to face the burdens of cognitive decline more severely,

like, in dementia. Many studies compare clinical differences among bilingual and monolingual seniors. The milestone work by Bialystok et al. (2007) showed an evident 4 years of delay in the onset of dementia for bilinguals when compared to monolingual peers. Alladi et al. (2013) replicated the study in India on a large sample of 648 individuals and found a 4–5 years delay in the onset of dementia in bilinguals compared to monolinguals.

Grundy et al. (2017) devised a model called bilingual anterior to posterior and subcortical shift (BAPSS), stating that compared to monolinguals, expert bilinguals rely less on the recruitment of frontal and executive regions and more on the posterior/subcortical regions to perform EC tasks. Bilingual elderly are better able to make a shift from late, more demanding, top-down processing, to more early-automatic processing because they have a lifelong experience of controlling two languages simultaneously. This line of work explains the mechanisms underlying the neuroprotective effect of bilingualism in aging: less dependence on frontal regions and more on subcortical/posterior regions would help bilinguals to maintain required cognitive performance by providing more resources that can be utilized with increasing task requirements.

16.6.2 Cognitive Interventions and Resilience

Psychological interventions can lead to positive changes. Cognitive interventions depend on rigorous exercise in cognitive operations like inductive reasoning and episodic memory. The aim is to enrich daily cognitive functioning and everyday competence (Willis et al. 2006). Importantly, the current cognitive interventions have been shown to promote specific cognitive skills (like mnemonic strategies) rather than fluid intelligence independently.

There is evidence to show that stimulating activities protect against dementia. For example, Wang et al. (2002) used data from 1987 to 1996, a longitudinal population-based study to examine whether engagement in different activities in the preceding 6.4 years leading up to dementia diagnosis was related to a decreased incidence of dementia. It was found that there existed an inverse relationship between frequent engagement in mental and social activities and dementia incidence. This finding also shows that resilience is not only a "naturally occurring" phenomenon, but that, it can be supported and strengthened by individual lifestyles, and by "age-friendly" social environment. In addition, physical activity interventions with muscle strength training and aerobic exercise prove to have positive effects on social and cognitive well-being. These interventions have proven to show an improvement at the brain level, particularly in the strength of synaptic connections (Colcombe et al. 2006).

In case of aging-related cognitive disorders, for instance, training with individuals with Mild cognitive impairment (MCI) using interactive imagery, the method of loci, face-name associations, hierarchical and semantic organization techniques showed positive effects of training in subjective memory and well-being (Belleville et al. 2011). Also, individuals with MCI were able to recruit new neural circuits to perform the demanding memory tasks compared to healthy older participants who did not

receive the intervention. In addition, clinical geropsychologists have contributed to neuro-rehabilitation programs for older people with cognitive linguistic therapies for deficits related to language after stroke in the left hemisphere. Interventions for those with traumatic brain injuries resulting in functional communication deficits, training in problem-solving, goal management, and self-monitoring strategies for the elderly with executive functioning deficits are also significant contribution (Kennedy et al. 2008).

16.6.3 Emotional Aging and Cognitive Reserve

Emotional aging (emotion regulation motivation) may serve as a protective factor as long as the cognitive control system is intact. Emotional well-being can enhance the control system by building resilience or compensation. Neuroimaging studies suggest that amygdala activity does not decline with age; however, amygdala reactivity to negative stimuli declines with age. This downregulation is mediated by control processes implemented by brain regions like Anterior Cingulate Cortex and medial prefrontal cortex (Mather 2012) suggesting the interaction between the underlying mechanisms of cognitive and affective aging.

Most of the work investigates the effect of cognition/cognitive reserve on emotion regulation in older adults. However, less is known about the relationship between cognitive reserve and emotional well-being. Researchers examined the effect of different CR proxies (education, literacy, occupation, stimulating activities) on unimodal and multimodal emotion recognition and found that CR proxies do not predict performance on emotion recognition tasks (Guerrini et al. 2022). However, a few earlier studies demonstrated a significant relationship between education as a CR proxy and emotion recognition in older adults (Demenescu et al. 2014) suggesting inconsistent findings related to CR and emotional aging. Further, it is not established yet if the positivity effect or enhanced emotion regulation motivation for positive affect could also aid in building cognitive reserve.

16.6.4 Implications of Emotional Aging for Cognitive Reserve

With growing age and especially after entering into the elderly phase, changes at the cognitive level are small and might not always result in impairment of function but participation in certain activities, trying to build cognitive reserve. However, it is found that the regulatory processes specifically with respect to cognitive control are sensitive to age-related changes. In the recent past, aging research has seen an enormous increase in the age-related strategic shift towards emotional goals, where, as age advances, emphasis on emotional life goals increases given the corresponding reduction in life expectancy (socioemotional selectivity theory, Carstensen 2006). This set of strategic adjustments in emotional processing has been argued to depend on the availability of cognitive resources particularly cognitive control (Mather and Knight 2005) in order to observe the age-related positivity

effect on cognitive performance. This idea is supported by the fact that cognitive reserve has an impact on the age-related positivity effect in memory. For instance, Bruno et al. (2021) found that age was negatively correlated with the recall of positive words in participants with fewer years of education. Positivity effect also influences emotion regulation motivation (prohedonic motivation for positive affect) and in terms of the use of emotion regulation strategies.

Emotion regulation involves choosing strategies that match emotion goals. People who are motivated to lessen emotional intensity in a situation, select strategies which aid in decreasing emotions (e.g., distraction), while those who are motivated to increase the emotional intensity, select strategies that aid in increasing emotions (e.g., rumination). In general, attention deployment/distraction (engaging more with positive/less with negative content) is a more preferred strategy for negative affect, used by the elderly compared to the reappraisal strategy which requires greater cognitive effort (Isaacowitz et al. 2009).

Allard and Kensinger (2014) also showed that some emotion regulation strategies are effective in aging. Older adults use selective attention and cognitive reappraisal for regulating their emotions, exhibiting greater dorsolateral prefrontal activity to passive viewing of negative video clips. Activity in dorsolateral prefrontal cortex was seen to be increased for reappraisal compared to selective attention strategy in older but not young adults, reflecting compensation for less efficient cognitive control processing in aging. In line with this interpretation, the timing of reappraisal-related activity in ventrolateral PFC was found to be delayed in older adults.

Spontaneous engagement of emotion control regions aid in emotion-regulatory benefits. Studies show that high-arousing information automatically captures attention (Armony and Dolan 2002), which is preserved in aging (Mather and Knight 2005) whereas low-arousing negative stimuli engage controlled processes (Kensinger and Corkin 2004). Dolcos et al. (2014) found that when older participants viewed emotional pictures, which varied in arousal, they engaged more automatic processes when evaluating high-arousing negative information, and more controlled processes in response to low-arousing negative information, also supported by the variations in amygdala and ventromedial prefrontal activity. Connecting brain and behavior, engagement of emotion control regions may result in the reduction in subjective experience of low-arousing negative information among the elderly, supporting the idea of activation of emotion regulation in aging. Such age-related changes in affective experience and regulation across the life span are expected to influence cognitive functioning and socioemotional well-being.

16.6.5 The Affective Reserve Hypothesis

Given the findings related to preserved emotional processing and emotion regulation also in the context of attention and memory in the elderly, the overall hypothesis is that cognition and affect show interdependent effects of aging as early as middle age until late adulthood. Therefore, we propose the *affective reserve hypothesis* based on

the findings in the literature and also observed in our work on positivity effect in the Indian context in terms of stability in positive affective experience and the effect of positive affect on cognitive processes such as attention, memory (Kennedy et al. 2020), and proactive cognitive control (Nigam and Kar 2021). If cognitive reserve builds resilience (Livingston et al. 2020), then affective reserve is also expected to build emotional resilience (ability to adapt to stressful situations).

Such resilience may also influence cognition and neuroplasticity by strengthening the connectivity between emotion networks and default mode networks (responsible for self-referential processing and autobiographical memory). There is evidence to show enhanced connectivity between amygdala and medial prefrontal cortex at rest for memory positivity (better learning for positive emotional faces) among older adults which was not observed in young adults (Sakaki et al. 2013). These findings suggest that top-down control processes mediated by the prefrontal cortex aid in shaping positivity effect in memory in the elderly. One possible reason for the enhanced connectivity between resting state networks and positivity effect could be that emotion regulation networks may be chronically activated in older adults to support emotion regulation goals towards positive stimuli.

The preference for positive affective experience, memory positivity, and motivation for emotion regulation is critical to adaptive aging similar to the cognitive reserve. The concept of affective reserve is not yet established in aging research; however, the existing findings across cultures and contexts suggest preserved affective processing and regulation motivation in the elderly. Future research in this direction needs to examine the causal mechanisms for affective reserve and explore ways to build affective reserve across the life span for successful aging.

16.7 Conclusion

Trajectory of emotion aging appears to be a life span effect. Locus of cognitive effects as they interact with emotion aging needs to be better understood. Convergence of quantitative and qualitative measures may strengthen the effects observed. Cognitive/neuroplasticity as a function of emotional aging may influence the cognitive reserve primarily through longitudinal studies. Adopting a life span perspective provides a more complete picture when cognitive costs of emotion regulation are observed. Motivation and long-term practice in regulating emotions can decrease the amount of resources necessary to maintain or regain emotional well-being, while performing well at other cognitive tasks. Growing older has the adaptive potential to reduce the cognitive costs of emotion regulation, further corroborating findings of higher emotional control with age.

Finally, an interesting question to answer is that how do older adults maintain high levels of well-being after they are confronted with bodily deterioration, increasingly frequent health problems and memory failures, and losses in mobility and in social worlds? One possible explanation as discussed in this chapter is related to an increasing motivation to regulate emotional states and increasing competence to do so. In addition, factors such as life experiences, education, occupation, bilingualism,

and positivity effect may moderate the effect of aging on one's cognitive-socio-affective well-being.

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Tissue Reconstructive and Regenerative Medicine Approach as an Anti-Aging Intervention: Relevance to Age-Induced Osteoarthritis

17

Parichita Mishra and Bhisham Narayan Singh

Abstract

Aging is a dynamic, irreversible, and inevitable physiological process associated with gradual deterioration in the overall performance of all the physiological system of human body. Everyone will undergo the process of aging, which makes the aged population more vulnerable to a plethora of age-associated disorders. One such prevalent disease is Osteoarthritis, which affects approximately 528 million people (as of 2019), and these numbers are predicted to increase as the population grows and ages. The progression of Osteoarthritis renders a great deal of pain and discomfort to the individual and limits their mobility significantly. This chapter contains four sections discussing the prevalence, causes, and prognostic options for age-induced Osteoarthritis. The underlying physical, biochemical, and molecular mechanisms leading up to the formation of an osteoarthritic joint are also explored. Furthermore, existing surgical, nonsurgical, and alternative treatment methods are discussed with an emphasis on the emergence of tissue reconstructive and regenerative medicine as a potential approach.

Keywords

Aging · Articular cartilage · Hydrogel · Osteoarthritis · Tissue engineering

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17.1 Introduction

Aging comes from the Latin word "*aevum*," meaning "lifetime," and is the gradual degradation of various biological functional systems essential for the sustenance of the human body. Poor coordination of the five vital senses alongside a slight decrease in height, sluggish metabolism, increased susceptibility to infections and diseases, wrinkling and sagging of skin, greying of hair, etc., (Urtamo et al. 2019) can also be seen. As of 2019, the United Nations has estimated that the average global life expectancy is 72.6 years. Hong Kong has the highest average life expectancy of 85.29 years, and the Central African Republic has the lowest average life expectancy of 54.36 years (Roser et al. 2013). The rate of progression of aging and an individual's average life expectancy depends on many factors such as gender, habitat, diet, access to healthcare, genetics, and lifestyle choices (Jang 2020). The onset of diseases such as atherosclerosis, hypertension, obesity, anemia, dementia, diabetes mellitus, cancer, cardiovascular disease, chronic obstructive pulmonary disease (COPD), gastrointestinal disorders, chronic kidney disease, cataract, osteoporosis, and Osteoarthritis accelerates with age (Zhang and Jordan 2010; Global RA Network 2021; Franceschi et al. 2018). Arthritis globally has more than 350 million as of 2021, with women being the most susceptible to developing Osteoarthritis beginning in their mid-40s (Global RA Network 2021). Osteoarthritis of the knee is twice as prevalent in women aged 60–79 years compared to their male counterparts (Global RA Network 2021). A future projection study conducted by the National Health Interview Survey between 2013 and 2015 predicts an increase in the incidence of Osteoarthritis as the population grows and ages, estimating that at least 78 million people will be diagnosed with Osteoarthritis by 2040 (Centers for Disease Control and Prevention 2003).

Cartilage tissue is a type of connective tissue that provides support and structure to the other vital internal organs in the body. It is also essential in developing long bones in children and adds to the mechanical strength of the different joints in the body by reducing friction between the bone surfaces (Benedek 2006). Joints such as the knees, hip, and thumbs, as seen in Fig. 17.1a, mainly contain hyaline or articular cartilage and are prone to developing osteoarthritis (Benedek 2006; Onishi et al. 2012). Some of the most typical symptoms of Osteoarthritis are pain during ambulation and limited or diminished range of flexibility during flexion, extension, and rotation of the joints, which causes extreme discomfort in the individual (Onishi et al. 2012). Such disparity in joint health could result from several factors such as aging, injuries, overloading and underloading of joints, and structural deformities during development, metabolic, and inflammatory diseases, infections (Onishi et al. 2012).

Diagnosis of Osteoarthritis is made by performing physical examinations, arthroscopies, and blood tests for inflammatory factors. X-rays of joints are evaluated to assess joint damage and degeneration. The clinical and laboratory diagnosis of knee osteoarthritis is made based on the standards set by the American College of Rheumatology (ACR) in 1981, and a potential patient must meet at least five of the nine characteristics other than chronic knee pain, as seen in Fig. 17.1b

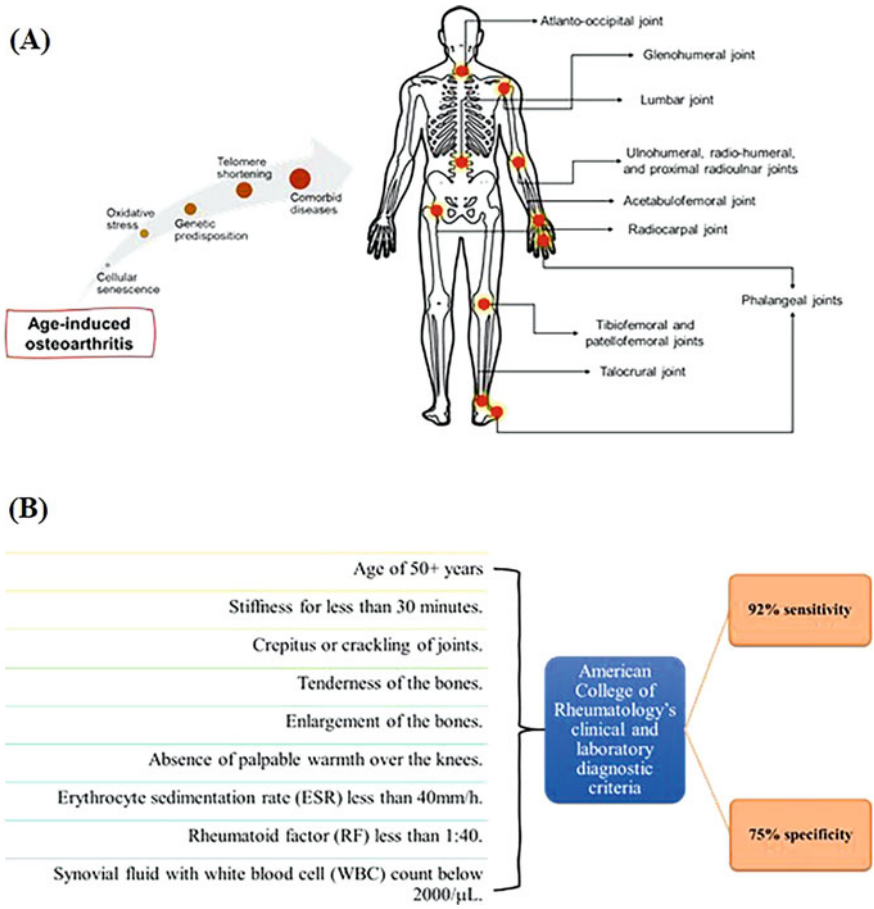

Fig. 17.1 (**a**) The various causes of age-induced Osteoarthritis and the numerous synovial joints (Benedek 2006; Auley et al. 2017) it can manifest in. (**b**) The clinical and laboratory diagnostic criteria as specified by the American College of Rheumatology (ACR) (Altman et al. 1986)

(Altman et al. 1986). A wide range of treatment options exists for targeting the pain inflicted on the joints. Patients can opt for changes in their lifestyle, surgical therapy, and medical intervention depending on the severity of their condition. A quickly evolving approach to combating Osteoarthritis is offered by the field of tissue reconstructive and regenerative medicine. It aims at providing a potential therapeutic translational outlook to generate suitable bio-substitute to restore the function of a lost tissue or organ. It also eliminates the dependency on donor organs and significantly reduced the risk of organ rejection. A tissue-engineering application also allows for creation of custom regenerative options for patients.

17.2 Changes in Articular Cartilage Joints Associated with Age-Induced Osteoarthritis

17.2.1 Structure, Component, and Function of Articular Cartilage

Although Osteoarthritis can affect people from all age groups but most prevalent in individuals above 50 years of age. Articular cartilage is a specialized type of avascular, aneural, and alymphatic hyaline cartilage found in diarthrodial joints or joints containing a synovial fluid reservoir (Fox et al. 2009). It is bluish, semi-transparent, and homogenous inconsistency with unique properties. Its superior load-bearing capacity, balanced weight distribution, and elevated shock absorption properties reduce friction between joints. The synovial fluid facilitates the growth and maintenance of articular cartilage by allowing sustained diffusion of nutrients and oxygen to the tissue (Fox et al. 2009).The ECM of articular cartilage is composed of water, collagen type-II, integrin, aggrecan, and small amounts of non-collagenous proteins, phospholipids, and chondronectin (Muir 1995). Water is the major component of the articular cartilage ECM and contributes up to 80% of the tissue's wet mass and is responsible for the tissue's superior load-bearing capacity. Water flow in the tissue is essential for the appropriate distribution of micronutrients to the chondrocytes and provides ample lubrication to the joint. Water is present inside a semi-solid, partially cross-linked gel in the interfibrillar space and does not exhibit any flow in a steady-state system. However, in a dynamic state system it exhibits a certain extent of flow via diffusion (Fox et al. 2009; Buckwalter et al. 1990). This gel-like characteristic is imparted by a combination of gradient pressure applied to the water and the frictional resistance offered by water flow in the ECM (Buckwalter and Mankin 1997; Maroudas et al. 1991).

Articular cartilage is rich in collagen type-II, which makes up about 90–95% of the total collagen mass in the ECM (Fox et al. 2009; Muir 1995). The COL2A1 gene codes for three pro-α1 type-II chains that form a homotrimer procollagen molecule (Ricard-Blum 2011). This procollagen molecule is processed to enable cross-linking and fibrillation, resulting in a mature collagen type-II molecule around the chondrocytes present in the articular cartilage ECM (Holmes et al. 2018). Collagen type-II molecules interact with proteoglycans and hyaluronan to provide the appropriate osmotic environment for normal joint function (Buckwalter et al. 1990; Eyre et al. 2006). Proteoglycans are glycosylated proteins made of multiple glycosaminoglycan (GAG) molecules covalently bonded to a core protein. GAGs are long polysaccharides with repeating disaccharide units joined by varying glycosidic bonds. GAGs have sulfate residues which make them highly polar and negatively charged (Hardingham and Bayliss 1990). Aggrecan enhances chondrocyte cell-cell and cell-ECM interactions, which are essential for the healthy growth and maintenance of tissue ECM. Leucine-rich proteoglycans like decorin and biglycan modulate the activity of crucial growth factors and other cell signaling molecules. Glycoproteins like chondronectin are also present, which mediates the binding of the collagen type-II molecule to the chondrocytes (Fox et al. 2009; Buckwalter et al. 1990; Hardingham and Bayliss 1990).

In human beings, articular cartilage thickness can range anywhere from 2 to 4 mm, supported by a distinctive zonal architecture (Fox et al. 2009). The articular cartilage is distinguished into three zones based on its unique architecture, namely—the superficial zone, the transitional zone, and the deep zone—which cover approximately 10–20%, 40–60%, and 30–40% of the total articular cartilage thickness, respectively (Fox et al. 2009). The superficial zone is preceded by the articular surface, which directly contacts the synovial fluid. In contrast, the deep zone is succeeded by the calcified zone of the tidemark, the subchondral bone, and the cancellous bone, respectively (Fox et al. 2009; Buckwalter et al. 1990).

The superficial zone has a dense tangential arrangement of supine chondrocytes and collagen type-II fibrils. Moreover, the superficial zone bears the major brunt of the various forces experienced by articular cartilage during articulation. The tangential arrangement of the collagen type-II fibrils imparts excellent tensile strength to the superficial zone (Eyre et al. 2006). The chondrocytes have a spherical morphology in the transitional zone. They are sparsely distributed surrounded by water molecules and thicker collagen type-II fibrils. The zonal architecture of the transitional zone's ECM is responsible for providing resistance to compressive forces. The deep zone has the highest proteoglycan content and negligible water concentration. The collagen type-II molecules are densely packed into thick fibrils and are arranged in a columnar fashion with the chondrocytes, anchored perpendicularly to the calcified zone of the tidemark. The chondrocyte population is nominal but highly hypertrophic. This unique orientation of the ECM molecules in the deep zone plays an integral role in providing the most significant amount of resistance to the various compressive forces experienced by the articular cartilage (Fox et al. 2009).

The progression of age-induced Osteoarthritis is marked by the observable structural changes in the articular cartilage matrix (Hunter and Felson 2006). These significant structural changes in the articular cartilage tissue can be attributed to the increased stiffness and brittle nature of the tissue matrix, which results in mechanical failure of the joint (Hunter and Felson 2006). Articular chondrocytes possess a poor mitotic index with poor regeneration potential. Once the cartilage damage becomes imminent, various pro-inflammatory pathways are activated in the synovial fluid to mitigate the debris leftover by the degenerating articular cartilage tissue (Loeser 2011). Although, synovial inflammation is a defense mechanism triggered by the body to limit further cartilage tissue matrix deterioration. However, this process is ultimately futile as it results in the activation of a chain reaction of inflammatory events that, in turn, causes further cartilage damage (Loeser 2011). The bones associated with articular joints undergo significant transformations, such as changes in sponginess of the trabecular bone and osteophyte formation. The friction between the joints increases significantly as the two adjacent ends of bones with osteophytes graze against each other, which leads to increased wear and tear of the articular cartilage tissue present in the joint (Chen et al. 2017).Some other symptoms of Osteoarthritis (Hunter and Felson 2006; Loeser 2011; Chen et al. 2017) include—crepitus or the popping and crunching of joints, sensory loss caused by the impingement of the nerve root and spinal stenosis, formation of Heberden and

Bouchard nodes (Thaper et al. 2005) in the hands and Baker cysts (Balik et al. 2019) in the knees, etc.

17.2.2 Cellular and Molecular Mechanisms of Articular Cartilage Formation and Its Degeneration in Osteoarthritic Joints

The establishment of the skeletal system in adult vertebrates is highly dependent on the molecular mechanisms of cartilage development at the embryonic stage (Hayes et al. 2001).The endoskeleton system creates parts like vertebrae, ribs, appendages, and the articular cartilage. In contrast, the dermal skeleton system develops into specialized bones like the clavicle, the skullcap or calvaria, flat bones of the skull and the mandible (Hayes et al. 2001). This compartmentalization brings to light the process of transdifferentiation, which links the development of the bone and cartilage. Transdifferentiation is the ability of a cell to differentiate fully/partly into different cell types without becoming pluripotent (Zhou et al. 2014).The development of the endoskeleton system is governed by a multitude of cell signaling molecules, which starts with the shapely accumulation of the skeletal progenitor cells into a "blueprint" resembling the future skeletal system, as represented in Fig. 17.2a. The differentiation of the mesenchyme into the skeletal system "blueprint" is modulated by a myriad of signaling pathways. Some include bone morphogenic proteins (BMPs), cadherin-2, and the SMAD family of transcription factors (Lim et al. 2015).

The systematic formation of cartilage is known as chondrogenesis. It is regulated mainly by the sex-determining region Y protein-related high mobility group-box (SOX) genes, specifically the transcription factors Sox 5, 6, and 9 (Kamachi 2013). Sox 9 prevents chondrocytes from differentiation into osteogenic lineages (Kamachi 2013). Furthermore, chondrogenic growth and maturation are regulated by fibroblast growth factor (FGF) (Deng et al. 1996), and prevention of chondrocyte hypertrophy is modulated by the Wnt/β-catenin signaling (Nusse and Clevers 2017) and Indian hedgehog (IHH) (Vortkamp et al. 1996).

In a healthy articular joint, the chondrocyte population maintains synthesis and degradation of the ECM (Fox et al. 2009). However, in an aged person, due to constant wear and tear of the cartilage matrix and poor regeneration capacity of the tissue, chondrocytes undergo a lot of stress at the molecular level (Hunter and Felson 2006). This stress leads to the expression of pro-inflammatory cytokines such as interferon-gamma (IFNγ), interleukin (IL) 1β and 6, tumor necrosis factor-alpha (TNFα), and ECM proteases, as shown in Fig. 17.2b (Sokolove and Lepus 2013). ECM proteases include aggrecanases-like disintegrin and metalloproteinase with thrombospondin motif (ADAMTS) 4 and 5, and matrix metalloproteinases (MMPs) allow for cartilage degeneration (Yang et al. 2017). This gives way to subsequent neurovascular invasion of the joint mediated by vascular endothelial growth factor (VEGF) and nerve growth factor (NGF) (Suri et al. 2007). TNFα also activates the nuclear factor kappa B (NFκB) pathway, which promotes the apoptosis of chondrocytes. It also increases the expression of catabolic molecules like

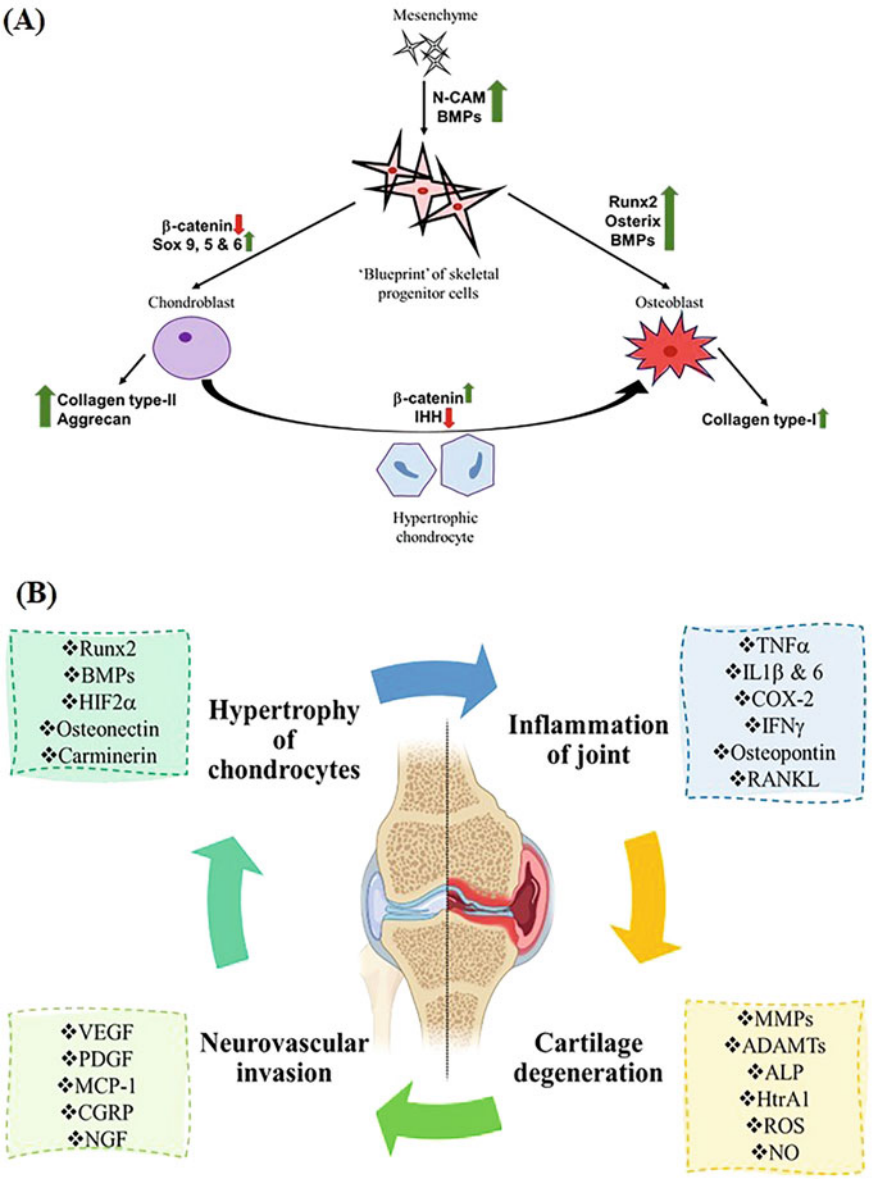

Fig. 17.2 (**a**) An overview of the essential signaling molecules governing chondrogenesis and osteogenesis from the "blueprint" of skeletal progenitor cells (Hayes et al. 2001). This signaling also highlights the transdifferentiation (Zhou et al. 2014) of chondroblasts into osteoblasts, mediated by the formation of a hypertrophic chondrocyte. (**b**) An outline of the four key features mediating the pathogenesis of Osteoarthritis in an individual alongside the salient signaling molecules associated with each feature (Hunter and Felson 2006; Loeser 2011; Chen et al. 2017) (*created with* BioRender.com)

cyclooxygenase-2 (COX-2), nitric oxide (NO), inducible nitric oxide synthase (iNOS), and prostaglandin E2 (PGE2) (Sokolove and Lepus 2013; Yang et al. 2017; Suri et al. 2007).

During this recurrent wear and tear, biomolecules like adenosine triphosphate (ATP), IL-6, nitric oxide (NO), prostaglandins, etc., are released, which sensitizes the nociceptors (Mease et al. 2011). The signals generated by the nociceptors further activate various neurotransmitters like—encephalin, noradrenaline, and serotonin. These neurotransmitters amplify the pain signals generated and experienced (Suri et al. 2007; Mease et al. 2011). Once the pain becomes unbearable, the individual may choose to reduce their mobility, which momentarily eases the pain. The static condition and vascular infiltration also influence the remodeling of ECM and the formation of fibrocartilage (Hunter and Felson 2006; Vincent and Wann 2019). The degradation of hyaline cartilage further reduces articulation in the affected individual. If the individual chooses to continue with their regular activities by bearing the discomfort, the degeneration of the joint rapidly progresses. The joint becomes highly inflamed and sensitized to pain thus becoming osteoarthritic (Vincent and Wann 2019).Some studies have also attributed mutations in the carbohydrate sulfotransferase 3 (CHST3) gene, fibrillin-2, latent transforming growth factor-beta binding protein 3 (LTBP3), matrix GLA protein (MGP), and vitamin D receptor (VDR) to genetically predispose an individual to develop Osteoarthritis with age (Boer et al. 2021).

17.3 Conventional Osteoarthritis Treatments and Their Limitations

The Kellgren-Lawrence grading scale was established in 1957 and accepted by the World Health Organization (WHO) in 1961 as the standard system for radiological characterization of the different grades of Osteoarthritis (Kellgren and Lawrence 1957). The Kellgren-Lawrence grading scale probes for the narrowing of joint space, formation of bone spurs or osteophytes, abnormal thickening or sclerosis, and deformities at the ends of bones (Kellgren and Lawrence 1957). Abundant prognostic choices are available to diagnose age-induced Osteoarthritis. Still, the available treatment options possess a limited therapeutic potential. They provide only a temporary solution to the problem, such as relief in pain and stiffness, with a high possibility of reoccurrence.

The nature of degeneration reported in age-induced Osteoarthritis is irreversible, and thus remedial treatment strategies have been considered the first line of approach. Moreover, the overloading of joints and the activation of pro-inflammatory pathways caused by obesity poses a significant risk to joint health. Therefore, obese are advised to lose weight by either improving their dietary choices or incorporating more physical exercise to combat their sedentary lifestyle (Connelly et al. 2015). Activities such as water aerobics (Silva et al. 2008), physiotherapy, and strength training are well known to increase the stability and strength of the affected joints and muscles (Bannuru et al. 2019; Hochberg et al. 2012). Intermittent cold and

hot therapy, when paired with more alternative therapeutic choices like acupuncture, yoga, and homeopathic remedies (Long and Ernst 2001) like arnica, bryonia, and calcium carbonate, can also supplement as treatment options to provide temporary relief for joint pain and stiffness (Connelly et al. 2015; Bannuru et al. 2019; Hochberg et al. 2012).

Age-induced Osteoarthritis can also be hereditary and present itself with a higher risk in post-menopausal women. Post-menopausal women usually exhibit decreased levels of estrogen, which significantly increases the oxidative stress on the articular cartilage tissue; therefore, increasing the amount of wear and tear (Connelly et al. 2015). In such cases, with somewhat advanced Osteoarthritis (above grade-2 on the Kellgren-Lawrence scale), patients are refrained from pursuing strenuous exercise and physiotherapy as it will most likely be counterproductive. Such patients are usually prescribed supplements like nonsteroidal anti-inflammatory drugs (NSAIDs), steroids, glucosamines, Food and Drug Administration (FDA) approved visco-supplementary hyaluronic acid (HA) injections, omega-3 fatty acids, etc. (Hochberg et al. 2012)

Glucosamine supplements can help counteract the drop of chondroitin levels in joints and provide temporary relief in patients with Osteoarthritis. However, long-term consumption of glucosamine comes with the added risk of heartburn, nausea, weight gain, and reduced insulin sensitivity (Reginster et al. 2012). Omega-3 fatty acids supplements can help in reducing the inflammation at the osteoarthritic joints but can increase the risk of bleeding (Goldberg and Katz 2007). Steroid creams and injections like cortisone can also counter inflammation and pain but should only be used as a last resort option in case of emergencies, as frequent usage can lead to further damage to the joint because of cortisone flares, elevated levels of blood sugar, allergies, infections, and atrophy of the adipose tissue (Jüni et al. 2015).

NSAIDs have been a popular choice of FDA-approved therapeutics to combat the pain and associated with osteoarthritic inflammation. Weak nonselective NSAIDs like ibuprofen, naproxen, etc., or potent nonselective NSAIDs like diclofenac, piroxicam, etc., are prescribed (Bannuru et al. 2019; Hochberg et al. 2012). NSAIDs work by preventing the production of prostaglandins. Prostaglandins are potent vasodilators, which interact with the prostaglandin receptors to cause increased sensitivity to pain, inflammation, and inhibit thrombogenesis (Mease et al. 2011). The production of prostaglandins is regulated by the signaling of cyclooxygenase (COX) 1 and 2 enzymes (also known as prostaglandin-endoperoxide synthase), which are selectively inhibited by NSAIDs. COX-2 selective NSAIDs like celecoxib, meloxicam, etoricoxib, etc., are commonly used (Lapane et al. 2015). Although the usage of NSAIDs in treating Osteoarthritis has been approved by the FDA, there are still many adverse side effects associated with the prolonged use of these drugs, such as indigestion, irritable bowel syndrome, formation of gastric ulcers, diarrhea, retention of excessive levels of sodium and potassium, high blood pressure, nausea, and photosensitivity (Lapane et al. 2015). Thus, NSAIDs must be prescribed with great precaution as it has been reported to lead to an increased risk for strokes, heart attacks, acute renal failure, unpredictable anaphylactic reactions, and miscarriages in pregnant women (Lapane et al. 2015). A relatively risk-free

approach for short-term pain relief for patients suffering from Osteoarthritis is visco-supplementation of the articular cartilage joints by injecting synthetic HA fluids to restore its deficiency in the joints (Balazs and Denlinger 1993). FDA-approved HA gel injections, likeEuflexxa™ and Gel-One®, are derivatives of hyaluronan and hylan, and can reduce inflammation in the affected joints for up to 6 months (Balazs and Denlinger 1993).

While the mentioned first-line treatment approaches can provide appreciable relief to the affected individuals however when the damage has progressed beyond grade 3 on the Kellgren-Lawrence scale, surgical intervention is unavoidable. Thereby, relatively conservative surgical procedures like abrasion chondroplasty, autologous chondrocyte implantation (ACI), microfracture, mosaicplasty, and spongialization can be an option for an osteoarthritic joint with moderate damage (Browne and Branch 2000). However, invasive surgical intervention becomes imperative for extreme articular cartilage degeneration with acute sclerosis and deformation of the bone. Surgical procedures like arthrodesis, arthroplasty, and osteotomy are employed based on the severity of the joint damage or osteoarthritic conditions (Browne and Branch 2000).

Abrasion chondroplasty and microfracture are arthroscopic surgical techniques that allow the body cells to stimulate the innate healing process. In arthroscopic surgeries, the damaged cartilage tissues are trimmed or removed to expose the joint. These techniques are often successful for lesions of around 2 cm. In abrasion chondroplasty, the subchondral bones are superficially rubbed to reveal the intraosseous space containing the mesenchymal stem cells (MSCs). However, abrasion chondroplasty may result in the loss of the mechanical strength imparted by the underlying bone (Browne and Branch 2000). Microfracture has a relatively lesser impact on the mechanical stability of the joint as only minuscule holes ranging from 0.5–1.0 mm in diameter are made in the cartilage to release the mesenchyme. However, the regenerated cartilage tissue is mostly fibrous with inferior load-bearing capabilities (Browne and Branch 2000). ACI is also a partially arthroscopic procedure that involves the isolation of the patient's autologous chondrocytes and then injecting under a patch of the periosteum surgically sewn under at the defect site (Peterson et al. 2010). Before implantation, the autologous chondrocytes are biopsied and cultured in vitro for 6–8 weeks to obtain a healthy culture of hyaline cartilage tissue. Mosaicplasty or osteochondral autograft transplantation (OATS) (Temenoff and Mikos 2000) is another popular surgical procedure mostly recommended for patients with full-thickness (both chondral and osteal) defects of up to 10–15 mm in diameter. Cylindrical osteochondral grafts are harvested from healthy donor sites such as the lateral intercondylar notch and transferred to the defect site. This procedure is advantageous as it shows a high union rate with a lower risk of tissue rejection and disease transmission. However, it is impractical for larger lesions as the supply of viable donor sites is limited (Browne and Branch 2000; Temenoff and Mikos 2000).

Arthroplasty is the total or partial replacement of a joint with an artificial joint replacement device of strong and biologically inert metals or alloys such as chromium, cobalt, and titanium. Auxiliary defects caused by extensive joint damage can

be corrected by performing an osteotomy in which bone tissue is physically realigned to shift the stress from the affected joint or by performing arthrodesis, wherein spinal bones are usually surgically fused to make up for the lack of depleted articular cartilage. Surgical procedures like arthrodesis, osteotomy, and arthroplasty results in stiffness of the replaced joints and reduced articulation (Browne and Branch 2000; Temenoff and Mikos 2000). All the arthroscopic techniques mentioned so far have finite success as the implants largely regenerate into transient fibrocartilage tissue instead of hyaline cartilage tissue, which is mechanically inferior and can result in periosteal hypertrophy (Temenoff and Mikos 2000).

17.4 Tissue Engineering Applications for Osteoarthritis

Tissue engineering is an interdisciplinary field of biomedical sciences, and Yuan-Cheng Fung first coined the term in the late 1980s. However, it was more popularized by the duo of Robert Langer and Joseph P. Vacanti in the early 1990s. Langer and Vacanti described tissue engineering as "an interdisciplinary field that applies the principles of engineering and the life sciences towards the development of biological substitutes with the potential to restore, maintain, or improve tissue function" (Langer and Vacanti 1993). The three fundamental pillars of tissue engineering are—cells, growth-inducing molecules, and matrices that provide physical and biochemical support for the regeneration of the tissue of interest (Temenoff and Mikos 2000; Langer and Vacanti 1993).

It is essential to understand the origin and characteristics of the tissue to select suitable cell sources to generate tissue graft. During the development of an embryo to an adult, each tissue can be broadly categorized into three different lineages based on cellular ancestry (Temenoff and Mikos 2000). Ectodermal-derived tissue forms the nervous system, endodermal-derived tissue gives rise to the liver, pancreas, and parathyroid glands, while mesodermal-derived tissue gives rise to the skeletal system, gonads, kidneys, heart, and adrenal cortex (Shahbazi 2020). Mesenchymal stem cells (MSCs) are dynamic multipotent cells with extraordinary self-renewal capacity and differentiation potential into cells type of mesodermal-derived tissue (Shahbazi 2020). MSCs can be found in fetal tissue such as cord blood, liver, lungs, and spleen or in adult tissue such as bone marrow, hair follicles, adipose tissue, peripheral blood, and deciduous teeth and are involved in immunomodulation, cell homing, and tissue repair (Hayes et al. 2001; Shahbazi 2020).

The main aim of tissue engineering is to provide ample substructure to the damaged tissue to accelerate the process of healing and regeneration (Temenoff and Mikos 2000). A suitable microenvironment needs to be provided for an extended period of tissue regeneration to facilitate the successful integration of the implanted cells or cell-scaffold construct. Incorporating suitable cells and biomolecules with artificially synthesized matrices can provide a favorable biocompatible microenvironment for successful tissue regeneration. Biocompatibility is defined as the ability of a particular material to provide an appropriate biological response upon application to a specific part of the body without eliciting any form of toxic, mutagenic, or

immunogenic reaction (Langer and Vacanti 1993). The matrix is designed to mimic the physical, chemical, and biological properties of the native tissue's ECM. Moreover, some of the principal characteristic requirements are porosity, pore size, swelling, hydrophilicity, stability, biodegradability, bioactivity, and mechanical strength (Temenoff and Mikos 2000).

Hydrogels are a three-dimensional network of polymers and have good potential in their ability to mimic the ECM of different tissues. Hydrogels made of copolymers of glycolmethacrylate were first reported by the Czechia chemists Otto Wichterle and DrahoslavLím (Wichterle and Lím 1960) in 1960. Hydrogels can be synthesized using natural, synthetic, or a unique blend of such polymers. While synthesizing hydrogels for articular cartilage, they are designed to be able to mimic the chondroitin sulfate, collagen, and hyaluronic acid content present in the ECM of the cartilage tissue (Temenoff and Mikos 2000). Naturally derived polymers such as alginate, chitosan, and gelatin are highly biocompatible but are prone to rapid degradation and are mechanically inferior as they are unable to withstand long periods of continual stress and strain (Ullah et al. 2015).

Therefore, the demand for synthetic polymers like polycaprolactone (PCL), poly (lactic-co-glycolic acid) (PLGA), and polyvinyl alcohol (PVA) in cartilage tissue engineering is on the rise as they are resistant to the hydrolysis of the covalent bonds due to the steric hindrance at the site of hydrolytic cleavage based on the degree of polymerization (Ullah et al. 2015; Ahmed 2015). Synthetic polymers are also more flexible in terms of chemical surface modification and fine-tuning mechanical properties. Hydrogels can be chemically, ionically, enzymatically, or photo cross-linked under varying conditions of pH, temperature, and electromagnetic field (Ahmed 2015). Table 17.1 lists FDA-approved products that are currently available for combating Osteoarthritis.

Although a diverse range of tissue-engineered products exists that target osteoarthritis, the search for a cure continues to be challenging and elusive. While products and surgical techniques such as NOVOCART® 3D and MACI® have made substantial progress in integrating the articular cartilage microenvironment as much as possible, they still fail in restoring native joint health in the long run. This happens because of the ultimate chondrogenic differentiation of the MSCs or autologous chondrocytes into fibrocartilage instead of hyaline cartilage. It is therefore of vital importance to further study the cross-play of the various risk factors involved in the pathogenesis of age-related Osteoarthritis. This is a herculean task that can only be undertaken by further optimization and customization of existing tissue-engineered products for every individual patient.

The impact of tissue engineering in the field of tissue reconstructive and regenerative medicine is undeniable. The global market for regenerative medicine is expected to grow by 16.3% before 2030, with the Asia Pacific region having the fastest growth (Regenerative Medicine Market Research Report 2030). With a rise in the usage of smart biomaterials which respond to physical, biological, and chemical stimuli the applications are endless. The highest demand for tissue-engineered products is in orthopedic, musculoskeletal, and skin regeneration with rising demand

Table 17.1 A tabulated summary of the various patented hydrogels and tissue-engineered constructs in chronological order approved by the FDA for treatment in patients diagnosed with Osteoarthritis

Name	Year of patent approval	Inventors	Description of the product
Atelocollagen®	1986	Teruo Miyata and Toshio Taira (Koken Co. Ltd., Japan)	Atelocollagen® is an injectable aqueous collagen solution (Miyata et al. 1986). This product could be safely used in living bodies as a medical material in the pH range of 6.5–8.0 and an osmolality range of 250–320 mOsm/ KgH_2O
HYAFF-11®	2000	Gloria Cialdi (Fidia Advanced Biopolymers SRL, Italy)	HYAFF-11® is a sulfated derivative of hyaluronic acid as hyaluronate esters and salts (Cialdi 2000). The number of sulfate groups ranged from anywhere between 0.5 and 3.5 groups per disaccharide unit. The sulfated products exhibit anticoagulant properties and reduce the adhesion of cells to themselves. Therefore, HYAFF-11® can be used to formulate more advanced biomaterials that can act as drug delivery systems
NeoCart®	2009	Shuichi Mizuno, Akihiko Kusanagi, Laurence J. B. Tarrant, ToshimasaTokuno, and Robert Lane Smith (Histogenics Corporation, USA)	NeoCart® is a construct that enabled the de novo formation of a superficial cartilage layer on its surface (Mizuno et al. 2009). The product claims to repair and restore the injured, damaged, diseased, or aged cartilage to its full functionality by implanting the neo-cartilage construct containing heterologous or autologous chondrocytes (cultured ex vivo) between two layers of biologically acceptable sealants. The first layer of the sealant would be introduced into the cartilage lesion, and the second layer of the sealant would be in contact with the neo-cartilage construct. Additionally, a space-holding thermo-

(continued)

Table 17.1 (continued)

Name	Year of patent approval	Inventors	Description of the product
			reversible gel and gelatin hydrogel would also be applied to the cavity before laying the first layer of the sealant
BST-CarGel®	2012	Noah Ben-Shalom, ZviNevo, Abraham Patchornik, and Dror Robinson (Chi2Gel Ltd., Israel)	BST-CarGel® is an injectable chitosan composition which forms a hydrogel under physiological conditions (Ben-Shalom et al. 2012). The product is said to have at least one type of chitosan that is acetylated anywhere in the range of from about 30% to about 60%, and at least one kind of chitosan that is 70% deacetylated. This unique composition of chitosan goes on to form a hydrogel at 37 °C and pH 7.4, with the acetylated and deacetylated chitosan molecules having a molecular weight of from 10–4000 kDa and 200–20,000 Da, respectively. This unique composition of the hydrogel provides it with excellent lubrication properties
NOVOCART® 3D	2013	Juergen Mollenhauer and Christoph Gaissmaier (TETEC-AG, Germany)	NOVOCART® 3D is a protocol for determining the purity and potency of chondrocytes in vitro (Mollenhauer et al. 2013). The protocol involved isolating and culturing chondrocytes to check for the expression of genetic markers like BSP-2, collagen type-I, FLT-1, and IL-1β, by checking for their mRNA and protein expression levels. This allows for the identification of an appropriate chondrocyte population that could then be used for a successful transplantation procedure
Bio-Seed® C	2014	Michael D. West, Hal Sternberg, and Karen	The patent encompasses methods and compositions

(continued)

Table 17.1 (continued)

Name	Year of patent approval	Inventors	Description of the product
		B. Chapman (BioTime Inc., USA)	related to the production, identification and use of competent embryonic progenitor cell lines that can successfully partake in chondrogenesis to produce a viable population of chondrocytes (West et al. 2014). A diverse set of primordial stem cells clonal cell lines associated and isolated from the mesenchyme expressing markers like GDF5, MSX1&2, and Sox9 were purified and expanded for more than 40 passages. The chondrogenic embryonic progenitor cell lines were then further isolated and were found to be negative for CD74, CD90, CD166, HOX genes, ITGA2, KCNK2, and PITX1. It was also claimed that the embryonic progenitor cell line could successfully generate cartilage in the absence of COL10A1 expression. The cell lines isolated via this protocol could further be used in the field of regenerative medicine by impregnating the selected cell lines into synthetic or biological matrices
MACI®	2016	Vericel Corporation, USA	MACI® or matrix autologous chondrocyte implantation (Carey et al. 2020) is a modification of the ACI technique that was developed by the Swedish scientist Lars Petersen (Peterson et al. 2010) in 1987. Autologous chondrocytes from the patient's own body are cultured into a collagen membrane of porcine origin. The chondrocytes are then expanded and deposited onto a

(continued)

Table 17.1 (continued)

Name	Year of patent approval	Inventors	Description of the product
			film that is implanted into the site of articular cartilage damage. Upon successful implantation, the membrane is integrated and absorbed back into the patient's tissue

in cardiovascular regeneration and oncology (Regenerative Medicine Market Research Report 2030).

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Age-Adjustment Expertise in Rat Models of Human Diseases

18

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Abstract

Rat models are the most frequently used models in preclinical and clinical trials because of having smaller body size, easier maintenance, presenting similar metabolomic and genomic characteristics with humans. It is much easier and more ethical to sight rats' life periods in laboratory husbandry conditions due to their higher reproductive capacities and adaptive abilities. However, in different life stages like prepubescence, adolescence, etc., rats' aging rates vary differently. Age-adjustment expertise is necessary to be taken into consideration if the research purpose is related to time correlation with the human life scale and disease stage due to mentioned reasons. When mimicking human diseases in rat models, it is crucial to determine proper durations of established pathology, adjust accurate administration periods of pharmacologic approach, and decide tissue sampling time. In this chapter, we have expressed how fast rats are in different life stages compared to humans, with the term we proposed Aging Rate Coefficient (ARC), which we have derived based on multiple statistical data. We have revealed the correlation errors in many conducted research. In this respect, our chapter, besides contributing to the literature with the new term, ARC, is also capable of guiding many future researches. In order to assess adjusted human-rat age algorithms for preclinical and clinical trials, it is necessary to conduct more studies for comparable outcome measures and future strategies.

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Keywords

Age-adjustment expertise · Human-rat age correlation · Life stages · Rat models · Disease models

18.1 Prologue

With the discovery of antibiotics and the increasing use of radiological imaging methods, human life expectancy has increased significantly during the last century (Christensen et al. 2009). The direct cause and consequence of this increase are efforts to understand the pathophysiological mechanisms of aging and age-related diseases in humans. In this context, it is tried to define aging, to elucidate its mechanisms, and to produce approaches to pathologies related to aging.

Aging is defined as progressive physiological changes in an organism that lead to gradual organ systems' failure, or a decline of biological functions and of the organism's ability to adapt to metabolic stress (Dziechciaż and Filip 2014). Although there is a common definition for aging, the life frames of each species, their aging patterns, and the effects of both biological and environmental factors on species' aging processes are unique (Holliday 2007).

Epigenetic changes are modifications to DNA that regulate whether genes are turned on or off without changing the sequence of DNA. Apart from the differences caused by genetic and biological factors, genetically almost identical individuals of the same species, such as identical twins, the random and independent difference in their levels of exposure to environmental conditions causes aging processes and health spans to differ from each other. With the revealing of this fact, the relationship between aging and epigenetics began to be examined more closely (Zhang et al. 2020a).

By the beginning of nineteenth century, albino rats became the most commonly preferred animal models in biomedical research, because of their recognition as the preeminent model for mammalian systems. However, the proper correlation between the age of rats and humans is still a subject of debate (Andreollo et al. 2012; Agoston 2017). All the differences between species should be taken into account in experimental animal studies in which one species is used to comment on another species or in which another species is mimicked.

Modeling animals for experimentation helps us to understand the pathological roots of human diseases. Rats are the most commonly used animals in experiments. Rat models have provided important insights into disease mechanisms and helped to identify drug targets (Agoston 2017). Hence, it is critical to know the human equivalent of rats' biological status and age at any given time. For this purpose, in this chapter, we will explain the approximate estimation of the rat-human age correlation and the biological differences between rats and humans.

18.1.1 Rats and Humans: Lifecycle of Two Species

There are more than 60 species of rats worldwide. However, in scientific studies, standardized rats produced in laboratories are used in order to prevent data differences arising from biological differences between species (Reinagel 2015). In recent years, Wistar rats and Sprague-Dawley rats have become the most widely used laboratory races worldwide (Hayward et al. 1997; Krinke et al. 2000). Therefore, all the information in this chapter refers these two rat species.

Estimation of the age correlation of rats with humans requires a wide variety of parameters. The main parameters to be considered are weight comparisons, staging of rat cerebral cortex development, and time to reach sexual maturity and musculoskeletal maturity. The lifespan of laboratory rats has been reported to be almost 3 years and the longevity is higher in female Wistar rats (2.2–3.7 years) than in male ones (1.7–3.2 years) (Ghasemi et al. 2021). To the calculations based on birth weight, it is accepted that the first 12 day of rat life are probably most comparable to the late gestational period of humans as rats' birth weight is approximately 1% of adult rat weight while human's is approximately 3% of adult human weight. Similar results are obtained by examining the development of the cerebral cortex. According to Romijn et al.'s multiparameter studies, the cerebral cortex of a newborn human is most developmentally comparable to that of a 12–13-day-old rat (Romijn et al. 1991). Calculations that take into account the time to reach sexual and musculoskeletal maturity are somewhat more complex. Since the prepubescence period of rats is very short, they reach sexual maturity very quickly. On average, rats possess the ability of giving birth in 6–8 weeks. It is known that female rats enter menopause around 18 months, this period corresponds to the age of 45–50 in humans. Considering musculoskeletal maturity to determine adulthood in rats is interestingly confusing because, unlike humans, rats' long bones do not have epiphyseal closure. According to studies, a period of reduced skeletal growth in Sprague-Dawley rats occurs approximately at 7–8 months. In humans, on average, epiphyseal closure occurs in the early 20s. The mean human equivalent of the rat ages, which are calculated via all these parameters, is illustrated in Fig. 18.1. The aging rate of rats is not stable in every period of the rat lifecycle. The fastest aging period is seen in prepubescence, it is followed by adolescence, early adulthood, and late adulthood periods, respectively. The slowest aging period in rats seen is during the nursing period. Table 18.1 describes how many rat days are required as equivalent to a human year at the same stage of life such as nursing period, preadolescence, adolescence, early adulthood, and late adulthood. Aging rate coefficient (ARC) statistically expresses how much faster the aging rate of rats is than humans at various life stages. We presented the ARC by dividing 365 human days into the equivalent of rat days, which are in the same life period. Each ARC we obtained gave us the coefficient for that period. We used this coefficient as an average multiplier for calculating the rat-human correlation for shorter periods (minutes, hours, days). When Table 18.1 is examined, it is seen that rats age faster than humans; 8.61 times faster in the nursing period, 84.88 times faster in prepubescence, 34.76 times faster in adolescence, 30.93 times faster in early adulthood, and 21.35

Fig. 18.1 This figure illustrates the mean human equivalent of the rat ages, calculated via parameters such as weight comparisons, staging of rat cerebral cortex development, and time to reach sexual maturity and musculoskeletal maturity

Table 18.1 Rat days equivalent to human year and Aging Rate Coefficient (ARC) at various life stages

Stages of life	Rat days equivalent to human year	ARC
Nursing period	42.4 rat days	8.61 AU
Prepubescence	4.3 rat days	84.88 AU
Adolescence	10.5 rat days	34.76 AU
Early adulthood	11.8 rat days	30.93 AU
Late adulthood	17.1 rat days	21.35 AU

This table describes how many rat days are required as equivalent to human year at the same stage of life such as nursing period, preadolescence, adolescence, early adulthood, and late adulthood. We presented ARC by dividing 365 human days into the equivalent of rat days, which are at the same lifecycle. Each ARC we obtained gave us the coefficient for that period. ARC statistically expresses how much faster the aging rate of rats than humans at various life stages
AU arbitrary unit

times faster in late adulthood (Table 18.1). The decreases in ARC values in early and late adulthood are remarkable which confirms the notion that aging is slower with advancing age. More detailed information can be seen on the subject given in the section titled "The phenomenon of late-life deceleration."

It is a fact that therapeutic interventions can be beneficial only when administered at appropriate time intervals in which they can be effective. Just as there are differences between human year and rat year, as we have shown in Fig. 18.1, there are also critical differences between human hours and rat hours. As pathological processes can change rapidly over time, particularly in the case of acute CNS disorders, the proper time intervals can easily be overlooked when conducting trials on animal models (Dromerick et al. 2021). The importance of all these ARC data emerges when conducting scientific research. Many critical computational errors are made in the human-rat correlation in many studies currently conducted. The state of these data obtained from rat models, when correlation is taken into account, is currently unknown.

In a study investigating the neuroprotective effects of the sonic hedgehog agonist in a rat neonatal stroke model, a 3-h model of ischemia was administered to postpartum 10-day-old rats (Nguyen et al. 2021). Considering the ADC for the nursing period, the human equivalent of postpartum 10-day-old rats is approximately 3 months old, whereas the term neonatal infant is a child under 28 days of age. In addition, the equivalent of 3 h of ischemia in rats during the nursing period means approximately 26 h of ischemia in human infants in the same period. In another study, examining the effects of pain on exercise-induced synaptogenesis in an experimental rat model of stroke, the age of rats was not shared, and exercise was initiated at an intensity of up to 30 min for a maximum of 14 days, 1 day after middle cerebral artery occlusion (Zhang et al. 2020b). In the mentioned study, in which the unknown age of the rat is a separate problem, for example, if we assumed the rat as an adolescent, the 30-min uninterrupted exercise is performed in the adolescent rat which corresponds to approximately 17.3 h of uninterrupted exercise in humans. Moreover, in the same study, 1 h of ischemia was applied to the rats, which corresponds to approximately 35 h in adolescent humans.

In another study titled "Calorie restriction changes muscle satellite cell proliferation in a manner independent of metabolic modulation," they used 12-week-old Sprague-Dawley rats and they performed a 40% calorie-restricted diet for 3 months or 6 months (Abreu et al. 2020). The equivalent of a 3-month diet in adolescent rats in humans is approximately 2.5 years; the equivalent of a 6-month diet is about 5 years.

The scientific reliability of the animal studies is questionable as are mostly not effectively translated to humans. Along with the necessity of paying attention to the timing of the simulated condition and the performed activity in all these exercise, hypoxia, ischemia, and similar models, another point to be considered is the timing of administrations or diets. Regardless of the content of the administered substance or performed diet program, it should be kept in mind that the human equivalent of a 5-rat minutes can vary from 21 min to 85 min, considering the ARC coefficients (Fig. 18.1, Table 18.1).

18.1.2 The Gompertz Makeham Law

Unexpected surprises are possible, even in a plan where every detail is taken into account with perfect care. These surprises can happen independently and spontaneously of the planner and implementer. While investigating aging and age-related pathologies, one of the most important surprises that may arise in animal models is that the animal model under optimal conditions dies for a reason other than the researched subject. When calculating these unexpected deaths according to the age of the animal model, Gompertz Makeham law should be taken into account to obtain more accurate data.

There is great interest in the question of the limits of the human lifespan. Attempts to answer this important question have spurred many studies on death trajectories in old age, often with contradictory results. Gompertz Makeham Law is an equation created by two different mathematical geniuses: Benjamin Gompertz and William Makeham (Golubev 2009).

Gompertz Makeham Law consists of two components that these two geniuses calculated separately: Gompertz and Makeham functions. Gompertz function was first proposed by Benjamin Gompertz in 1825. Function can be mathematically expressed as $R_m = R_0\, e^{at}$. R_m is the rate of mortality, a and R_0 are constants and t is the time parameter. Gompertz function is the age-dependent component of the human death rate and increases exponentially with age. In fact, it was first used in insurance calculations. It was created by statistically calculating and bringing together the deterioration of mechanical tools in closed areas (home, workplace) independently of an external factor (accident, sabotage). In a protected environment where external causes of death are rare (laboratory conditions, countries with low mortality, etc.), the age-independent mortality component is usually negligible (Golubev 2004, 2009). In this situation, the formula can be simplified according to a Gompertz function. In models using animals produced in the laboratory, the hazard function is practically omitted and calculations are solely made according to Gompertz function (Missov and Lenart 2013).

Makeham function is the age-independent component of the Gompertz–Makeham law. Contrary to the Gompertz fiction, Makeham function calculates the hazard functions that will affect the mechanism in question in an open environment (Castellares et al. 2020).

The graph obtained from the data derived from Gompertz function is called the Gompertz curve (Golubev 2009; Castellares et al. 2020) (Fig. 18.2). When the Gompertz curve, which is composed of demographic data obtained from humans, is examined, it is seen that the probability of death in the neonatal period is higher than in many other periods of life. It is quite possible that the causes of this tragic height are congenital complications and losses in the neonatal period. We included two shaded areas in the Gompertz curve in Fig. 18.2, one in the 0–20 age group and the other in the 95–100 age group to draw attention to the change in death rates. In the graph, which accepts the beginning of life as the moment of birth, the probability of death at the time of birth is expressed approximately with a person in their late 50s. Considering birth complications and neonatal diseases, this height is reasonable.

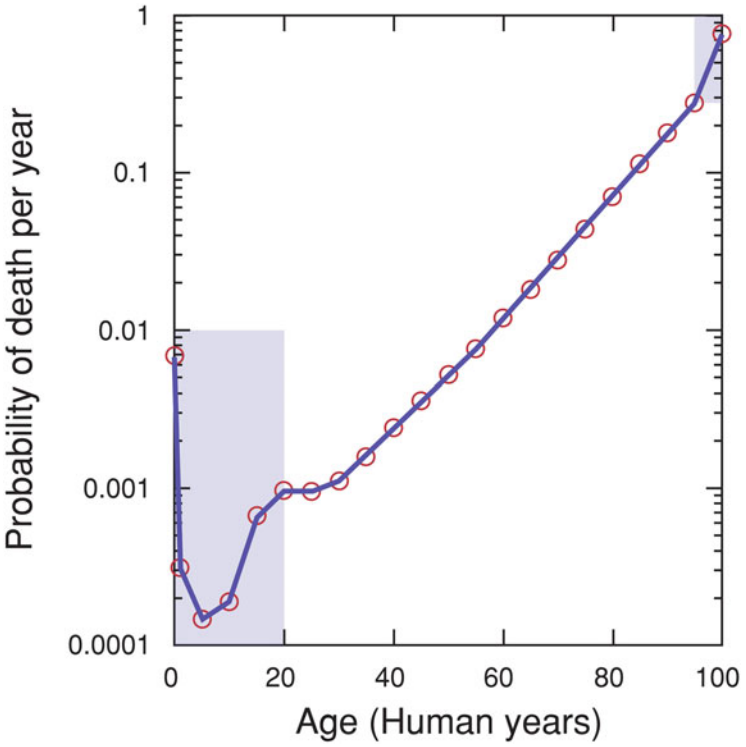

Fig. 18.2 The Gompertz Curve, which is composed of demographic data, shows probability of death by age. Two shaded areas are included in the Gompertz curve in Fig. 18.2, one in the 0–20 age group and the other in the 95–100 age group to draw attention to the acceleration changes in death rates

A sharp decrease is observed in probability of date in the period up to 3 years of age following birth. This is the first drop on the chart. In the period from 3 to 5 years old, there is a slight decrease compared to the first decrease. This is the second and last negative curve in the Gompertz curve. There is no decrease in probability of death on the curve from the age of 5 onwards. The trend with sharp rise seen between the ages of 10–18 starts to decrease between the ages of 18–20. Between the ages of 20–25, the change in the curve is almost unchanged. From the age of 30 to the age of 90–95, the probability of death increases almost linearly. There is a remarkable increase in probability of death at the age of 95–100, which is the second shaded area of the Curve (Golubev 2009; Missov and Lenart 2013).

18.1.3 The Phenomenon of Late-Life Mortality Deceleration

The phenomenon of late-life mortality deceleration states that mortality rates increase more slowly in older ages (Chen et al. 2013; Gavrilov and Gavrilova

2019). Many organisms, including humans, show a deceleration in mortality rates in later life. This mortality deceleration is rapid enough to cause a marked cessation of biological aging, allowing advanced age mortality in a few species to remain constant in old age (Gavrilov and Gavrilova 2019). There are two main theories involved in explaining late-life mortality deceleration: the heterogeneity of frailty theory and the Hamiltonian theory (Zajitschek et al. 2014).

The heterogeneity of frailty states that all populations have a higher degree of heterogeneity than is explained by the observed covariates because individuals differ from each other for many other unobserved traits called frailty. Frailty is a general concept that combines in a single measure all factors that increase or decrease the risk of death for a given individual, regardless of the source of heterogeneity such as acquired weakness, lifestyle factors, environmental risks, and innate biological frailty (Zarulli 2016). The accumulation of clinical symptoms and signs leads to frailty and has both an age-independent (background) component and an age-dependent (exponential) component, akin to the Gompertz Makeham model for the risk of mortality (Mitnitski et al. 2002). Frailty can be measured in relation to the accumulation of those deficits by using a frailty index developed from databases of aging (Searle et al. 2008). The rapid growth of the aging human population highlights the need for laboratory animal models to study the fundamental biological processes of aging and susceptibility to heterogeneity. There is a need for methods for assessing and monitoring the health of aging models over time. A systematic process for creating a frailty index, which relates deficit accumulation to the individual risk of death may aid the understanding of frailty-related disorders in older adults.

The Hamiltonian theory is a function used to solve the optimal control problem for a dynamic system. It can be understood as an instantaneous increment of the Lagrangian expression (the strategy of finding the local maximums and minimums of a function subject to equality constraints) of the problem to be optimized over a given time period. Inspired by, but different from, the Hamiltonian of classical mechanics, the Hamiltonian of optimal control theory was developed by Lev Pontryagin as part of the principle of maximum. Pontryagin proved that a necessary condition for solving the optimal control problem is that the control must be chosen in such a way as to optimize the Hamiltonian (Schäfer and Jaranowski 2018).

It is known that frailty has a significant effect on probability of death in humans (Kojima et al. 2018). Despite the fact that Frailty affects the hazard function in Gompertz Makeham, the failure to take into account the frailty index in animals—especially in laboratory animal models—and the lack of sufficient research in the relevant fields constitute an obstacle to refining the reliability of the data obtained in animal experiments and models.

In this chapter, we have expressed how fast rats are in different life frames compared to humans, with the term we proposed the term ARC, which we have derived based on multiple statistical data. We have revealed the correlation errors in many conducted research. The scientific validity of the animal studies is questionable as are mostly not effectively translated to humans. Regardless of the performed action, it should be kept in mind that the human equivalent of 5-rat min can vary

from 21 min to 85 min, considering the ARC coefficients. Also, it is necessary to calculate the hazard function in Gompertz Makeham Law, taking into account the frailty values for each type of animal model. It is clear that a Gompertz curve to be prepared for experimental animal models will significantly contribute to animal studies with the our suggested term ARC.

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