Syed Ibrahim Rizvi · Ufuk Çakatay
*Editors*

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# Foreword

Is aging a disease? Are age-related diseases distinct from aging? Is aging a bad thing? Is aging a solvable medical problem?

These questions are highly divisive. To most people, it is extraordinary that the questions would even be asked, because the answers are so self-evident – but, of course, that is true both of people whose answers would be "yes" and of those whose answers would be "no." And that, itself, is unequivocally a problem – a BIG problem.

It turns out, furthermore, that the tenor of the debate around these questions varies considerably according to culture. I have lived most of my life in England, but now I live in California, where I find that there is far more agreement with my own answers to the above questions (which are "no," "no," "yes," and "yes," in case you were wondering) than elsewhere. Conversely, I find that the consensus in Asian countries is extraordinarily opposed to this way of thinking and wedded instead to the view that aging is a natural, inevitable, and welcome process that is utterly off-limits to medicine. This attitude to aging has something of a silver lining, in that it also underpins the deep-seated respect for the elderly of which Asian cultures are legitimately proud: the far better integration of the elderly in society, the encouragement to remain active late in life, and so on. But in the long run, it is a huge problem. It prevents Asian countries from contributing, to the extent that they could, to medical research efforts directed at keeping the elderly truly healthy, let alone achieving the ultimate goal of restoring them to genuinely youthful mental and physical performance.

I will lay my cards on the table here: I believe that this is the wrong kind of respect for the elderly. Even in the West, and though things are gradually improving, a seriously problematic level of ambivalence persists with regard to these questions – a degree of doubt as to the wisdom or practicality of efforts to bring aging under medical control – that powerfully limits access to funding for such work, thereby slowing it and thereby costing vast numbers of lives in the future. But this lack of enlightenment in Asia is far more severe.

This volume has the potential to help change that. The research teams that have authored these chapters are mostly based in Asia (I'm going to count Turkey as Asia for this purpose!) or originate from there, and as a result I expect (and hope) that the book will attract a strong audience in that part of the world, though without doubt it will also appeal to a worldwide audience. By providing scientists and interested

laypeople with authoritative, up-to-date information concerning the status and progress of research into aging, this book will raise the quality of debate around the questions with which I began this foreword. And there can only be one outcome of that: a broader and more crystallized understanding that aging is indeed a solvable medical problem and one to which all nations and cultures have the opportunity, and the humanitarian duty, to contribute.

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# Preface

Since the dawn of civilization, man has always been fascinated by the thought of living longer. Every system of medicine around the world has tried to provide some intervention for a longer life-span. The ancient Indian text, Rigveda (> 1000 BC), mentions a drink "amrita" which can bestow immortality. However, until 1950s, scientists had little understanding of aging, which is evident from the lecture of Sir Peter Medawar delivered at University College London in 1951, entitled "An Unsolved Problem in Biology."

The last few decades have seen tremendous advances in the understanding of molecular events which underline the process of aging. It is indeed a big achievement of science that we now have a better view of the hallmarks of aging. This understanding has provided gerontologists with "targets" which can be exploited for possible anti-aging interventions.

Finding an anti-aging intervention is far more difficult than finding a cure to any disease. Aging per se is not a disease; however, with age, the body becomes predisposed to a host of ailments affecting different organs, which culminate into loss of function and ultimately death. Interestingly, while the rate of aging for a given species remains the same, the aging process is highly heterochronic.

Intervening into aging is the next frontier in contemporary medicine and will remain to be of increasing importance over time as other sources of poor health are addressed more and more successfully. Aging being a highly complex event throws up a huge array of scientific explanations, all of which provide, to some extent, convincing arguments. In the light of such variation in possible theories which explain the process of aging, the strategies being experimented for anti-aging interventions are also highly diverse.

Literature is scattered for possible anti-aging interventions. Moreover the plurality of the events which constitute the aging mechanism makes it extremely challenging to find an intervention which may be considered "anti-aging" in a holistic sense. Despite the complexities, new scientific evidence emerging with continuous research continues to present interesting targets for devising anti-aging strategies. This book is an attempt to provide a compact source of emerging anti-aging interventions which offer hope for a longer healthspan, based on our current understanding of the aging process.

A huge array of literature exists which espouses the role of dietary antioxidants as possible anti-aging agents. Although this presumption is largely due to the role of

polyphenols in counteracting oxidative damages which accompany aging, several large-scale clinical trials have failed to come up with concurring results. We however feel that the dietary efficacy of antioxidants may have cultural/geographical differences. Regions where the diet is largely deficient in antioxidants may benefit from an intervention strategy based on dietary polyphenols. Keeping this aspect in view, this book offers three chapters (Chaps. 15, 18, and 21) which provide a detailed overview of the role of polyphenols in aging.

Chapters 2, 3, and 4 highlight approaches that include noncoding RNAs, stem cell reprogramming, and tissue engineering, which have potential to provide anti-aging strategies based on highly specialized techniques. Senescent cells are known to contribute to disease onset and progression through complex cell and non-cell-autonomous effects; as a result, cellular senescence is being increasingly associated with aging. Chapters 5 and 6 deal with senotherapeutics.

The understanding of the signaling pathways has provided molecular targets which can be targeted for anti-aging effects. Chapters 9 and 10 are focused on mTOR inhibition and sirtuin modulation. Age-related diseases and frailty syndromes share some common features which converge on inflammation. Chapters 8 and 23 provide an insight into the role of inflammation in aging and anti-aging interventions based anti-inflammatory approaches.

Important topics providing anti-aging approaches based on telomerase activity, intermittent fasting, melatonin, and phytochemicals have been included in Chaps. 7, 13, 14, and 17. The activation of plasma membrane redox system (PMRS) has been suggested as a novel strategy for anti-aging intervention (Chap. 19). An interesting approach involves the use of computational methods (Chap. 12). Interventions against sarcopenia (Chap. 20) and brain injury-induced aging (Chap. 22) are also included in our book.

We would like to thank all our contributors who provided us with excellent chapters making possible the compilation of this book.

Allahabad, Uttar Pradesh, India — Syed Ibrahim Rizvi
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# About the Editors

**Dr. Syed Ibrahim Rizvi** is a professor of Biochemistry at the University of Allahabad, India. He has been a visiting professor at several universities including the University of Athens (Greece); University of Nice (France); University of Pisa; University of Milan; University of Rome (Italy); Istanbul University (Turkey); Research Centre for Natural Sciences, Budapest (Hungary); and Bangladesh University of Health Sciences, Dhaka. Prof. Rizvi is a grantee of the International Foundation for Science, Sweden. His research interests include aging mechanisms, antiaging interventions, metabolic diseases, and natural products. To date, he has published 145 research papers, 10 book chapters, and a large number of popular science articles. He has been awarded fellowships/grants by the European Molecular Biology Organization (EMBO); Federation of European Biochemical Societies (FEBS); International Cell Research Organization; Organisation for the Prohibition of Chemical Weapons (OPCW), The Hague (Netherlands); Department of Science and Technology, Government of India; and University Grants Commission, India. He has received the Young Scientist Project Award from the Department of Science and Technology, Government of India, and was conferred the Academic Excellence Award by the University of Allahabad in 2016. Prof. Rizvi serves on the editorial/review boards of several leading research journals, and his work has been highlighted in the BBC (London) and other print and TV media. Prof. Rizvi has 30 years of teaching/research experience.

**Dr. Ufuk Çakatay** is a senior scientist in the Department of Medical Biochemistry, Cerrahpaşa Faculty of Medicine, Istanbul University. Previously, he worked as an associate professor at the Central Laboratory of Clinical Biochemistry, Istanbul Faculty of Medicine. He graduated from the Department of Biology, Faculty of Science, and received his PhD degree from the Department of Medical Biochemistry, Cerrahpaşa Faculty of Medicine. His research interests include the optimization of bioanalytic methods for measuring oxidative stress, experimental animal models of aging, and age-related disorders. He has a long-standing interest in various protein oxidation biomarkers such as protein carbonyl groups, protein hydroperoxides, advanced oxidation protein products, dityrosine, and nitrotyrosine. Dr. Çakatay has authored more than 80 publications, which include research papers, book chapters, editorials, and invited reviews on free radical biology, aging, diabetes, and

biological activity of alpha-lipoic acid. He has served as a guest editor, referee, or member of the editorial board for more than 50 journals. He has received various prestigious awards such as the top reviewer award from Elsevier Science Publishing (2008) and serves on Istanbul University's Committee on Animal Research and Ethics.


# Aging Principles and Perspectives for Intervention

1

Suresh I. S. Rattan

**Abstract**

The evolutionary and the biological principles of aging are now well established, and these show that aging is not determined by any specific gerontogenes. Instead, it is the imperfect maintenance and repair systems that lead to a progressive failure of homeodynamics, aging and eventual death. Gene therapy, stem cell therapy, hormonal replenishment and nutritional supplementations, tested mostly in experimental model systems, have achieved limited success for humans. The complex trait of aging requires wholistic approaches for maintaining or improving health in old age. A promising approach for health maintenance and improvement is that of mild stress-induced physiological hormesis. Physical and mental exercise, various non-nutritional food components, such as polyphenols, flavonoids and terpenoids in spices, oils and other formulations are hormetins, which have health beneficial effects through physiological hormesis. The future scenarios for aging intervention include intelligent redesigning and transhumanistic enhancements through robots and cyborgs combining both organic and biomechatronic body parts.

**Keywords**

Biogerontology · Gerontogenes · Healthspan · Homeostasis · Homeodynamics · Hormetin

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## 1.1 Introduction

Improving health, preventing aging and extending lifespan is one of the longest running dreams of human beings. While searching for an elixir for eternal life may still occupy the minds of some, modern biogerontology has shifted the focus towards developing and utilizing more realistic, rational and evidence-based approaches. Therefore, in order to fully appreciate and evaluate such approaches, it is important to have an overview and understanding of the current status of aging research, especially that of the study of the biological basis of aging. The aim of this article is threefold: (1) to provide a general review of the evolutionary, cellular and molecular bases of aging, (2) to discuss homeodynamics of survival and (3) to present a critical appraisal of various approaches towards modulating aging, including its prevention or reversion, enhancement of health and extension of healthspan.

It is now generally accepted that the biological basis of aging are well understood (Holliday 2006; Hayflick 2007a). As a result of this achievement of biogerontology, a conceptual framework and general principles of aging and longevity have been formulated. The three main biological principles of aging and longevity are summarized in Table 1.1.

In accordance with the above principles, aging is an epigenetic, emergent and a meta-phenomenon, which is affected by numerous factors. While no tissue, organ or system becomes functionally exhausted even in very old organisms, it is their collective interaction and interdependence at all levels that is decisive of overall health and survival. The contribution of genes to the lifespan of an individual is considered to be about 25%, as calculated from the longevity-correlation analyses performed on the data for the lifespan variance among siblings and monozygotic and dizygotic twins (Herskind et al. 1996). This means that non-genetic, epigenetic and environmental factors, including lifestyle, have much larger influence in determining the health, quality and the length of lifespan of an individual. This also implies that aging, healthspan and lifespan are not predetermined and can be affected by various methods of intervention.

**Table 1.1** Principles of biological aging and longevity

| |
|---|
| 1. *Aging starts after essential lifespan:* Biological aging is a progressive loss of physical function and fitness, which occurs during the extended period of survival beyond the natural lifespan of a species, termed "essential lifespan" (ELS) (Rattan 2000a, b; Rattan and Clark 2005) |
| 2. *Aging is a post-genetic emergent phenomenon:* Aging phenotype is an emergent phenomenon observed in highly protected environments allowing survival beyond ELS. There is no genetic programme for determining the exact duration of survival of an individual; and there are no gerontogenes whose evolutionary function is to cause aging and limit the lifespan (Rattan 1995; Holliday and Rattan 2010) |
| 3. *Heterogeneity of the aging phenotype:* The rate of progression and phenotype of aging are different in different species, in organisms within a species, in organs and tissues within an individual, in cell types within a tissue, in subcellular compartments within a cell type and in macromolecules within a cell (Rattan 2012a, 2016b) |

## 1.2 Basis of Survival: Homeostasis Versus Homeodynamics

What makes living systems different from the inorganic and nonliving systems is their intrinsic ability to respond, to counteract and to adapt to the external and internal sources of disturbance. The traditional term to describe this ability is homeostasis, which, however, is not totally correct. The main reason for the incompleteness of the homeostasis model is its notion of "stability through constancy", which does not take into account the dynamic nature of information and interaction networks that underlie the complexity of the biological systems. Therefore, the term homeodynamics encompasses the fact that, unlike machines, the internal conditions of biological systems are not permanently fixed, are not at equilibrium and are under constant dynamic regulation and interaction among various levels of organization (Yates 1994).

The property of homeodynamics of the living systems is founded in a wide range of maintenance and repair processes at all levels of organization (Table 1.2). All these processes are governed by hundreds of survival-assurance genes, which give rise to a "homeodynamic space", as the ultimate determinant of an individual's chance and ability to survive and maintain health (Rattan 2006, 2012a). Aging, age-related diseases and eventual death are the result of a failure of homeodynamics. This fact is also reflected in the definition of aging as a progressive shrinkage of the homeodynamic space (Rattan 2006, 2012a).

## 1.3 Genetics and Epigenetics of Aging

Since all molecular processes in living systems are based in and regulated by genes and gene products, discovering genes for aging has been an important theme in biogerontology. However, evolutionary theories of aging and longevity discount the notions of any specific genes for aging (Kowald and Kirkwood 2016).

**Table 1.2** Main maintenance and repair pathways in biological systems arranged from molecular to whole body level

| |
|---|
| Nuclear and mitochondrial DNA repair |
| Anti-oxidative enzymes and free radical scavengers |
| Degradation of damaged DNA and RNA |
| Protein repair |
| Degradation of damaged proteins |
| Degradation of damaged organelles |
| Programmed cell death – apoptosis |
| Intracellular stress responses |
| Detoxification of harmful chemicals and metabolites |
| Immune responses |
| Wound healing and tissue regeneration |
| Other higher-order defences, thermal regulation, neuroendocrine balance and circadian rhythms |

Furthermore, the strong heterogeneity of the aging phenotype is indicative of the fact that the progression of aging is neither programmed nor deterministic but mostly mediated by stochastic events (Holliday 2007, 2009). On the other hand, aging does appear to have a genetic component, and the role of genes in aging is indicated by (1) an apparent limit to lifespan within a species (Carnes et al. 2003; Dong et al. 2016), (2) some heritability of lifespan as evident from studies on twins (Tan et al. 2013), (3) presence of human genetic mutants of premature aging syndromes (Kipling et al. 2004; Martin et al. 2007) and (4) association of some gene polymorphisms with extreme longevity (de Magalhaes 2014b).

In order to resolve the paradox of stochastic nature of the progression of the aging and the genetic aspects of longevity, a novel view about the nature of aging genes, termed gerontogenes, has been put forward, and a modified term "virtual gerontogenes" has been suggested implying the altered state of survival genes as giving the appearance of being the real aging genes (Rattan 1985, 1995). This notion of virtual genes also applies to several so-called disease-causing genes. For example, the Werner gene, which is considered to "cause" the premature aging syndrome, is in reality a DNA helicase gene whose normal role in DNA replication and repair prevents the emergence of the Werner's syndrome, and it is only when this gene is altered by mutation that the disease phenotype emerges (Goldstein et al. 1990). The same applies to most of the so-called oncogenes, which are cancer-causing only when they are mutated and cannot perform their normal function (Tacutu et al. 2011).

The nature of virtual gerontogenes is considered to be of two types: (1) genes with mutations already present at the time of fertilization and birth and that manifest any deleterious effects after the period of growth, development and maturation (Partridge 2001; de Magalhaes 2012) and (2) the antagonistic pleiotropic genes, which were selected for survival benefits during early development but which can have potentially harmful effects in post-reproductive life when they are no longer under the force of natural selection (Kirkwood and Rose 1991; Holliday and Rattan 2010).

There is a large body of evidence showing that the genes involved in the maintenance and repair pathways are the main determinants of species' longevity (Rattan 2015a). Experimental extension of lifespan of various organisms and comparative studies of species with widely varying lifespans provide such evidence. Such genes are commonly known as the longevity assurance genes (LAG) or vitagenes that determine the ELS of a species (Rattan 2007). These longevity assurance genetic pathways include the efficiency of deoxyribonucleic acid (DNA) repair (Rattan 1989; Park et al. 2011), the fidelity of genetic information transfer (Kirkwood et al. 1984), the efficiency of protein degradation (Schmidt and Finley 2013), cellular responsiveness to stress (Kapahi et al. 1999) and the capacity to protect from free radical- and oxidation-induced molecular damage (Jones 2015). A very important understanding to emerge from the above studies is that the diversity of genes associated with aging and longevity of different organisms implies that there is no single and universal pathway affecting these phenotypes. It seems that whereas from an evolutionary point of view the genes involved in repair and maintenance pathways are important as the LAG, each species has also evolved additional species-specific

pathways of aging. Such genetic pathways have been termed as public and private pathways, respectively (Martin 2007).

In addition to the genetic aspects of aging and longevity, there is a lot of interest in understanding the epigenetic aspects of aging (Pal and Tyler 2016; Sen et al. 2016). Methylated cytosines, oxidatively modified nucleotides, alternatively spliced RNAs and post-translationally modified proteins, including protein folding, comprise the main intracellular epigenetic markers (Lund and van Lohuizen 2004). Since the full spectrum of epigenetics of aging is yet to be unraveled, it is one of the most attractive and challenging areas of research in biogerontology (Johnson et al. 2012; Heyn et al. 2012; Hannum et al. 2013). A major reason for the apparent difficulties in fully understanding the epigenetics of aging is the existence of several orders higher complexity and diversity of the constituting components, such as physical, chemical, biological and environmental factors, including psychological factors in human beings. Furthermore, a lot of epigenetic modifications can occur reversibly on a daily basis, depending on several lifestyle factors (Gensous et al. 2017; Chaleckis et al. 2016).

## 1.4 Molecular Mechanisms of Aging

The theories of the molecular mechanisms of aging are mostly centred on the occurrence and accumulation of damage (Yin and Chen 2005; Rattan 2006, 2008b). Although other views, such as continuous growth leading to a kind of quasi-programme (Blagosklonny 2012), and progressive increase in entropy (Hayflick 2007b) are also discussed as the mechanisms of aging, the occurrence and accumulation of molecular damage are the most studied aspects of molecular gerontology.

There are three main types of sources for the origin of macromolecular damage:

1. Chemical species (e.g. reactive oxygen species (ROS) and other free radicals (FR)) formed due to external inducers of oxidative damage and as a consequence of cellular metabolism involving oxygen, metals and other metabolites (Forman 2016).
2. Nutritional glucose and its metabolites and their biochemical interactions with ROS and FR (Nedic et al. 2015; Tanase et al. 2016).
3. Spontaneous errors in biochemical processes, such as DNA duplication, transcription, post-transcriptional processing, translation and post-translational modifications (Nyström 2002).

An age-related increase in the levels of various types of macromolecular damage, including DNA, RNA, protein, carbohydrates and lipid damage, is well documented (Holliday 2007; Rattan 2006, 2012a). Often, the mechanistic theories of biological aging have focused on a single category of damage inducers as a universal explanation. For example, the free radical theory of aging (FRTA), proposed by Denham Harman in 1954, is based on the premise that a single biochemical process

of FR-induced damage may be responsible for the aging and death of all living beings (for an update, see Harman 2006). In support of this idea, there is a significant amount of evidence that shows that ROS and other FR are indeed involved in the occurrence of damage and can lead to structural and functional disorders, diseases and death. However, a lack of incorporation of the essential role of FR in the normal functioning and survival of biological systems has raised several points of criticism about FRTA (Gruber et al. 2008; Halliwell 2009). Furthermore, FRTA presents FR as the ultimate cause of damage while ignoring the fact that there are large differences in the range of FR-counteracting mechanisms in different species (Vina et al. 2013; Jones 2015). In addition, contrary and/or lack of beneficial results of antioxidant and FR-scavenging therapies as predicted by FRTA have restricted FRTA to being only a partial explanation of aging (Le Bourg and Fournier 2004; Le Bourg 2005; Howes 2006).

The biological consequences of increased levels of molecular damage are wide-ranging and include mutations, altered gene expression, cell cycle arrest, cell death, loss of intercellular communication, disorganization of the tissues, dysfunctioning of the organs, reduced stress tolerance and reduced ability to adapt (Rattan 2008b): Each of these biological consequences has, historically, been used as the basis of developing other so-called theories of aging, such as pineal gland theory, neuroendocrine theory, immunological theory, replicative senescence theory, etc. However, at present, the occurrence and accumulation of molecular damage as the basis of age-related failure of homeodynamics are considered as a unified explanation for biological aging (Rattan 2006, 2008b).

## 1.5 Aging Interventions: Treatment, Prevention or Management

One's approach towards intervention in aging can be influenced by one's understanding of aging either being a disease that needs to be treated or being a condition emerging from the basic life processes, which can be modulated to some extent. Since aging is an emergent phenotype due to the failure of homeodynamics and not due to the action of any life-limiting and death-causing mechanisms, it changes aging interventional approach from "anti-aging" to "healthy aging". Aging occurs in spite of the presence of complex pathways of maintenance, repair and defence, and there is no "enemy within" that needs to be eliminated. Even the diseases of old age, such as Alzheimer, Parkinson, type 2 diabetes and cancers, have no simple causative agents except for the life processes themselves.

Table 1.3 presents the rationale behind the present and future strategies for aging interventions, which are briefly discussed below.

**Table 1.3** The present and future strategies for aging intervention

| Strategy | Interventions |
|---|---|
| Piecemeal remedy – "fix what is broken" | Cosmetics, tissue and organ repair, organ transplantation, senescent cell removal, young blood/plasma transfusion, stem cells |
| Replenishment and supplementation | Hormones, nutritional supplements with synthetic and natural molecules including antioxidants, vitamins and phytochemicals |
| Strengthening the homeodynamics | Hormesis through nutritional hormetins, food physical activity, immunological challenge and social and cognitive engagement |
| Gene therapy and intelligent redesigning | Gene therapy, genetic and bodily enhancements, trans-humanistic cyborgs and robotics |

### 1.5.1 Piecemeal Remedies

One of the most common and prevalent biomedical approaches to aging intervention is the so-called piecemeal remedies. The basic logic behind this approach is to "fix what is broke"; and it ranges from cosmetics to the tissue/organ repair or transplantation, targeted treatments with stem cells, and rejuvenation with young blood/plasma transfusion (Goodell and Rando 2015; Rebo et al. 2016; Castellano et al. 2015). More recently, elimination of senescent cells by potential senolytic compounds is becoming an increasingly appealing approach (Naylor et al. 2013; Cortese and Santostasi 2016; He and Sharpless 2017; de Keizer 2017). Although such interventions often have life-saving effects in acute situations, these benefits are often transient, limited and require recurring interventions (Kyriazis 2014).

### 1.5.2 Replenishment and Supplementation

One of the most widely used aging interventional strategies, tested mostly in animal model systems, is that of replenishing the loss. However, the naïve premise of this approach is that age-related decline in the levels of hormones, enzymes and other metabolites is always harmful and that these declined levels should be brought back to the youthful levels. This view almost totally ignores the biogerontological understanding that many changes occurring during aging are often the sign of remodelling and adaptation for survival and health (Davies 2016; Martin et al. 2015). For example, a reduction in the levels of various hormones and their intermediates and receptors seems to be a co-requirement for the extension of lifespan of organisms, as determined by genetic and non-genetic interventions (Rattan and Sharma 2017). Similarly, unexpectedly long-living naked mole rats and bats generally have much lower levels of hormones than short-lived species (Gorbunova et al. 2014; Brunet-Rossinni and Austad 2004). Furthermore, some claims have been made that the increased longevity of eunuchs and castrated men could be due to their low levels of growth hormone and sex steroids (Min et al. 2012). Therefore,

several biogerontologists have cautioned that hormonal and nutritional supplementation as replenishments may have little, none or even harmful effects in normal healthy situations (Le Bourg 2005; Rizvi and Jha 2011; Sadowska-Bartosz and Bartosz 2014; Conti et al. 2016; Vaiserman et al. 2016).

### 1.5.3 Strengthening the Homeodynamics

Biogerontologists are increasingly realizing that "single-molecule, single-target" oriented approaches for aging intervention are severely limited because these neglect the highly dynamic, interactive and networking nature of life. Therefore, whole body level holistic or more accurately "wholistic" (in order to distinguish science-based approaches from the "everything goes" holistic claims) approaches are being tested and developed as promising aging interventions. Food, physical activity and mental engagement come under such wholistic interventions, which strengthen the homeodynamics (Rattan 2015b, 2017). One such wholistic interventionary approach is that of hormesis.

Physiological hormesis in health maintenance and improvement is defined as the life-supporting beneficial effects resulting from the cellular and organismic responses to repeated and transient exposure to mild stress (Le Bourg and Rattan 2008; Mattson and Calabrese 2010; Rattan 2014). Moderate physical exercise is the paradigm for stress-induced physiological hormesis (Sen et al. 2000; Radak et al. 2005; Williamson and Pahor 2010). Other stress inducers which have been shown to affect aging of cells and animals include acetaldehyde, alcohols, dietary restriction, flavonoids, heat shock, heavy metals, hypergravity, intermittent fasting, infections, irradiation, pro-oxidants, polyphenols and terpenoids (Le Bourg and Rattan 2008; Mattson and Calabrese 2010; Rattan 2014; Weis et al. 2017). An important observation in studies of physiological hormesis is that a single stressor, such as heat shock or exercise, can strengthen the overall homeodynamics and enhance other abilities, such as adaptability, cognition, immune response, memory, resilience and overall robustness. These systemic and wholistic effects are generally achieved by initiating a cascade of processes that result in a biological amplification of effects.

All such conditions, which bring about health beneficial effects by initially causing low-level stress, are termed as hormetins (Rattan and Demirovic 2009, 2010a, b). Hormetins can be further categorized as (1) physical hormetins, such as heat, radiation and physical exercise; (2) nutritional hormetins, such as phytochemicals in spices, micronutrients and other natural and synthetic food components; and (3) psychological or mental hormetins, such as brain exercise through cognitive games and challenges, including solving puzzles, social engagement, focused attention and meditation (Brewer et al. 2011; Stark 2012; Duraimani et al. 2015).

The molecular basis of hormesis lies in the activation of stress response pathways on exposure to single or multiple rounds of mild stress (Rattan 2008a; Demirovic et al. 2014). Whereas severe and chronic stress results in the weakening of homeodynamics and can lead to functional impairments, diseases and death,

transient and mild stress strengthens the homeodynamic ability of a biological system (Demirovic and Rattan 2013). It is important to recount that although the measurable effects after a single round of mild stress exposure are usually small, a repeated exposure results in the biological consequences which are cumulative, amplified and physiologically significant, as exemplified by the health beneficial effects of repeated moderate exercise.

It should also be pointed out that several so-called antioxidants, including numerous plant components, some vitamins and micronutrients, are actually stress-inducing hormetins and that their biological effects as being antioxidants are not due to the compounds themselves being direct antioxidants (Panossian 2017; Qi et al. 2017; Linnane et al. 2007; Mocchegiani et al. 2011; Martucci et al. 2017; Li et al. 2017; Camandola and Mattson 2017; Pallauf et al. 2016). Discovering novel hormetins is a developing area of research, which is also drawing significant attention of the aesthetic, healthcare and food industry (Rattan 2012b; Rattan et al. 2013).

Some possibilities of discovering novel hormetins by activating different SR pathways are food-restriction mimetics and other inducers of autophagy (Ingram and Roth 2015; Darzynkiewicz et al. 2014), antidiabetic drug metformin (Barzilai et al. 2012; Campbell et al. 2017), DNA repair response inducers (Darzynkiewicz et al. 2014), resveratrol and its analogues as inducers of sirtuin stress response, inducers of Nrf2-mediated oxidative stress response (Kumar et al. 2014) and NF-kB-mediated anti-inflammatory response (Haas 2009; Martucci et al. 2017). Diet-microbiota interactions may also involve stress response-mediated hormesis for their health beneficial effects (Sonnenburg and Backhed 2016). A detailed database for aging-related drugs has also been developed (Barardo et al. 2017).

### 1.5.4 Gene Therapy and Intelligent Redesigning

Biogerontologists have identified hundreds of putative gerontogenes as potential targets for gene therapy against aging (for the latest information on such genes, refer to various online databases, such as http://genomics.senescence.info/genes/) (de Magalhaes 2014b). However, it is important to realize that in almost all such studies, the extension of lifespan by gene therapy was observed when a significant reduction or total inhibition of the activity of one or more genes was achieved. For example, one of the earliest experimental studies performed on the nematode *C. elegans* demonstrated that a chemically induced mutation in a single gene *age-1* resulted in a significant increase in the lifespan of the mutated worms (Friedman and Johnson 1988a, b). Other examples of such "loss of function" gene therapies associated with extended period of survival are (1) nutrition and hormonal sensing and signalling including insulin/insulin-like growth factor-1 and its target forkhead transcription factor (FOXO), (2) energy generation and utilization in mitochondrial respiratory chain and (3) translational interference through target of rapamycin (TOR) (North and Sinclair 2007; Chen et al. 2005; Kenyon 2001, 2005; Hipkiss 2007, 2008; Vellai et al. 2003). Similarly, several mutant mice strains with defects in growth hormone (GH) pathways in terms of deficiencies of GH levels and GH

receptor have extended lifespans (Napoli et al. 2003; Purdom and Chen 2003; Longo and Finch 2003). Application of RNAi technology, together with the role of circulating RNAs, and small noncoding RNAs, has also identified numerous genes whose normal levels of activities are lifespan restricting and can be a target for gene therapy (de Magalhaes 2014b).

In contrast to the above studies on the longevity-promoting effects of the lost or reduced activities of various genes, studies have also been performed on testing the effects of adding one or multiple copies of some genes on aging and longevity of model systems. These include the addition of gene(s) for one of the protein elongation factors (Shepherd et al. 1989), antioxidant genes superoxide dismutase and catalase (Orr and Sohal 1994; Sun et al. 2004; Parkes et al. 1998; Schriner and Linford 2006), sirtuin (Rogina and Helfand 2004), FOXO (Giannakou et al. 2004), heat shock proteins (Yokohama et al. 2002; Morrow et al. 2004; Walker and Lithgow 2003), heat shock factor, (Hsu et al. 2003; Morley and Morimoto 2004), protein repair methyltransferase (Chavous et al. 2001) and klotho, which is an inhibitor of insulin and IGF-1 signalling (Kurosu et al. 2005).

One of the challenges for these gene therapy-oriented aging interventions is that very little is known about the physiological price paid for inactivating or overstimulating genes whose normal function is a part of the general metabolism and signalling (Rincon et al. 2004; Van Voorhies et al. 2006). For example, laboratory-protected longevity mutants in *C. elegans* have reduced Darwinian fitness when competing with the wild-type worms under nutritionally challenging conditions (Walker et al. 2000; Chen et al. 2007; Van Voorhies 2003). Similarly, extension of murine lifespan by the addition of *klotho* gene induces insulin resistance and disruption of insulin/IGF-1 signalling pathway (Rincon et al. 2004; Van Voorhies et al. 2006; UNGER 2006; Wang and Sun 2009).

Another experimental model system used for testing potential gene-based aging interventions is the Hayflick system of limited proliferative lifespan of normal diploid differentiated cells in culture (Rattan and Hayflick 2016). Most of these interventions are mediated by transient or permanent transfection and ectopic expression of different genes and have focused on extending the replicative lifespan of cells by bypassing the cell cycle checkpoints (Campisi and D'Adda Di Fagagna 2007; Itahana et al. 2004; Collado et al. 2007). The ectopic expression of telomerase is one such widely used genetic intervention (Simonsen et al. 2002; Davis and Kipling 2005). However, these studies have raised an important point of caution that continuous proliferation of such genetically modified non-aging cells often leads to their genomic instability, transformation and carcinogenic activity (Wang et al. 2000; Serakinci et al. 2004). Similarly, in the case of animals, although telomerase-negative mice had reduced lifespan and several other abnormalities, overexpression of telomerase in their skin increased myc-induced hyperplasia (Lansdorp 1997; Flores et al. 2006).

In the case of humans, although several single gene mutations are known which lead to accelerated aging and significantly reduced lifespan (Martin 2005; Martin et al. 2007), no gene mutations have yet been identified which increase the human lifespan. A strategy that has been used extensively to identify potential longevity

genes is by gene association analysis of genetic polymorphisms with human longevity (Singh et al. 2007). The full list of genes associated with human longevity, generally identified by both single nucleotide polymorphism (SNP) analysis or by genome-wide association studies (GWAS), can be retrieved from http://genomics.senescence.info/genes/. To what extent this information can be used to develop gene-based aging interventions in humans is not yet clear.

Some future scenarios for aging interventions include intelligent redesigning either by the so-called strategies for engineered negligible senescence (SENS) (De Grey 2006) or by post-humanistic or trans-humanistic enhancements through robots and cyborgs combining both organic and biomechatronic body parts (Palese 2012). Such interventions, if successful, raise several ethical issues such as the social and environmental consequences of extreme longevity and the basic understanding of what it means to be human (Chan 2008; Seppet et al. 2011).

## 1.6 Recapitulation

The principles of aging and longevity, as described in Table 1.1, indicate that the occurrence of aging in the period beyond ELS of the species is inevitable owing to the imperfections of the survival mechanisms. Aging in itself is not a disease but is the universal cause of age-related diseases. Therefore, whereas optimal treatment of each and every disease, irrespective of age, is a social and moral necessity, maintaining health and improving the quality of human life in old age require a shift in approach from aging as a disease to aging as a life condition that can be modulated.

Although "aging is a disease" label may have some role to play in attracting the attention of big business and investors (de Magalhaes et al. 2017), it totally disregards the scientific history and understanding of the biological basis of aging. If aging is a disease, then it is our own fault – we breathe, we eat food, and we have complex but imperfect biochemistry (Rattan 2016a). The so-called war against aging and any other similar rhetoric are totally misplaced, because there is no enemy within or without. Aging must be approached as a stage in life history of an individual, which is served best by biomedical, technological and social interventions, which could diminish the severity of age-related frailty, along with a possible extension of healthspan and lifespan.

Biogerontologists are beginning to narrow down the potential aging pathways, including insulin/IGF-1 growth axis, mTOR activity and stress resistance, which could be amenable to manipulation (de Magalhaes 2014a, b). There is evidence that those and other metabolic pathways can be effectively modulated by lifestyle alterations, such as intermittent food restriction, exercise and nutritional and pharmacological interventions (Vaiserman et al. 2016). However, one major challenge still is to translate the information gathered from studies performed on experimental model systems of insects, nematodes, rodents and others to human beings. After all, human are perhaps our ultimate target for such interventions!

Another challenge for biogerontologists trying to develop effective means of aging intervention is to come out of the reductionistic mode of doing experiments. The three pillars of health – food, physical activity, and mental and social engagement – require a change in the way the experiments are designed and performed. The history of aging intervention research has shown that taking this or that single compound of natural or synthetic origin, force-feeding it to some experimental model system and analysing one or few molecular targets have, so far, not led to any really useful practical interventions for human beings – whatever the hype by the media or the cosmetic industry.

Furthermore, if we want to curtail the mushroomic growth of self-proclaimed specialists and longevity gurus making false promises, muddling the thinking and promoting impractical and even harmful interventions, then cross-disciplinary collaborations among biologists, engineers, sociologists, philosophers and other scholars from humanities and sciences must be developed (Le Bourg 2013). We also need to ask ourselves as to what is the ultimate aim of aging research: is it to eliminate aging and death forever? And even more importantly, could we, would we and should we do that?

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# 2 Non-coding RNAs as Potential Targets for Treatment and Early Diagnosis of Age-Associated Neurodegenerative Diseases

Shamsuzzama, Lalit Kumar, Rizwanul Haque, and Aamir Nazir

**Abstract**

Neurodegenerative diseases (NDs) are debilitating disorders affecting a significant portion of the world's rapidly growing aging population. Alzheimer's disease (AD), Parkinson's disease (PD), Huntington disease (HD), and amyotrophic lateral sclerosis (ALS) are the most common NDs. These diseases constitute a group of disorders, wherein aggregation of misfolded proteins, mitochondrial function, disruption of cellular signaling, and neuronal cell death occurs. The exact etiology is still unknown, and hence a complete cure to these diseases is yet to be found, partly because these diseases are multifactorial in nature and a single factor responsible for cause and progression of these ailments is not known to exist. Recent studies indicate that non-coding RNAs (particularly miRNAs and circRNAs) are possibly involved in progression of various neurodegenerative diseases. Precisely, miRNAs are highly expressed in the neurons of central nervous system where they play pivotal role during neuronal differentiation and neuronal plasticity. The nature of miRNAs to regulate hundreds of genes, thereby multiple pathways simultaneously, makes it possible that any common miRNA may trigger multiple pathways associated with NDs. The ability of circRNAs to regulate the function of miRNAs by sponging has emerged as interesting possibility, thus being explored as biomarker and as potential novel target for therapeutic intervention against these ailments. Here, we provide an overview on the potential target of non-coding RNAs (miRNAs and circRNAs) in various NDs.

**Keywords**

Non-coding RNA · Neurodegenerative disease · Aging

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## 2.1 Introduction

Age-associated neurodegenerative diseases (NDs) are a major public health challenge to researchers and healthcare providers because a complete cure to these ailments does not exist. The available drugs only provide symptomatic relief leading to worsening of the conditions over a longer period. The characteristic features of NDs include accumulation of misfolded proteins inside and outside of neurons in the major brain regions. Alzheimer's disease (AD) and Parkinson's disease (PD) are the most pervasive among the various NDs reported till date. Over the previous decades, genetic studies have provided clues toward the role of non-coding RNAs in progression of various disease conditions. With recent studies within the field of RNA biology, a class of non-coding RNA, miRNAs, and circular RNAs (circRNA) has emerged as major RNA regulatory molecules. miRNAs regulate gene expression by recognizing untranslated region (UTR) of mRNAs and suppress their function either by inhibition or degradation of mRNA translation. The circRNA molecules are known to be formed by scrambling of exons during splicing and regulate gene expression by sponging miRNAs. The ability of non-coding RNA (particularly miRNAs and circRNA) toward regulating complex gene networks and their specificity has made these molecules immensely interesting toward being explored as novel targets for potential therapeutic intercessions in many disease conditions including NDs. This chapter endeavors to highlight certain critical aspects of non-coding RNAs as potential early diagnostic markers and as possible therapeutic targets; their limitations in such use are also discussed.

## 2.2 Neurodegenerative Diseases

Age-associated neurodegenerative diseases (NDs) are emerging as a major social problem and are posing huge cost to the healthcare providers and caregivers due to increased life expectancy and associated changes. NDs are characterized by progressive loss of neurons and synapses in the nervous system. These diseases are multifactorial in nature ranging from environmental, genetic, endogenous factors to age of the organism. The prominent hallmark of neurodegenerative diseases is age-related accumulation of disease-specific misfolded proteins, for example, Aβ and tau in case of Alzheimer's disease (AD), α-synuclein in Parkinson's disease (PD), huntingtin in Huntington disease (HD), and superoxide dismutase in amyotrophic lateral sclerosis (ALS) (Croese and Furlan 2017). More than 600 neurological disorders have been reported, and the most common among them are AD, PD, HD, ALS, prion disease, and schizophrenia. In India approximately 30 million people are affected from neurological disorders (Gourie-Devi 2014).

### 2.2.1 Alzheimer's Disease

Alzheimer's disease (AD) is the most widely recognized neurodegenerative ailment, ranked as the sixth driving cause of death in the United States, characterized by decline in memory and thinking skill. German physician Dr. Alois Alzheimer, who first time described the symptoms of focal symptoms, progressive cognitive impairment, hallucinations, delusions, and psychosocial incompetence on 4 November 1906 at the 37th Conference of South-West German Psychiatrists in Tübingen. The cause of AD depends upon geography, age, and even ethnicity. Worldwide incidence of AD was 44 million in 2016, and it is expected that it will be reached 65 million by 2030. The worldwide cost of Alzheimer's is assessed to be $605 billion, which is equal to 1% of the entire world's gross production (https://www.alz.org/documents_custom/2016-facts-and-figures.pdfs).

In AD beta-amyloid plaques aggregate outside, while neurofibrillary tangles are formed inside neurons. Beta-amyloid plaques are made up of 40–42 amino acid fragments, which are derived from membranes spanning amyloid precursor protein (APP). These fragments are formed by sequential proteolytic cleavage of β and γ secretase. Initially, α-secretase and β- secretase compete with each other for cleaving APP. If APP is cleaved by α-secretase, then there is no formation of Aβ plaque, but if APP is cleaved by β-secretase, then it is further cleaved by γ-secretase resulting into formation of soluble Aβ-40 and insoluble Aβ-42 fragment. Aβ-42 fragment is chemically stickier than the other lengths and therefore gets accumulated into clumps or plaques.

Tau is a microtubule-associated protein which contains more than 80 potential sites for phosphorylation. Optimal level of phosphorylation of tau proteins is required for microtubule binding. Abnormal hyperphosphorylation of tau leads to self-association rather than binding microtubule, resulting in production of paired helical structure and neurofibrillary tangles (NFTs). Due to accumulation of these plaques and tangles, the communication between neurons as well as transportation of nutrients is hampered, resulting in degeneration of neuronal cells (Schonrock et al. 2012; Brandt and Leschik 2004).

### 2.2.2 Parkinson's Disease

PD is the second most common NDs after AD, which was first reported in great details by an English physician named James Parkinson in "An Essay on the Shaking Palsy" in 1817. The pathological hallmark of the PD was first described by Frederic Lewy. A loss of function of the basal ganglial neurons of substantia nigra pars compacta which leads to depletion of dopamine in the striatum which controls a person's body movement is being reported in Parkinson's patients (Lees 2007). PD is characterized clinically by motor symptoms like tremor, bradykinesia, and rigidity as well as non-motor symptoms like depression, cognitive decline, anxiety, difficulty in memorizing, and sleep disturbances (Massano and Bhatia 2012). PD like other NDs is caused by improper folding of a protein, namely, α-Syn which gets

aggregated and leads to neuronal toxicity and formation of inclusions. These inclusions are termed as Lewy bodies whose major component is α-syn in its misfolded state (Dikiy and Eliezer 2012). Environmental toxins, oxidative stress, and aging play an important role in the case of sporadic PD. Around 20% cases is due to genetic mutation of Parkinson's-related genes explicitly PARK1/PARK4 (α-synuclein), PARK2 (Parkin), PARK6 (PINK1), PARK7 (DJ-1), PARK8 (LRRK2), and PARK9 (ATP13A2) (Coppede 2012).

As indicated by Parkinson's Disease Foundation, more than ten million individuals worldwide are living with Parkinson's ailment till now, and this number is expected to get doubled by 2030. Frequency of Parkinson's increases with age, but an estimated 4% of individuals with PD are diagnosed before the age of 50. The estimated cost of Parkinson's is nearly $25 billion per year in the United States alone which includes both direct and indirect cost (http://www.parkinson.org/Understanding-Parkinsons/Causes-and-Statistics/Statistics).

## 2.3 Challenges in Treating the Neurodegenerative Diseases

The exact mechanisms behind the age-related neurodegeneration of AD and PD are still unknown, and hence a complete cure to these diseases is yet to be found. The discovery of an effective therapy against these diseases is quite challenging due to complex etiology, multifactorial nature, and blood-brain barrier:

1. *Complex etiology of the disease*: Researcher across the world is trying to understand the actual cause of AD and PD. The exact etiology of AD and PD is still unknown, although over the last three decades there has been great progress with respect to understanding the molecular mechanisms behind the initiation and progression of AD and PD. Nonetheless, the inability to interlink the group of abnormalities under a primary pathogenic mechanism of NDs still exists.
2. *Multifactorial nature of the disease*: The main challenge for drug discovery of AD and PD is to choose the right biochemical target. It is known that multiple factor is associated in the development and progression of these diseases. These disease conditions present with aggregated proteins, reduced neurotransmitter levels, elevated reactive oxygen species levels, and neuronal cell death. All of them or any of these may contribute to the development of these ailments, so we require a drug that could interact with several molecular targets of the cascade. All the drugs which are prescribed to patients provide only symptomatic relief, yet none of them inhibits disease progression and hence remains ineffective.
3. *Blood-Brain Barrier*: Healthy human brain has 100 billion neurons, which are connected to each other via process called synapses. Our body provides additional protection of the brain by creating a selective semipermeable membrane barrier called blood-brain barrier. Blood-brain barriers help to maintain the integrity and microenvironment of the brain by inhibiting the entry of almost every molecule except the entry of essential nutrients like glucose, some amino acid, insulin, and other precursor molecules. Although blood-brain barriers play

pivotal role to protect the brain from most pathogen and other fluctuation of ions in the blood, they also create problem for delivering the new drugs into the brain to cure neurological disorder.

## 2.4 Treatments Available

Till now, there is no complete cure for neurodegenerative diseases (particularly AD and PD). However FDA-approved drugs are prescribed by doctors to AD and PD patients; these drugs provide only symptomatic relief, and their effectiveness varies from person to person (Table 2.1).

**Table 2.1** List of drugs approved for AD and PD

| Drug name | | Brand name | Function | For |
|---|---|---|---|---|
| Donepezil | | Aricept | Cholinesterase inhibitor | AD |
| Galantamine | | Razadyne | Cholinesterase inhibitor | AD |
| Memantine | | Namenda | NMDA (N-methyl-D aspartate) receptor antagonist | AD |
| Rivastigmine | | Exelon | Cholinesterase inhibitor | AD |
| Donepezil and memantine | | Namzaric | Cholinesterase inhibitor + NMDA (N-methyl-D aspartate) receptor antagonist | AD |
| Levodopa | | Sinemet | Natural chemical that is converted to dopamine in the brain | PD |
| Carbidopa-levodopa | | Duopa | Levodopa is changed over to dopamine in the brain. Carbidopa keeps the breakdown of levodopa before it can reach the brain and induce its effect | PD |
| Dopamine agonists | Pramipexole | Mirapex | Dopamine agonists actually mimic the effects of dopamine without having to be converted | PD |
| | Ropinirole | Requip | | |
| | Rotigotine | Neupro | | |
| | Apomorphine | Apokyn | | |
| Glutamate antagonist (amantadine) | | Symmetrel | The exact function is unknown; it is given along with other drugs of PD | PD |
| MAO-B inhibitors | Selegiline | Eldepryl, Zelapar | They keep the breakdown of brain dopamine by deactivating the brain enzyme monoamine oxidase B (MAO-B) | PD |
| | Rasagiline | Azilect | | |
| Anticholinergics (benztropine) | | Cogentin | Reduce symptoms of tremor in people | PD |
| COMT inhibitors (entacapone) | | Comtan | They mildly prolong the impact of levodopa by inhibiting an enzyme that breaks down dopamine | PD |
| Pimavanserin | | Nuplazid | They reduce hallucinations and delusions associated with Parkinson's disease psychosis by acting as an inverse agonist and antagonist of serotonin 5-HT2A receptors | PD |

## 2.5 Junk DNA Hypothesis

A major portion of human genome is transcribed but less than 1.5% of genes encode proteins. In ancient day it was believed that the portion of genomic DNA which was not translated to any protein is the junk DNA and has no role in the survival of the cell. But now the next generation of geneticists revealed that some pieces of junk DNA play important role to our survival as our more familiar genes. Many of them may transcribe into molecule that participates in development and other biological process. If these pieces of junk DNA become damaged, we may suffer devastating consequences like cancer, brain damage, and neurodegenerative disease depending on what pieces are affected. Genetic studies have provided clues toward the role of non-coding RNA (ncRNA) in various processes of cell survival and different diseases conditions including NDs.

## 2.6 Non-coding RNAs

Non-coding RNAs are the major class of regulatory RNAs, known to play key role in gene regulation at the posttranscriptional and transcriptional level. MicroRNAs (miRNAs) are endogenous 20–23 nt long, non-coding RNAs, found along the taxa. miRNAs are known to involve in important regulatory functions in course of gene expression. miRNAs recognize untranslated region (UTR) of mRNAs and block their function either by cleavage or inhibiting the translation of mRNA. Mature miRNAs are generated either by canonical or noncanonical pathways (Meza-Sosa et al. 2012).

## 2.7 History of miRNA

Lin-4 was the first miRNA to be described in a model system *Caenorhabditis elegans* (*C. elegans*) by Victor Ambros' group in 1993 (Lee et al. 1993). Lin-4 function as a regulator of lin-14 genes, a nuclear factor that negatively regulates the transition to larval stage. Thus lin-4 miRNA controls the embryonic cell lineage patterns by decreasing the expression of lin-14 mRNA (Wightman et al. 1993). In 2000, a second miRNA named let-7 was discovered by two separate groups, which play important roles in the development of a later larval stage to adult in *C. elegans* (Reinhart et al. 2000; Slack et al. 2000).

## 2.8 Role of Non-coding RNAs in Various Disease Progressions

Expression of non-coding RNAs (particularly miRNAs) greatly varies from organ to organ, and also their expression levels have been changed in normal and disease condition (Liang et al. 2007). Brains of vertebrates have a greater number of

miRNAs than any other organ. Alterations in miRNAs expression level have been seen in the brain of neurodegenerative disease patients (Adlakha and Saini 2014). Recent studies suggest that miRNA-regulating pathways may be playing a central role in the development of various disease progressions including Alzheimer's, Parkinson's, Huntington's, and amyotrophic lateral sclerosis.

## 2.9 Non-coding RNAs and Alzheimer's Disease

The pathological hallmarks of AD are the accumulation of extracellular amyloid plaque and intracellular neurofibrillary tangles. The etiology of the AD is still poorly understood, and complete treatment is unavailable till now. Over the past decade, non-coding RNA (including miRNA) has arisen as a major class of regulatory molecules which participated in various physiological processes and disease condition. Growing evidences suggest that alteration of non-coding RNA network could contribute to risks for the development of AD (Weinberg and Wood 2009). Among all non-coding RNAs, miRNAs are extensively studied. A number of specific miRNAs are dysregulated in AD and cerebral spinal fluid (CSF). A study reported that miR-124a, miR-125b, miR-128, miR-132, and miR-219 were abundantly altered in AD brain (Kumar et al. 2017). Subsequently, many groups have showed that expression of miR-29 family which included miR-29a, miR-29b, and miR-29c changed in brains of AD patients. As compared to normal elder-age levels, miR-34a and 181b are significantly upregulated in AD subjects. As miR-29a and miR-29b-1 were found to regulate the expression of BACE1 mRNA, the miR-29a/b-1 cluster was significantly declined in AD patients showing strangely high BACE1 protein levels (Hebert et al. 2008). miR-124 and miR-9 could control endogenous tau exon 10 splicing in neuronal cells by regulating specific splicing factors (Hebert et al. 2012). miR-103 and miR-107 repressed the translation of cofilin mRNA (Yao et al. 2010). Cofilin forms Hirano bodies which are also present in AD brain patients in addition to amyloid plaques and neurofibrillary tangles (Hirano 1994). miR-107 regulates beta-amyloid precursor protein cleavage enzyme (BACE1), and it is found that post-mortem AD human brain has low levels of miR-107 (Wang et al. 2008a).

BACE1 antisense transcript (BACE1-AS), an lncRNA, transcribed by the antisense strand of BACE1, could control BACE1 expression, and BACE1-AS concentrations were elevated in APP transgenic mouse (Modarresi et al. 2011). Another lncRNA BC200 level was increased in those areas in parallel with the progression of AD (Mus et al. 2007). Other ncRNAs are little known in the role of AD, such as 17A siRNA, which was embedded in the GABA B receptor and deregulated in brain tissue in AD patients. Furthermore, 17A promoted Aß secretion and increase the accumulation of Aß (Massone et al. 2011).

## 2.10 Non-coding RNAs and Parkinson's Disease

Selective degeneration of dopaminergic neurons and accumulation of α-synuclein in substantia nigra lead to Parkinson's disease. α-Synuclein is controlled posttranscriptionally by miR-7 and miR-153 (Doxakis 2010). A single-nucleotide polymorphism in the promoter region of fibroblast growth factor 20 (FGF20) disrupts the binding of miR-433, resulted increase the expression of FGF20. Increased expression of FGF20 protein correlated with increased expression of α-synuclein protein (Wang et al. 2008b). LRRK2, the most affected gene in PD functions in the dopaminergic neurons, negatively regulates let-7 and mir-184, and silencing of let-7 leads to neuroprotection through reducing α-synuclein aggregation (Gehrke et al. 2010; Shamsuzzama et al. 2017). Expression of miR-133b is observed in the midbrain dopaminergic neurons, and decreased expression of mir-133b may play a neuroprotective role (Wang et al. 2008b). Scientist reported that lncRNAs and their expression levels have been increased in neurodegenerative disease, for example, increased level of RP11-462G22.1 and RP11-79P5.3. lncRNA has been seen in PD (Soreq et al. 2014). Recently, C. Carrieri et al. identified AS Uchl1 as an antisense to the mouse ubiquitin carboxy-terminal hydrolase L1 (AS Uchl1). Mutation of AS Uchl1 gene has been reported in early-onset familiar PD. Also, loss of UCHL1 activity has been accounted in numerous neurodegenerative disorders (Carrieri et al. 2015).

## 2.11 Non-coding RNAs and Huntington's Disease

Huntington's disease (HD) is an autosomal dominant neurological disorder, caused by mutation in the gene that encodes huntingtin protein (HTT) (Bilen et al. 2006). Recent studies suggest that posttranscriptional regulations of genes by miRNAs are also altered in HD. Expression of neuronal miRNAs, namely, mir-132, mir-124, and mir-9/9*, is downregulated in mouse models and human HD patients (Johnson and Buckley 2009). Downregulation of miRNAs let-7a, let-7c, let-7d, and let 7e was observed in HD and upregulation of miR-30a, miR-30b, miR-30c, and miR-30e in HD (Marti et al. 2010). A few miRNAs, for example, miR-9, miR-29b, miR-29a, miR-129a, miR-132, miR-330, miR-17, miR-196, miR-222, miR-485, and miR-486 are affected in HD, and in addition, previous reports suggest that MiR-34b is elevated in plasma of Huntington's disease patients (Conaco et al. 2006; Packer et al. 2008). lncRNAs have important roles in progression of diseases; TUG1, LINC00341, RPS20P22, and NEAT1 lncRNAs are upregulated, and MEG3, DGCR5, LINC00342, and DGCR5 lncRNAs were downregulated in HD (Johnson et al. 2009; Smeenk et al. 2011).

## 2.12 Non-coding RNAs and Amyotrophic Lateral Sclerosis (ALS)

The much related to non-coding RNAs is not explored yet in ALS. The importance of miRNAs in ALS was observed through synapses, neurofilaments, neurogenesis, and neuroinflammation. In synapses miR-206, miR-29a, miR-29b, miR-455, and miR-338-3p were upregulated, and miR-149, miR-328, miR-451, miR-583, miR-638, miR-665, and miR-1275 were downregulated (Toivonen et al. 2014; Williams et al. 2009; Valdez et al. 2014; Russell et al. 2013). miR-146a, miR-524-5p, miR-1, and miR-582-3p were found upregulated, and miR-9, miR-124a, miR-134, and miR-125 were downregulated in neurogenesis (Zhang et al. 2013; Marcuzzo et al. 2014; Zhou et al. 2013; Nolan et al. 2014). In case of neuroinflammation, miR-155, let-7, miR-223, and miR-365 were found upregulated, and miR-148b-5p, miR-577, miR133b, and miR-140-3p were downregulated (Koval et al. 2013; Parisi et al. 2013).

## 2.13 miRNAs and circRNAs as Potential Targets for Neurodegenerative Diseases

Regulatory function of miRNAs and circRNAs have significant role in neuronal development, differentiation, and maturation. Dysregulations of miRNAs and circRNA expression are known to involve in the development of neurodegenerative Alzheimer's and Parkinson's disease (Gehrke et al. 2010; Hoss et al. 2016; Femminella et al. 2015; Kumar et al. 2016).

circRNAs are normally expressed in the mammalian cells, and it is estimated that their expressions were modulated in disease conditions. circRNAs might have pivotal roles in the development and progression of numerous human diseases including NDs. In NDs the various functions of circRNAs are proposed, but precise mechanistic understanding is not yet explored. Researchers across the world give a vision about the involvement of miRNA and circRNA in neurological ailments like AD and PD. It is known that miR-7 miRNA regulates the expression of α-synuclein protein (Junn et al. 2009). ciRS-7/CDR1, a circRNA, acts as a regulator of miR-7, which may provide strong evidence toward the association of ciRS-7/CDR1-AS in PD. Different miRNAs such as let-7, miR-34a/b, and miR-153 are likewise observed to be reduced in PD (Doxakis 2010; Minones-Moyano et al. 2011), which provide a clue that there are possibilities of association of other unknown circRNAs in the progression of PD.

Alzheimer's disease, the most common NDs aggregation of β-amyloid protein, leads the main cause of disease (Ambros 2004) and has been accounted for to be related with miRNAs like let-7i, miR-9, miR-15, miR-146b, miR-181c, miR-210, miR-338, and miR-451. These miRNAs are recorded as downregulated in patients suffering from AD (Hebert et al. 2008; Maes et al. 2008). The age-related studies reported that expression of miR-34 was likewise observed to be decreased (Dimmeler and Nicotera 2013). Increased expressions of circRNAs have been seen in neuronal

tissue at the time of development and during CNS aging. In *Drosophila*, it has been found that expressions of circRNAs increase with the increment of age. Increasing the accumulation of circRNAs with age provides a clue for the establishment of circRNA molecules as biomarker of aging (Westholm et al. 2014). Huge numbers of circRNAs are described in neuronal tissues (Ashwal-Fluss et al. 2014). Moreover, a large number of circRNAs was identified as part of mammalian brains (Rybak-Wolf et al. 2015). Expressions of circRNAs are stage specific, and it has been reported that circRNAs were upregulated constantly during development (You et al. 2015). Involvements of miRNAs are also seen in the progression and development of Huntington's disease, multiple sclerosis, and amyotrophic lateral sclerosis (ALS). Recent study suggests that there is a plausibility that expression of these miRNAs may get blocked by some unidentified circRNAs which makes circRNAs a novel target for the treatment of neurological disorder.

## 2.14 Progress Being Made

Till date there is hardly any disease in which expression of miRNAs is not known to have any role in the progression and development of disease. The huge associations of miRNAs across human diseases have shown that miRNA can be used as new therapeutic strategies. To date there are two approaches that have been used for developing miRNA-based therapeutics: miRNA antagonist and miRNA mimics. miRNA antagonist was generally used to create loss of miRNA function. In this strategy, a highly modified miRNA passenger strand is introduced that binds with the active miRNA strand. The binding of miRNA with antagomir is irreversible, so that miRNA duplex is unable to be processed by RISC which resulted in degradation by dicer enzyme. miRNA mimics, also known as miRNA replacement therapy, are used for gaining of miRNA function. Introduction of miRNA mimics leads to a reactivation of pathways that are needed for normal biological process and blocks those protein synthesis that leads to disease (Pereira et al. 2017; Bader et al. 2010; Kota et al. 2009; Wiggins et al. 2010).

Dysregulation of miRNAs and circRNAs has been reported in a variety of diseases. Very little is known about the function of individual circRNAs and the biological implications in progression of NDs. With the identification of miRNAs biomarkers in serum or plasma, the clinical development of pharmaceutical drugs based on miRNA might be possible in the near future. miRNA as new biomarkers for complex neurodegenerative Alzheimer's and Parkinson's might be used as early diagnostic tools and prediction of drug response and side effects.

## 2.15 Strategies Ahead

There are many challenges faced for targeted delivery of molecule; many of them include half-life of miRNA mimics/inhibitor and its potential off-target effects. However, in 2011, Alvarez-Erviti et al. reported that by using exosome, siRNA and

protein can deliver into the brain of mice when it was injected intravenously (Alvarez-Erviti et al. 2011). Despite many challenges, several miRNA molecule mimics/antagomirs have progressed into product and clinical development. According to previous report, the most advanced miRNA, which are used as therapeutics candidates, are shown in Table 2.2 (Lages et al. 2012).

## 2.16 Advent of Non-coding RNAs as an Early Diagnostic Tool

The circulatory nature of miRNAs in the blood and its regulatory function to regulate hundreds of genes simultaneously make it a potential early diagnostic tool and therapeutic target for neurodegenerative diseases. For example, Wang et al. reported that miR-146 can be use as biomarker for the early diagnosis of AD, because it can be detected in human blood monocytes. And the upregulatory effect of miR-146 can be reduced by using therapeutic potential of miRNA, i.e., anti-miR-146a (Wang et al. 2012). Another separate study, which was conducted in a transgenic mice model of AD (Tg-19959), demonstrated that the aggregation of soluble beta-amyloid was significantly decreased by using LNA-modified siRNA targeting BACE1 and BACE-1-AS (Modarresi et al. 2011). Large number of miRNAs like miR-7, let-7, miR-153, miR-133b, miR184, and miR-433 might be associated with the pathophysiology of PD, which suggests a novel therapeutic target (Harraz et al. 2011). In general, literature survey recommends that different non-coding RNAs particularly miRNAs could serve as early diagnostic biomarkers and therapeutic targets for neurological diseases.

## 2.17 *C. elegans* as Model Organism to Study the Role of Non-coding RNAs for Neurodegenerative Diseases

*C. elegans* is good genetic model system to exploring the function of non-coding RNAs. It has conserved pathways with powerful molecular and genetic tools that enable cost-effective discovery of new non-coding RNAs. Deep-sequencing technologies such as next-generation sequencing (NGS) provide a great opportunity in accelerating the rate of novel miRNAs discovery. Till now miRNAs database, miRBase-22, illustrated that *C. elegans* genome has 253 miRNAs precursor and 437 mature miRNAs, although this number might be higher (Coppede 2012).

**Table 2.2** Therapeutic potential of miRNAs

| miRNA | Diseases | Status of development |
|---|---|---|
| miR-122 (antagonist) | Hepatitis C virus | Phase 2 clinical trials |
| miR-208/499 (antagonist) | Chronic heart failure | Preclinical development |
| miR-195 (antagonist) | Post-myocardial infarction remodeling | Preclinical development |
| miR-34 (mimic) | Cancer | Preclinical development |
| Let-7 (mimic) | Cancer | Preclinical development |

Cortes-Lopez et al. reported that 1166 circRNAs are accumulated in *C. elegans* during aging. These circRNAs are derived from 797 genes that have diverse function (Cortes-Lopez et al. 2018).

## 2.18 Future Directions

From the past few years, the interest in the contribution of ncRNAs to the development and progression of neurodegenerative disease is booming, but much effort is wanted toward determination of the full extent of this contribution and the mechanism by which ncRNAs may exert their pathological effects. The emerging genomic, epigenomic, and bioinformatic approached will be crucial in this context. The important challenge is to identify and characterize their mechanism to all ncRNAs encoded in the human genome. The nature of ncRNAs especially miRNAs to regulate hundreds of genes simultaneously thereby it could regulate multiple pathways simultaneously, that make it possible that any common miRNA may trigger multiple pathways associated with neurological disorders. The ability of miRNA molecules to regulate complex gene networks and specificity of miRNA sequences led these molecules to be regarded as exciting novel targets for potential therapeutic interventions in disease conditions.

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# The Potential Role of Stem Cell Reprogramming in Antiaging

3

Banu Orta Yilmaz and Melike Erkan

**Abstract**

Aging is a natural process defined as a progressive decline in physiological functions which lead to increased risk of diseases and death. Recent advances in antiaging intervention have focused on stem cell-based therapies and cell reprogramming. The development of stem cell reprogramming to fight the aging process has recently become important issue in antiaging strategies. Stem cell-based therapies and cell reprogramming have provided various strategies to alter somatic cell identity into induced-pluripotent stem cell. Stem cells are defined as pluripotent cells that possess both the abilities of self-renewal and differentiation toward numerous cell types. Cell reprogramming is simply composed of deleting cell memory and rewriting new identity of somatic cell. Stem cell reprogramming has provided enormous insight on regenerative medicine for antiaging. This chapter has focused on potential role of stem cell reprogramming to slow down aging process.

**Keywords**

Stem cell reprogramming · Stem cell therapy · Antiaging · Induced-pluripotent stem cells · Transcriptional factors

## 3.1 Stem Cells and Aging

Stem cells are functionally undifferentiated biological cells that can be transformed into different types of cells during embryonic and adult period (Jeevani 2011). These cells have a role in the repair and renewal of various tissues and organs and

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have the ability to self-renew to produce more stem cells (Avasthi et al. 2008). A stem cell has two different roles. One of them is to provide new stem cells for the stability of stem cell reserves. The other is to differentiate into cells with a special function, such as muscle, bone, brain, or red blood cells, in case of deficiency (Bindu and Srilatha 2011).

Stem cells are classified into five main classes according to their ability to transform into different cell types (Table 3.1). These five main classes are totipotent, pluripotent, multipotent, oligopotent, and unipotent (Kalra and Tomar 2014). *Totipotent* stem cells have the potential to differentiate into all cells in the body. These cells occur after the fertilization of the sperm and the egg and include the cells formed after the first few divisions of the fertilized egg (zygote). The fertilized egg is the only stem cell known for its totipotent property (Blau et al. 2001). *Pluripotent* stem cells can be transformed into nearly all cell types, but these cells are not competent to constitute the all organism. Pluripotent stem cells can generate all differentiated cell types that are derived from the mesoderm, endoderm, and ectoderm germ layers in the body and also have the potential to self-renew (Gardner 2002). *Multipotent* stem cells are found in adult tissues, but only those of a closely related family of cells. These stem cells can only be transformed into a limited number of cell types. Multipotent stem cells can only form into cells of the organ from which they originate. For example, a blood multipotent stem cell can be differentiated into all kinds of blood cells, but not a brain or skin cell (Verfaillie et al. 2002). *Oligopotent* stem cells are differentiated into only a few cell groups. For example, vascular stem cells, which have the ability to differentiate into both endothelial and smooth muscle cells, depending on the requirement, are oligopotent stem cells (Majo et al. 2008). *Unipotent* stem cells are only capable of differentiating into a single cell type. Muscle stem cells are example for unipotent cells, which are also known as precursor cells. Unipotent stem cells are distinguished from non-stem cells by their ability to self-renew (Blanpain et al. 2007).

Stem cells based on their sources are embryonal and adult stem cells (Snykers et al. 2009) (Table 3.1). *Embryonic stem cells* (ESC) are pluripotent stem cells derived from the inner cell mass of the blastocyst, which occurs immediately after fertilization of eggs and sperm in oviduct. In the early stages of embryonic development, the cells remain partially undifferentiated and have the ability to become almost any tissue in the body. Human embryonic stem cells are derived from embryos that are typically 4 or 5 days and consist of approximately 100–200 cells. Embryonic stem cells derived from early embryos have two important characteristics: self-renewal and pluripotency (Smith 2001). *Adult stem cells* are multipotent and often produce cell types of the tissue which they are present. There are also pluripotent adult stem cells with fewer numbers and are found in various tissues, including umbilical cord blood. These cells are undifferentiated cells, and they can self-renew indefinitely, have the potential to transform into specialized cells of other tissues, and provide continuity and repair of tissue (Young and Black 2004). When recent studies were examined, it was found that the tissues reported to contain stem cells are increasing. These tissues can be briefly described as: bone marrow, peripheral blood, brain, spinal cord, dental pulp, blood vessels, skeletal muscle,

**Table 3.1** Types and classifications of stem cells

| Types and classification of stem cells | | | | |
|---|---|---|---|---|
| Potency | | Description | Example | |
| | Totipotent | Differentiate into all possible cell types | Morula stage cell | |
| | Pluripotent | Differentiate into almost all cell types | Inner mass cell | |
| | Multipotent | Differentiate into a closely related family of cells | Adipose tissue cell | |
| | Oligopotent | Differentiate into a few cells | Corneal epithelium cell | |
| | Unipotent | Only produce cells of their own type | Muscle stem cell | |
| Sources | Embryonic stem cells | Form any differentiated cell of the body | Blastocyst stage cells | |
| | Fetal stem cells | Primitive cell types found in fetus | Cord blood stem cell | |
| | Adult stem cells | Undifferentiated cells which maintain and repair the tissue that are found | Hematopoietic | Mesenchymal |
| | | | Myeloid stem cell | Bone marrow stromal stem cell |
| | | | Lymphoid stem cell | |
| | Induced pluripotent stem cells | Reprogrammed somatic cells | All possible types of specialized cells | |

epithelium of the skin and digestive system, cornea, retina, liver, and pancreas (Valarmathi and Fuseler 2011).

Aging is a disease that occurs in tissues and organs, depending on time, with genetic and environmental factors, and continues throughout the process, from birth to death (Carmona and Michan 2016). Primary aging is disruption of the molecular mechanism of structural and functional integrity of cells and tissues by genetic factors. Secondary aging is observed by the effects of diseases and environmental factors. Aging is a very complicated phenomenon, which is affected by many molecular mechanisms. Based on the studies in recent years, the mechanisms of aging can be categorized as follows: telomere hypothesis, premature aging syndromes, epigenetic factors, oxidative stress and mitochondrial damage, growth hormone deficiency, and somatic mutations (Collins et al. 2007; Dykstra et al. 2011; Bernet et al. 2014; Cosgrove et al. 2014). In the last decades, it is clear that aging of an organ is linked to regression associated with aging in somatic stem cell function in various animal models (Akanuru and Geiger 2016). Studies have shown that the reduction of numbers of mammalian adult stem cells and the loss of function are closely related to aging (Oh et al. 2014). Loss of stem cells play a major role in age-related diseases such as osteoporosis, Alzheimer's, atherosclerosis, progressive Parkinson's disease, type 2 diabetes, anemia, and cancer (Vilchez et al. 2013). Many important physiological, functional, and molecular parameters are involved in stem cell senescence. These parameters can be briefly summarized as follows: typical Hayflick

phenomenon of cellular aging, decrease of proliferation potential, shortening telomeres, DNA damage, epigenetic changes, increased oxidative stress, and mitochondrial dysfunction (Vilchez et al. 2013; Noda et al. 2009).

## 3.2 Recent Advances in Cellular Reprogramming Era

In the beginning, stem cells could only be taken in the embryonic stage, but as seen in recent studies, mature cells were converted into primitive cells by a process called reprogramming, which made it possible to transform into all kinds of cells in the human body. In 2006, Takahashi and Yamanaka identified induced pluripotent stem cells (iPSCs) to be used in stem cell research and treatments (Takahashi and Yamanaka 2006). They have shown that in the study, stem cells can be obtained by reprogramming differentiated fibroblast cells from an adult mouse. The same study also showed that these cells transformed from fibroblast cells when injected into mouse embryos and differentiated into many cells other than post-growth fibroblasts. The reprogramming of these fibroblast cells to acquire stem cell characterization has been achieved using four transcription factors (Octamer-binding transcription factor 4 (Oct4), sex determining region Y-box 2 (Sox2), Kruppel-like factor 4 (Klf-4) and cMyc) (Takahashi et al. 2007; Aoi et al. 2008). From the iPSCs obtained by reprogramming, highly differentiated cells with different roles and characteristics such as cardiovascular, retina, and macrophage were produced in different cell culture medium (Narazaki et al. 2008; Hirami et al. 2009; Senju et al. 2009). Reprogramming can be induced not only by Oct3/4, Sox2, Klf4, and c-Myc but also by combinations of other genes that provide transcriptional control of stem cells such as Nanog, Lin28, ESRRB, and NR5A2 (Ichida et al. 2009; Yu et al. 2007). Recent studies have shown that adult somatic cells can be transformed into specialized cell types using various transcription factors (Table 3.2). Because of their self-renewal and pluripotency capacities, human iPSCs have been shown to be used in many disease models such as osteoporosis, Alzheimer's, progressive Parkinson's disease, type 2 diabetes, and cancer (Yang et al. 2016; Hallett et al. 2015; Qi et al. 2016; Kudva et al. 2012; Griscelli et al. 2017; Jones et al. 2017; Toustrup et al. 2017; Heman-Ackah et al. 2017).

## 3.3 Molecular Mechanism of Stem Cell Reprogramming

Aging is related to the disruption of the homeostatic mechanisms that support the structure and function of adult tissues. The growing number of mutations due to aging causes increased possibility of cellular apoptosis, senescence, and malignancy, and thus, aging is a risk factor for many diseases (Rando and Chang 2012).

The discovery of reprogramming mechanisms that redefine the transcriptional program in adult cells, not only with regard to potential but also telomere maintenance, oxidative damage, and senescence signaling, has made it easier to maintain the viability of adult stem cells in culture and to protect these cells in vivo (Boyette

**Table 3.2** Cellular reprogramming with transcription factor expression

| | Cell source | Induced cells | Transcription factors | References |
|---|---|---|---|---|
| In vivo *studies* | Exocrine cells | β-cells | Pdx1, Neurog3, Mafa | Zhou et al. (2008) |
| | Cardiofibroblasts | Cardiomyocytes | Gata4, Mef2c, Tbx5 | Qian et al. (2012) |
| | Astrocytes | Neurons | Ascl1, Brn2, Myt1l | Torper et al. (2013) |
| | Astrocytes | Neuroblast | Sox2 | Niu et al. (2015) |
| | Myofibroblasts | Hepatocytes | Foxa3, Gata4, Hnf1a, Hnf4a | Song et al. (2016) |
| | Myofibroblasts | Hepatocytes | Foxa1, Foxa2, Foxa3, Gata4, Hnf1a, Hnf4a | Rezvani et al. (2016) |
| | Granulosa and theca cells | Sertoli and Leydig cells | Foxl2 | Uhlenhaut et al. (2009) |
| In vitro *studies* | B cells, T cells, fibroblasts | Macrophage-like cells | C/EBPα, PU1 | Feng et al. (2008) |
| | Fibroblasts | Neuron-like cells | Ascı1, Brn2, Myt1l | Ieda et al. (2010) |
| | B cells | Macrophages, T cells | Pax5 | Vierbuchen et al. (2010) |
| | Fibroblasts | Neurons | Ascı1, Brn2, Myt1l | Pfisterer et al. (2011) |
| | B cells, T cells, fibroblasts | Macrophage-like cells | C/EBPα, PU1 | Xie et al. (2004) |

and Tuan 2014). Particularly in mouse embryonic stem cells, the regulation of stem cell pluripotency and differentiation has been studied both transcriptionally and epigenetically. Nowadays, highly efficient sequencing techniques are used to characterize the regulatory networks in all embryonic stem cells. In determining the fate of stem cells, the roles of regulatory networks, including the function of microRNAs and epigenetic markers, are analyzed thoroughly.

Regulatory networks in the reprogrammed cells are also investigated through analytical processes involving the whole genome. Studies on induced pluripotent stem cells have shown that many diseases related to aging can be treated (Hallett et al. 2015; Qi et al. 2016; Kudva et al. 2012; Griscelli et al. 2017). However, it has also been observed in studies that the resistance of cells against reprogramming by classic Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc) increases in parallel with the age of individuals (Kasper et al. 2009). There are seven Sir2 homologues in mammalian cells, which are SIRT1 to SIRT7 and act on many cellular metabolic pathways (Lavu et al. 2008; Donmez and Guarente 2010). Sirtuin 6, or SIRT6, which has been identified as a critical regulator of transcription, genomic stability, and telomere integrity, was shown to upregulate transcription in an adult stem cell reprogramming process. Although SIRT1 has been shown to be the most effective sirtuin for aging, it has been shown not to extend the life span of transgenic mice which overexpressed SIRT1. On the other hand, studies on

male mice have shown that SIRT6 extends life span (Hall et al. 2013). Therefore, SIRT6 can indicate the relationship between aging, rejuvenation, and epigenetics in the reprogramming processes (Sharma et al. 2013). Studies have shown that SIRT7 deacetylates p53 and promotes transcription of RNA polymerase; also in SIRT7 knockout mice it has been shown to shorten life span with aging-related diseases (Vakhrusheva et al. 2008).

Determining the transcriptional networks and epigenetic profiles between cells of different ages and species will help to reveal the general characteristics of aging. As with the relationship between the pluripotent and differentiated state, these transcriptional networks and epigenetic profiles make it possible to directly test whether it is possible to program a cell to be young or old. In conclusion, it can be said that cell aging, which is a cellular recycling process, is characterized by progressive epigenetics rather than permanent genetic mutations (Rando and Chang 2012).

## 3.4 Antiaging Strategy with In Vivo and In Vitro Stem Cell Reprogramming

Diseases that increase with aging are a major factor in the shortening of human life span. Therefore, scientists are looking for various ways to delay aging. One of these is the reprogramming of the cells. Any dividing cells in the body could be reprogrammed into iPSCs. Therefore, iPSCs-based therapies have become popular in recent years as tools that shed light on the field antiaging medicine. In this regard, some studies have revealed that iPSCs from elderly people have been reprogrammed to treat age-related disease (Ohmine et al. 2012; Somers et al. 2010; Yagi et al. 2012). For this purpose, human keratinocytes derived from 56 to 78 years old individuals were reprogrammed using Oct4, Sox2, Klf4, and c-Myc. According to results of this study, reprogrammed human keratinocytes demonstrated states associated with antiaging such as morphological changes, induction of pluripotency genes, telomere elongation, and downregulation of senescence and apoptotic genes (Ohmine et al. 2012). Somers et al. (2010) reported that the use of humanized version of a single lentiviral "stem cell cassette" vector for reprogramming fibroblasts obtained from humans may be utilized for regenerative medicine applications. In another study, fibroblasts obtained from centenarian donors (106 and 109 years old) were reprogrammed via Yamanaka factors and then it was found that obtained iPSCs showed exceptional longevity with no serious disease risk factors (Yagi et al. 2012). It has been shown that senescent fibroblasts from 74-year-old donor can be reprogrammed using reprogramming cocktail and thus some aspects of aging are rejuvenated by cellular reprogramming (Lapasset et al. 2011).

Additionally, some in vitro studies have indicated that reprogramming by Yamanaka factors can reset epigenetic signs associated with cellular damage, stress, and senescence (Liu et al. 2011; Zhang et al. 2011). In the light of the results obtained from these in vitro studies, short-term induction with Yamanaka factors (2 days) in vivo ameliorates aging marks and promotes tissue regeneration, and thus extends life span of mice (Ocampo et al. 2016). Furthermore, regenerative capacity of β-cells

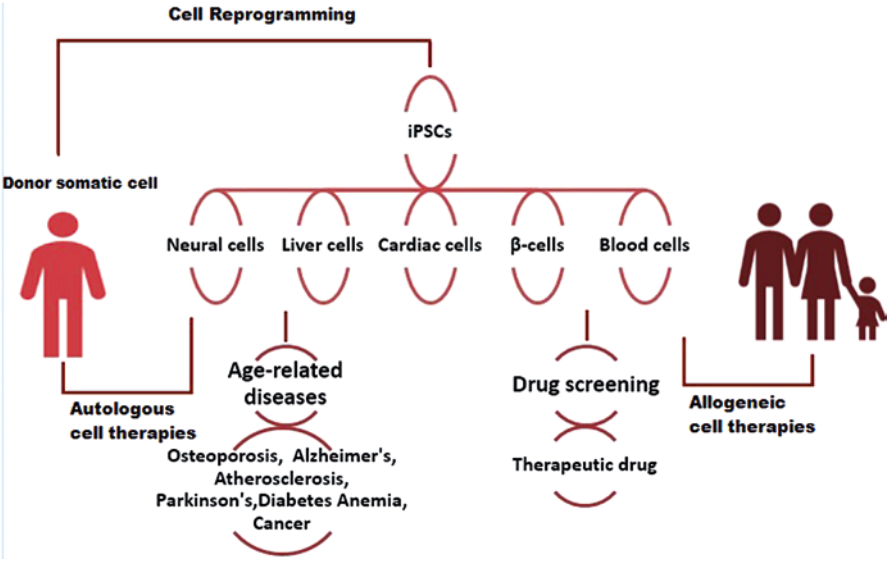


**Fig. 3.1** Summary of potential antiaging therapies based on stem cell reprogramming

or skeletal muscle after pancreatic or muscle injury improved in short-term Yamanaka factors-induced old-aged mice (Taguchi and Yamada 2017). Recently, in vivo cell reprogramming with Yamanaka factors provided benefits in acceleration of drug development and clinical human trials for treated diseases (Fig. 3.1).

## 3.5 Therapeutic Approaches of Aging

The growing knowledge of stem cell biology and the ability to regulate the ex vivo and in vivo differentiation capacities of stem cells are promising for the regeneration of damaged tissues and organs (Barrilleaux et al. 2006). The fact that many aging diseases are due to the exhaustion of adult stem cells has led to the necessity of repairing adult stem cell function for regeneration and healing of aged tissues (Kasper et al. 2009). Adult stem cell transplantation in humans has responded positively to the treatment of many diseases such as ischemic heart diseases, rejuvenation for the aging brain, vascular system disorders, erectile dysfunction, and stroke (Behfar et al. 2007; Qiu et al. 2012; McGuckin et al. 2013; Lopez-Leon et al. 2017). The appearance of these diseases in the elderly and loss of function in stem cells depending on age make the treatment process difficult (Lepperdinger et al. 2008). In order to demonstrate age-related changes in the functional behavior of mesenchymal stem cells, migration rates, differentiation, and proliferation capacities of cells from young and old donors were compared. Researchers concluded that depending on age the migration capacity decreased and senescence rate increased in mesenchymal stem cells (Kasper et al. 2009).

Transplantation of adult stem cells or endogenous regulation of adult stem cells in vivo with specific growth factors has allowed the development of new stem cell-based therapeutic approaches for regenerative medicine. Two main findings of mesenchymal stem cell senescence are reduced defense against reactive oxygen species and impaired actin dynamics (Kasper et al. 2009; Yagi et al. 2013).

Researchers have been able to improve stem cell function and thus increase therapeutic capacity by using various antioxidants and growth factors to increase the resistance of stem cells to reactive oxygen species. Regulation of the balance between the stability and division of adult stem cells is provided through the activation of various developmental signals (Mimeault et al. 2007). Hormones, fibroblast growth factor, epidermal growth factor, sonic hedgehog, Wnt/β-catenin, Notch, and bone morphogenic proteins may upregulate the self-renewal and differentiation capacities of adult stem cells under certain physiological and pathological conditions (Moore and Lemischka 2006).

## 3.6 Conclusion

Although there are many studies on the relationship of stem cells to aging, there are many other important questions and technical difficulties. In 2006, reprogramming mouse somatic cells with a small number of transcription factors led to accelerated stem cell studies (Takahashi and Yamanaka 2006). Induced pluripotent stem cells obtained as a result of this study have enabled the use of induced pluripotent stem cells in the identification of age-related human diseases and have encouraged studies in this regard. Recently, studies have been conducted to convert somatic body cells into stem cells in vivo and in vitro. Reprogramming with the use of lineage-specific transcription factors in vivo has become an advantage for regenerative medicine. In vivo programming is more useful because of many reasons such as genetic changes that can occur in long-term in vitro culture. All these developments in cellular reprogramming allow the development of a therapeutic model for human diseases by using the relevant human cell types. Despite in recent studies, there are still difficulties in reprogramming stem cells in age-related diseases. More research is needed on the mechanisms described to identify the barriers to somatic cell reprogramming during aging. However, these technologies offer exciting new potential approaches to many human diseases.

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# Tissue Engineering and Regenerative Medicine: A Translational Research for Antiaging Strategy

4

Bhisham Narayan Singh, Anubha Joshi, Sarada Prasanna Mallick, and Pradeep Srivastava

**Abstract**

Aging is a natural and progressive process which is manifested by structural and functional damage to various body organs. Tissue engineering and regenerative medicine are considered as one of the most advanced modern strategies to understand the complexity of aging and restore the functionalities of organ systems which worsen due to aging. The scope of such an advanced biomedical technology was unearthed several decades ago, and even drastic progress has been achieved in the field of graft development for the skin, bone, cartilage, etc. to regenerate damaged/diseased tissue/organ. Tissue engineering involves fabrication of biomimetic graft which recruits stem cells, allowing them to proliferate or populate the graft to facilitate integration with surrounding tissues and regenerate damaged/diseased tissue. Currently, tissue engineering-based approaches for the treatment of various diseases caused by deterioration of tissues/organ through aging are in either preclinical or initial clinical stages for the development of alternative commercial medical product. This chapter covers various appropriate tissue engineering and regenerative medicinal approaches adopted to develop functional graft and potential stem cell therapy to restore damaged or diseased tissue/organ to address the issues of aging.

**Keywords**

Aging · Biomimetics · Regenerative medicine · Stem cells · Tissue engineering

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## 4.1 Introduction

Various theories has been proposed to understand aging mechanism at cellular level and its advancement from microscopic failure of cells to macroscopic failure of tissues and finally failure of organ followed by death. Aging is a progressive phenomenon and reported to be governed by various factors such as malfunctioning of cellular macromolecular factories, which further cascades from molecular level to the cellular level. The major hallmarks associated with the aging are genomic instability, stem cell exhaustion, mitochondrial dysfunction, loss of proteostasis, epigenetic alteration, and cellular senescence (López-Otín et al. 2013). These hallmarks alone or in association lead to the malfunctioning of cellular activities, which may propagate from cell to cell at varying degree, and complete tissue/organ. Damages at genetic level including loss of integrity and stability of genetic material or defects in nuclear lamina are due to exogenous factors such as physical, chemical, and biological agent, endogenous error in DNA replication, and occurrence of hydrolytic reaction, and generation of reactive oxygen species may lead to genetic instability (Hoeijmakers 2009; Dechat et al. 2008). Also, mutations in mitochondrial DNA are due to error, while DNA replication in adult or aged cells causes respiratory dysfunction in various tissues (Ameur et al. 2011). Furthermore, all cells and tissues involve alterations in DNA methylation patterns and remodeling of chromatin and posttranslational modification of histones throughout the life and thereby lead to epigenetic alteration with aging (Talens et al. 2012; López-Otín et al. 2013). However, gradual accumulation of such genetic damages with advancement of age leads to aging through cascade effect from microscopic level to macroscopic level. Apart from genetic instability, imbalanced protein homeostasis leads to failure of maintaining structural and functional property of misfolded protein, and thereby accumulation of such misfolded protein gradually increases with aging, and thus, age-related diseases such as Alzimer's, Parkinson's, and cataracts occur (Powers et al. 2009). Impaired cells due to genetic instability and epigenetic alteration and impaired proteome homeostasis are regularly cleaned through well-known mechanism of cellular senescence. However, with aging rate, accumulation of senescent cells increases, and rate of clearance decreases. This might be due to impaired regenerative potential or stem cell exhaustion, and thus, tissue damages are aggravated with aging (López-Otín et al. 2013; Cerletti et al. 2012).

Stem cells or regenerative cells are located in various tissues, which play distinctive role in tissue repair, remodeling, and regeneration. Tissue-specific stem cells possess higher potential to proliferate and generate tissue-specific precursor cells in order to replace damaged cells through lineage-specific terminal differentiations. Thereby, stem cells maintain appropriate balance between cellular senescence and proliferative activity of tissues (Oh et al. 2014). Moreover, stem cells are also prone to undergo aging, and thereby with aging tissue, reparative potential diminishes. Thereby, stem cells isolated at young stage such as cord blood-derived stem cells were reported to be a much more attractive source for regenerative medicine due to its higher stemness, multilineage differentiation potential, and immunomodulatory potential as compared to adult stem cells (Nagamura-Inoue and He 2014). Thus,

stem cells provide an alternative route to treat various aging-associated or degenerative diseases. Stem cells could be used as cell therapy or in combination with artificial extracellular matrix to regenerate damaged/diseased tissues. Regenerative medicine or tissue engineering technology offers an innovative approach to generate artificial tissues/organ for patients suffering from injuries or aging-associated organ failure. At present mostly patients are treated with donor organ; however, scarcity of donor organ remains a great challenge. To overcome such challenges, various researchers have been involved in the generation of functional tissue-engineered construct using stem cells, biomaterials, and implant fabrication technology such as bioprinting, freeze drying, freeze gelation, gas foaming, electrospinning, etc. to replace or repair damaged/diseased tissues/organ. In the last few decades, various tissue-engineered products for treatment of damaged/diseased tissues are approved by the Food and Drug Administration (FDA) for commercial applications. Such tissue-engineered products loaded and cultured with specific cell type to generate tissue-engineered construct such as Carticel are available in the market for commercial applications in case of articular cartilage defects (Dewan et al. 2014). Also, various tissue-engineered products are available for the regeneration of diseased or damaged skin and bone tissues. Furthermore, yet a long way need to be cover in the feild of tissue engineering and regenerative medicine to generate a functional complex organ for the treatment of patients suffering from end-stage organ failure and to meet the need for lack of donor organ supplies.

## 4.2 Stem Cells, Sources, and Its Therapeutic Potential

Autologous cells from patients are more preferable to generate tissue-engineered construct. However, due to aging adult cells are less susceptible to proliferate and populate. Thereby, the use of stem cells due to its higher proliferation and differentiation potential is considered as a major choice for cell therapy and generation of artificial tissue-engineered construct. Stem cells are undifferentiated cells having significantly higher self-renewal potential to differentiate into both non-renewing progenitor cells and terminally differentiated effector cells of all the three germ layers (Watt and Hogan 2000). Stem cells derived from inner cell mass of blastocysts are pluripotent embryonic stem cells, and stem cells derived from organs such as bone marrow, dental pulp, etc. are multipotent adult stem cells (Fig. 4.1) (Odorico et al. 2001).

Moreover, human stem cells are further categorized as human hematopoietic stem cells (hHSCs) capable to proliferate and differentiate into nonadherent blood cells and human mesenchymal stem cells (hMSCs) capable to proliferate and differentiate in various adherent cells. Human mesenchymal stem cells are reported to be isolated and propagated from stroma of various sources (Fig. 4.1) such as bone marrow, dental pulp, umbilical cord, adipose tissue, peripheral blood, etc. Furthermore, hMSCs are having the potential to differentiate under suitable conditions into various cell types such as chondrocytes, osteoblasts, adipocytes, neurons, cardiomyocytes, other mesodermal cell types, etc. (Kassem 2006). Among various

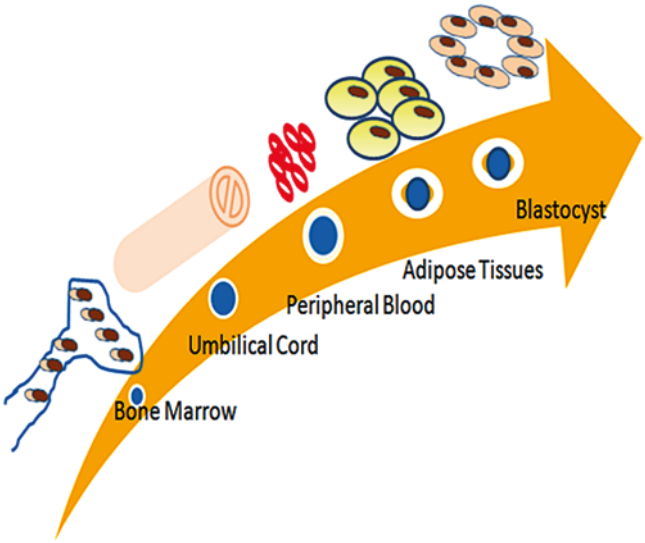


**Fig. 4.1** Stem cells and progenitor cell sources considered for cell isolation and its cultivation for cellular therapy and generation of tissue-engineered construct

stem cells depending upon their sources, embryonic stem cells are confirmed to be the most potential stem cells with highest stemness and ability to differentiate into all types of cells. However, in spite of superior therapeutic potential due to ethical restriction, embryonic stem cells are not considered for cell therapy. Therefore, bone marrow-derived mesenchymal stem cells (BM-MSCs) and umbilical cord blood-derived mesenchymal stem cells (UC-MSCs) are more preferable choices for stem cell therapy. However, limited numbers of autologous hMSCs and poor growth and differentiation potential due to aging limit the clinical application of BM-MSCs. Therefore, limitations associated with both embryonic stem cells and BM-MSCs have led to design more potential and clinically significant alternative source of stem cells. UC-MSCs show closer gene expression profile as of embryonic stem cells and thus exhibit superior self-renewal potential in comparison with BM-MSCs (Hsieh et al. 2010; Fong et al. 2011). Thereby, UC-MSCs are more preferable as compared to other sources, and this has led to significant rise in the healthcare sector such as cord blood banking for medical application as regenerative medicine. As discussed earlier, stem cell technology possesses the potential to generate a large number of tissue-specific cells in standardized condition, and this could be useful to treat various age-associated diseases. Also, aging-related Parkinson disease due to progressive loss or changes in motor function including bradykinesia, rigidity, and gait disorder may be treated with stem cell-derived dopaminergic neurons. However, stem cell-derived dopaminergic neurons should release host-specific dopamine, able to reverse the changes in motor function, able to survive for long term in human putamen, possess ability to establish a dense network across the striatum, and should functionally integrate with host neural circuitries (Lindvall et al. 2004). Researchers

reported the formation of stem cell-derived dopaminergic neurons; few of them observed glial response and failed to detect neurogenesis following dopaminergic lesions (Lindvall et al. 2004). Thus, stem cell-based cellular therapy might be useful to treat aging-associated Parkinson disease; however, still detailed clinical investigation is required to be performed before developing a potential stem cell-based therapy in the future. Apart from neurodegenerative diseases, stem cell-based therapy may prevent or even reverse progression of heart failure. Obstruction of coronary arteries and high blood pressure leads to gradual loss of cardiomyocytes and thereby causes heart failure. Cardiac transplantation is the only standard therapy for heart failure and provides solution to address loss of cardiomyocyte. However, stem cell technology might be a potential option to overcome the lack of cardiac transplant availability as stem cells possess superior potential to regenerate the myocardium (Segers and Lee 2008). Thus, stem cells from various sources show enormous potential to generate various cell types under specific culture condition and provide alternative approach toward the treatment of various diseases or aging-associated disorders.

## 4.3 Tissue Engineering for Aging-Associated Disorder

Aging is an unavoidable condition and imposes difficulty in maintaining homeostasis, leading to dysfunction and defects in a number of everyday life functions. Tissue engineering looks out for an alternative way to provide permanent solution to various aging-associated chronic diseases and defects in the tissue and organs of individuals. Although tissue engineering is a relatively newly conceived technology, however, it holds great therapeutic potential. When talking about tissue engineering, the first and foremost concept is to develop a bioengineered matrix (scaffold) inspired from the natural extracellular matrix (ECM) using suitable biomaterials. The desired physicochemical and biological properties of the developed matrix may be as follows:

- *Biocompatibility*
- The most important property of a scaffold is its biocompatibility. It should facilitate cell adherence, proliferation, and migration through the scaffold, and eventually cell proliferation should take place that ultimately leads to the deposition of a regenerated natural matrix. Also, it should not elicit any detrimental immunological reaction that leads to the rejection of the scaffold.
- *Biodegradability*
- Scaffolds are not intended to be used as permanent implants, and thus they should degrade over time, while the body's cells must replace the implanted scaffold with natural ECM. During degradation, the scaffold should not produce any by-products which are toxic to the body.
- *Mechanical Properties*
- The scaffold should be optimized for its mechanical strength according to the site of insertion and should be robust enough for surgical handling (Hutmacher

2000). For bone and cartilage tissue regeneration, the scaffold should have sufficient mechanical strength, which is a challenge in the field of orthopedic tissue engineering.

- *Scaffold Architecture*
- Scaffold architecture is one of the important parameters considered when dealing with the development of tissue-engineered products. The scaffold should have highly porous structure, and the pores should be interconnected, which is important for mainly two reasons. Firstly, porous structures allow diffusion of nutrients to the cells present in core of the scaffold. Secondly, they allow the removal of the degraded or waste products out of the scaffold while tissue regeneration phenomenon occurs. The mean pore size of a scaffold is an important criterion for successful scaffold fabrication, where the optimal pore size of a scaffold varies with the type of tissue being engineered.
- *Manufacturing Technology*
- One should aim to use the technology for the manufacturing (freeze drying, freeze gelation, phase separation, electrospinning, and bioprinting) of scaffolds which can be scaled up for industrial production or mass production of tissue-engineered products.

Apart from the abovementioned criteria, selection of suitable biomaterial for the fabrication of scaffold plays a critical role in tissue regeneration.

## 4.4 Biomaterials

The European Society for Biomaterials (ESB) in 1976 defined biomaterial as "a nonviable material used in a medical device and intended to interact with biological systems," while the current definition is "a material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body." The role of biomaterials has changed drastically from just interacting with the body toward making tissue regeneration possible. In tissue engineering for the fabrication of scaffolds, mostly three types of biomaterials are used: ceramics, synthetic polymers, and natural polymers (Table 4.1).

- Ceramic has been widely used for bone tissue regeneration application because of its good mechanical strength, biocompatibility, and structural similarity to the mineral phase of the bone. Also, it helps the osteoblast cell or hMSCs to differentiate and proliferate (Chen et al. 2002). However, their main disadvantage is brittleness, low elasticity, and difficulties in providing desired shape to the implants. Hydroxyapatite (HA), a bioceramic, is the main constituent of the bone, but it faces some problems in bone tissue engineering because of its poor degradation rate.
- Synthetic polymers have numerous advantages like controlled degradation kinetics, can be easily fabricated into various shapes, and provide high mechanical strength to the scaffold (Lu et al. 2000). Synthetic biopolymers which don't

**Table 4.1** Classification of biomaterial commonly used for generation of artificial extracellular matrix (scaffold)

| Materials | Examples |
|---|---|
| Ceramic | Hydroxyapatite |
| | Tricalcium phosphate |
| | Bioglass |
| Synthetic polymer | Polystyrene |
| | Poly-l-lactic acid (plla) |
| | Polyglycolic acid (pga) |
| | Poly (dl-lactic-co-glycolic) acid (plga) |
| | Polycaprolactone(PCL) |
| Natural polymer | Collagen |
| | Alginate-based substrates |
| | Chitosan |
| | Various proteoglycans |
| | Glycosaminoglycans |
| | Silk fibroin |
| | Gelatin |
| | Elastin |
| | Hyaluronic acid |
| | Chitin |

release toxic by-products on degradation are mostly preferred for scaffold fabrication. The main disadvantage associated with using synthetic polymers is either low bioactivity or no bioactivity which leads to poor tissue integration potential of the scaffold.

- The most suitable biomaterial for tissue engineering applications is natural polymers. Natural polymers are mainly biodegradable, which doesn't produce toxic by-products, and over the time replaced by host cells and natural ECM. The main disadvantage of using natural polymer is its poor mechanical strength.

All the materials mentioned above when used in a single phase have one or other problem associated with them; hence the research is shifted toward developing composite scaffolds. Composite scaffolds are scaffolds containing a number of phases and containing different compositions of ceramic, synthetic, or natural polymers amalgamated together having a synergistic effect on the scaffold. Overall, tissue engineering aims to generate potential therapeutic approaches using regenerative cells and artificial extracellular matrix (scaffold) for various defects and diseases. Applications of tissue engineering in the regeneration of some of the aged or diseased tissues are discussed for following cases:

- Hepatic disorder
- Cardiac defects
- Orthopedic defects
- Dental defects

## 4.5 Tissue Engineering Strategies for Aging-Associated Liver Disorders

The liver mainly consists of parenchymal cells such as Kupffer cells, epithelial cells, sinusoidal epithelial cells, biliary epithelial cells, hepatocytes, hepatocyte precursor cells, and fibroblasts (Arias et al. 2011). About 70% of cellular population is of hepatocytes, which play an important role in metabolic functions of the hepatic tissues (Oertel and Shafritz 2008). The hepatic structure and function of liver cells alter slowly with aging, and the aged population is susceptible to diseases like cirrhosis. For the last-stage patients of chronic liver disease, liver transplantation is mostly preferred to save the life of a patient, but the shortage of donor remains a problem. Moreover, hepatic tissue engineering is the best alternative therapeutic strategy using an appropriate tissue-engineered construct with which one can deal with the shortage of hepatic donors.

Different biomaterials, such as hydrogels, porous scaffolds, microcapsules, and hollow fibers, have been explored by various researchers across the globe to mimic higher levels of liver-specific functions and mechanical stability (Underhill et al. 2007; Sullivan et al. 2007). Natural polymers such as collagen, fibronectin, gelatin, and Matrigel used for fabrication of scaffolds have been used in many studies for hepatogenic differentiation of stem cells (Ong et al. 2006; Schwartz et al. 2002). The following are some of the natural polymers used in hepatic tissue engineering:

- Chitosan has reactive amino and hydroxyl groups similar to glycosaminoglycans (GAGs), such as chondroitin sulfate and keratin sulfate (main liver ECM component), making it the most suitable natural polymer for hepatic tissue engineering (Jiankang et al. 2009; Yamane et al. 2005; Chen et al. 2008).
- Gelatin, the partially hydrolyzed form of collagen, is another biomaterial having superior cell attachment property used in hepatocyte tissue engineering (Jiankang et al. 2009). Normal physiology of hepatocyte is maintained for a period of about 2 months in a chitosan-gelatin scaffold (Yan et al. 2005).
- Type I collagen is used for fabricating scaffolds for soft tissues and organs, which helps in cell proliferation in hepatic tissue engineering (Nehrer et al. 1997).
- Hyaluronic acid provides viscoelasticity, biodegradability, and biocompatibility to the scaffold. It is the first molecule to be secreted in tissue repair along with collagen. Hyaluronic acid enhances cell division of fibroblasts (one of the constituents of liver cells) (Lin and Liu 2007).

Scaffolds made from only natural polymers are bioactive but have poor mechanical strength and are difficult to handle (Badylak et al. 2009). Researchers to curb the disadvantages of natural polymers went ahead with synthetic polymers for fabrication of scaffolds which provided optimal manipulative mechanical properties and degradation rate.

- Polyethylene glycol (PEG) is a nontoxic and non-immunogenic synthetic polymer, and through specific surface modification, one can overcome its inability of cell adhesion. It can be easily modified with functional groups, and therefore PEG hydrogels are used extensively for 3D cultures (Zhu 2010; DeVolder and Kong 2012; Nuttelman et al. 2005). It has been reported that PEG hydrogels favor the growth and functioning of hepatocytes and hepatoblasts (Underhill et al. 2007; Itle et al. 2005).
- Poly-l-lactic acid (PLLA) and poly(lactic-co-glycolic acid) (PLGA) are the widely used biocompatible and biodegradable aliphatic polyesters for hepatic tissue engineering (Mooney et al. 1995). When combined with poly (vinyl alcohol), primary rat hepatocyte seeding on the scaffold was reported to be enhanced (Mooney et al. 1995).

Synthetic polymers despite having a number of advantages lack bioactivity and cell recognition signals which create hurdles for generation of functional tissue-engineered construct. Therefore, the focus has shifted toward the development of hybrid and composite scaffold, integrating the advantages of both natural and synthetic polymers (Zhang et al. 2005; Venugopal et al. 2005).

The design architecture for hepatic tissue depends on the following:

- *Material properties*: The material used must be stiff enough to support the fibroblast cell to differentiate into myoblasts and porous enough to facilitate optimal mass transfer activities.
- *Biofunctionalization*: Surface modification of developed scaffold with bioactive proteins such as RGD, LGPA, or YIGSR enhances cellular adhesions through interaction between hepatocyte and functional moieties over matrix (Park et al. 2005; Patel et al. 2005).
- *Architecture*: The liver is made up of functional hepatic lobules having central vein and portal triads. Hepatocytes are present between central vein and portal triads in a platelike structure. The liver has a complex 3D structure, and for successful development of hepatic tissue-engineered construct, an appropriate 3D scaffold needs to be developed which mimics natural tissue architecture and provides superior cell-cell interaction, cell- ECM interaction, and macroscale arrangement in essential (Fig. 4.2).

Hepatocytes are known to function better in their three-dimensional aggregates or spheroids than in monolayer culture (Glicklis et al. 2000; Hamamoto et al. 1998). Hepatocytes grown in aggregation have more cell to cell contact and thereby support the formation of gap junctions and bile canaliculi reinforcing phenotype of the hepatocytes (Landry et al. 1985; Abu-Absi et al. 2002). Thus, through advanced tissue engineering technology, we can develop artificial 3D functional hepatic construct to restore the function of aging-associated damaged/diseased hepatic tissue or organ.

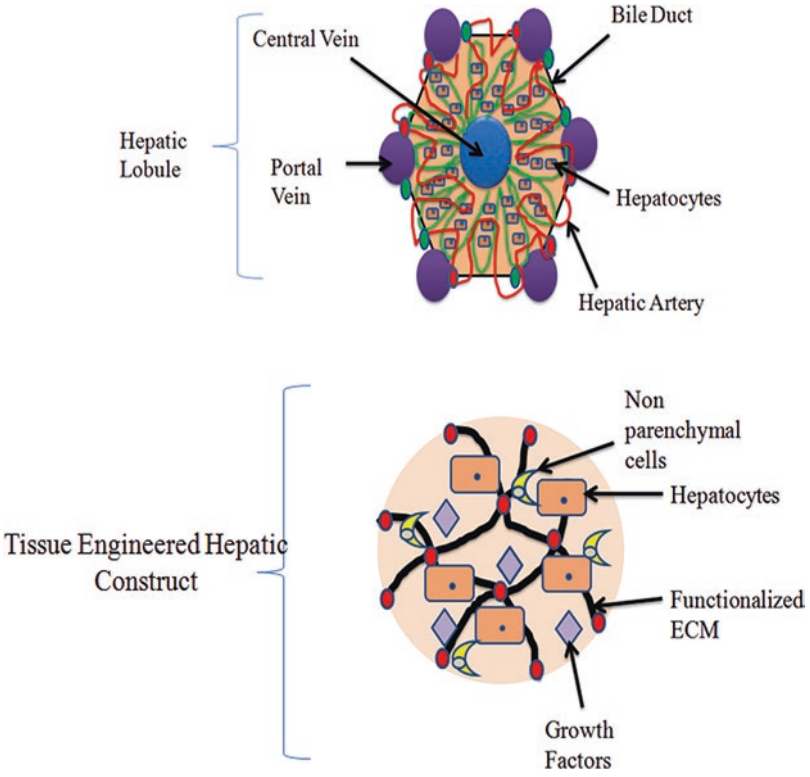


**Fig. 4.2** This figure shows generation of hepatic tissue-engineered construct platforms inspired from functional unit of liver tissue. Lobule is the functional unit of the liver, which is composed of a central vein connected to the portal triads including hepatic artery, portal vein, and bile duct. Non-parenchymal cells and hepatocytes are between portal triad and central vein. The functional lobule inspires generation of functional tissue-engineered hepatic construct

## 4.6 Tissue Engineering Strategies for Aging-Associated Cardiovascular Disorders

With the advent of aging, cardiovascular problems such as heart valve diseases/dysfunction are the major causes of casualties in the Western world. Bioprosthetic, mechanical heart valves, and cryopreserved homograft valves are widely used in valve replacement (Schoen 2011), which has a number of limitations associated with them. Bioprosthetic valves are antithrombogenic but are not durable, while mechanical valves have good durability, but lifelong anticoagulation treatment is required to restore the functionality. Both of these valves are susceptible to infections and thereby prone to further replacement surgery as the complication arises. These limitations can be overcome by engineering biomimetic functional tissue valves facilitating superior cell adhesion, growth, maintenance of tissue homeostasis, and nonobstructive and non-thrombogenic advantages. Heart valve tissue

engineering mainly focus on designing a tubular valve with leaflets using synthetic polymeric scaffold, and cells are seeded to generate tissue-engineered construct before implantation. The use of natural valve material such as decellularized valve or the use of fabricated ECM is also in practice. For successful heart valve engineering, conditions like scaffold material, cell source, in vitro manipulation, and clinical evaluation using appropriate animal models must be met (Hjortnaes et al. 2009).

A number of synthetic polymers like poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(lactic-co-glycolic acid) (PLGA) have been reported to be useful in engineering artificial heart valves (Hoerstrup et al. 2000; Zund et al. 1997). In the use of PLA, PGA scaffolds lacked mechanical strength, and addition of poly-4-hydroxybutyrate (P4HB) shows enhanced structural molding of the valve (Sodian et al. 2000). However, there is a great need of performing clinical trial of polymeric scaffold for successful tissue-engineered heart valve generation (Schaefermeier et al. 2009).

- An alternative to creating three-dimensional scaffolds is the use of decellularized biological-based scaffold. Decellularization of allogenic or xenogenic valves are done through using mainly four techniques such as enzymatic cell removal, freeze drying, osmotic gradients, and their combinations to engineer heart valve. During decellularization, it must be made sure that all the cells and genetic materials are removed to avoid any immunogenic response or zoonotic disease transfer after implantation and the process should retain the structural component of the ECM. The decellularized valve can then be reseeded inside a bioreactor and then implanted inside the patient, or the valve can be directly put inside the patient's body which itself acts as a bioreactor (Fig. 4.3) (Cheung et al. 2015). This should support growth of the cells and should guide cell to cell interaction leading to the formation of required tissue.

## 4.7 Tissue Engineering Strategies for Aging-Associated Orthopedic Defects

Aging has a major effect on bone tissue both at macroscopic and microscopic level. With aging, the population is prone to diseases like osteoporosis and arthritis, which is expected to continuously rises worldwide in the future (Dall et al. 2013). Currently, clinical treatments for orthopedic reconstructive surgery mainly include uses of autografts, allografts, and xenografts to restore or repair the diseased/damaged tissues (Bauer and Muschler 2000; Popat et al. 2007). However, these treatments have certain limitations such as limited availability, immunogenicity, and zoonotic disease transfer, which are major concerns and lead to the development of an alternative translational medicine through bone tissue engineering applications (Oryan et al. 2014; Manivasagam et al. 2010). Tissue engineering mainly involves in the development of bioactive, biodegradable, biocompatible, and biomimetic artificial ECM to replace, repair, and restore damaged tissues. Bone ECM is mostly composed of organic component mainly collagen and mineralized inorganic component

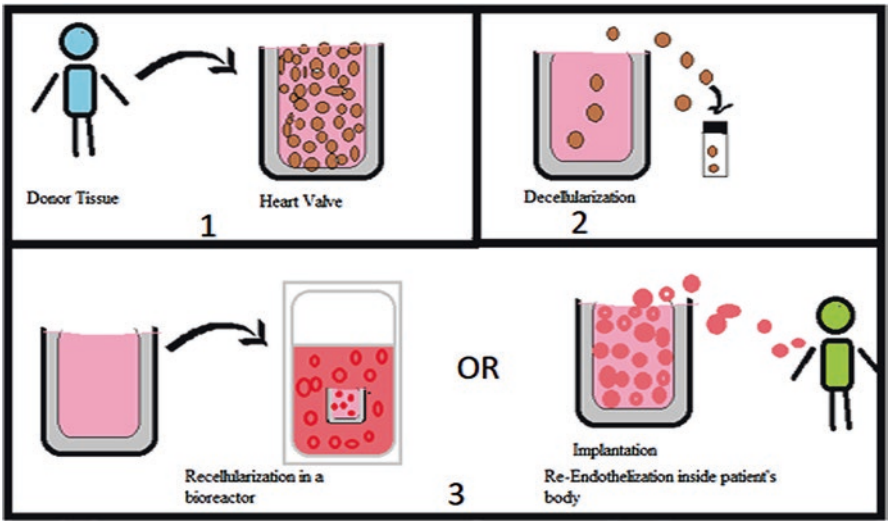


**Fig. 4.3** Herein heart valve (1) is extracted from a donor tissue, and it is then decellularized (2), using various techniques, and then the recellularization of heart valve can be done by using a bioreactor (3) or implanted inside the patient where the patient's body act as a bioreactor

(carbonated apatite) (Weiner and Wagner 1998), and thereby scaffold engineered for bone tissue engineering applications should mimic the natural ECM of bone tissues mainly with respect to its composition, physicochemical properties, structural architecture, and biological properties. The materials considered for the development of bone tissue construct should be the following:

- Osteoinductive: Able to promote the differentiation of progenitor cells
- Osteoconductive: Able to support bone growth and osteoblasts are able to migrate and adhere to the scaffold
- Osseointegration: Able to integrate with the surrounding bone

In bone tissue engineering, the tissue regeneration strategy involves the development of 3D porous composite scaffolds with similar composition to the bone ECM. The materials mainly used for scaffold fabrication of bone tissue engineering are the following:

- Bioactive inorganic materials: Mineral phase of the bone is mimicked by a range of bioactive inorganic materials like tricalcium phosphate, hydroxyapatite (HA), and bioactive glass (bioglass) (Hench and Polak 2002; Zijderveld et al. 2005). When bioactive glass is immersed in a biological fluid, the bioglass produces carbonated hydroxyapatite layer which promotes integration of scaffold with the adjoining bones leading to cell differentiation and osteogenesis (Jell and Stevens 2006; Tsigkou et al. 2007). Moreover, bioactive inorganic materials cannot be used alone, because of its brittle nature and difficulties in fabrication of scaffold

with complex architecture. Thus, polymeric composite using bioceramic provides an appropriate biomaterial for bone tissue-engineered construct development.

- Natural polymers like collagen, hyaluronic acid, chitosan, silk fibroin, cellulose and its derivatives, and gelatine are suitable materials for the development of bone scaffold, providing cell adhesion, spreading, and proliferation properties.
- Synthetic polymers polylactic acid (PLA), polyglycolic acid (PGA), and polycaprolactone (PCL) can help in the fabrication of scaffold with enhanced mechanical strength.

It is important to develop manufacturing processes that guarantee automated bone production (Marcacci et al. 2007; Dalby et al. 2007), where bioreactors and computer modeling are utilized. Apart from bone tissue regeneration, orthopedic tissue engineering also involves in the development of scaffold for regeneration of damaged cartilage as cartilage tissues are nonvascularized and possess poor regenerative potential; thus tissue engineering play a distinctive role in treatment of various aging-associated diseases such as osteoarthritis (OA), relapsing polychondritis, costochondritis, chondrosarcoma, ochronosis, traumatic rupture of the cartilage, etc. The cartilage is a connective tissue which is generally found in the joint of the bones, rib cage, ear, nose, etc. It is not as hard and rigid as the bone, but is stiffer and less flexible than muscle. Articular cartilage is a complex avascular tissue which consists of cells called chondrocytes suspended in a collagenous matrix (Fig. 4.4). Aging associated with the knee joints includes joint stiffness, loss of cartilage, loss of joint contour, angular deformities, loss of hyaline cartilage, decreased water content with increased calcium salts, etc. With aging collagen fibers which show increased fiber size similar to that seen in OA, articular cartilage ECM changes in entire quantity and structure and goes through proteolysis and other posttranslational modifications. Chondrocytes provide support, structure, and flexibility in the adult and show reduced functional activity with aging.

## 4.8 Clinical Aspects Toward Cartilage Tissue Regeneration

Cartilage tissue contains a specialized cells named as chondrocytes producing a large amount of extracellular matrix composed of collagen fibers, abundant ground substance rich in proteoglycan, and elastin fibers containing no blood vessels. There are various aging-related problems that occur in cartilage tissue like joint pain, tenderness, swelling, stiffness, reduced motion, ligament injury, etc. The problems were identified as imaging tests like X-ray, MRI, arthroscopy, and laboratory tests include joint fluid and blood analysis. Tissue engineering scaffold for cartilage defect is more suitable than metal implant in the current scenario. In the present situation, natural and synthetic scaffold materials such as ceramics, polymers, etc. are being used, which are being further used to cellularize with stem cells and autologous chondrocytes. Various studies reported that human umbilical cord MSCs, a primitive source of chondrocytes, showing superior pluripotency (OCT4 and

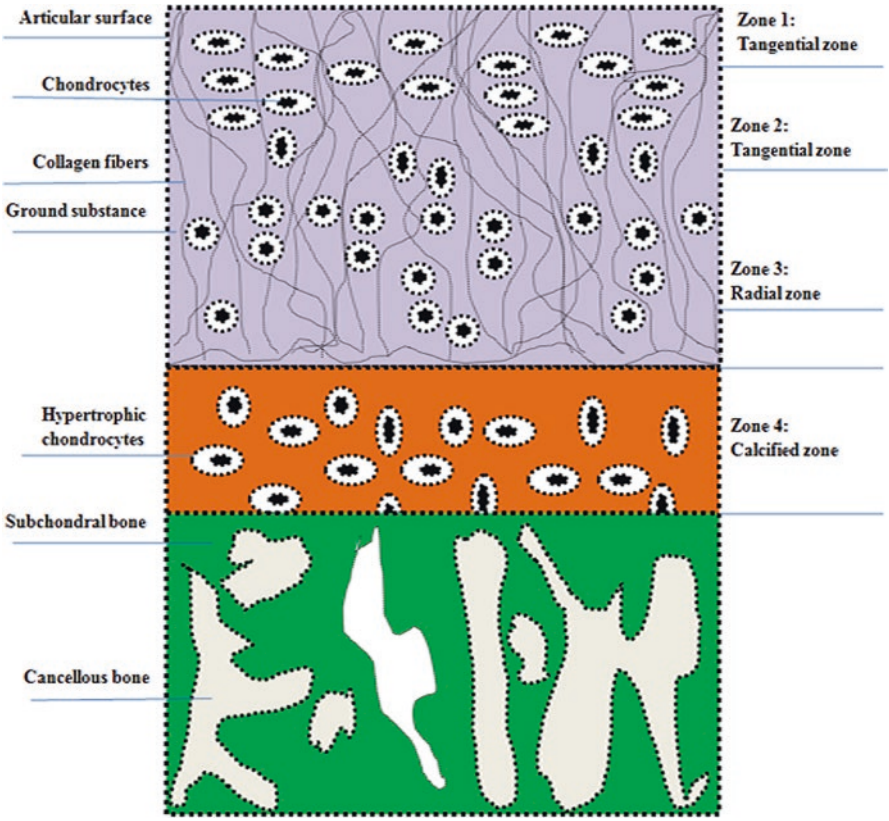


**Fig. 4.4** Pathogenesis pathways in established osteoarthritis

NANOG) gene activity have greater proliferation and differentiation capabilities (Toh et al. 2016). Induced pluripotent stem cells (iPSCs) are also reported to be a potential cell source with significant self-regeneration ability and the potential to differentiate into ecto-, meso-, and endodermal origin (Driessen et al. 2017). IPSCs are appropriate tools for modeling soft tissue development and disease and correspond to promising specific cell source for the regeneration of articular cartilage (Guzzo and Drissi 2015). Current cartilage repair technology is based on three types of surgery techniques like marrow stimulation, osteochondral autografts, and autologous chondrocyte implantation. Stem cell cartilage regeneration therapy is one of the most effective treatments for osteoarthritis damage in knee joint. Advanced technologies and regenerative medicine lead to the development of "cartilage autograft implantation system"; in this process patients own healthy cartilage obtained from a low weight-bearing region for surgical treatment (Engelhart et al. 2012; Kon et al. 2012). Arthro Kinetics AG developed an advanced technique for regenerating joint mobility called as "CaReS®" (cartilage regeneration system), which includes collagen type I matrix colonized with autologous cartilage (chondroblast and

chondrocyte) cells and used for the regeneration of articular cartilage defects. CellGenix established "CartiGro ACT" product for autologous chondrocyte transplantation, and the outcomes are really well. Genzyme Biosurgery introduced a product recently, "Carticel ACI." ProChon Biotech is also an autologous cartilage regeneration system which uses a variant of fibroblast growth factor to expand dedifferentiated cartilage cells. 3D printing-based platform technology can be effectively exploited for regeneration of various heterogeneous tissues as well as osteochondral tissue (Shim et al. 2016). Nanofibrous hollow microspheres are an excellent injectable cell carrier for cartilage regeneration (Liu et al. 2011). Some of the FDA-approved orthopedic products developed and intended to use for the repair of diseased or defected tissues associated with skeletal system of human being are listed in Table 4.2 (Hellman 2008). Thus tremendous work is going on in the field of tissue engineering to develop potential product to repair diseased/damaged cartilage tissues.

## 4.9 Tissue Engineering Application in Dental Problems

Tissue engineering dealing with regeneration of lost or damaged tissue can also be used for solving dental problems associated with aging population. Tooth engineering with tools like stem cells seeded on scaffolds is the novel approach to restore the damaged tissue with bioengineered tooth. Two different tissues are involved in the

**Table 4.2** FDA-approved tissue-engineered products for orthopedic applications

| Orthopedic products | Sponsors | Intended application |
|---|---|---|
| GEM 21STM (growth factor and synthetic beta-tricalcium phosphate-enriched matrix) | Biomimetic Pharmaceuticals, Inc. | Suitable for treatment of periodontally related defects such as intrabony, gingival recession associated with periodontal and furcation defects |
| OP-1 putty (type-1 bovine bone collagen matrix loaded with recombinant human osteogenic protein used with putty additive like carboxymethyl cellulose sodium) | Stryker Biotech | Suitable for posterolateral lumbar spinal fusion |
| Infuse bone graft/LT-cage<br>Lumbar tapered fusion<br>Device (type-1 bovine bone collagen matrix loaded with recombinant human bone morphogenetic protein-2, titanium alloy cage) | Medtronic | Suitable for spinal fusion in case of degenerative disk disease |
| Carticel (autologous cultured chondrocytes) | Genzyme Corporation | Suitable for regeneration of femoral condyle |
| OP-1 implant (type-1 bovine bone collagen matrix loaded with recombinant human osteogenic protein) | Stryker Biotech | Used for treatment of long bone non-union defects |

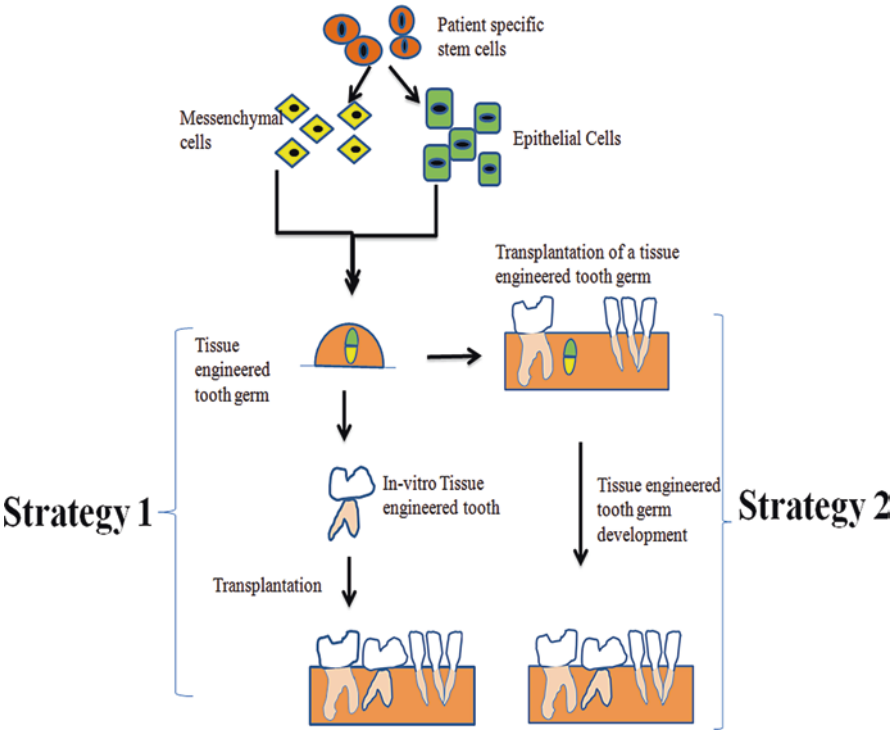


**Fig. 4.5** Articular cartilage stratification zones correlate to the cell differentiation stages

formation of the tooth; the fabrication of tissue-engineered tooth requires dental mesenchymal and epithelial cells (Fig. 4.5) (Amar et al. 1989; Yoshiba et al. 1998). Mesenchymal cells and epithelial cells on a drop of collagen gel were seeded and placed in the tooth cavity of the mouse [63]. This bioengineered tooth germ was seen to form well-structured tooth when implanted in the jawbone (Nakao et al. 2007). Another strategy could be the development of a bioengineered tooth in vitro and transplanting the tooth in place of the missing one (Ikeda et al. 2009).

Three main points to be considered for dental tissue engineering are the cells used, the scaffolding biomaterial, and the growth factors.

Latest clinical trials in humans have demonstrated that cells attached on collagen helped in the regeneration of the bone in the lower jaw. Dental tissue engineering is promising for the patients who have dental diseases and defects.

## 4.10 Conclusion

Great expectations are there from regenerative medicine or tissue engineering, even when human clinical trials are very few. Like successful dermal implants for treating skin defects, tissue engineering of organs like the liver and pancreas have not

been easy, because hepatocytes or pancreatic cell cultures are more difficult than keratinocytes, and likewise it is difficult to engineer whole complex structure of an organ. And therefore tissue engineering, started during the late 1980s, fails to produce many tissue-engineered products to the market. The delay of clinical trials might be because of the gap in communication between the tissue engineer, the researcher of academic institutions, and the surgeon who conduct implantation. Collaboration between different groups from different fields is a major requirement for successful operation of tissue engineering. Thus in spite of recent achievements and advances in tissue engineering, tissue engineering has yet much to deliver.

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# Advances in Senotherapies

5

Janice Wong, Anam Qudrat, Abdullah Al Mosabbir, and Kevin Truong

**Abstract**

Aging brings about many risks factors that can lead to age-related chronic disorders such as atherosclerosis, osteoporosis, and neurodegenerative diseases. Implicated in aging and age-related pathologies, the accumulation of senescent cells can prevent tissue repair and regeneration, leading to loss of physiological function. Cellular senescence is an age-related process in which cells cease to divide permanently, resist apoptosis, and can secrete harmful substances to adjacent cells. Senescent cells exert a larger degenerative effect on neighboring cells by acquiring senescence-associated secretory phenotypes (SASP) that release inflammatory cytokines, growth factors, and proteases. Studies in model organisms have shown that the clearance of senescent cells in model organisms lead to an increase in healthy lifespan as measured by delays in the onset of age-related dysfunctions and pathologies. *Senolytics* are a class of senotherapies that use molecular compounds to selectively and efficiently induce cell death in senescent cells. In some cases, senolytics targeting pro-survival networks including p53/p21, Bcl-2, PI3K/Akt, and serpin pathways have shown promising results in in vivo mouse studies such as extended health span and restoration of tissue function. In this chapter, we will discuss the development of senolytic strategies,

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specifically therapeutic agents that target cellular senescence and pro-survival pathways as well as offer insights into strategy improvements and alternatives.

**Keywords**
Senolytic · Senescence · SASP · Senotherapies · Biomarkers

## Abbreviations

| | |
|---|---|
| 3MR | Trimodal reporter protein |
| Akt | Protein kinase B |
| ASK1 | Apoptosis signaling regulating kinase 1 |
| CDKN2A | Cyclin-dependent kinase inhibitor 2A |
| DRI | D-retro-inverso |
| EFNB1 | Ephrin B1 protein |
| EFNB3 | Ephrin B3 protein |
| FOXO4 | Forkhead box protein O4 |
| HSP90 | Heat shock protein 90 |
| HSV-TK | Herpes simplex virus 1 (HSV-1) thymidine kinase |
| HUVE | Human umbilical vein endothelial cell line |
| IL-6 | Interleukin-6 |
| IMR90 | Human lung IMR90 cell line |
| LUC | *Renilla* luciferase |
| mRFP | Monomeric red fluorescent protein |
| PAI-1 | Plasminogen activator inhibitor 1 |
| p-Akt | Phosphorylated protein kinase B |
| PDK1 | 3′-Phosphoinositide-dependent kinase 1 |
| PH | Pleckstrin homology |
| PI3K | Phosphatidylinositol-3 kinases |
| PIP2 | Phosphatidylinositol-4,5-bisphosphate |
| PIP3 | Phosphatidylinositol-3,4,5-trisphosphate |
| PTEN | Phosphatase and tensin homologue |
| ROS | Reactive-oxygen species |
| SASP | Senescence-associated secretory phenotype |
| SA-β-gal | Senescence-associated β-galactosidase |
| SH2 | Src homology 2 |
| siRNA | Small interfering ribonucleic acid, silencing ribonucleic acid |
| TM | Transmembrane |
| WI-38 | Human fetal lung cell line |

## 5.1 Introduction

Senescence is defined as the gradual deterioration of function leading to increased mortality from disease and injury, also referred to as "biological aging." Although the concept of death may seem as an inevitability, reducing the rate of senescence has shown the possibility of delaying death or promoting regeneration. For example, as early as 1934, rats undergoing dietary restriction have shown to increase their lifespan by 14–45% (Swindell 2012). Alternatively, some fish, turtles, and invertebrates achieve a state of "negligible senescence," which occurs when there is no measurable decline in function or reproductive capabilities with age (Guerin 2004). In humans, changes to senescence come in the form of diseases that hijack natural senescent processes, such as the rare accelerated aging syndrome (Dreesen and Stewart 2011) or "Syndrome X," a disease in which a person remains physically and mentally an infant throughout their life (Walker et al. 2015). Since age is the major risk factor for prevalent diseases in the developed world (e.g., cancer, cardiovascular disease, and neurodegeneration), as well as the fact that the aging process can be hijacked, the possibility of reversing the aging process through manipulating cellular senescence pathways is an area of active research.

Cellular senescence, which leads to organismal senescence, specifically refers to the irreversible arrest of cell proliferation that starts due to stressful stimuli (Campisi and D'Adda Di Fagagna 2007). These can include DNA damage, dysfunctional telomeres, disrupted chromatin, or oncogenesis (Campisi et al. 2001; Serrano and Blasco 2001). Not only are senescent cells irreversibly arrested, but they also secrete an assortment of cytokines, chemokines, growth factors, and proteinases that are collectively termed the senescence-associated secretory phenotype (SASP) (Campisi 2013). The SASP act as paracrine signals that produce a myriad of effects depending on the physiological context. With age, the number of senescent cells increases and consequently leads to an accumulation of SASP cytokines and proteins which in turn can accelerate pathology. This condition in cells is thought to be irreversible due to the absence of any known physiological conditions that can reverse senescence. However, biological manipulations targeting cellular senescence pathways have shown a renewal of proliferative function. For example, inactivation of the p53 gene in senescent fibroblasts caused a return to robust growth (Beausejour et al. 2003). This finding is one of many that attempt to uncover therapeutic strategies for age-related diseases by selectively eliminating the disease-causing features of senescent cells, collectively termed *senotherapies. Senolytics* are a class of senotherapies that uses molecular compounds to selectively and efficiently induce cell death in senescent cells. For example, Baker et al. (2011) showed that the removal of p16(Ink4a)-positive senescent cells in mice delayed age-associated diseases such as osteoporosis. In this chapter, we review the literature contributing to the development of senolytic strategies, specifically therapeutic agents that target cellular senescence pathways.

## 5.2 Senolytics Targeting Pro-survival Networks

Cellular senescence is described as having a double-edged influence on cellular proliferation. On the one hand, senescence can be seen as an anticancer response that turns potentially cancerous cells into benign tumors. On the other hand, senescence of healthy cells or large amounts of cells can reduce the ability for tissue to regenerate and repair itself. Thus, most senolytic agents are currently being developed to target pro-survival networks due to the observation that cellular senescence is preceded by some form of tumorigenic stress such as DNA damage. Although harboring DNA damage and being immersed in local SASP, senescent cells have the remarkable ability to withstand stress. Common markers of cellular senescence include decreased cellular proliferation and increased cell size and volume (Fuhrmann-Stroissnigg et al. 2017). Senescent cells also tend to have increased expression of cell cycle inhibitors (e.g., p21(Cip1) and p16(Ink4a)) and the SASP factor interleukin-6 (IL-6) (Fuhrmann-Stroissnigg et al. 2017). Consequently, it is hypothesized that they have upregulated pro-survival/anti-apoptotic networks. Senolytics targeting pro-survival networks have shown efficacy against atherosclerosis, osteoporosis, cancer, and other age-related disorders (Campisi and D'Adda Di Fagagna 2007). The major pro-survival/anti-apoptotic pathways to be discussed in this chapter include p53/p21, Bcl-2/Bcl-xL, PI3K/Akt, and serpin pathways (Fig. 5.1).

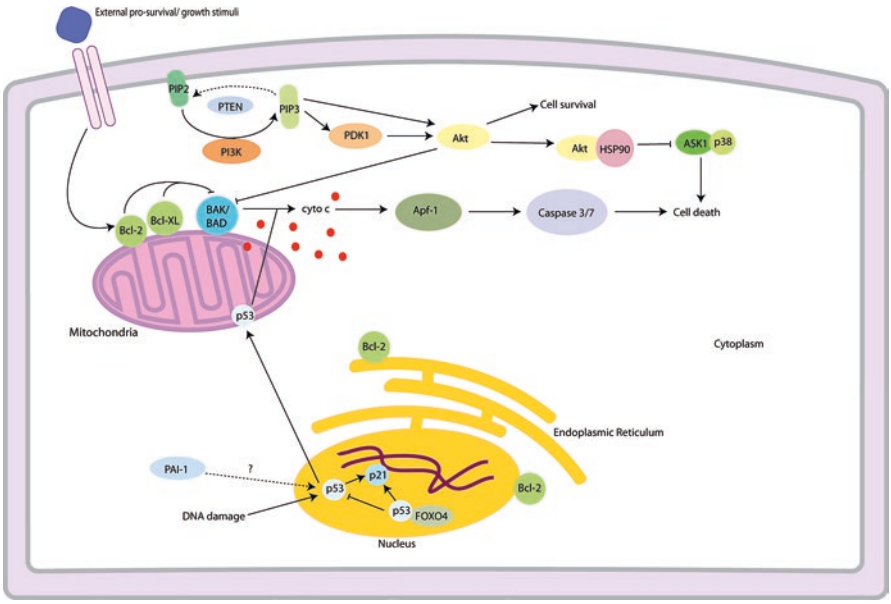


**Fig. 5.1** The p53/p21, Bcl-2, and PI3K/Akt pathways all have regulatory roles in cell survival. Although it is known that PAI-1 upregulates p53/p21 activity, the mechanism of the serpin pathway still remains unknown

## 5.3 p53/p21 Pathway: FOXO4-Interacting Peptide

Permanent growth arrest is initiated with the p53/p21 pathway. Activated p53 leads to the induction of p21, which in turn inhibits the cyclin/cyclin-dependent kinase complexes involved in cell cycle progression (Harris and Levine 2005). Activation of p53 also leads to cell death as p53 is translocated to the mitochondria, which is important in the release of cytochrome *c* and protease activation (Harris and Levine 2005). However, in senescent cells, while cyclin/cyclin-dependent kinase activity is inhibited, cells do not undergo apoptosis.

In a study with human IMR90 fibroblast cells, despite elevated levels of pro-apoptotic initiators and reduced levels of anti-apoptotic factors, cells resisted death (Baar et al. 2017). The group also observed that senescent cells had elevated mRNA and protein expression of FOXO4, a protein that had not been previously linked to senescent cell death.

Interestingly, FOXO4 was able to induce IMR90 cells to senesce rather than apoptose, despite the high levels of pro-apoptotic and low levels of anti-apoptotic factors priming the cells. FOXO4's mechanism involves its association with p53 in the nucleus, preventing nuclear exclusion and p53-mediated apoptosis. By inhibiting FOXO4, there was release of cytochrome *c* and caspase activity in pre-senescent cells, while there was decreased cell viability and density in senescent cell cultures.

In response, Baar et al. (2017) aimed to disrupt the FOXO4-p53 interaction, designing a D-retro-inverso (DRI)-modified peptide containing part of the p53-interaction region found in FOXO4 which they called FOXO4-DRI. DRI-modified peptides can increase peptide potency in vitro and in vivo (Borsello et al. 2003). FOXO4-DRI was capable of binding to p53 with higher affinity than FOXO4, enabling p53 nuclear exclusion and translocation to the mitochondria where it induced apoptosis. In fast-aging mice, FOXO4-DRI treatment reduced the effects of doxorubicin-induced senescence, counteracted hair loss, improved renal function, and improved fitness such as increased voluntary running wheel activity.

## 5.4 Bcl-2/Bcl-xL Pathway Inhibitors

The B-cell lymphoma-2 (Bcl-2)-related family constitutes important apoptosis-regulatory genes that usually act on the mitochondrial and nuclear membrane and endoplasmic reticulum due to a carboxy-terminal transmembrane (TM) region limiting their subcellular distribution (Muchmore et al. 1996; Wang et al. 2001). While most Bcl-2 proteins are death-inhibiting (e.g., Bcl-2, Bcl-xL, Bcl-w, Mcl-1, Bfl1/A-1, Bcl-B), containing all four Bcl-2 homology domains, there are also Bcl-2 homologues that comprise of death-inducers, subdivided into proteins containing Bcl-2 homology 1–3 domains (e.g., Bax, Bak, Bok) (Wolter et al. 1997; Chittenden et al. 1995; Hsu et al. 1997) and proteins containing only the BH3 domain (e.g., Bid, Bim, Bad) (Oltersdorf et al. 2005; O'Connor et al. 1998; Yang et al. 1995). Notably, the ratio of pro- and anti-apoptotic Bcl-2 family proteins influences the fate of a cell.

When the expression of anti-apoptotic Bcl-2 proteins overwhelms the levels of pro-apoptotic Bcl-2 proteins, the cell can escape apoptosis, thus resisting drugs and therapeutic agents (Del Poeta et al. 2003; Minn et al. 1995).

Constitutively high levels of the pro-survival Bcl-2 proteins have been associated with aggressive malignancies, drug resistance toward chemotherapeutic agents, and cellular senescence (Reed 2008; Davis et al. 2003). Hence, there has been a significant effort in targeting Bcl-2 family proteins with senolytic agents, such as TW-37, which is a nonpeptide Bcl-2 inhibitor (Zhu et al. 2016); Navitoclax (ABT-263), which has shown preferential elimination of senescent cells by inducing apoptosis via caspase 3 and 7 activation (Zhu et al. 2016); and ABT-737, a Bcl-2/Bcl-w/Bcl-xL inhibitor, which has shown in vivo preferential elimination of senescent cells and increased hair-follicle stem cell proliferation in the epidermis (Yosef et al. 2016).

However, the effectiveness of Bcl-2 inhibitors is cell-type dependent. For instance, Navitoclax (ABT-263) has shown to selectively induce apoptosis in radiation-induced senescent human umbilical vein endothelial cells (HUVECs) and IMR90 cells, whereas TW-37 has no senolytic activity in these cell types (Zhu et al. 2017). Another issue posed by inhibitors like ABT-263 and ABT-737 is their cause of severe thrombocytopenia (Schoenwaelder et al. 2011; Schoenwaelder and Jackson 2012).

## 5.5 PI3K/Akt Pathway: HSP90 Inhibitors and Fisetin

Phosphatidylinositol-3 kinases, PI3Ks, are lipid kinases capable of phosphorylating inositol ring 3'-OH group found in inositol phospholipids (Fruman et al. 1998). Through one of their SH2 domains in the adaptor subunit, PI3Ks are recruited to the membrane, binding to phosphotyrosine residues on growth factor receptors/adaptor proteins. Activation of PI3K leads to the conversion of phosphatidylinositol-4,5-bisphosphate (PIP2) to the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3), in which PIP3 recruits signaling proteins with the pleckstrin homology (PH) domains to the inner membrane such as PDK1 and Akt (Pawson and Nash 2000). PDK1 (3′-phosphoinositide-dependent kinase 1) is thought to be a constitutively active protein that phosphorylates Akt at T308, enabling stabilization of phosphorylated Akt (p-Akt) (Alessi et al. 1996). Through several mechanisms, active p-Akt activates and inhibits several substrates involved in regulating cell survival, cell cycle progression, and cell growth (Fresno Vara et al. 2004). The PI3K/Akt pathway has been associated with inducing an apoptosis-resistant phenotype and senescence in several cell types (Lorenzini et al. 2002; Astle et al. 2012).

It has been suggested that heat shock protein 90 (HSP90) binds to p-Akt and apoptosis signaling regulating kinase 1 (ASK1) which stabilize p-Akt, encouraging cellular survival and senescence. This binding prevents ASK1 from forming an interaction with p38 to induce signaling for apoptosis (Watanabe et al. 2015; Zhang et al. 2005). It has also been suggested that HSP90 and Akt need to function together

in order to inhibit ASK1-p38 signaling. With that being said, disruption of the HSP90-Akt interaction would lead to destabilization of active/phosphorylated Akt and subsequent apoptosis.

Several HSP90 inhibitors have been identified as potential senolytics including tanespimycin (17-AAG), geldanamycin, and 17-DMAG (Fuhrmann-Stroissnigg et al. 2017).The group considered chemical compounds to have senolytic potential if they significantly reduced senescent cells. In the same study, 17-DMAG was able to downregulate the level of p-Akt in senescent Ercc1$^{-/-}$mouse embryonic fibroblast (MEF) cells in vitro, while another HSP90 inhibitor, namely, ganetespib, showed senolytic activity specifically in HUVECs. This illustrates that not all HSP90 inhibitors work in a similar fashion on all cell types.

Using a human progeroid syndrome mice model, Fuhrmann-Stroissnigg et al. (2017) found that 17-DMAG extended health span by assessing reduction in age-related symptoms such as kyphosis, dystonia, tremor, loss of forelimb grip strength, coat condition, ataxia, gait disorder, and body condition.

Another chemical that functions through the PI3K/Akt pathway specifically is a naturally occurring flavone called fisetin (3,3′,4′,7-tetrahydroxyflavone), which is found in high concentrations in strawberries (160 μg/g) (Khan et al. 2013). Fisetin is a hydrophobic molecule that accumulates in cells and has shown selective apoptosis induction of human breast cancer MCF-7 cells via caspases 7, 8, and 9 (Yang et al. 2012). In both in vitro and in vivo studies, fisetin demonstrated senolytic activity in HUVECs, but not IMR90 and primary human preadipocytes, as shown by caspase 3 and 7 activity assays (Zhu et al. 2017). Fisetin is a widely available nutritional supplement and has very little known side effects, demonstrating its potential to act as an orally administered senolytic agent (Zhu et al. 2017).

## 5.6 Serpin Pathway Inhibitors

Serpin genes encode for serine protease inhibitor (serpin) superfamily proteins that include serpin B2 (PAI-2), serpin E1 (PAI-1), and serpin E2 (Potempa et al. 1994; Kortlever et al. 2006). Among these, there has been considerable interest in plasminogen activator inhibitor 1 (PAI-1) as it has been identified as a senescence-associated gene given its increased expression in senescent cells (Kortlever et al. 2006; Suzuki et al. 2001; Dimri et al. 2000; Elzi et al. 2012). There has been increasing evidence to support that PAI-1 is not only a biomarker but also a mediator of cellular senescence (Perez et al. 2010; Eren et al. 2014a, b; Ghosh et al. 2016). In rat idiopathic pulmonary fibrosis (IPF) alveolar type II (ATII) cells, treatment with bleomycin caused an increase in PAI-1, as well as other senescence biomarkers p53, p21, and senescence-associated beta-galactosidase (SA-β-gal) (Jiang et al. 2017). On the other hand, silencing PAI-1 using siRNA reduced p53 and p21 expressions (Jiang et al. 2017). This suggests that PAI-1 positively regulates p53 and p21 levels in the p53-p21 pathway. However, the mechanism PAI-1 uses to regulate p53 activity is still unknown.

## 5.7 Screening for Senolytic Agents

### 5.7.1 β-Galactosidase Assay

The β-galactosidase assay is a widely used screening platform to identify senotherapeutic drugs in vitro and in vivo due to its simplistic method and apparent specificity toward senescent cells (Krishnamurthy et al. 2004; Cao et al. 2003; Castro et al. 2003; Itahana et al. 2007). The assay measures the expression levels of senescence-associated β-galactosidase activity (SA-β-gal) which is expressed predominantly by senescent cells, occurring at higher frequency in older tissues (Dimri et al. 1995). SA-β-gal is detectable by colorimetric X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) staining at pH 6.0 and/or by using the fluorescent substrate $C_{12}$FDG (5-dodecanoylaminofluorescein-di-b-D-galactopyranoside) staining (Dimri et al. 1995). There are, however, a few criticisms of using SA-β-gal as a surrogate marker of senescent cells. It is important to consider that SA-β-gal has also been highly expressed in non-senescent states (Severino et al. 2000; Untergasser et al. 2003) and in confluent cultures maintained for prolonged periods in vitro (Dimri et al. 1995). Additionally, despite mRNA knockdown of the gene *GLB1*, which codes for SA-β-gal expression, cells still entered senescence (Lee et al. 2006). This suggests that SA-β-gal is not required for senescence. Hence, measuring SA-β-gal is sometimes coupled with measuring other biomarkers of senescence such as p16 gene products and the SASP inflammatory cytokine IL-6 (Kuilman et al. 2008; Capparelli et al. 2012; Marcoux et al. 2013).

## 5.8 Animal Models for Senescent Studies

Engineered by Demaria et al. (2014), the p16-3MR mouse model allows observation and manipulation of senescent cells in vivo. Using their p16-3MR model, the group demonstrated that although senescent cells have mostly inflammatory and detrimental effects through SASP, the total elimination of senescent cells can hinder wound healing and tissue differentiation (Demaria et al. 2014).The p16-3MR mouse strain expresses a trimodal reporter protein (3MR) that is under control by the p16(Ink4a) promoter and contains the functional domains of a synthetic *Renilla* luciferase (LUC), monomeric red fluorescent protein (mRFP), and truncated herpes simplex virus 1 (HSV-1) thymidine kinase (HSV-TK) (Demaria et al. 2014; Ray et al. 2004).Senescent cells, both in vivo and in vitro, often express p16(Ink4a), a cyclin-dependent kinase inhibitor that is also known as CDKN2A. Expression of p16(Ink4a) causes the growth arrest associated with irreversible senescence (Coppe et al. 2011; Baker et al. 2011). Hence, the amount of p16 gene products and the SASP (the most common cytokine being IL-6) would reflect the number of senescent cells. Cells were also engineered to be fluorescent via mRFP to allow easy identification of senescent cells from tissue samples and to be bioluminescent via LUC to allow traceability in vivo (Chang et al. 2016). HSV-TK converts the ganciclovir prodrug into a toxic form that subsequently induces apoptosis (Laberge et al. 2013; Gao et al. 1999).

## 5.9 Assessing Current Senolytic Agents

Developing drugs for targeted senolytics is currently being researched as a viable treatment modality to alleviate disease symptoms. Markers of drug effectiveness include expression levels of survival gene networks together with apoptotic resistance. Small molecule drugs would significantly inform current medicinal approaches aimed to relieve the harmful effects of aging diseases as senolytics drugs have the capacity to selectively target and kill senescent cells. Common target genes converge through various pathways to cell cycle inhibitors such as p21, which in turn inhibits cyclins/cyclin-dependent kinases eventually leading to senescence.

One key pathway that current tested drugs target for senescence are the ephrin-dependent receptor ligands, called EFNB1 or EFNB3 (Hwang et al. 2018). These are the largest receptor tyrosine kinases and coordinate cell survival during development. Like other ligand-receptor interactions, the ephrin receptors can interact with ligands on adjacent cells to stimulate downstream cell signaling. Specifically, EFNB3 has been known to induce the SASP when the gene is overexpressed (Hwang et al. 2018). Table 5.1 highlights the pro-survival pathways together with their targeted senolytics for reference.

In vitro testing of drugs targets gene products that protect senescent cells. Both dasatinib and quercetin are two drugs that are known to clear senescent cells (Hwang et al. 2018). The drug, dasatinib, is an inhibitor of multiple tyrosine kinases which was originally used for treating cancers. Specifically, it is known to inhibit the suppression of apoptosis in human fat cell progenitors. Similarly, quercetin inhibits another class of kinases called PI3K and serpins. This drug was particularly effective against heart and umbilical vein endothelial cells (HUVEC). This presents evidence for cell-specific targeted therapy. In addition, combinations of both drugs showed the selective killing of both senescent fat cell progenitors as well as HUVECs.

Post in vitro testing, the drugs were tested in reducing the viability of senescent murine cells. The murine cells specifically tested were mouse embryonic fibroblasts (MEFs) which showed a significant reduction in number post treatment with both dasatinib and quercetin (Demaria et al. 2014). In these mice, when the

**Table 5.1** Mapping drugs to potential targeted pathways

| Potential pathway | Targeted senolytics | References |
|---|---|---|
| p53/p21 | Quercetin, dasatinib + quercetin, piperlongumine, FOXO4-related peptide | Hwang et al. (2018), Wang et al. (2016), and Baar et al. (2017) |
| Bcl-2/ Bcl-xL | Quercetin, Navitoclax (ABT-263), ABT-737, piperlongumine, A1331852, A1155463 | Hwang et al. (2018), Wang et al. (2016), Baar et al. (2017), and Zhu et al. (2017) |
| PI3K/Akt | Quercetin, fisetin, geldanamycin, tanespimycin, ganetespib | Hwang et al. (2018), Khan et al. (2013), and Fuhrmann-Stroissnigg et al. (2017) |
| Serpin | Quercetin, dasatinib + quercetin | Hwang et al. (2018) |

cardiovascular function was tested, there was substantial impairment in vascular reactivity seen in aged mice. This further uncovered a link between senescent cells and cardiovascular dysfunction in humans.

However, not all formulations were successful in drug testing. For example, a selective prodrug in targeting senescent cells is the quercetin derivative quercetin 3D galactoside (Q3G; hyperoside). Hyperoside is a natural derivative of quercetin and structurally identical except that it contains a cleavable galactoside group. However, it was found to be ineffective in targeting senescent endothelial cells in vitro (Hwang et al. 2018).

Navitoclax, a Bcl-2 inhibitor, was similar in action to quercetin and dasatinib and also eliminated cells via apoptosis in similar human and mouse cell types (Zhu et al. 2017). In addition to targeting multiple Bcl-2 family target proteins, Navitoclax acted non-specifically. To illustrate, administration of Navitoclax mice led to the effective depletion of senescent bone marrow hematopoietic stem cells (HSCs) as well as senescent muscle stem cells (MuSCs) (Chang et al. 2016). Similarly, Bcl-xL inhibitors, namely, A1331852 and A1155463, were found to be senolytic in HUVECs and IMR90 cells, but not preadipocytes (Zhu et al. 2017). This activity occurred through apoptosis as tested by caspase 3/7 activity in vitro.

Piperlongumine is a natural product, isolated from the genus *Piper* and demonstrated to have senolytic properties (Wang et al. 2016). Piperlongumine was shown to selectively kill human WI-38 fibroblasts by several means: reducing viability in IR-induced as well as Ras-induced WI-38 senescent cells. It was uncovered that the selective killing occurred by apoptosis through a reactive-oxygen species (ROS)-independent mechanism. Further, a synergistic effect of piperlongumine was seen when administered together with Navitoclax.

Other drugs on the market such as metformin, rapamycin, and ruxolitinib have shown promising effects to suppress SASP, specifically alleviating symptoms of age-related disorders causing metabolic dysfunction (Huffman et al. 2016). For example, ruxolitinib, a JAK 1/2 inhibitor, alleviates insulin resistance and tissue dysfunction (Xu et al. 2015). The intermittent administration of these drugs has been shown to mitigate effects of cellular senescence. However, since the specific mechanism of action remains unknown, it is difficult to identify exactly how these SASP inhibitors function. Moreover, administering them in conjunction with the pathway-specific drugs described above may prove a difficult, if not impossible, task.

## 5.10 Future Direction of Senolytics

Although the potential to translate senolytics into clinical treatments shows promise, there are some concerns moving forward. Perhaps the biggest challenge lies in the fact that cellular senescence has a dual nature, and creating anti-aging treatments may not be as simple as accentuating or attenuating core senescence pathways. The inhibition of growth, for example, acts as a natural anticancer mechanism that prevents tumors from progressing past benign stages. Given that tumorigenic

effects largely begin by mutations in the genome, removing the senescence capabilities of rapidly dividing cells altogether would simultaneously increase the chances of developing tumors. This is a major risk with regard to "off-target" effects of senolytic drugs. On the other hand, the benefits of removing senescent cells in animal models have clearly shown promise. For example, the removal of p16(Ink4a)-positive senescent cells in mice delays age-associated diseases such as osteoporosis (Baker et al. 2011). One strategy to approach dealing with issues regarding the duality of senescent effects is to engineer therapies to target specific cellular contexts. A recent innovative approach involved using engineered proteins expressed in cells to target senescent cells releasing IL-6 cytokines (Fig. 5.2) (Qudrat et al. 2017).

Understanding the specific contexts of harmful senescence states and targeting therapeutics to that context may provide a better approach to selectively remove harmful senescent cells. This cellular-based approach to senotherapies involves the creation of a stable cell line expressing a synthetic chimeric receptor for a SASP cytokine and calcium-activated RhoA (CaRQ) to enable migration toward SASP sources (Qudrat et al. 2017). In Qudrat et al. (2017), IL-6 targeting cells were engineered to express a chimeric receptor called IL6Rchi, which is comprised of the extracellular portion of the IL-6 receptor to bind to the SASP cytokine IL-6, and the transmembrane and cytoplasmic domains of VEGFR2 to generate calcium signals. The calcium signals generated by IL-6 binding to IL6Rchi activate CaRQ-mediated cell migration, enabling the IL-6 targeting cell to migrate toward areas/sources of high IL-6 expression (i.e., senescent cells expressing the SASP). Once at the targeted SASP site, the HSV-TK system can be used to convert ganciclovir into its toxic form to induce apoptosis (Gao et al. 1999; Qudrat et al. 2017). The results have been promising in vitro in targeting directed SASP engineered cells. Additionally, through the use of antibody components in the chimeric receptors, cell migration can be rewired to nearly any SASP cytokine (Qudrat and Truong 2018).

Although the potential to translate senolytics into clinical treatments is present, there are clear obstacles. For one, the main difficulty is in determining potential endpoints in clinical trials (Kirkland and Tchkonia 2017). Further, treatments that

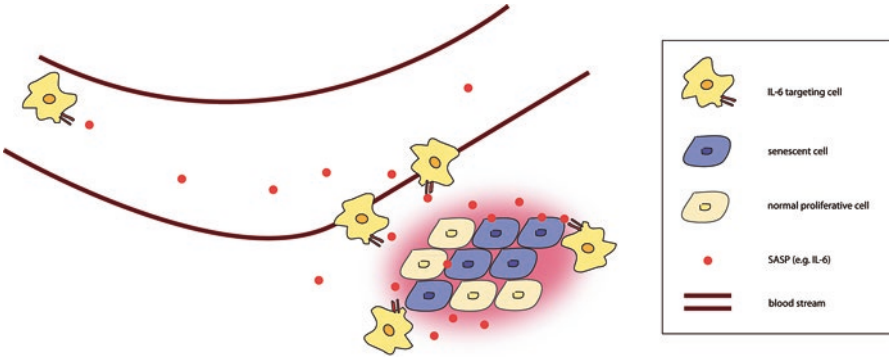


**Fig. 5.2** Concept of IL-6 targeting cells responding and migrating toward IL-6 sources (e.g., senescent cells)

appear effective in mice may prove altogether ineffective in humans. This is particularly true when attempting to translate from genetically induced mice models to humans. And since not all senescent cells are harmful as they play an important role in wound healing and tissue repair, the need for targeted therapeutics is ever present to eliminate harmful effects and maintain beneficial effects of cellular senescence. On the contrary, generating targeted therapeutics to remove senescent cells may have decreased rates of drug resistance and recurrence since these cells no longer divide (Kirkland and Tchkonia 2017).

Looking ahead, there are many questions that still need to be addressed before senolytics find their way to the market. First, there is a need for characterizing potential drug side effects, in addition to delays in wound healing, as well as off-target effects (Demaria et al. 2014). Secondly, to optimize the frequency of drug administration, rates of senescent cell re-accumulation need to be explored. Thirdly, the additive and synergistic effects of drugs, specifically in conjunction with SASP inhibitors, need to be uncovered. Fourthly, definitive studies need to be performed to expound the realized effect of senolytics on lifespan. Lastly, a multiplex approach needs to be evaluated coupling drug delivery and cell-based therapeutics to maximize proficiency.

## 5.11 Conclusion

Age is a condition associated with decreasing physiological and psychological capabilities in humans. This deterioration gives rise to vulnerability to a host of diseases as well as a lower quality of life. Over the past century, decreased mortality rates and increased life expectancy has been a major advancement of the human condition, followed closely however by a domination of age-related diseases. The discovery of cellular senescence pathways and their link to aging and the development of animal models that show little signs of accelerated aging have been a major step to a potential treatment for aging conditions. Understanding that cellular senescence can be a beneficial mechanism for cancer protection has also deepened our understanding of the benefits of aging and the complexity of human physiology. Senolytic therapies have attempted to reduce age-related pathologies by the removal of harmful senescent cells. Senolytics targeting pro-survival networks have shown efficacy against atherosclerosis, osteoporosis, cancer, and other age-related disorders in cell and animal-based studies. Developing targeted approaches to remove harmful senescence while maintaining the body's natural defense against uncontrolled proliferation may be the next major challenge. Greater testing in clinical trials and well-defined outcomes are also needed, but nevertheless, the current outlook in senolytics seems bright. The past century has seen a decrease in mortality rates and increased life expectancy, and although more research is needed, significant steps are being made toward understanding the complexity of aging to move forward into the next century.

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# Novel Classification Perspective of Geroprotective and Senolytic Drugs as an Antiaging Strategy

6

Karolin Yanar

**Abstract**

Aging is an inevitable, physiologically irreversible, and progressive process. It involves various detrimental changes in the ability to maintain cellular homeostasis. During the aging period, senescent cells are accumulated. Due to the significant medical advances in the treatment of various life-threatening diseases, life expectancy is rising day by day. Thus, higher speed of population aging brings enhanced prevalence of age-related disorders. Increasing mid-life quality and extending the life span of aging individuals seem possible by decreasing the rate of aging process with the help of various pharmacologically active substances called as geroprotective or senolytic drugs. Several numbers of naturally found and synthetic substances may provide a source of therapeutic drugs which are proposed to have some geroprotective or senolytic effects, reducing the rate of aging and extending the life span. These therapeutic drugs have some beneficial effects on cellular metabolism such as antioxidant, free radical scavenger, immunomodulator, and metal chelator activities. Some of the aforementioned drugs are called as smart molecules because of their pluripotency effects. Attributed to their properties, these drugs may overcome impaired cellular metabolic homeostasis. This chapter aimed to classify geroprotective and senolytic drugs via their structural properties and pharmacological mechanisms.

**Keywords**

Antiaging · Antioxidant · Caloric restriction · Immunomodulator · Geroprotective drugs

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## 6.1 Dawn of Geroprotective Drugs

Global aging and increasing prevalence of age-related disorders attract the attention of researchers worldwide. Finally, gerontology as a discipline in scientific field is proposed by Russian and French biologist Ilya Mechnikov (Moskalev et al. 2017).

Gerontologists dedicated themselves to slow down degenerative outcomes of aging process. More than 300 theories have been proposed, and aforementioned theories interrelated with each other (Medvedev 1990). It is currently not impossible to slow down detrimental outcomes of aging process because of the discovery of more than 200 substances as geroprotective drugs (Moskalev et al. 2016). Geroprotective drugs may affect the root cause of aging and age-related pathologies via preventing or delaying onset or progress of age-related disorders (Moskalev et al. 2016; Ito et al. 2012). Many of the aforementioned drugs are called as "smart molecules" because of their pluripotency effects.

## 6.2 Definition of Geroprotective Drugs

Geroprotective drugs need to have some of the following selection criteria:

- Having the ability to increase life span.
- Diminish rate of the progression of age-related disorders.
- Have maximum benefit and accessible toxicity.
- Should enhance health quality of elderly life.
- Action mechanisms or their targets should evolutionarily be preserved.
- Enhance the organism resistance to adverse environmental factors (Moskalev et al. 2017).

## 6.3 Classification of Geroprotective Drugs

It can be suggested that classification of geroprotective drugs may be divided into two major groups, their chemical structure and action mechanism.

### 6.3.1 Classification of Geroprotective Drugs According to Their Chemical Structure

Classification of geroprotective drugs according to chemical structure is given in Table 6.1.

**Table 6.1** Classifications of geroprotective drugs according to chemical structure

| Chemical structure | Example of geroprotective drugs | Chemical structure | Example of geroprotective drugs |
|---|---|---|---|
| Amines | D-Glucosamine, spermidine | Minerals | Magnesium, cooper, selenium, zinc |
| Amino acid derivatives | N-Acetyl L-cysteine, S-adenosylmethionine, carnosine, histidyl hydrazine, methionine | Vitamins | Ascorbic acid, tocopherol, vitamin B3, vitamin B5, vitamin B6, vitamin D, alpha lipoic acid |
| Sugar and sugar derivatives | Trehalose, 2-deoxy-D-glucose, mannoheptulose | Polyphenol | Resveratrol, quercetin, gallic acid, catechin, ellagic acid, curcumin, caffeic acid |
| Hormones | Melatonin, dehydroepiandrostenedione, estrogen, testosterone | Peptides | Carnosine, glutathione |
| Organic acids | Alpha-ketoglutarate, malate, fumarate | | |

### 6.3.2 Classification of Geroprotective Drugs According to Action Mechanism

Even though various candidate substances that ameliorate the higher rate of age-related degenerative changes have been currently proposed, there is still no global consensus of classification issue of these substances in current literature.

Recently extended classification of geroprotectors:

- Mitochondria-targeted antioxidants
- Advanced glycation end product (AGE) and advanced lipid peroxidation end product (ALE) inhibitors
- Mimetics of caloric restriction
- Epigenetic modulators
- Immunomodulators
- Hormones and hormonelike substances

#### 6.3.2.1 Mitochondria-Targeted Antioxidants

Various theories have been proposed to clarify homeostatic regulation mechanisms of aging, including mitochondrial and free radical theories (Loenen 2010). Antioxidant supplementation draws attention to overcome excessive free radical formation and age-related degenerative pathologies (Fusco et al. 2007; Erdoğan et al. 2017). Supplemented antioxidants mainly act as free radical scavengers depending on their dosage. They play important role in direct or indirect neutralization of free radicals, reducing the peroxide concentration and repairing oxidized cell membranes, quenching iron to decrease free radical production (Berger 2005).

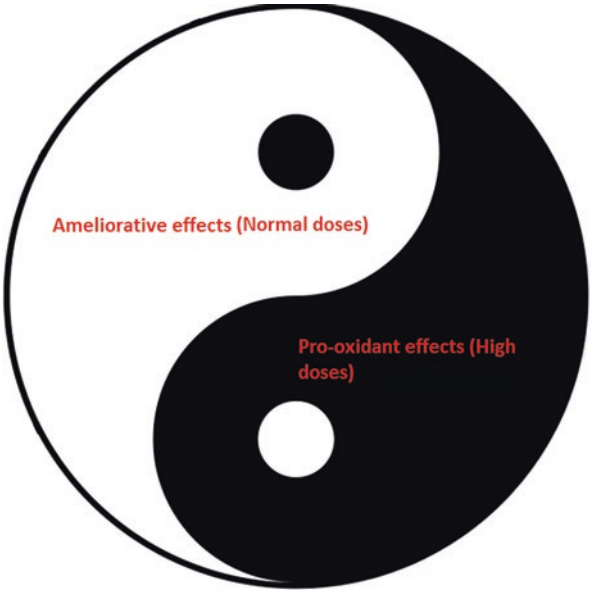


**Fig 6.1** Effects of substances depend on their dosage: at high concentration substances lose their antioxidant abilities and represent opposite effect as prooxidant. The black and white areas symbolize prooxidant and ameliorative effects of substances called as antioxidant depending on their dosage

**Table 6.2** Summary of mitochondria-targeted antioxidants and their geroprotective properties

| Mitochondria-targeted antioxidants | Geroprotective properties |
|---|---|
| Nitroxides | Superoxide radical scavengers |
| Lipoic acid | Metal chelator activities, AGE inhibitor, anti-inflammatory |
| Plastoquinone | Free radical scavenger, antibacterial activity |
| Mitoquinone | Free radical scavenger, anti-inflammatory, antihypoxic activity |
| MitoVitE | Mitochondrial redox regulator |
| Ebselen | Mimics glutathione peroxidase activity, ALE inhibitor |
| Latrepirdine | Antihistaminic, neuroprotector, monoamine oxidase inhibitor, acetylcholine esterase inhibitor |
| Szeto-Schiller peptides | Free radical scavenger, ALE inhibitor |
| N-Acetyl cysteine | Antioxidant, anti-inflammatory, ALE inhibitor |

*AGEs* advanced glycation end products, *ALEs* advanced lipid end products

At high levels, antioxidants not only failed to ameliorate age-related pathologies, but they may also cause adverse events due to their prooxidant activity (Howes 2006; Kayali et al. 2007) (Fig. 6.1).

Antioxidant targeted therapy is based on two main strategies; one of them is conjugation to lipophilic cations as triphenylphosphonium ($TPP^+$) (Murphy 2008) and the other is incorporation into mitochondria-targeted peptides as Szeto-Schiller (SS) peptides.

Geroprotectors as mitochondria-targeted antioxidants are given in Table 6.2.

### Lipoic Acid

Lipoic acid (LA) is an amphipathic, sulfur-containing metabolic antioxidant molecule which acts as a metal chelator and glycation inhibitor (Muellenbach et al. 2008; Packer et al. 1995). It is also synthesized endogenously by LA synthase in mitochondria and also it is supplied nutritionally. Atukeren et al. showed metal chelator activities of both LA and its reduced form dihydrolipoic acid (DHLA) on free radical-induced human albumin oxidation at in vitro conditions (Atukeren et al. 2010).

### Szeto-Schiller Peptides

Szeto-Schiller (SS) peptides are synthesized from basic and aromatic amino acids; thus, they are called as aromatic-cationic peptides (Zhao et al. 2004). SS peptides are comprised of four alternating aromatic/basic D-amino acids in the first or second position of their rings with three positive charges at physiological pH. Despite three positive charges, their structure allows SS peptides to freely penetrate aging cells. Uptake of these peptides occurs in energy-independent, dose-dependent, non-saturable manner (Zhao et al. 2003). The presence of a D-amino acid in either the first or second position of the sequence provides them resistance against aminopeptidase activity, and amidation of the C-terminus reduces hydrolysis from the C-terminus. Four different SS peptides identified (Zhao et al. 2004). These peptides are called as SS-01 (H-Tyr-D-Arg-Phe-Lys-$NH_2$), SS-02 (H-Dmt-D-Arg-Phe-Lys-$NH_2$), SS-31 (H-D-Arg-Dmt-Lys-Phe-$NH_2$), and SS-20 (H-Phe-D-Arg-Phe-Lys-$NH_2$). SS peptides act as free radical scavengers as $H_2O_2$ and $ONOO^-$. These peptides inhibit lipid peroxidation via the inhibition of oxidative modification of linoleic acid and low-density lipoprotein (LDL). Their scavenging action can be attributed to the tyrosine or mainly dimethyltyrosine residues (Zhao et al. 2004).

### N-Acetyl Cysteine

N-Acetyl cysteine is the acetylated form of cysteine and serves as a hydrophilic antioxidant, free radical scavenger and anti-inflammatory, glutamate-modulating agent (Avantaggiato et al. 2014; Oliver et al. 2015). Its antioxidant activity appears against hypochlorous acid, $OH^{\cdot}$ radicals, and $H_2O_2$ via its thiol (-SH) groups (Aruoma et al. 1989; Avantaggiato et al. 2014). N-Acetyl cysteine increases the intracellular levels of -SH groups of cysteine. Cysteine is the primary amino acid of glutathione synthesis and necessary to ensure optimum cellular reduced glutathione levels (Oliver et al. 2015). Glutathione reacts with peroxynitrite to form S-nitrosothiols. S-Nitrosothiols protect the accumulation of excessive amount of peroxynitrites and prevent adverse effects of nitrosative stress (Loscalzo 2001). N-Acetyl cysteine inhibits protein and lipid peroxidation (Negre-Salvayre et al. 2008; Arakawa et al. 2007); ameliorates deteriorated membrane integrity, cellular dysfunction, and apoptosis; and ensures restoration of excess amount of malondialdehyde (MDA), acetylcholine esterase, choline acetyltransferase, and acetylcholine to physiological levels (Fu et al. 2006).

#### 6.3.2.2 Advanced Glycation End Product and Advanced Lipid End Product Inhibitors

Advanced glycation end products (AGEs) are very heterogenous compounds which are formed by Maillard reaction. Protein turnover rate is one of the limiting factors of AGE accumulation. Excessive AGE accumulation is seen most prominently in long half-life of proteins such as crystalline (Nowotny et al. 2015; Dalle-Donne et al. 2003). Not only protein turnover rate but also accumulation of glycating agent and glyoxidative stress may form AGEs (Nowotny et al. 2015). The steady-state level of systemic AGEs is determined by the function of a balance between their formation and removal. AGE inhibitors may serve as relatively nonspecific nucleophiles. Their nucleophilic features give them an advantage in affecting ALE formation (Onorato et al. 2000). Considerable evidence suggests that ALE inhibitors may be considered as useful markers to evaluate initiation and progression of age-related disorders (Aronson 2003). Currently, various ALE and AGE inhibitors have been used for prevention of related disorders (Table 6.3). These inhibitors inhibit related pathways in different steps (Fig. 6.2).

##### Angiotensin-Converting Enzyme Inhibitors

Angiotensin-converting enzyme (ACE) inhibitors exhibit various protective effects: inhibit LDL oxidation and the formation of MDA and 4-HNE (Kornatowski et al. 2006; Saeidnia and Abdollahi 2013). It was reported that captopril, a thiol-containing ACE inhibitor, was able to significantly decrease the oxidative modification of LDL particles via scavenging hypochlorous acid (Van Antwerpen et al. 2006).

##### Carnosine

A natural product, carnosine (β-alanyl-L-histidine), increases the chronological life span of human fibroblast cells and effectively postpones cellular senescence (McFarland and Holliday 1994). AGE and ALE inhibitors generally considered as antiaging compounds (Hipkiss 2017) form adducts with various aldehydes and ketones (Vistoli et al. 2009). Carnosine has also metal chelator free radical scavenger activities and affects gene expression (Fontana et al. 2002; Boldyrev et al. 1994; Hipkiss 2017).

**Table 6.3** Summary of geroprotectors that act as AGE and ALE inhibitors and their geroprotective properties

| AGE and ALE inhibitors | Geroprotective properties |
|---|---|
| Aminoguanidine | Prevents against AGE and lipid peroxidation products production |
| Pyridoxamine | Inhibits LDL oxidation and AGE formation |
| Angiotensin-converting enzyme inhibitors | Inhibits 4-HNE and MDA formation, free radical scavenger |
| Carnosine | AGE and ALE inhibitor, free radical scavenger, metal chelator |
| Cortagen | ALE inhibitor, activation of antioxidant defense system |

*AGE* advanced glycation end product, *ALE* advanced lipid end product, *HNE* hydroxynonenal, *MDA* malondialdehyde

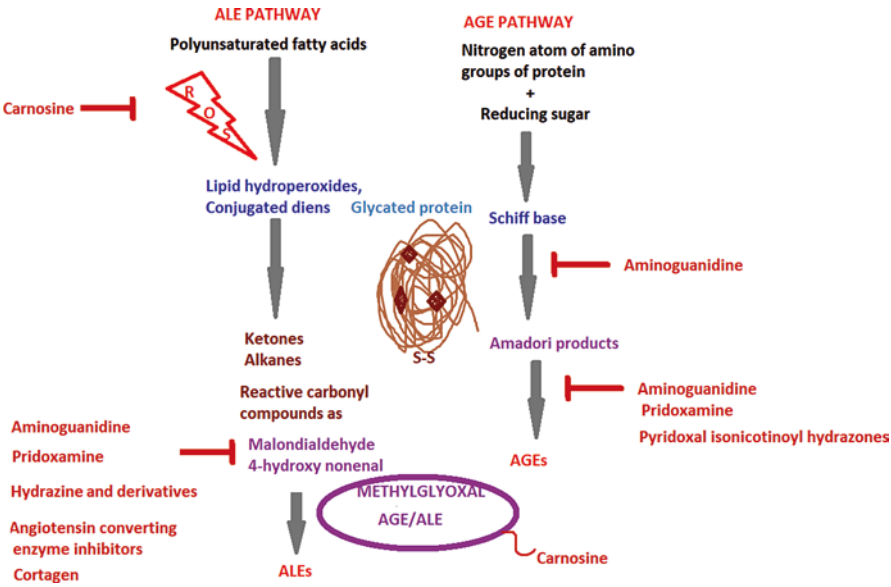


**Fig. 6.2** Effects of AGE and ALE inhibitors. Reactive oxygen radicals affect polyunsaturated fatty acids and lead to lipid peroxidation. This pathway is called as ALE pathway. On the other hand, increased oxidative stress leads to structural protein modification and impaired function of protein. Reaction between high concentration of reducing sugar and protein results in Schiff base formation and further reactions. Amadori products are formed and finally advanced glycation end products are formed. AGE and ALE inhibitors such as carnosine, aminoguanidine, pyridoxamine, angiotensin-converting enzyme inhibitors, and cortagen block ALE and AGE formation at different steps

### 6.3.2.3 Caloric Restriction Mimetics

The caloric restriction (CR) theory of aging was proposed by Professor David Sinclair (Sinclair 2005). Expected CRM characteristics are proposed by Ingram et al. as follows:

- Imitates the metabolic, hormonal, and physiological effects of CR
- Activates stress response pathways noticed in CR and increases stress protection
- Reduces the incidence of age-related disorders and maintains more healthful life span (López-Lluch and Navas 2016)

Proposed strategies of caloric restriction mimetics are shown (Table 6.4):

- Inhibits glycolytic pathway (Ingram and Roth 2011)
- Activates silent information regulator 2 (SIR2) gene (Chen and Guarente 2007), induces sirtuins (Guarente 2013)

**Table 6.4** Summary of geroprotective drugs acting as caloric restriction mimetics

| Geroprotectors acting as caloric restriction mimetics | Geroprotective properties |
|---|---|
| 2-Deoxy-D-glucose | Glycolytic inhibitor |
| Mannoheptulose | Glycolytic inhibitor |
| Metformin | Enhances antioxidant defense system, mimics CR |
| Resveratrol | Activates SIRT-2 |
| Rapamycin | mTOR inhibitor |
| Methionine | Inhibits ROS formation, mimics CR |

*CR* caloric restriction, *SIRT2* silent mating type information regulation-2 homolog, *mTOR* mammalian target of rapamycin, *ROS* reactive oxygen species

- Inhibits serine threonine kinase pathway known as nutrient-sensing mammalian target of rapamycin (mTOR) (Ma et al. 2015)
- Reduces mitochondrial free radical formation and improved antioxidant system activity (López-Lluch and Navas 2016)
- Inhibits AMP-activated protein kinase (AMPK) activity (To et al. 2007)

### Biguanide (Metformin)

Metformin enhances the sensitivity of insulin receptors and antioxidant defense and activates genes that inhibit gluconeogenesis. It induces glycolysis in hepatocytes, thus reducing the risk of nonenzymatic glycation of structural proteins, cellular macromolecules, and other age-related disorders (Dhahbi et al. 2005; Schramm et al. 2011).

### Resveratrol

Resveratrol is a polyphenol stilbene and synthesized naturally by several edible fruits (Mohar and Malik 2012). Resveratrol activates SIRT-2, and the associated improvement in energy utilization and insulin sensitivity closely resembles the benefits of CR (Mohar and Malik 2012; Lam et al. 2013). In vitro application of resveratrol prevents deleterious effects of oxidative damage in erythrocytes from human donors of all ages (Pandey and Rizvi 2014).

### Rapamycin

The Food and Drug Administration-approved compound rapamycin was the first pharmacological agent shown to extend maximal life span in mammalians (Ehninger et al. 2014).Rapamycin acts on mTOR pathway, which is a nutrient-sensing protein that modulates the response to starvation. Rapamycin inhibits mTOR, by AMPK-dependent and AMPK-independent pathways, inhibits translation, and stimulates autophagy like CR. This process leads to the extension of life span (Magon et al. 2012). Its use prevents new tumor formation and leads to regression of already existing tumors. Rapamycin also reduces progression of atherosclerosis but causes hyperlipidemia (Magon et al. 2012).

#### 6.3.2.4 Epigenetic Regulators

**Histone Deacetylase Inhibitors**

Histone deacetylase (HDAC) inhibitors are classified into four groups as classes I, II, III, and IV. Class I and class III are related with aging process (Ferguson and McKinsey 2015). Histone deacetylase inhibitors are able to restore redox homeostasis. Administration of valproic acid as a weak albeit selective inhibitor of class I HDACs inhibits cardiac hypertrophy and fibrosis, which is associated with reduced levels of reactive oxygen species (ROS) and pro-inflammatory cytokines (Fass et al. 2010). Valproic acid-mediated lowering of ROS levels may be explained as diminished expression of a component of the superoxide-generating NADPH oxidase complex (Wang et al. 2010). Class I HDAC inhibitor, MS-275, is related to increased expression of the mitochondrial ROS scavengers such as superoxide dismutase (Mn-SOD) and catalase (CAT) in myocardial tissue (Aune et al. 2014).

**S-Adenosylmethionine**

S-Adenosylmethionine is a sulfur-containing molecule. It is eminent as the methyl donor for the majority of methyl transferases that modify macro- and micromolecules including toxic metals, such as arsenic (Loenen 2010). It also acts as an inhibitor of 4-HNE adduct formation (Valentovic et al. 2004).

#### 6.3.2.5 Immunomodulators

According to immunologic theory of aging, immune function impairs with advancing age. Impairment of immune function may lead to increased risk of infections and tumor progression and also tendency of autoimmune disease (Anisimov 2001).

**Thymoquinone**

Thymoquinone (TQ) is known as active pharmacological constituent of *Nigella sativa* seeds. It has potent anti-inflammatory, immunomodulator, anti-histaminic, antimicrobial, and anti-tumor effects (Darakhshan et al. 2015; Khader and Eckl 2014; Woo et al. 2012; Farkhondeh et al. 2017; Bargi et al. 2017). TQ inhibits proliferation and migration via decreased nuclear factor kappa B (NfκB) and thus decreased tumor necrosis factor- alpha (TNF-α) and interleukin-8 (IL-8) levels and also decreases matrix metalloproteinases 2 and 9 (Farkhondeh et al. 2017). Additionally, thymoquinone acts as ALE inhibitor and enhances expressions and activities of antioxidant enzymes such as glutathione reductase, glutathione peroxidase, CAT, and SOD (Bargi et al. 2017). TQ also normalizes age-related dysregulations in angiotensin system (Idris-Khodja and Schini-Kerth 2012).

**Peptides and Polypeptides**

A polypeptide complex epithalamin is able to decrease the threshold of hypothalamic estrogen sensitivity and restores regular estrous cycles in elderly animals (Khavinson et al. 2013). It increases melatonin production, improves immunological parameters, and also exhibits anticarcinogenic effects. Administration of

epithalamin may suppress the formation of early lipid peroxidation products such as diene conjugates. Epithalamin administration causes reliable increase in the activity of blood total antioxidant status (Khavinson et al. 2013).

Delta-sleep-inducing peptide exhibits a wide range of positive regulator properties on aging and represents antioxidant, immunomodulator, and antistressor effects (Odin et al. 2004; Aggarwal and Razvi 2013).

#### 6.3.2.6 Hormones and Hormonelike Substances

Endocrinological variations occur during aging period (Aggarwal and Razvi 2013; Jonas et al. 2015; Diamanti-Kandarakis et al. 2017). Hormonal variations may be associated with oxidative and nitrosative damage (Diamanti-Kandarakis et al. 2017).

##### Melatonin

It has been well known that melatonin and its metabolites are able to represent antioxidant effects via their free radical scavenger and immunomodulator activities (Korkmaz et al. 2012; Diamanti-Kandarakis et al. 2017; Karaaslan and Suzen 2015).

Protective actions of melatonin ensure maintenance of electron flux and diminish electron leakage and structural integrity of the mitochondria (Hardeland et al. 2015).

##### Thyroid Hormones

Thyroid hormones, triiodothyronine (T3) and thyroxine (T4), regulate cellular differentiation and maintain metabolic homeostasis. Impaired metabolic homeostasis may cause increased tendency of neurodegenerative disorders (Fu et al. 2014). Thyroid dysfunction is commonly seen in elderly individuals especially in females (Aggarwal and Razvi 2013). As serum thyroid-stimulating hormones, free T4 and T3 levels change with aging. Selenium plays an essential role in thyroid function and resistance to oxidative stress. Selenium depletion is seen in aging period (Diamanti-Kandarakis et al. 2017). Thyroxine administration significantly increases the levels of choline acetyltransferase, nerve growth factor, SOD, CAT, and glutathione peroxidase activities (Fu et al. 2014).

##### Sex Hormones

One of the milestones of antiaging therapy is sex hormone replacement therapy, in both male and female elderly individuals (Samaras et al. 2014). It was previously shown that dehydroepiandrosterone (DHEA) inhibits DNA synthesis and production of superoxides in aging tissues, decreases body weight, and represents considerably antiatherogenic, antidiabetic, and antiautoimmune properties (Valenti 1997).

Estriol is a widely used antiaging drug. It is a weak estrogen and potentially safer in terms of breast cancer (Samaras et al. 2014).

Testosterone biosynthesis gradually diminished with advancing age. Testosterone replacement therapy has been studied in aging populations regarding age-related disorders (Kenny et al. 2001; Storer et al. 2008; Yanar et al. 2015)

## 6.4 Conclusion

Chronological aging includes many complicated mechanisms. Gerontologists have suggested many heterogenous groups of therapeutics with different molecular mechanisms. Currently used geroprotectors have aimed to reduce the rate of aging, postpone onset of age-related disorders, and extend the healthy life span. However, they have some adverse effects depending on their usage, dosage, and application intervals. Further investigations need to focus on antiaging therapeutic strategies and alleviate the degenerative changes during aging period.

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# Antiaging Strategies Based on Telomerase Activity

7

Yasemin Aydin

**Abstract**

Telomeres are DNA sequence that are repeated at the end of the linear chromosomes and ensure chromosome stability during replication. Telomere length shortened each cell division and during oxidative stress. When telomeres lose their length critically, cell division can no longer occur which causes cells to enter senescence. Besides, telomeres are sensitive to oxidative stress which can cause telomere shortening. Telomerase, which consists of a structural RNA and two proteins, is a cellular reverse transcriptase. This reverse transcriptase adds new DNA onto the telomeres and thus it is responsible for telomere length. There is no telomerase activity in human somatic cells due to the lack of the human telomerase reverse transcriptase (hTERT) expression; because of this situation, telomeres progressively shortened and finally exhausted with aging process. Telomerase activation is a potentially helpful technique for anti-aging strategy and to combat age-related diseases. Telomerase activators that are chemical molecules activate telomerase, or hTERT is used as an antiaging supplement that is a new era of antiaging nutritional science. This chapter has discussed antiaging strategies based on telomerase activity to combat the aging process.

**Keywords**

Antiaging · Cell life span · Telomerase · Telomerase therapeutics · Telomere

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## 7.1 Telomere and Telomerase Activity

### 7.1.1 Telomere

Telomeres are defined as dynamic nucleoprotein complexes that are located at the ends of the linear chromosome. They shield chrosomal ends from biodegredation, DNA damage response initiation, chromosomal fusions, and chromosomal instability (Blackburn 1991). Telomeres consist of G-rich nucleotide (TTAGGG) repeats associated with a multiprotein complex called shelterin (Palm and de Lange 2008). Shelterin is comprised of six proteins, telomeric repeat binding 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 repressor/activator protein 1 (RAP1) (De Lange 2005). While TRF1 and TRF2 are directly bound to telomeric double-stranded DNA, POT1 is bound the telomeric single-stranded DNA (van Steensel and de Lange 1997). TRF 1 promotes telomeric replication and prevents telomere fusions (Sfeir et al. 2009). TRF2 plays an important role in theprotection of chromosomal ends (van Steensel et al. 1998) and also helps form T-loops which are known as higher telomeric structures (Griffith et al. 1999). TIN2, another protein that constitutes shelterin, is a key constituent of the shelterin complex, binding TRF1 and TRF2 simultaneously and assuring structural integrity of the complex (Kim et al. 1999). POT1, which interacts directly with TPP1 protein, and TPP1 are responsible for the protection of the single-stranded tract of the telomere (Hockemeyer et al. 2007). RAP1, which is also known as TRF2-interacting protein, is a stabilizing protein that interacts with TRF2 (Celli and de Lange 2005). The shelterin complex performs two important functions, which are the prevention of recognition of the chromosomal ends by the DNA damage machinery and recruitment of telomerase (Palm and de Lange 2008).

### 7.1.2 Telomerase Activity

Telomerase, also termed as telomere terminal transferase, is an enzyme that uses RNA template to synthesize single-stranded TTAGGG sequence of telomere (Blackburn 1990). Telomerase comprised of two major components, which are a telomerase reverse transcriptase (TERT) protein and a noncoding telomerase RNA component (TERC). Although telomerase activity in extracts from the ciliate *Tetrahymena* was discovered in 1985 by Greider and Blackburn, TERT, the catalytic subunit of the enzyme, was not identified until 1997 (Greider and Blackburn 1985). Apart from TERC and TERT component of telomerase, it consists of the accessory protein like dyskerins, TCAB1, NHP2, NOP10, and GAR1, which are required for telomerase biogenesis and localization activity (Cohen et al. 2007).Telomerase is responsible for adding G-rich nucleotide (TTAGGG) sequence to preserve the lenghts of telomeres, thus compensating for the continuous telomere attrition at each cell division (Blackburn and Collins 2011). Telomere replication is a multistep

process which consists of three stages: telomere binding, polymerization, and translocation. In the first step of telomere replication, telomerase binds to 3′ overhang of telomere, mediated by the TPP1-TERT interaction, which is complementary to the telomerase RNA. The second step of telomere replication is polymerization or telomere stretching that continues to the 5′ terminus of the template region of DNA. When the elongation of telomere repeat is complete, telomerase can be displaced to start synthesis of another telomere repeat sequence (Harley and Villeponteau 1995).

Telomerase gene is expressed during early development, and its expression in adults is limited to cells with highly proliferating capacity such as germ cells, hemotopoietic stem cells, and progenitor/stem cells (Weng et al. 1997). In addition, telomerase activity is also high in embryonic stem cells (ESC) to avoid significant telomere shortening and to enhance self-renewal of ESC (Yang et al. 2008). Telomerase is also upregulated in immortalized cells and many tumor cells (Kim et al. 1994). It is known that the amount of telomerase activity is not adequate to overcome the continuous renewal in adult stem cell, and therefore, telomeres shorten with aging (Batista 2014).

The regulation of telomerase and its activity occurs in cells with various ways, which are transcriptional control of TERT, alternative splicing variant of TERT, sex and growth hormones such as estrogen, androgens, proteins that are found in telomere and associated with telomerase complex, and other factors involving in the phosphorylation and assembly of telomerase or transporting its complex subunits (Bayne et al. 2008; Gonzalez-Suarez et al. 2005; Liu et al. 2001; Villa et al. 2001; Xin et al. 2007). TERT is a primary factor in the regulation of telomerase activity at various levels, including both direct and indirect regulation of gene expression, alternative splicing, protein tertiary folding, and posttranslational modification (Liu et al. 2010). Alternative splicing variants are also important factors for telomerase action, for instance, many cells have different TERT variants from each other, and this allows the cells to have various life spans (Yi et al. 2000). Posttranslational modification of telomerase occurs through phosphorylation of serine/threonine or tyrosine residues, a specific region of catalytic subunit of TERT (Cong et al. 2002). Shelterin proteins are closely related with action of telomerase with increased telomerase activity associated with interactive relation between TPP1 and the TEN domain of TERT (Xin et al. 2007). Other proteins are required for the telomerase activity as well, like chaperone proteins p23 and hsp90 which are associated with effective assembly of the telomerase holoenzyme (Holt et al. 1999).

Another mechanism to regulate the activity of telomerase is intracellular trafficking into the Cajal bodies providing the nuclear location for the enzyme to assemble and/or maturate the telomerase holoenzyme (Jady et al. 2006). TERC accumulation in Cajal bodies requires the association of TCAB1 to CAB box, which is a short sequence motif of TERC (Cristofari et al. 2007). It has been reported that once telomerase enzyme is functional, Cajal bodies coexist with telomeres during S phase of cell cycle (Venteicher et al. 2009).

## 7.2 The Hayflick Limit

Telomeres shortened at each cell division and become dysfunctional with age as they lack the telomerase activity in human somatic cells (Aubert and Lansdorp 2008). When telomeres lose their length critically, a cellular response is triggered, and cell division can no longer occur which causes cells to exit the cell cycle and enter senescence or programmed cell death. The fact, known as Hayflick limit, pointed out that the cells reach their maximum proliferative capacity (Hayflick 1965). In 1961, Leonard Hayflick and Paul Sidney Moorhead discovered the cultured fetal human fibroblasts have restricted capacity to divide in the cultured condition (Hayflick and Moorhead 1961). In 1965, Hayflick suggested that the life of a cell consisted of three phases. According to his study, phase one is started with healthy cell division, and then, cell division become slow in the phase two. Finally, cells reach phase three where cell division stops and senescence begins (Hayflick 1965). Research on Hayflick limit helps scientist understand cellular aging and prevent cell senescence. In the early 1970s, it was found that the replication of linear DNA by DNA polymerase resulted in the loss of terminal sequences of chromosome, termed as telomere. DNA polymerase cannot extend the 3′ end of linear DNA due to lagging strand synthesis and this is called as end-replication problem (Olovnikov 1973). This end-replication problem is improved by telomerase and shelterin complex of telomere (Greider and Blackburn 1987). However, telomerase is inactive in all human cells except for stem cell niches and germ cells (Shay and Wright 2010). Eventually, the end-replication problem causes telomeres to shorten in human cells with aging (Harley et al. 1990).

## 7.3 Telomerase and Aging

Telomere shortening is sufficient to provoke age-related disease such as cardiovascular disease, liver cirrhosis, atherosclerosis (Minamino and Komuro 2007), diabetes (Sone and Kagawa 2005), infectious diseases, and Alzheimer's disease (Fig. 7.1) (Cawthon et al. 2003; Mattson 2000). Moreover, telomere shortening is regarding to the premature aging syndrome, bone marrow failure syndrome, and human chronic diseases like hypertension (Serrano and Andres 2004). A connection between shortening of telomeres and cellular senescence or aging was reported by Harley et al. (1990). Studies have shown that telomere shortening during aging in human somatic cells occurs by reason of absence of telomerase activity. Studies carried out with telomerase knockout (mTERC−/−) mice indicate that a generation-dependent telomere shortening leads to cell cycle arrest and apoptosis (Blasco et al. 1997). Although telomerase is highly expressed and found in embryonic stem cells, germ cells, hematopoietic cells, and epithelial cells like the skin, liver, and spleen that possess highly regenerative features (Counter et al. 1995; Hiyama et al. 1995; Wright et al. 1996), it is repressed in many somatic cells to reduce the probability of cancer (Harley et al. 1994). The telomerase repression process is accomplished by tightly regulating the expression of TERT, which results in the absence of activators

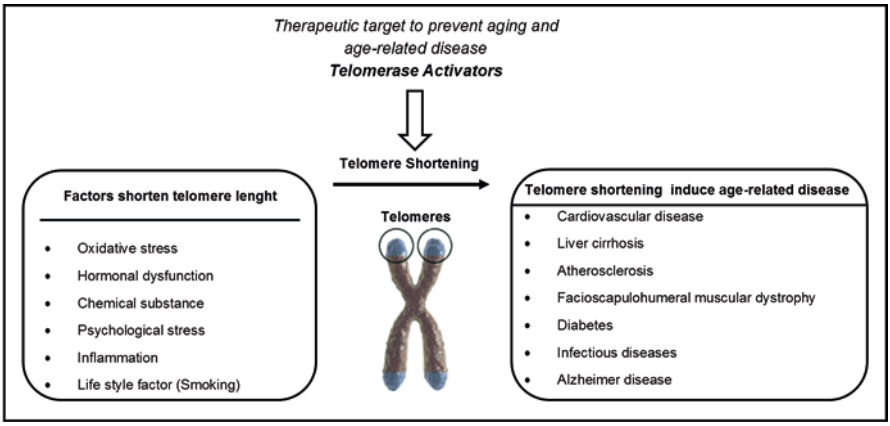


**Fig. 7.1** Telomere shortening in aging

rather than the presence of repressors (Djojosubroto et al. 2003). The high expression and activity of telomerase enzyme is found in most of human cancers. Telomerase activity may be required for normal cells to be transformed cancer cells and is essential for cellular immortalization in human cells (Kim et al. 1994). Being transformed cancer cells are accomplished by not only telomerase activity, but also coupled with deactivating tumor supressor genes or activating oncogenes (Stewart and Bertuch 2010). Tumor growth is suppressed and programmed cell death is increased in cancer cells in which telomerase activity is inhibited (Hahn et al. 1999).

Reactive oxygen species (ROS) causing oxidative stress are responsible for both the induction and maintenance of cellular senescence. Several studies have demonstrated that ROS can expedite telomere shortening in vivo and in vitro and can also damage DNA directly, especially telomere structure (Chen et al. 1995; Ren et al. 2001; Rubio et al. 2004). Many studies have shown that several diseases are associated with ROS-mediated telomere shortening such as Fanconi anemia, respiratory chain disorder, Leber hereditary optic neuropathy (LHON), and mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke (MELAS) syndrome (Adelfalk et al. 2001; Oexle and Zwirner 1997). Moreover, it is known that ROS-mediated oxidative stress triggers nuclear export and mitochondrial import of telomerase, which causes shortened telomere (Haendeler et al. 2003).

On the contrary, telomere length positively correlates with longevity (Njajou et al. 2009). Thus, the approach to enhance telomere length via increasing telomerase activity is very important for antiaging strategies (Tomas-Loba et al. 2008). Several studies showed that aging process can be reversed by telomerase activity in mice and rats (Bernardes de Jesus et al. 2012). One study showed that telomerase activation with overexpression of TERT in various tissues brought about a prolonged lifespan up to 10% when compared to wild-type mice (Gonzalez-Suarez et al. 2005).Another study indicated that increased TERT expression in cancer-resistant mice delayed aging and extend longevity by 40% (Tomas-Loba et al. 2008). Reactivated telomerase by using adenoviruses extends life span up to 24% in

1-year-old mice and up to 13% in 2-year-old mice without cancer risk (Bernardes de Jesus et al. 2012). In addition, telomerase reactivation in telomerase-deficient mice results in the extension of telomeres, reduction of DNA damage signaling and related cellular checkpoint responses, and reversion of degenerative phenotypes including the testes, spleen, intestines, neuronal progenitors, newborn neurons, and oligodendrocyte (Jaskelioff et al. 2011). In bovine keratin 5 mouse model, transgenic mice exhibit more efficient wound healing and higher proliferation rate in keratinocytes than wild-type mice due to expression of TERT (Gonzalez-Suarez et al. 2001).

Telomerase promotes cell survival to protect cells from programmed cell death. In cardiac myocytes, TERT expression can retard the cell cycle exit, induce hypertrophy, and promote cardiac muscle survival (Oh et al. 2001). Apart from that, neuronal cells do not show telomere shortening because of their TERT expression (Mattson et al. 2001). TERT also has therapeutic importance in the central nervous system (Gonzalez-Giraldo et al. 2016).

## 7.4 The Potential Role of Telomerase in Antiaging Therapies

Aging is described as a process that involves time-dependent anatomical and physiological changes which are responsible for the increased risk of disease and death (Ahmed and Tollefsbol 2001). The aging process of the cells and individuals begins to emerge immediately after birth and accelerate as age progress. According to this idea, telomerase gene present in inactive form in most human somatic cells after the embryonic stage, and eventually telomere shortening occurs with age (Skulachev 1997). The cells expressing telomerase have been a potential field of study for the "antiaging" interventions, because they can sustain a youthful condition and proliferative indefinitely (Bodnar et al. 1998). In this respect, it was thought that immortalized cells could be crucial to the replacement of damaged tissues and organs during aging. According to this treatment, cells with short telomeres will be isolated from a patient, and telomere length in the treated cells increased via expression and activation of TERT. The cells would be reproduced in culture condition, and then cells would be transplanted instead of damaged or aging tissue and organ (Shay and Wright 2000). Experiments conducted with telomerase-deficient mice have indicated that telomerase gene therapy is potentially usable in impaired organ regeneration induced by telomerase shortening (Rudolph et al. 2000). Recent study has indicated that telomerase gene therapy provides increased telomerase expression in a mouse model with aplastic anemia, which result in telomere elongation and ultimately the reversal of aplastic anemia phenotypes without increased cancer susceptibility (Bär et al. 2016).

The concept of immortalization of cells by TERT sheds light on scientist in using cell therapy to prevent cell senescence. TERT-modified cells are used in classical gene therapy for in vitro optimization of stem cell transplantation, for tissue

engineering like construction of new blood vessel, for the treatment of chronic disease such as atherosclerosis, and for the treatment of cancers such as hepatocellular carcinoma (Klinger et al. 2006; Nazari-Shafti and Cooke 2015; Shay and Wright 2007). Various human cell types including skin keratinocytes, dermal fibroblasts, muscle cells, endothelial cells, bone marrow stromal cells, osteoblasts, odontoblasts, retinal-pigmented epithelial cells, and corneal epithelial cells are immortalized by TERT to extend the life span of cells (Darimont et al. 2002; Oh et al. 2001; Robertson et al. 2005; Simonsen et al. 2002; Vaughan et al. 2004). It is reported that telomerase-negative normal human cells like retinal pigment epithelial cells express telomerase and exhibit reduction of replicative senescence, when transfected with vectors encoding to TERT (Bodnar et al. 1998). Except for transfection of TERT gene, transcriptional downregulation of telomerase can be reversed by various substances like histone deacetylase inhibitors and estrogen receptor agonists (Doshida et al. 2006). Furthermore, protection of intracellular localization of telomerase, which locates in nucleus and cytosol, is a potential therapy for antiaging intervention (Stewart 2002). Androgen therapies are used for the treatment of aplastic anemia because androgens can activate transcription of TERT (Calado et al. 2009). Treatment of telomeropathic patients with a synthetic androgen, danazol, has shown an increase in the length of telomeres in leukocytes (Townsley et al. 2016).

## 7.5 Telomerase Activators: Therapeutic Value and Future Perspectives

The utilization of telomerase activators in the treatment of aging-related phenotypes are of interest in recent years for the scientists who study with antiaging interventions. Although telomerase chemical activators have been reported such as TA-65, there are limited studies about their mechanism of action in the cells. The first potential telomerase activator is TA-65, which is a small molecule derived from an extract of the root of *Astragalus membranaceus*. The study conducted with TA-65 has shown that dietary intake in mice increases telomerase level in tissue and consequently causes elongation in critically short telomeres. This study suggests that TA-65 improves health-span indicators including osteoporosis, glucose tolerance, and skin fitness, and thus, it should be used in antiaging therapies (Bernardes de Jesus et al. 2011). Another study is stated that telomerase activation occurs in low nanomolar level of TA-65 in human keratinocytes, fibroblasts, and immune cells in culture (Harley et al. 2011). In human T cells administrated with TA-65, MAPK-specific telomerase activation and significant increase in proliferation activity were observed (Molgora et al. 2013). TA-65 supplementation also caused improvement of markers of bone, metabolic, and cardiovascular health in human trials (Harley et al. 2013). Recently, an efficiency of TA-65 in treatment of early age-related macular degeneration was reported in a randomized placebo-controlled interventional study (Dow and Harley 2016).

TAT2 is a single chemical substance extracted from the roots of *Astragalus membranaceus* (Liu et al. 2017). TAT2, also known as cycloastragenol, was purposed as a therapeutic for HIV patients to increase the number of senescent memory CD8 T cells, which is found that it elongates human telomeres (Dock and Effros 2011). Recent study has shown that treatment with GRN510, a new small telomerase activator derived from GGNR665/TAT2, in murine model activates telomerase both in hematopoietic progenitor cells ex vivo and in the bone marrow and lung tissue in vivo (Le Saux et al. 2013).

AGS-499 and AGS-500, which are chemical compounds, increase telomerase activity and TERT level in time- and dose-dependent manner in human bone marrow mesenchymal stem cells. Prolonged treatment of AGS protected cell from apoptosis and DNA damages induced by $H_2O_2$ (Tichon et al. 2013). Another study conducted with AGS-499 showed that this novel compound increased expression and activity of telomerase in brain and spinal cord of mice (Eitan et al. 2012).

Genistein is a controversial molecule on telomerase activation due to its bilateral effects on telomerase activity. Genistein had a telomerase activator role at low concentrations (0.5 μM) in DU-145 and LNCaP prostate, MCF-7 breast, and SKOV-3 ovarian cancer cells; however, its inhibition of telomerase was observed at higher concentrations (50 μM) in all cell lines (Chau et al. 2007). Resveratrol, which is a natural phytoalexin present in grapes, fruits, and root extracts, makes the telomerase gene in active in human mammary and endothelial progenitor cells (Pearce et al. 2008).

There are several promising telomerase activators except for those mentioned above, which are summarized in Table 7.1. However, there is almost no sufficient study about these telomerase activators so far. This means that scientist should detail studies about telomerase activator to maintain healthy and long life span.

## 7.6 Conclusion

Over the last century, a long, healthy life expectancy has increased rapidly and thus, researchers have tried to find a way to extend human life span. In this context, telomeres are very important DNA regions of chromosomes. Telomere shortening is an indicator of biological aging and is associated with age-related disease such cognitive decline, diabetes, and chronic liver disease. Telomeres become shorter as age progress and shortening of telomeres constantly leads to senescence and/or apoptosis. Determining the pathways that regulate longevity is critical to develop new strategies for prolonged the life span in human being. Therefore, various molecules have been investigated for this respect, which can affect telomere length through telomerase expression and activation. Telomerase activators appear to be a promising candidate for antiaging interventions. Progress in this field and future studies with telomerase activators may be helpful to determine which molecules and mechanism effectively target telomeres and extend human life.

**Table 7.1** Various telomerase activators summarized

| Telomerase activator name | Source | Study model | Reference |
|---|---|---|---|
| TA-65 | *Astragalus membranaceus* | Mice | Bernardes de Jesus et al. (2011) |
| | | Cell lines | Harley et al. (2011) |
| | | Human trials | Harley et al. (2013) |
| Cycloastragenol (TAT2 or GRN665) | *Astragalus membranaceus* | Cell lines | Dock and Effros (2011) |
| GRN510 | Derived from GRN665/TAT2 | Murine | Le Saux et al. (2013) |
| AGS-499 and AGS-500 | Synthesized triaryl compounds | Cell line | Tichon et al. (2013) |
| | | Mice | Eitan et al. (2012) |
| Genistein | Soy bean | Cell lines | Chau et al. 2007 |
| Resveratrol | Red wine | Cell lines | Pearce et al. (2008) |
| Selenium | Nuts, vegetables, fruits, grains | Rat | Yu et al. (2009) |
| *Cynomorium* species (Maltese mushroom) | *Cynomorium* species | Mice | Ma et al. (2009) |
| Purslane | Purslane herb | – | No study |
| Omega-3 | Fish, oyster, cod liver oil | Human | Farzaneh-Far et al. (2010) |
| Vitamin D | Sunlight, cod liver oil, salmon, sardines, tuna | Human | Zhu et al. (2012) |
| Ginkgo Biloba extract | *Ginkgo Biloba* | Cell line | Dong et al. (2007) |

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# Immune Modulation and Its Role in Antiaging

8

Mustafa Erinc Sitar, Elcin Akduman Alasehir, and Belkiz Ongen Ipek

**Abstract**

Life expectancy of the communities is constantly increasing every minute, including less developed countries. That's why application of studies to overcome and/or slow aging has always been an important and attractive issue for professional healthcare workers throughout history. There are numerous studies on different theories for explaining the aging process, but none of them can fully explain the cause. As it is known already, aging of different organs differs in a large extent. Fundamentals of this difference are mitotic activity of the tissues and resistance degree to deleterious damages. Cellular and molecular defense mechanisms clearly define resistance degree as "immunity." Decline in immunity may cause a progressive step in aging. Inflammaging, which is low-grade chronic inflammatory status that is characteristic of the aging process, can be taught to be a biological factor responsible for the age-related diseases in the elderly. Possibility to decrease inflammaging without compromising the physiological role of inflammation can be a strategy for future perspectives. In this chapter we will focus on aging and immune system regulation together with therapies and/or modulations to rehabilitate aging.

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**Keywords**
Aging · Autoimmunity · Immune modulation · Immunosenescence · Inflammaging

## Abbreviations

| | |
|---|---|
| ANA | Antinuclear antibodies |
| CMV | Cytomegalovirus |
| DCs | Dendritic cells |
| DHEA | Dehydroepiandrosterone |
| ds | Double stranded |
| FMLP | N-formyl-methionyl-leucyl-phenylalanine |
| GM-CSF | G-macrophage colony-stimulating factor |
| Htert | Human telomerase |
| IFN-γ | Interferon-γ |
| Ig | Immunoglobulin |
| IKK | IκB kinase |
| JAK | Janus kinase |
| MHC | Major histocompatibility complex |
| NF-κB | Nuclear factor-κB |
| NK | Natural killer |
| NLR | NOD-like receptors |
| NO | Nitric oxide |
| NOD | Nnucleotid oligomerization domain |
| PI3K | Phosphoinositide 3-kinase |
| PMN | Polymorphonuclear neutrophil |
| RA | Rheumatoid arthritis |
| RF | Rheumatoid factor |
| RLR | RIG-like receptors |
| ROS | Reactive oxygen species |
| SLE | Systemic lupus erythomatosus |
| ss | Single stranded |
| STAT | Signal transducer and activator of transcription |
| TCR | T-cell receptor |
| TLRs | Toll-like receptors |
| TNF-α | Tumor necrosis factor α |
| Tregs | Regulatory T cells |

## 8.1 Introduction and History of Immune Theory of Aging

What is the main reason of aging? When does aging start chronologically and biologically in separate ways? Is it only a chronological issue? The society is being populated by the elderly with an increasing momentum in the twenty-first century. Lifespan of human being (biological limit of life) has stayed stable (~125 years) for the past decades. On the other hand, due to daily advancements in state-of-the-art medical practice and better healthcare modalities, mortality rates are decreasing and mean life expectancy (71 years for global population) is increasing (WHO 2015). Slow and/or healthier aging will be a realistic purpose to healthcare systems for the entire world to minimize burdens of the increasingly surviving population into seventh, eighth, and even ninth decades. In this chapter, we will focus on issues related to autoimmune diseases in elderly and inflammaging that will try to help biogerontologists to understand immunosenescence. We will also focus on some possible modulation strategies to overcome age-related degenerative changes in both innate and adaptive systems.

Aging is a complicated process and should be investigated in physiological, metabolic, physical capability, cognitive function, and psychological and social points of views. The parameters that evaluate cardiovascular function, endocrine function, inflammation, immune function, and metabolic processes have been used as metabolic biomarkers in aging (Lara et al. 2013). For centuries, aging was an unsolved and unavoidable problem for scholars. Defining mechanisms of a process should always be the first step in solving a scientific problem. From this point of view, there has always been a network of theories related to aging. Biological clock, time-based longevity, endocrine alterations, mitochondria-originated oxidative damage, mitochondria-lysosome organelle turnover relations, immunological alterations, opposite pleiotropic effects of special genes, cross-linking reactions, and wearing out of tissues are substantial instances for the aforementioned issue (Sitar et al. 2013; Jin 2010). The process of aging is an incredibly complex phenomenon, and it is impossible to understand it from only one or two perspectives. It is better to assume it as a multifactorial process. Many data in current literature support the idea that every original theory may at least in part explain the aging process. It is obvious that these networks of theories are associated with each other. For instance, reactive oxygen species, which are also fundamental substances of oxidative stress theory of aging, can also initiate a cellular immune response within the organism (Harman 1983). Even though there are lots of evidences which support the oxidative stress theory of aging, the mechanism of immune aging is not completely understood in details (Cakatay et al. 2013). Systematic, irreversible, and intrinsic roles of immune system on aging were first reported by Roy Walford in the late 1960s (Walford 1969; Fulop et al. 2014). It was presented as a time-based programmed theory postulating immunosenescence, which leads to increased vulnerability and frailty to diseases (Watad et al. 2017). It is attested that immune defense system reaches its peak capacity at puberty and then starts to decline with the passing of time (Jin 2010). There are lots of contributors to this phenomenon such as stress, oxidative damage, less effective antibodies, proinflammatory cytokines, increased incidence

of infectious diseases, point mutations, chromosomal rearrangements, and adrenocortical hormone secretion changes (Ferrari et al. 2001; Xia et al. 2016). Milestone of immune theory attraction was a term "inflammaging." It was first named by Franceschi et al. to attract increasingly important role of chronic progressive increase in the proinflammatory nonresolving status which develops gradually through the continuous antigenic stimulation (Franceschi et al. 2000; Fulop et al. 2014). It is also defined to be an ongoing low-grade chronic inflammation, which causes minute, gradual and progressive functional decline in the entire organism. Mitochondrial dysfunction and chronic inflammation can be considered as hallmarks of immunosenescence connecting aging and age-related degenerative diseases such as cardiovascular diseases, osteoporosis, depression, Alzheimer's disease, Parkinson's disease, malignancies, and type II diabetes (López-Otín et al. 2013). Immune theory of aging also tries to explain a very important and common clinical entity in elderly, increased rate of infections, and high asymptomatic autoimmune antibody rates.

## 8.2 Immunosenescence

The immune system and its functions are negatively affected by aging. These age-related degenerative changes are called immunosenescence (Martorana et al. 2012; Montgomery and Shaw 2015). Although immunosenescence was hypothesized to be a random deteriorative phenomenon (Franceschi and Cossarizza 1995), thymus involution, limitation of the T-cell pool and oligoclonal proliferation of memory/effector cells against common pathogens, and enhancement of proinflammatory cytokines causing a chronic inflammatory condition have been shown to be involved (Candore et al. 2008; Krabbe et al. 2004; Bruunsgaard 2006).

### 8.2.1 Changes in Adaptive Immunity

Aging leads to an increase in antigen-experienced B and T cells, while naive cell populations decrease (Montgomery and Shaw 2015). With aging, naive CD8+ T cells decrease within peripheral blood CD3+ population, a higher amount of memory CD8+ T cells are found, and the effect of aging is less on CD4+ cells (Fagnoni et al. 2000; Saule et al. 2006; Henson and Akbar 2010; Nikolich-Žugich and Rudd 2010). The antigen encountered lymphocyte pool increases, and interleukin-4 and IL-10 become the predominant cytokines (Appay and Sauce 2014; Rink et al. 1998; Bektas et al. 2017). Thymic involution, regression in size due to decreased functional tissue, takes place around puberty due to hormonal changes. However, various assays that demonstrate thymic output, such as T-cell receptor excision circle assays, have shown that the thymus continues to function until old age (Douek et al. 1998; Ferrando-Martínez et al. 2010). The deterioration of thymus functions is thought to be a triggering event for the reduction of immune surveillance in the older population (Linton and Dorshkind 2004). In spite of this deterioration, lymphocyte numbers remain

constant over decades, as partial lymphopenia resulting from thymic function loss increases naive T-cell numbers (Kohler and Thiel 2009; Nikolich-Žugich 2008; Sauce et al. 2012). An overwhelmingly large percentage of naive CD4+ T cells are produced through peripheral T-cell proliferation (den Braber et al. 2012). The steady number of peripheral naive T cells is due to both thymic output and homeostatic proliferation, unlike that observed in mice (Nikolich-Žugich and Rudd 2010). There is a large variety of T-cell receptor (TCR) in which the T-cell pool resides. This allows a response to an almost infinite number of antigens and is extremely important for the immune system. Therefore, decreased precursors will negatively affect T-cell responses (Naylor et al. 2005). Oligoclonal cell populations may increase with age as TCR diversity decreases (Vallejo 2007; Olsson et al. 2001).

Inflammaging and immunosencescense are affected by pathogens that shape T-cell function (Bektas et al. 2017), such as cytomegalovirus (CMV) which leads to a reduction of TCR repertoire and clonally expanding T-cell subsets and consequently T-cell senescence. This effect is especially observed in CMV-specific memory CD8+ T cells especially CD8+ CD28+ T cells (Bauer and De la Fuente 2016). This process is called "memory inflation" (Klenerman and Oxenius 2016; Cao et al. 2010). T cells and TCR signaling are also affected by age, as shown by gene expression profiles. Comparison of CD8+ T cells of younger and older individuals has demonstrated up- or downregulation of different genes (Fessler et al. 2013). Exaggerated immune reactions and immune homeostasis are primarily controlled by regulatory T cells (Tregs) (Jagger et al. 2014), which also decrease in number, distribution, and function by age (Li and Zheng 2015). Treg lineage is affected by FoxP3 transcription factor, and its suppressive function can only continue with its expression (Fessler et al. 2013). With age, FoxP3+ CD4+ Tregs increase in mice and humans leading to immunosenescence (Raynor et al. 2012, 2015).

Age is reported to lead to phenotypical and functional changes in B cells (Frasca et al. 2011). B-cell antigen receptor-related activation decreases and this in return causes lower antibody titers and affinity. Additionally, evidence suggests that the functionally exhausted memory B cells accumulate followed by a decrease in naive B cells. This is similar to the affect aging has on T cells. Both these affects impact immune competence (Bancos and Phipps 2010).

### 8.2.2 Changes in Innate Immunity

Increased age leads to reduced recruitment, phagocytosis, and granule release of polymorphonuclear neutrophils (PMN) or macrophages. This leads to a deficiency of the immune system and suggests an age-related dysfunction of signal transduction leading to immunosenescence (Solana et al. 2012; Shaw et al. 2013).

#### 8.2.2.1 Neutrophils

Neutrophils are the first defense mechanism at sites of inflammation (Jaillon et al. 2013). While the total number of neutrophils does not change with age (Solana et al. 2012), chemotaxis and phagocytosis are impaired and free radical production

decreases (Fortin et al. 2008). This is reported to be due to altered signal transduction through surface receptors by their specific ligands, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and N-formyl-methionyl-leucyl-phenylalanine (FMLP) (Hajishengallis 2014). Anti-apoptotic responses to GM-CSF mediated through JAK (Janus kinase)-STAT (signal transducer and activator of transcription) tyrosine kinase (Fortin et al. 2007) and phosphoinositide 3-kinase (PI3K)-AKT pathways (Tortorella et al. 2006) are examples for altered signal transduction. These alterations of the lipid membrane structure and lipid rafts may result in inappropriate localization or retention in membrane signaling domains (Shaw et al. 2013).

#### 8.2.2.2 Monocytes and Macrophages

Increased age also causes changes in monocytes and macrophages. A shift occurs in the proportion of monocyte subsets and therefore inflammatory profiles. Increased age leads to some monocyte and macrophage functions to be compromised. These functions include chemotaxis, phagocytosis, production of some cytokines/chemokines, reactive oxygen or nitrogen species, and expression of major histocompatibility complex (MHC) class II and costimulatory molecules. Plasticity of macrophages allows them to switch between a state of equilibrium to inflammation and vice versa (Glezeva et al. 2015; Malyshev and Malyshev 2015). This switch requires several microenvironmental factors. When a macrophage is removed from an inflammaging microenvironment, macrophage response is restored to a similar young macrophage, leading to the conclusion that "aged phenotype" of macrophages may be reversible. This may lead to therapeutic applications in the future (Albright et al. 2016).

#### 8.2.2.3 Natural Killer (NK) Cells

NK cells act on virus-infected and cancerous cells leading to their destruction. They are divided into a $CD56^{low}$ and $CD56^{hi}$ population that has cytotoxic activity and is responsible for cytokine production respectfully (Caligiuri 2008). As age increases, cytotoxicity decreases with an expansion of $CD56^{low}$ cytotoxic NK cell compartment (Almeida-Oliveira et al. 2011; Chidrawar et al. 2006; Garff-Tavernier et al. 2010). Cytokines and chemokines produced by NK cells such as IL-8 and interferon-γ (IFN-γ) also decrease with age (Mocchegiani et al. 2009).

#### 8.2.2.4 Dendritic Cells (DCs)

Changes in the basic functions of DCs, such as phagocytosis, chemotaxis, IL-12, and IFN-α production and antigen presentation, which can occur without changes in number and structure, may result in the suppression of naive CD4 + T cells (Agrawal et al. 2007, 2010; Della Bella et al. 2007; Panda et al. 2010; Sridharan et al. 2011; Jing et al. 2009). Alterations in the expression and signaling mechanisms of Toll-like receptors (TLRs), NOD (nucleotid oligomerization domain)-like receptors (NLR), RIG-like receptors (RLR), and C-type lectin receptors known as "pattern recognition receptors" are associated with these changes (Agrawal et al. 2010). The

most significant changes are shown to be the reduced PI3K signal pathways changing TLR signaling (Agrawal et al. 2007).

Recognition of microbial structures, especially TLRs activate innate immunity. Signals generated by engagement of pathogen-derived ligands and specific TLRs activate nuclear factor-κB (NF-κB) and stimulate expression of antiviral and proinflammatory response genes (Olivieri et al. 2013). Age-related changes in the function of the murine TLR have been shown that they reduce TLR-induced cytokine production in macrophages of elderly mice involving differences within the relevant TLRs that may be the result of differing genetic inheritance (Boehmer et al. 2004, 2005; Renshaw et al. 2002). Studies demonstrate reduced TLR2 and TLR4 function in macrophages and depressed TLR1, TLR2, and TLR4 protein expression in lung homogenates of elderly mice, with concomitant reduced proinflammatory cytokine production in response to treatment with bacterial pathogen-associated molecular patterns and decreased NF-κB activation (Hinojosa et al. 2009).

## 8.3 Inflammaging

High levels of acute phase reactants and proinflammatory cytokines in elderly people without any immune stimulant and underlying disease are termed inflammaging (Shaw et al. 2013; Franceschi et al. 2000). Several posttranslationally modified macromolecules, parasites, pathogens (CMV, herpes simplex virus-1, Epstein-Barr virus), and tumor antigens can induce the innate immunity, which is especially achieved through macrophages, in particular by TLRs and may cause low-grade antigenic stimulation (De Martinis et al. 2005; Fulop et al. 2011). Like the continuous formation of tumor cells, these changes contribute to the environment of an immune state with diminishing activity that does not respond appropriately to new antigens (Fulop et al. 2011). In addition, inflammaging may begin with altered intestinal permeability leading to changes in microbiota of the gastrointestinal tract (Kim et al. 2016).

Family of the NF-κB are necessary for the transcription of genes that take part in innate and adaptive immune systems (Oeckinghaus and Ghosh 2009). Pathogens activate NF-κB via TLR-induced signals, proinflammatory cytokines (TNF and IL-1), TCR and B-cell receptor, and growth factors (Tilstra et al. 2012). Aging mediators like reactive oxygen species, cellular senescence, and DNA damage stimulate NF-κB by activating IκB kinase (IKK) and phosphorylating IκB, thus affecting the expression of several cytokines, chemokines, growth factors, endothelial adhesion molecules, and interferon regulatory factors. Activation of NF-κB increase the production of inflammatory cytokines and thus stimulates NF-κB. This homeostatic imbalance may lead to proposed chronic inflammatory state of aging. The chronic suppression of IKK/NF-κB has been shown to postpone the chronic aging-related diseases in DNA repair-deficient *Ercc1*$^{-/\Delta}$ mice (Tilstra et al. 2012; Salminen et al. 2008) (Fig. 8.1).

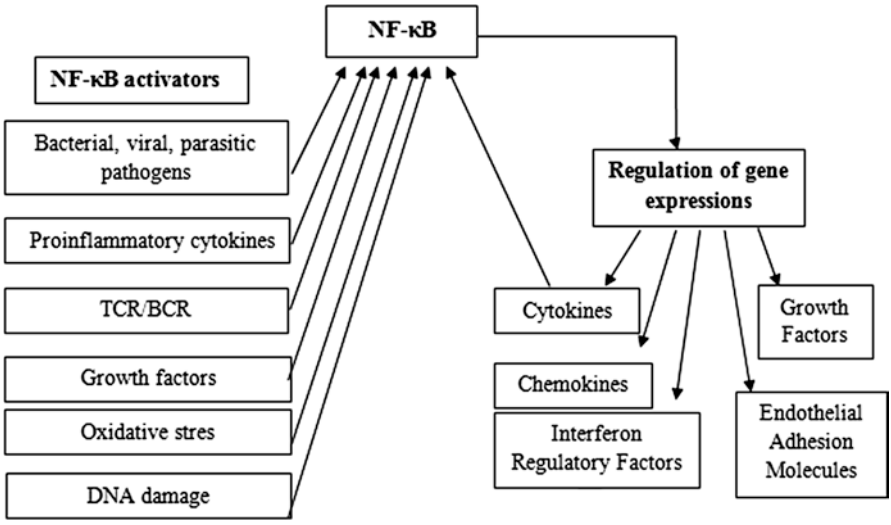


**Fig. 8.1** NF-κB interactions in immune responses

## 8.4 Autoimmune Diseases and Their Relationship with Age

Chronological and/or biological age of the patients is an important informative tool which is used for differential diagnosis of autoimmune and immune genetic diseases in daily routine practice. Many autoimmune diseases preferentially occur in a specific time of the adulthood (Goronzy and Weyand 2012). Autoimmune diseases, except giant cell arteritis and primary biliary cirrhosis, are predominantly seen in females of childbearing age. Older people carry an undeniable risk of autoimmune diseases as well. Contributing factors to this phenomenon are:

(a) Premature T cell senescence
(b) Alterations in apoptosis in T cells and high exposure to apoptotic cells (Candore et al. 1997; Grolleau-Julius et al. 2010)
(c) Production of less protective antibodies which have lower affinity to antigens (Stacy et al. 2002)

In Table 8.1, detailed information about common autoimmune diseases and their relationship with age is summarized. Giant cell arteritis is quite commonly seen in elderly people. It relates to age-induced remodeling of the vascular wall and age-related immune system alteration which results in decreasing and narrowing of T-cell diversity. Granulomatous lesions are localized on large- or medium-sized arteries together with multisystemic inflammation. The pathology of the initiating inflammatory process couldn't be displayed, but abnormalities in innate and adaptive immunity play an important role in the starting and continuing process of the vasculitis (Mohan et al. 2011).

**Table 8.1** Common autoimmune diseases which are seen in elderly and their relationship with aging

| Disease | Important points in pathogenesis | Relation with aging | References |
|---|---|---|---|
| Polymyalgia rheumatica and temporal arteritis (giant cell arteritis) | Periarticular inflammation and vasculopathy | Almost nonexistent in younger persons | Grolleau-Julius et al. (2010) |
| Rheumatoid arthritis | Chronic and symmetrical polyarthritis | Elderly RA patients with geriatric syndrome had a longer disease duration and more patients surviving into old ages progressively | Song et al. (2009) |
| Systemic lupus erythematosus | Antibody production and complement fixing immune complex deposition | Insidious clinic and longer duration from disease onset to diagnosis in elderly | Chen et al. (2009) |
| Ankylosing spondylitis | More severe inflammation | Difficult-to-interpret radiological aspects because of age-related degenerations | Toussirot and Wendling (2005) |
| Graves disease | Genetic clonal lack of suppressor T cells | Atypical and longer presentation in elderly | Ginsberg (2003) |
| Hashimoto disease | Lymphocytic infiltration, fibrosis, and autoantibodies to thyroglobulin | Unique treatment and diagnostic modalities are needed for elderly | Ju and Zhang (2017) and Caleo et al. (2013) |
| Celiac disease | Loss of villi leading to malabsorption | More prominent micronutrient deficiencies than intestinal symptoms in elderly | Rashtak and Murray (2009) |
| Inflammatory bowel disease | Aberrant immune system reacting inappropriately to gut organisms and their by-products | Dysbiosis and dysregulation of the immune system playing more significant role than genetics | Taleban et al. (2015) |
| Multiple sclerosis | Central nervous system inflammation, demyelination, axonal degeneration, and gliosis | Accelerated clinic causing irreversible disability in elderly | Sorkin et al. (2012) |
| Primary biliary cirrhosis | Chronic cholestatic hepatic disease which can progress into cirrhosis by time | Low degree of initial histological stage and high frequency of asymptomaticity in elderly | Muratori et al. (2008) |
| Autoimmune gastritis (pernicious anemia) | Autoimmune disorder which leads to vitamin B 12 deficiency due to presence of gastric autoantibodies | More common polyautoimmunity and multiple autoimmune syndrome in elderly | Ohara et al. (2015) and Kalkan and Soykan (2017) |

Rheumatoid arthritis (RA), which is a systemic and inflammatory disease, affects primarily women between ages of 30 and 50. Patients with RA have premature immunaging. T cells in RA patients are identified by telomeric shortening in CD34 hematopoietic precursors, loss of telomeres activity in T cells, and restriction in clonal proliferation capacity. The biologic effect of T cells in elderly is associated with reduction in functionality, declined vaccine responses and increased risk of infection, neurocognitive problems, and cardiovascular diseases (Weyand et al. 2014).

SLE, which is a chronic autoimmune disease, is described by multisystem involvement. It is diagnosed by multiple laboratory and clinical evidences. SLE is frequently diagnosed in women who are in their 20–40s. Late-onset SLE, which means the diagnosis was made above 50–65 years of age, affects 12–18% of total SLE population.

As it is understood from the Table 8.1 and aforementioned information, there is a general ambiguous clinical spectrum in elderly, which can lead to significant delays in diagnosis. It should always be kept in mind that there are patients who are passing to geriatric age and new patients who are diagnosed at geriatric age. Chronic and nonfatal nature of the diseases together with more effective treatment modalities are the main reasons for this circumstance. Both clinical approach and methodology in research studies should be planned according to this significant difference. Fewer amounts of researchers have recruited aged populations in their studies and mostly being over 65 has been used as an exclusion criterion. Because of variation in tolerance, response to treatments, pathogenic mechanisms contributing to the disease itself, and natural age-related degenerative differences in clinical course will be crucial during management of the disease. Marked or complicated elevation of laboratory parameters, usage of polypharmacy, and lacking/inappropriate diagnostic criteria specific to old age groups will provide extra burden to the issue. As a conclusion more studies are needed to enlighten diagnostic and therapeutical issues in elderly especially regarding long-term side effects of pharmaceutical agents. Extensive and multidisciplinary approach is needed to minimize morbidity and mortality.

## 8.5 Biomarker of Aging and Diagnostic Tools of Autoimmune Diseases in Elderly

The American Federation for Aging Research had proposed some criteria for an analytical test to be a marker of biological and/or chronological aging in both clinical and research concepts. Aforementioned analytical parameter must estimate physiological, cognitive, and physical function of the individual independently of chorological age. It must be testable easily and efficiently that most clinical laboratories could perform the test accurately and reproducibly. It also should work in laboratory experimental animals as well as humans. Many important findings have been developed over the 50 years, but no ideal biomarker of aging had been identified which can fulfill all the criteria above.

Elementary analysis of autoimmune diseases starts with searching autoantibodies circulating in the serum of the individuals. When compared to young individuals, older people have increased levels of autoantibodies, but they have a decreased frequency of autoimmune diseases. The occurrence of some non-organ-specific antibodies was detected positive in healthy people who are over than 80 years old. The increase of these autoantibodies was associated with the result of a damaged tissue or high exposure to apoptotic cells instead of an autoimmune response (Vadasz et al. 2013). Rheumatoid factor (RF), antinuclear antibodies (ANA), and anticardiolipin antibodies were found positive with the frequency of 14%, 31%, and 51%, respectively, in the healthy elderly people (Manoussakis et al. 1987). In another study, increased ANA and anticardiolipin and antithyroid antibodies were found in healthy people who were 101–106 years old when compared with younger people who were 26–60 years old (Candore et al. 1997). Additionally, it was reported that higher rates of rheumatoid factors, anti-Ro and anti-La, lower rates of anti-RNP and anti-dsDNA antibodies, and hypocomplementemia were detected for late-onset SLE. None of these antibodies were correlated with organ complications of this systemic and highly variable disease (Rovenský and Tuchyňová 2008).

Anti-thyroglobulin or antithyroid peroxidase levels are usually found high in autoimmune Hashimato thyroiditis, but these autoantibodies are also found in healthy elderly people with a frequency of 14,6% in older women and 10,4% in the elderly people (Bryl and Witkowski 2009).

Anti-double-stranded (ds)DNA antibodies were found positive in about 14% of elderly people who were older than 80 years, but some studies found no relation with anti-dsDNA levels in older people (Manoussakis et al. 1987; Candore et al. 1997). Anti-single-stranded (ss)DNA antibodies was found positive with the frequency of 17% in the elderly people who are older than 81 years old (Candore et al. 1997). Anticardiolipin antibodies were also detected in healthy elderly people's sera in about 50% (Manoussakis et al. 1987). Similar results were detected for the RF which is an autoantibody directed against immunoglobulins (Ig). RF IgM and RF IgA levels were found positive with the frequency of 26,6% and 18.7%, respectively, in elderly people (Andersen-Ranberg et al. 2004).

## 8.6 Improvement of Immune Functions in Aging

People have always tried to find a way to improve and extend their lifespan and their quality of life in history. Aging and longevity are very complex phenomena because genetic, behavioral, dietary, environmental, and social factors can affect life expectancy. Proposed therapies for aging which are caloric restriction (Lu et al. 2011), spermidine (Eisenberg et al. 2009), metformin (Anisimov 2013), resveratrol (Chung et al. 2012), and rapamycin (Wilkinson et al. 2012) showed to have side effects such as malnutrition (Yang et al. 2016), nausea, gastrointestinal discomfort, nephrotoxicity (De Cabo et al. 2014), and adverse effects (Lamming et al. 2012).

Melatonin can be an effective immunoenhancing agent in the elderly. It augments monocytes possibly via enhancement of monocytes sensitivity to GM-CSF,

increases production of CD4+ lymphocytes and NK cells, and also decreases CD8+ lymphocytes (Currier et al. 2000; Srinivasan et al. 2005; Castrillon et al. 2001). The production and release of various cytokines like IL-2, IL-6, IFN-γ, and IL-12 from T-helper lymphocytes and NK cells also are increased by melatonin (Carrillo-Vico et al. 2006; García-Mauriño et al. 2000). Melatonin probably modulates immune function by acting on the G protein-cAMP signal pathway and intracellular glutathione levels (Wei et al. 2003; Urata et al. 1999). Melatonin production decreases with age. Orally administered melatonin can change mRNA levels and control the attenuated immune responses in the elderly (Bondy et al. 2004).

Wang et al. showed the treatment of aged SAMR1 mice with LW-AFC, an herbal medicine, reversed the immunosenescence status by reversing the reduced rates of helper and suppressor T cells and B cells; besides, the increased rates of regulatory T cells in the peripheral blood could make alterations in the levels of IL-1β, IL-2, IL-6, IL-17, IL-23, IL-4, IL-5, IL-10, eotaxin, G-CSF, GM-CSF, IFN-γ, RANTES, TNF-α, TNF-β, and MCP-1. These effects of LW-AFC were found out to be superior to melatonin (Wang et al. 2016).

IL-7 involves in maintenance of naive and memory CD8 T cells which are necessary for host defense (Campos and Godson 2003; Schluns et al. 2000). IL-7 by binding to the IL-7R complex stimulates sequential phosphorylation of Jak1, Jak3, and STAT5, leading to the upregulation of Bcl-2, which promotes cell survival (Jiang et al. 2004). Melchionda F et al. found out that IL-7 improves CD8 T-cell responses and increases survival of memory CD8 T cells (Melchionda et al. 2005). Kim et al. demonstrated an age-associated decrease in IL-7Rα expression by $EM_{CD45RA}$ CD8 T cells that impairs cell signaling and survival responses to IL-7, and they stated that possible effects of IL-7 therapy in the elderly should be evaluated as it may not be as efficient and useful as in the young because of the decreased IL-7R expression and limited TCR pool (Kim et al. 2006).

In replicative senescence, CD8 T cells are unable to upregulate telomerase in case of chronic antigenic stimulations with latent viruses like HIV-1. Cell division without any telomerase activity leads to progressive telomere shortening. High amounts of senescent T cells are responsible for weak vaccine responses, bone loss, and elevated levels of proinflammatory cytokines. Telomerase-based gene therapy studies have suggested that replicative senescence can be delayed or prevented. The catalytic component of human telomerase (hTERT) gene has been shown to make unlimited proliferation and telomere length stabilization possible. Providing continuance of telomerase activity in virus-specific CD8 T cells may be a beneficial therapeutic approach for HIV (Effros 2007). Tert activation has been reported to cause safety problems due to close association with many cancers and reactivation of endogenous telomerase. For this reason, as well as with ongoing Tert-based studies in mice, development of safe treatment strategies for controllable telomerase activation in humans is an important challenge (Bär and Blasco 2016).

Nutrition has always been recognized with overall mortality and morbidity, and its role in improving quality of life and extending the lifespan has always attracted attention in scientific interest. Several foods and foodstuffs which have

anti-inflammatory and anti-oxidative properties have been identified as antiaging foods such as berries, dark chocolate, beans, fish, vegetables, nuts, garlic, whole grains, avocado, green tea, curcumin, etc. Curcumin is an Indian curry derived from *Curcuma longa*. Curcumin has been confirmed by its effect on anti-inflammatory and antioxidant actions in vivo and in vitro. It inhibits the activity of the transcription factor NF-κB that activates the expression of TNF-α, IL-1β, and IL-6. Curcumin's effect on inflammatory process suggests that it can delay the process of aging (Sikora et al. 2010). Dietary intake of curcumin also increased the lifespan of *Drosophila melanogaster* and *Caenorhabditis elegans* (Si and Liu 2014).

Cocoa contains a lot of epicatechin, a flavanol which can also be found in green tea, apples, berries, grapes, and pears. Dietary intake of cocoa can extend the life expectancy for 4 years in humans (Kirschbaum 1997). These effects can be attributed to epicatechin which can improve blood vessel function, insulin sensitivity, and hepatic antioxidant glutathione concentration and reduce systemic inflammation markers, serum low-density lipoprotein cholesterol, and insulin-like growth factor-1. It has been shown that dietary intake of epicatechin also increased the lifespan of *Drosophila melanogaster* and *Caenorhabditis elegans* (Si and Liu 2014).

Mediterranean diet which is characterized by the consumption of olive oil, fruits, vegetables, cereals, fish, poultry, and wine was shown to be associated with longevity in HALE project (Knoops et al. 2004) and also with a 21% reduction in mortality in EPIC-Spain (Buckland et al. 2011). Marie-Paule Vasson et al. evaluated biomarkers of immune status in healthy old people in three European countries and found that NK cells were increased in Spanish population. They concluded that NK cell status may predict morbidity and mortality in old people which can be attributed to Mediterranean diet (Vasson et al. 2013).

Besides nutrition, calorie restriction was also found to be associated with longevity and decreased incidence of chronic diseases like cardiovascular and neurological diseases, cancer, atherosclerosis, diabetes mellitus, and obesity. Adiponectin levels are increased by calorie restriction, and adiponectin suppresses the formation of inflammatory factors such as adhesion molecules and TNF-alpha (Chrysohoou and Stefanadis 2013). Dietary restriction improves healthy lifespan in *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Drosophila melanogaster* (Fontana and Partridge 2015). Calorie restriction has a lot of beneficial effects on health and mortality, but it is difficult to implement in real life.

Several studies have interpreted the role of microbial balance of colon, which is called microbiota, contributes to digestion by fermentation (saccharolytic) and putrefaction (proteolytic) (Fig. 8.2) (Kumar et al. 2016; Giorgetti et al. 2015). Microbiota also attributes to host immunity, metabolism, and health status by decreasing the activity of enzymes involved in the production of carcinogenesis, increasing mucosal cell proliferation and healing process in inflammatory bowel disease (Montemurno et al. 2014; Giorgetti et al. 2015). A healthy and balanced microbiota primarily consists of saccharolytic effect which bifidobacteria and lactobacilli has (Chrysohoou and Stefanadis 2013). These bacteria hydrolyze polysaccharides to its monomers, and then these monomers are converted to short-chain

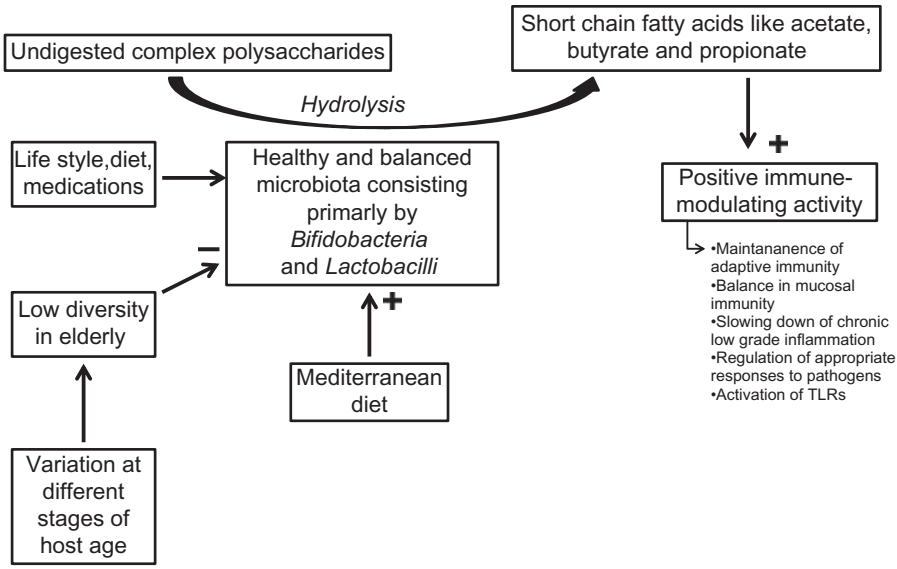


**Fig. 8.2** Microbiota and its relations with aging in elderly

fatty acids which are known by their protective affects and positive immune-modulating activity (Montemurno et al. 2014). Mediterranean diet can support the beneficial replacement of saccharolytic profile which can act as positive microbes. Complex carbohydrates fermentation ends with immune modulating activity by supporting intestinal barrier unity and by revealing direct transcriptional responses in immune cells (Kau et al. 2011). The bifidobacteria numbers in the gut decline prominently after the age of 55–60; therefore probiotic products can have important effects on prevention of age-related diseases (Nova et al. 2007). Gill HS et al. and Chiang BL et al. found that the phagocytic activity of monocytes and polymorphonuclear cells and tumoricidal activity of NK cells can be improved by supplementation of Bifidobacterium lactis in elderly people (Gill et al. 2001; Chiang et al. 2000). Bifidobacterium lactis was also shown with increase phagocytic activity and improve IFN-alpha production by peripheral blood mononuclear cells in elderly people (Chiang et al. 2000; Gill et al. 2001). The same results concerning phagocytic and NK activities were also obtained with the supplementation of *Lactobacillus rhamnosus* in middle-aged and elderly subjects (Nova et al. 2007).

Among all the essential micronutrients in human diet, zinc is accepted to be one of the most abundant trace elements which should be taken regularly for a healthy life. The sources of zinc are red meat, animal protein sources, sea foods, cereals, and nuts. It is not stored in the body; therefore daily intake is essential. Zinc is required for several metabolic pathways that take part in structural and functional integrity of transcription factors and more than 300 enzymes. NF-κB transcription factor

contains Zinc which has a critical role in expression of several immune and inflammatory cytokines. The effect of Zn is suppression of phosphorylation and degradation of the inhibitory proteins which separate NF-κB in the cytoplasm. Zinc plays a key role in inflammatory signal transduction and proinflammatory cascade. Proinflammatory cytokines are believed to play an important role in age and age-related disease; therefore zinc can be assumed as an important trace element in aging (Vasto et al. 2006).

Synthetic drugs have been used for antiaging benefits due to their antioxidant, anti-inflammatory, antidiabetic, or immunostimulant effect (Kapoor et al. 2009). Aspirin or acetylsalicylic acid is an anti-inflammatory drug that inhibits cyclooxygenase enzyme. Cyclooxygenase is responsible of production of prostaglandins and thromboxanes from arachidonic acid. In this way aspirin inhibits platelet aggregation, and aspirin also delays the start of endothelial senescence by preventing reduction of nitric oxide (NO) formation (Kapoor et al. 2009). Inosine pranobex or isoprinosine is an immunostimulant complex which has antiviral feature and can repair immune responses in aging (Kapoor et al. 2009).

Some hormones like sex hormones, human growth hormone, pineal hormone, melatonin, and dehydroepiandrosterone (DHEA) have been shown to have antiaging effect. These hormones can improve vitality, strength, vigor, and sense of well-being. DHEA which is a steroid prohormone produced by the adrenal glands has antiaging effect that has greater potential than other hormones. DHEA levels decreases with age. DHEA levels are more important in elderly due to the decreased production of estrogens in postmenopausal women and decreased testosterone levels in men. DHEA's effects are seen frequently by its hormone end products. DHEA has both beneficial effects on the immune system as well as on the cardiovascular and neurological system (Kapoor et al. 2009).

## 8.7 Conclusion and Future Directions

Prolongation of mean lifespan is a well-accepted and ongoing victory for the health status of our society. But together with this progressing triumph, geriatric health problems will be focus of attention more and more importantly as time passes. There are promising antiaging dietary modulation options in modern medicine like curcumin, zinc, cocoa, and calorie restriction. Melatonin, telomerase-based gene therapy, fecal microbiota transplantation, DHEAS replacement, and even routine medications are considered as good candidates to prevent or delay age-related degenerations and vulnerability. As new bridges between immune system and aging are constructed, there will be new hopes for these achievements. Multidisciplinary, future-directed researches involving high number of patients are needed for new treatment discoveries to improve immunity and maintain reasonably good health in aged populations.

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# Sirtuin Modulators and Brain Aging

9

Hale Z. Toklu and Almari Ginory

**Abstract**

The sirtuins are proteins with enzymatic activity, which regulate diverse cellular processes including aging, longevity, inflammation, obesity, and stress resistance. There are seven sirtuins in mammals with varied subcellular localization and enzymatic activity. Of these, SIRT1 exhibits NAD-dependent deacetylase activity, and it has been the most studied isoform to target aging-related neurodegenerative disorders and longevity due to caloric restriction. SIRT activation can exert positive effects in aging-related disorders such as metabolic, cardiovascular, and neurodegenerative diseases; while SIRT1 inhibitors have anticancer properties. Currently, a number of clinical trials are conveyed with modulators of SIRT. This chapter focuses on SIRT activators and their effects on brain aging.

**Keywords**

Sirtuin · SIRT1 · Brain · Aging · Senescence · SIRT activators, SIRT inhibitors, central nervous system

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## Abbreviations

| | |
|---|---|
| ALS | Amyotrophic lateral sclerosis |
| FoxO | Forkhead box O |
| mTOR | Mammalian target of rapamycin |
| NAD | Nicotinamide adenine dinucleotide |
| SIRT | Sirtuin |

## 9.1 Sirtuins, Types and General Function

Sirtuins were initially described as transcription-silencing histone deacetylase enzymes in yeast (Satoh and Imai 2014). In mammals, there are seven sirtuins (SIRT1–SIRT7). All have a similar catalytic NAD+-binding domain, but each has diverse enzymatic activities, substrates, and cellular functions (Watroba et al. 2017). Although they are initially defined as deacetylases, they can also have deacylase and O-ADP-ribosylase activities (Houtkooper et al. 2012).

All these seven sirtuins have significant roles in physiological and pathological processes such as metabolism, longevity, senescence, cell survival, proliferation, apoptosis, DNA repair, and aging. Therefore, they are the potential targets for the treatment of neurodegenerative diseases, cardiovascular diseases, cancer, and aging (Carafa et al. 2016).

### 9.1.1 SIRTs in the Brain

Although different types of sirtuins are found in the peripheral tissues, SIRT1 is widely studied in the brain; however, there are limited studies with other subtypes. All subtypes are expressed in neurons. SIRT1, SIR5, SIRT6, and SIRT7 are expressed in astrocytes, while SIRT2 is detected in myelin-producing cells (Anamika et al., 2017). Table 9.1 summarizes the localization and function of sirtuins in the brain.

### 9.1.2 Brain Aging and SIRTs

SIRTs have been shown to have role in the pathogenesis of a number of brain disorders. These include traumatic brain injuries (closed-head trauma, ischemia, stroke), neurodegenerative disorders (Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS)), psychiatric disorders (depression, anxiety, sleep disorders), and aging (Satoh et al. 2017).

The process of normal brain aging includes areas of brain atrophy, reduced neurogenesis, decreased neurotransmitter production, increased myelination, and changes in neuronal synaptic structures. It has been shown that reduced

**Table 9.1** The distribution and function of sirtuins in the brain

| Species (human) | Intracellular location | Cell type | Region in the brain | Activity | Function | References |
|---|---|---|---|---|---|---|
| SIRT1 | Nucleus, cytoplasm | Neurons and astrocytes | Brain stem, cortex, cerebellum, hypothalamus, hippocampus, olfactory bulb, striatum | Deacetylase | Metabolism inflammation | Morris (2013), Satoh et al. (2017), and Sidorova-Darmos et al. (2014) |
| SIRT2 | Cytoplasm | Myelin-producing cells (oligodendrocytes and Schwann cells) | Brain stem, cortex, cerebellum, hypothalamus, hippocampus, olfactory bulb, striatum | Deacetylase | Cell cycle, tumorigenesis | Morris (2013), Satoh et al. (2017), and Sidorova-Darmos et al. (2014) |
| SIRT3 | Mitochondria | Neurons | Brain stem, cortex, cerebellum, hypothalamus, hippocampus, olfactory bulb, striatum | Deacetylase and ADP-ribosyl transferase | Metabolism | Morris (2013), Satoh et al. (2017), Yin et al. (2015), and Sidorova-Darmos et al. (2014) |
| SIRT4 | Mitochondria | Astrocytes | Brain stem, cortex, cerebellum, hypothalamus, hippocampus, olfactory bulb, preoptic area, striatum | ADP-ribosyl transferase | Insulin secretion | Morris (2013), Satoh et al. (2017), Pusalkar et al. (2016), Sidorova-Darmos et al. (2014), and Wong et al. (2015) |
| SIRT5 | Mitochondria | Neurons and astrocytes | Brain stem, cortex, cerebellum, hypothalamus, hippocampus, olfactory bulb, preoptic area, striatum | Demalonylase, desuccinylase, and deacetylase | Ammonia detoxification | Morris (2013), Satoh et al. (2017), Sidorova-Darmos et al. (2014), and Wong et al. (2015) |
| SIRT6 | Nucleus | Neurons and astrocytes | Amygdala, brain stem, cortex, cerebellum, hypothalamus, hippocampus, olfactory bulb, striatum | ADP-ribosyl transferase and deacetylase | DNA repair, metabolism, TNF-alpha secretion, neurogenesis | Morris (2013), Satoh et al. (2017), Sidorova-Darmos et al. (2014), Zhang et al. (2017b), and Okun et al. (2017) |
| SIRT7 | Nucleus | Neurons and astrocytes | Amygdala, cortex, hippocampus, striatum, thalamus | Deacetylase | rRNA transcription, memory | Morris (2013), Satoh et al. (2017), Braidy et al. (2015), and Islam et al. (2018) |

neurogenesis plays a role in the development of neurodegenerative diseases. Sirtuins have been shown to regulate neurogenesis and synaptic plasticity (Satoh et al. 2017). Sirtuin activation has also been shown to slow down the cognitive decline associated with aging (Morris 2013).

SIRT1 overexpression helps to protect against Alzheimer's disease, Huntington's disease, and ALS via reductions in cell death, B-amyloid production, and plaque formation (Paraiso et al. 2013). Alzheimer's disease has been associated with increased tau protein, a component of neurofibrillary tangles. SIRT1 can deacetylate tau thus decreasing the presence of neurofibrillary tangles (Morris 2013). In mouse models, SIRT1 deficiency causes impaired synaptic plasticity and increased neurogenesis which leads to memory impairment (Satoh et al. 2017). In addition, SIRT1 overexpression slows neurodegeneration associated with Parkinson's disease (Paraiso et al. 2013). In mouse models, SIRT1 leads to breakdown of alpha-synuclein which is involved in the pathogenesis of both Parkinson's disease and Lewy body dementia (Morris 2013). Further research will be needed regarding the protective effects of SIRT1 in neurodegenerative diseases in humans.

The exact mechanism by which SIRT2 and SIRT6 contribute to brain aging is not yet fully known. SIRT2 overexpression and caloric restriction have been shown to increase the life span of yeast, but this has not yet been proven in humans (Carafa et al. 2012). In other models, SIRT2 is theorized to be neurotoxic. SIRT2 inhibition decreases apoptosis and is considered neuroprotective in models of Parkinson's disease and Huntington's disease (Morris 2013). SIRT6 may have a role in decreasing the destruction of telomeres, which has been associated with aging (Carafa et al. 2012).

There is still much research that needs to be done in the area of SIRT activation/inhibition and the role in neurodegenerative disease. Current research shows promise for future SIRT modulating agents as being beneficial for the treatment of neurodegenerative diseases and aging (Carafa et al. 2016).

## 9.2 Modulation of SIRT as a Therapeutic Target in Diseases

During recent years, with the discovery of the SIRTs in brain function and brain disorders, studies focused on targeting modifying SIRT function to alter pathophysiological processes.

Especially during past decade, experimental molecules were synthesized to modify SIRT function. These ligands are mostly selective for SIRT1/2 as inhibitors or activators (Fig. 9.1).

### 9.2.1 SIRT Activators

#### 9.2.1.1 Caloric Restriction

Dietary interventions such as caloric restriction (CR) extend life span and health span (Libert and Guarente 2013). CR refers to dietary regimens that reduce daily

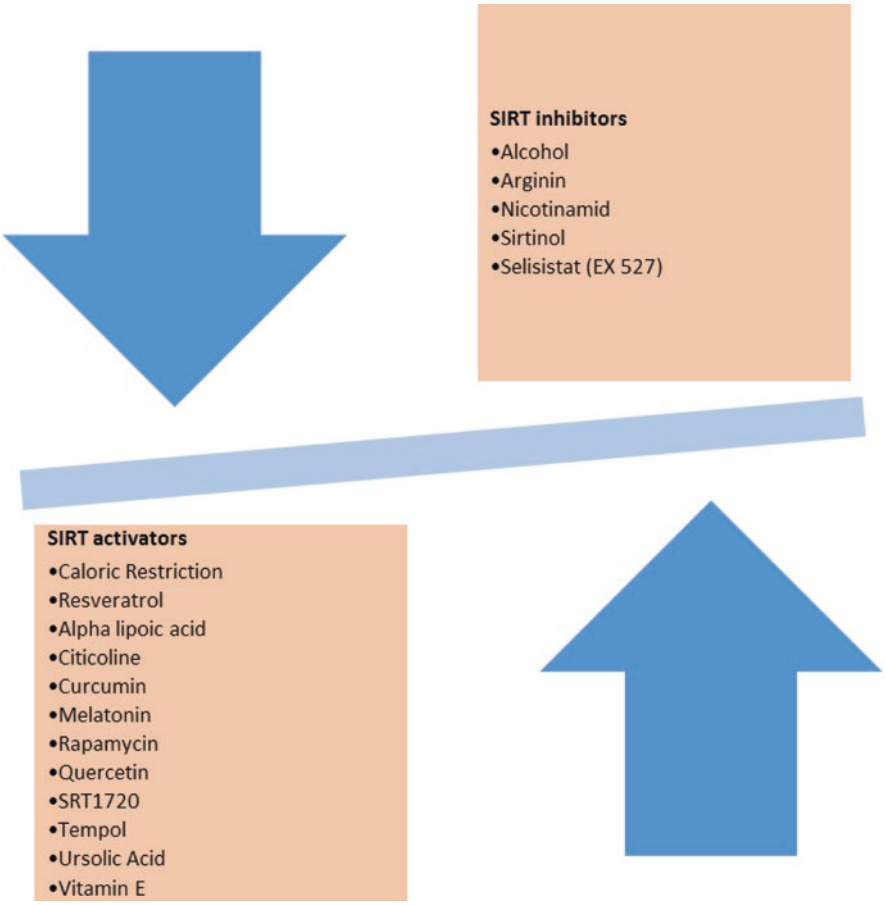


**Fig. 9.1** Modulation of sirtuins (SIRT) with inhibitors and activators

calorie intake without incurring malnutrition (i.e., ≥10% in humans, ≥20% in animal) (Bales and Kraus 2013).

Recent data from animal and human studies demonstrate that CR slows down the aging and cognitive decline. Caloric restriction reduces the rate of metabolism, improves insulin sensitivity, and protects against oxidative stress and inflammation induced by aging [20]. It was shown that caloric restriction-induced longevity in rodents was mediated by SIRTs (Libert and Guarente 2013; Nikolai et al. 2015).

SIRT1 deficiency results in elevated mTOR (mammalian target of rapamycin) signaling. mTOR is a key kinase enzyme in modulating energy metabolism, nutrient sensing, aging, and longevity. Excessive mTOR activity is inhibited by caloric restriction and several agents like rapamycin. Both activate SIRT1 and increase life span (Ehninger et al. 2014). SIRT1 activator resveratrol has also been shown to inhibit mTOR activity, whereas SIRT1 inhibitor nicotinamide enhanced it in a SIRT1-dependent manner (Ghosh et al. 2010).

#### 9.2.1.2 Citicoline

Citicoline (cytidine 5′-diphosphocholine) is an essential intermediate in the biosynthetic pathway of structural phospholipids in cell membranes, particularly phosphatidylcholine (Secades and Lorenzo 2006). It has been widely studied for its neuroprotective effects as well as the effects on improving memory. Citicoline increased SIRT1 protein expression in the brain following stroke; and this increase was prevented by pretreatment with SIRT inhibitor sirtinol (Hurtado et al. 2013).

#### 9.2.1.3 Curcumin

Curcumin ((1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is the major active ingredient of turmeric (*Curcuma longa*) root. It has antioxidant, anti-inflammatory, and anticancer activities (Aggarwal et al. 2007; Jurenka 2009). A recent evidence suggested that curcumin has been shown to upregulate SIRT3 expression in the skeletal muscle tissues (Zhang et al. 2017a). In addition to periphery, curcumin also upregulated SIRT1 expression in the brain following stroke (Miao et al. 2016). The effects of curcumin (reduced infarct size, reduced edema, and improved neurological scores) were attenuated by sirtinol (Miao et al. 2016).

#### 9.2.1.4 α-Lipoic Acid

Alpha-lipoic acid (6,8-thioctic acid) is a dithiol compound derived from octanoic acid, which acts as a coenzyme for several redox reactions. It is synthesized in body naturally and is essential for aerobic metabolism. Many experimental and some clinical trials have been carried out to study its efficacy in diseases related to aging-induced oxidative stress (Skibska and Goraca 2015; Tibullo et al. 2017; Shay et al. 2009) and neurotrauma (Ekiz et al. 2017; Toklu et al. 2010a; Ersahin et al. 2010; Toklu et al. 2009). Lipoic acid was shown to SIRt1 and SIRT3 in peripheral tissues (Valdecantos et al. 2012; Zhang et al. 2014).

#### 9.2.1.5 Melatonin

Melatonin (N-acetyl-5-methoxy tryptamine) is a hormone that is secreted by the pineal gland and regulates sleep/wake cycles. It is a popular antioxidant supplement to support healthy aging. Its endogenous levels decrease in neurodegenerative disorders such as Alzheimer's disease (Hardeland et al. 2015). Several mechanisms were suggested for the actions of melatonin; however there are only few studies which show its effect on SIRT1 levels. Chang et al. (2009) have shown that melatonin treatment efficaciously retained the relative protein levels of SIRT1 in the hippocampus of completely sleep-deprived rats (Chang et al. 2009). Recently, other studies supported melatonin's ability to increase SIRT levels against brain aging (Cristofol et al. 2012; Kireev et al. 2013).

#### 9.2.1.6 Quercetin

Quercetin (3,3′,4′5,7-pentahydroxyflavone) is a polyphenolic compound found in a variety of plants. It has antioxidant, anti-inflammatory, immuno-protective, and even anticarcinogenic effects (Andres et al. 2018). Like resveratrol, quercetin is also

an indirect activator of SIRT1 (Chung et al. 2010). Quercetin has been shown to delay postovulatory aging of mouse oocytes by regulating SIRT expression (Wang et al. 2017). However, its effect in the brain to activate SIRTs is unclear. In one study, quercetin increased hippocampal SIRT1 levels and improved cognitive function in aged rats (Sarubbo et al. 2018).

#### 9.2.1.7 Rapamycin

Rapamycin, also called sirolimus is an immunosuppressant drug used for preventing rejection of organ transplants. It is the first pharmacological agent shown to extend life span in mammalian species (Carter et al. 2016; Ehninger et al. 2014). As mentioned earlier, caloric restriction has been shown to enhance longevity via the activation of SIRT pathway (Zhang et al. 2011), and SIRT1 deficiency results in elevated mammalian mTOR signaling (Ghosh et al. 2010). mTOR is a kinase which has key role in longevity and energy metabolism. Rapamycin inhibits mTOR activity, mimics caloric restriction, and activates SIRT1. Thus, it has been shown to improve aging-related hypothalamic insulin resistance in rat models (Carter et al. 2016; Scarpace et al. 2016; Toklu et al. 2016) besides its effect on increasing life span (Ehninger et al. 2014).

#### 9.2.1.8 Resveratrol

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a plant polyphenol, found abundantly in grape skin, blueberries, peanuts, and pistachios. In addition to its potential therapeutic effects in cancers and cardiovascular diseases, its neuroprotective effects in various neurotrauma models have been widely studied over the years (Lopez et al. 2015; Toklu et al. 2010b). Resveratrol is claimed to be a promising agent in epilepsy, brain trauma, Alzheimer's disease, and other neurodegenerative diseases because of its effects in improving cognitive function and neuronal plasticity (Lange and Li 2018; Sarubbo et al. 2017; Poulose et al. 2015; Dias et al. 2016; Toklu et al. 2010b).

SIRT1 has emerged as an attractive therapeutic target for many aging-related diseases; however, how its activity is modulated by resveratrol has been poorly understood. Resveratrol is a SIRT1 activator and mTOR inhibitor (Carafa et al. 2016; Ghosh et al. 2010). Therefore, it mimics the beneficial effects of dietary restriction because of this common mechanism (Dolinsky and Dyck 2011; Villalba and Alcain 2012). However, resveratrol was not proven to extend life span like CR. On the other hand, it was demonstrated that resveratrol slows down the onset of age-related diseases (McCubrey et al. 2017).

Enhanced cerebral microvascular circulation, neurogenesis, mitochondrial function, neuroprotection, and neuronal survival are achieved with resveratrol treatment in experimental studies. In addition, resveratrol treatment decreased macular degeneration, retinal aging, and aging-induced hearing loss in rats (McCubrey et al. 2017).

#### 9.2.1.9 SRT1720

SRT1720 is an experimental compound which is recently synthesized as selective activator of SIRT1. It is more potent than the prototype activator resveratrol. It

stimulates 750% SIRT1 activity at a concentration of 10 μM (Villalba and Alcain 2012). It activates SIRT2 and SIRT3 with a lower efficacy. Like resveratrol, SRT1720 increased life span in mice (Mitchell et al. 2014) and preserved aging-induced vascular dysfunction (Gano et al. 2014). Its efficacy was demonstrated in reversing the adverse effects of obesity and insulin resistance, restoring metabolic function in mice (Nguyen et al. 2018). Another study showed that intravenous SRT1720 treatment attenuated systemic inflammatory response in mice sepsis model (Khader et al. 2017). Lahusen and Deng (2015) have suggested that SRT1720 could be potential therapeutic agent for cancer treatment due to its effect on enhancing lysosomal-dependent necrosis in breast cancer cells (Lahusen and Deng 2015).

#### 9.2.1.10 Tempol

4-Hydroxy-tempol (4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl) is a membrane permeable free radical scavenger. Tempol has been investigated in a number of experimental hypertension, neuronal injury, aging, and obesity models (Dornas et al. 2015; Hamel 2015; Wilcox 2010; Toklu et al. 2017).

In vitro tempol treatment was demonstrated to revert the downregulation of SIRT2 and SIRT6 in neural stem cells exposed to high glucose (Yu et al. 2016).

Decreased SIRT1 activity has been claimed to be responsible for aging-induced hypothalamic degeneration, metabolic disorders, and obesity. To test this hypothesis, we infused tempol into the brains of aged rats and measured SIRT1, p53, and AMPK proteins. Old rats had significantly lower levels of SIRT1 in the hypothalamus. However, centrally given tempol failed to modulate SIRT pathway in the hypothalamus and improve aging-induced obesity (Toklu et al. 2017).

#### 9.2.1.11 Ursolic Acid

Ursolic acid (3β-hydroxy-12-ursen-28-ic acid) is a pentacyclic triterpenoid found in peels of fruits (apples, prunes) and in herbs like rosemary, lavender, basil, peppermint, eucalyptus, oregano, and thyme (Wozniak et al. 2015). Ursolic acid and its analogs are studied to treat various cancers, inflammation, diabetes, Parkinson's disease, Alzheimer's disease, hepatitis B, hepatitis C, and AIDS (Hussain et al. 2017).

Ursolic acid has been shown to regulate aging by the activation of SIRT1 and SIRT6 in hypothalamus. It attenuated mitochondrial dysfunction in aged animals (Bahrami and Bakhtiari 2016). In an earlier study, it protected against D-galactose (D-gal)-induced neurotoxicity and improved cognitive function (Lu et al. 2007).

#### 9.2.1.12 Vitamin E

Vitamin E includes a group of lipid-soluble tocopherols and tocotrienols. α-Tocopherol is the most plentiful and bioavailable form of vitamin E for humans (La Fata et al. 2014). It is a well-known antioxidant. Vitamin E deficiency causes neuronal dysfunction due to unmanaged oxidative stress. Hence, vitamin E was widely studied for its effect on brain aging and cognitive function (La Fata et al. 2014; Tucker 2016).

A recent study has demonstrated that long-term deficiency of vitamin E remarkably decreased the expression of silent mating-type information regulation (SIRT)-2

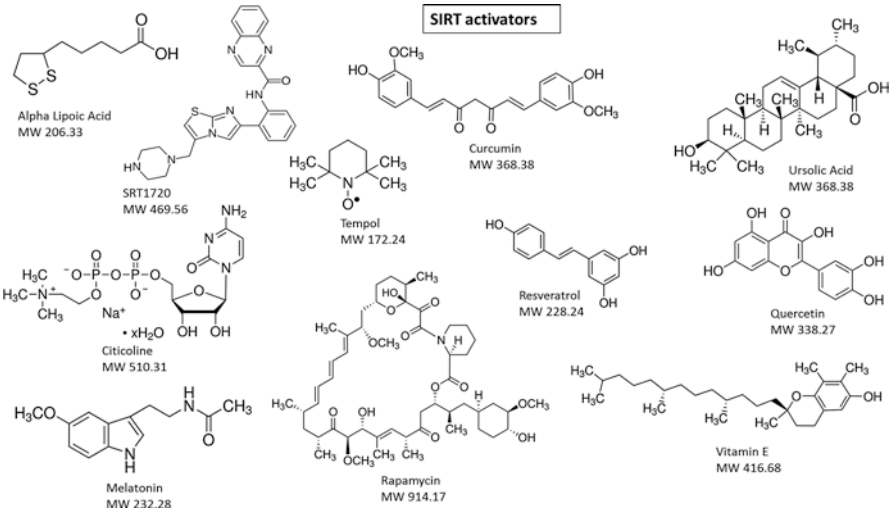


**Fig. 9.2** Chemical structures of SIRT activators

mRNA compared to short-term deficiency (Fukui et al. 2014). However, further studies are required to elucidate the SIRT-related mechanisms underlying the neuroprotective effect of vitamin E.

The studies involving these vitamin supplements have controversial results on their benefit, the evidence suggests that healthy brain aging may be achieved by healthy nutrition which contains balanced combination of these vitamins and fatty acids as well as reduced dietary sugar (Tucker 2016) (Fig. 9.2).

## 9.2.2 SIRT Inhibitors

There are no publications in literature which directly study the effect of SIRT inhibitors in brain aging. However, there are few studies published which studied the physiological effects of SIRT1 inhibition in the brain. Therefore, in this section, the studies conducted with SIRT1 inhibitors will be discussed for their effects on the brain. Since most of the neurodegenerative brain disorders are known to enhance brain aging due to neuronal injury, it is critical to recognize the consequences of SIRT inhibition in the brain in experimental neurotrauma models.

### 9.2.2.1 Alcohol

Ethanol downregulates SIRT1 in hepatic cells in humans and experimental animal models. The ethanol-mediated disruption of SIRT1 signaling leads to excess fat accumulation and inflammation (You et al. 2015). On the other hand, Mediterranean-type diet and moderate alcohol consumption with food have been suggested to be protective against cardiovascular diseases and increase longevity. Hence, the Mediterranean way of drinking refers to drinking wine, up to two glasses a day for

men and one glass for women. The efficacy is the result of polyphenolic substances such as catechin and epicatechin, proanthocyanidin, anthocyanin, various phenolic acids, quercetin, and the stilbene resveratrol. Resveratrol is the most widely studied compound for its effects on increasing the expression of Sirt1 (Giacosa et al. 2016).

Acute ethanol inebriation was demonstrated to reduce Sir2 levels and increase histone H3 acetylation in the brain. This leads to neuroadaptive changes in synapsin levels, which is a protein required for ethanol sensitivity and tolerance (Engel et al. 2016). Furthermore, binge ethanol consumption during puberty has been demonstrated to alter microRNA expression in the hippocampus and cause long-term changes in brain-derived neurotrophic factor (BDNF) and sirtuin-1 (SIRT1) levels (Prins et al. 2014).

#### 9.2.2.2 Cambinol

Cambinol is a chemically stable compound that shares a β-naphthol moiety with sirtinol and inhibits both SIRT1 and SIRT2 in vitro. It has weak inhibitory activity against SIRT5 (Villalba and Alcain 2012). However, inhibitory activity of cambinol for neutral sphingomyelinase 2 (nSMase2) in the brain was tenfold more potent than its effect on SIRT1/2. It was suggested that its neuroprotective effects in primary neurons were via nSMase2 inhibition (Figuera-Losada et al. 2015).

#### 9.2.2.3 L-Arginine

L-Arginine is an amino acid which plays vital role in nutrition and health (Wu and Meininger 2000). L-Arginine is a precursor of nitric oxide, ornithine, citrulline, agmatine, polyamines, creatine, and proteins. While aging is often associated with L-arginine deficiency, supplementation for aging-related cardiovascular disorders was suggested. However, clinical and experimental studies demonstrate controversial data on its beneficial effects (Moretto et al. 2017).

It has been recently demonstrated that SIRT1 mediates L-arginine protection against diabetic myocardial fibrosis via equilibrating the balance between profibrotic and antifibrotic mediators (Rizk et al. 2014).

#### 9.2.2.4 Nicotinamide (Vitamin B3)

Niacin is the precursor to two important cofactors, NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate), which are necessary for many enzymes catalyzing redox reactions in the human organism. Nicotinamide, also known as niacinamide, or vitamin B3, is an important compound functioning as a component of the coenzyme NAD.

In an in vitro study, antimycin A-induced increase ROS levels and apoptosis in the brain was enhanced by nicotinamide due to inhibition of SIRT (Hori et al. 2013). Even though nicotinamide is an inhibitor of SIRT1 in vitro, it may activate SIRT pathway in vivo (Hwang and Song 2017). Thus this may explain its protective effect on neuronal function after severe hypoxia (Shetty et al. 2014).

As mentioned earlier in this chapter, NAD+ acts as a metabolic sensor and SIRT1 activity is increased in energy/nutrient stress (Houtkooper et al. 2012). SIRT1 has also been reported to improve insulin sensitivity in vitro or in vivo. Long-term

treatment with the SIRT1 inhibitor nicotinamide significantly impaired glucose tolerance in nicotinamide-treated mice (Qi et al. 2016). Furthermore, another study has demonstrated that leptin's ability to improve glucose metabolism in the brain and reduce tau phosphorylation/β-amyloid production was prevented by nicotinamide (Greco et al. 2011).

#### 9.2.2.5 Salermide

Salermide is a sirtuin inhibitor that acts on SIRT1 and SIRT2. Salermide is a reverse amide of sirtinol and it is more potent than sirtinol (Lara et al. 2009). It was shown that the inhibition of Sirt1 with salermide decreased BBB permeability, attenuated apoptosis in both normal and ischemic conditions in vitro (Chen et al. 2017). Sirt3 expression was also partially prevented by salermide in this study, suggesting its role in the regulation of SIRT1/SIRT3 pathway in the brain. In contrary to its anti-apoptotic effect in in vitro ischemia model (Chen et al. 2017), an in vivo study with mice using a traumatic brain injury model demonstrated that salermide promoted neuronal apoptosis (Zhao et al. 2012). Moreover, blood glucose levels were significantly elevated in the salermide-treated mice compared to controls, providing evidence for the role of SIRT1 in regulating insulin sensitivity (Zhao et al. 2012).

#### 9.2.2.6 Selisistat (EX-527)

Recent studies have demonstrated that SIRT1 mediates depression (Kim et al. 2016) and anxiety by activating MAO-A in the brain (Libert et al. 2011). Thus, SIRT1 inhibition is expected to decrease MAO-A activity and increase monoamine levels. As known, MAO-A inhibitors are pharmacological agents which are clinically used to treat depression. Consistent with this hypothesis, our recent findings have demonstrated that continuous infusion of EX-527 to the brain of aged rats increased locomotor activity by altering norepinephrine turnover in brain regions like the hypothalamus, pituitary, and nucleus tractus solitarius (NTS) [unpublished data].

SIRT1 pathway was also thought to be a potential target for the prevention and treatment of epilepsy and epileptic damage. Although earlier findings pointed out to the involvement of SIRT1-related pathway in epileptogenesis (Wang et al. 2016), a recent study with EX-527 did not support this hypothesis (Hall et al. 2017).

#### 9.2.2.7 Sirtinol

Sirtinol, is a cell-permeable 2-hydroxy-1-naphthaldehyde derivative that acts as a selective inhibitor on SIRT1 and SIRT2 (Villalba and Alcain 2012).

SIRT1 was reported to be involved in the pathogenesis of cerebral ischemia, subarachnoid hemorrhage, and brain tumors via p53 deacetylation (Chen et al. 2017; Zhang et al. 2016). Hence, resveratrol, SIRT1 activator, exerts protective effects on the brain in experimental trauma models (Qian et al. 2017; Toklu et al. 2010b). Protective effects of resveratrol in subarachnoid hemorrhage were blocked by sirtinol, suggesting the role of SIRT1 in the formation of brain edema and preservation of blood brain barrier integrity (Cristofol et al. 2012).

In another study, endothelium-dependent vasodilation was impaired with sirtinol incubation in both young and older mice (Donato et al. 2011).

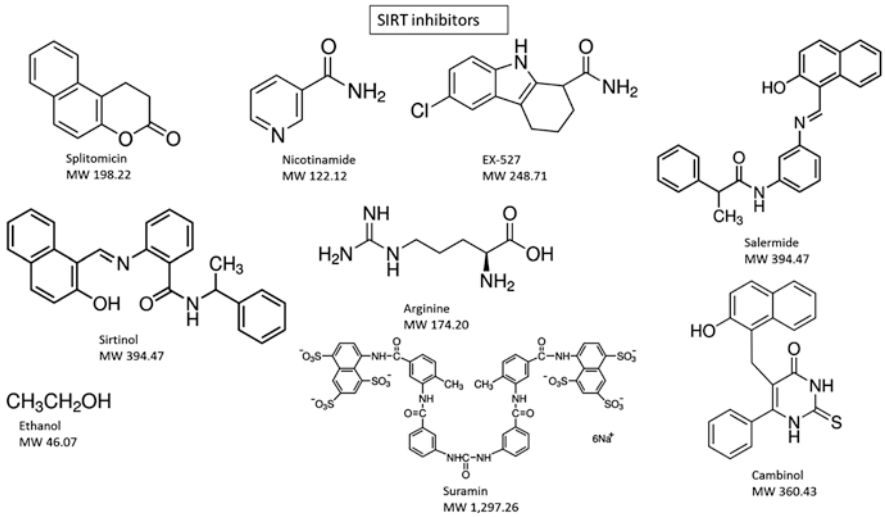


**Fig. 9.3** Chemical structures of SIRT inhibitors

### 9.2.2.8 Splitomicin

Splitomicin is a β-naphthol derivative and is an inhibitor of SIR2. It inhibits platelet aggregation by increasing cyclic AMP via inhibition of phosphodiesterase enzyme (Liu et al. 2009).

In an in vitro study, antimycin A-induced increase ROS levels and apoptosis was enhanced by splitomicin (Hori et al. 2013). Its effect was attributed to modification of FOXOs and p53 under oxidative stress.

### 9.2.2.9 Suramin

Suramin is anaphthylurea derivative that is used against trypanosoma infections. Suramin analogs have been shown to have inhibitory effects on SIRT1, SIRT2, and SIRT5 (Trapp et al. 2007; Villalba and Alcain 2012). In an earlier study, suramin abolished the ability of amyloid-β to increase the amplitude and velocity of calcium wave propagation in astrocytes (Haughey and Mattson 2003). However, the effects in the brain were attributed to its direct inhibition on adenylyl cyclase enzyme (Stohr et al. 2005). No studies were carried out to specifically evaluate suramin effects and SIRT inhibition in the brain (Fig. 9.3).

## 9.3 Conclusion

Biological senescence is a loss of integration and resilience. Resilience is the ability to achieve a positive outcome when facing adversity. Even though aging is inevitable, the evidence suggests that a healthy lifestyle has a crucial role to achieve brain resilience during aging.

The relationship between SIRT function and extended life span is widely studied. With the advances in our understanding of SIRT function in the brain, and its role in pathological process, the specific targeting in aging brain will become more important. Either synthetic or natural SIRT-modulating ligands (for their potential in resilience) may be clinically used in the future to help healthy aging of the brain.

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# Inhibition of mTOR Signalling: A Potential Anti-aging Drug Strategy

10

Komal Saraswat, Raushan Kumar, and Syed Ibrahim Rizvi

**Abstract**

Maintenance of cellular energy homeostasis, counterbalanced by cellular stress response and an adequate cellular housekeeping are the hallmarks of improved healthspan and lifespan. Mammalian target of rapamycin (mTOR) is a central cell growth regulator that integrates cellular growth and proliferation with nutrient status of the cell. It is an intracellular nutrient sensor that controls protein synthesis, cell growth and metabolism. mTOR turns off stress resistance and autophagy and activates translation. Available scientific evidence endorses that molecular inhibition of this pathway slows aging, extends lifespan and improves symptoms of diverse array of age-related diseases in wide range of species. Rapamycin, a small molecule inhibitor of the protein kinase mTOR, has been found to extend the lifespan of model organisms including mice. Many of such inhibitors of this pathway are already characterized and clinically approved. In the present chapter, we summarize current understanding of mTOR and its role in aging and age-related disease progression.

**Keywords**

Aging · Autophagy · Mammalian target of rapamycin · Protein synthesis · Rapamycin

## 10.1 Introduction

Aging is an inevitable process accompanied by multiple molecular changes in gene expression, altered metabolite levels and accumulation of molecular damages (Lee et al. 2017). Thus interventions that target these molecular changes provide a novel

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entrance for evolution of innovative preventatives and therapeutics that delay the onset and progression of aging and age-related ailments (Saraswat and Rizvi 2017).

Mechanistic target of rapamycin (mTOR) is an integrated signalling network that converges various upstream signalling stimulations to regulate various cellular processes such as cell proliferation, growth, survival, metabolism, autophagy, protein synthesis and apoptosis (Johnson et al. 2013). The ability of mTOR to regulate various cellular processes has attracted great interest. Understanding this molecular pathway has led to the use of mTOR inhibitors (such as rapamycin and rapalogs) in the treatment against mouse models of age-related diseases, such as cancer, neurodegenerative diseases, cognitive decline, rheumatoid arthritis, organ transplantation and coronary restenosis (Ehninger et al. 2014).

The story of mTOR began in the 1970s, when soil samples from a Polynesian island Rapa Nui was found to have antifungal activity. This was attributed to be due to the presence of the bacteria *Streptomyces hygroscopicus* that produces a natural macrocyclic lactone, rapamycin. Since then, rapamycin (also called sirolimus) is used as antifungal, anti-cell proliferative drug that possesses strong immunosuppressant properties (Morris 1992). It has also been approved by the FDA as an anticancer drug. In 2009, Harrison and co-workers (2009) reported that rapamycin, when fed late in life could extend mean and median lifespan of male and female mice. Since then, rapamycin and other rapalogs have come into intense scrutiny for their potential as an anti-aging drug. In the 1990s, genetic screening of *Saccharomyces cerevisiae* revealed two genes, namely, TOR1 and TOR2, that are the mediators of the toxic effects of rapamycin in yeast (Kunz et al. 1993). Soon after this discovery, the mTOR was identified in mammalian cells too and designated as mechanistic target of rapamycin (Sabatini et al. 1994; Sabers et al. 1995). In the past few years, our understanding of this kinase has increased by leaps and bounds. Now it is known that mTOR is involved in regulating a diverse set of cellular functions and is sensitive to many environmental and endocrine stimuli. Thus, mTOR functions not only as a master regulator of cellular functions but also for metabolism and aging.

mTOR is a 289-kDa protein that belongs to a class of serine/threonine protein kinase of phosphoinositide 3-kinase (PI3K)-related kinase family. mTOR nucleates around two distinct multi-protein complexes: mTORC1 and mTORC2 that differ in function, substrates and their sensitivity to rapamycin.

## 10.2 mTORC1: Central Regulator of Cellular Functions

Complex 1 of mTOR has five distinct components; a catalytic subunit, a regulatory subunit (raptor) which regulates mTORC1 assembly and recruits it to substrate and another unit named mammalian lethal with Sec13 protein 8 (mLST8 also known as GβL) of unknown function. Other two components are proline-rich AKT substrate (PRAS40) and mTOR-interacting protein known as deptor, both of which have a negative role in regulation of the complex (Peterson et al. 2009). When the activity of mTORC1 is reduced, PRAS40 acts as a direct inhibitor of substrate binding (Wang et al. 2007). When activated, mTORC1 phosphorylates PRAS40 and deptor,

thereby removing their direct interaction with mTORC1. Bacterial macrolide rapamycin played a major role in understanding how mTORC1 functions. When rapamycin enters the cell, it inhibits the activity of mTORC1 by binding to the FK506-binding protein of 12 kDa (FKBP12) and interacting with the FKBP12-rapamycin-binding domain (FRB) of mTOR.

mTORC1 is well positioned to coordinate various cellular growth processes and the availability of nutrients, energy and growth factors (Wullschleger et al. 2006). Signals from various upstream components that activate mTORC1 include the PI3K/AKT, Ras/MAPK and AMPK pathway (Mendoza et al. 2011; Mihaylova and Shaw 2011). These upstream signalling networks are activated in response to various intracellular and extracellular stimuli (such as availability of oxygen, nutrients, cellular energy status, growth factors and stress) and converge onto mTORC1-related signalling network to control essential cellular processes including protein and lipid synthesis and autophagy (Laplante and Sabatini 2012). The key upstream regulator of mTORC1 is heterodimer TSC1 (or hamartin) and TSC2 (or tuberin). This dimer acts as a GTPase-activating protein (GAP) for Rheb (stand for Ras homolog enriched in brain) GTPase. Once bound with GTP, Rheb directly phosphorylates mTORC1 to activate its kinase activity. TSC1/TSC12 negatively controls the activity of mTORC1 by acting as Rheb GAP, i.e. it converts active Rheb into its inactive form by converting its GTP bound form to GDP bound form (Inoki et al. 2005; Tee et al. 2003).

Growth factors such as insulin and insulin-like growth factor1 stimulate PI3K and Ras signalling networks which in turn phosphorylate and inactivate TSC1/TSC12, thus integrating the signals to the mTORC1 (Sengupta et al. 2010).

Pro-inflammatory cytokines, such as tumour necrosis factor-α (TNFα), also activate mTOR in a similar fashion (Lee et al. 2007). Akt, either in integration to PI3K pathway or independent of TSC1/TSC12, can also signal to mTORC1 by phosphorylating and disengaging its regulatory subunit (raptor) from its inhibitory subunit (PRAS40) (Sancak et al. 2007; Thedieck et al. 2007).

Canonical Wnt pathway that directs cell proliferation, cell polarity and cell fate determination during embryonic development and tissue homeostasis also play a role in regulating mTORC1. It suppresses TSC1/TSC12 by phosphorylating glycogen synthase kinase 3β (GSK3-β), directly phosphorylating and activating TSC2 (Inoki et al. 2006).

Intracellular energy status is also a crucial factor in regulation of mTOR pathway. When cellular energy status is low (i.e. lower ATP/ADP ratio), the activity of mTORC1 is kept in check through AMP-activated protein kinase (AMPK) pathway (Xu et al. 2012). During energy deprivation, AMPK phosphorylates TSC2 which acts as a GAP for Rheb, thus inhibiting the activation of mTORC1. AMPK also regulates mTORC1 activity independent of TSC1/TSC12 by directly phosphorylating raptor, the regulatory subunit of mTOR (Gwinn et al. 2008). Other stress conditions such as mild hypoxia also inhibit mTOR pathway by activating AMPK (Schneider et al. 2008).

Hypoxia can also activate TSC1/TSC12 through transcriptional regulation of DNA damage response 1 (REDD1) by releasing TSC2 from its

growth-factor-induced association with 14-3-3 proteins (DeYoung et al. 2008). This response is evolved to restrain any energy consuming process when only oxygen but not nutrient is a limiting factor.

High intracellular concentration of amino acid, most specifically leucine and arginine, also activates mTORC1 and is required by several upstream signalling networks to activate mTORC1 (Goberdhan et al. 2016). It has been known for many years that amino acids activate mTOR pathway independent of TSC1/TSC12. In 2008, it was discovered that a protein named Rag GTPase is required for amino acid-dependent activation for mTORC1. Four of these proteins (Rag A to Rag D) exist in a heterodimeric form (Rag A/Rag B bind with Rag C/Rag D). These heterodimers function in contrast to nucleotide loading state, i.e. if Rag A/Rag B binds with GTP, Rag C/Rag D will be bound to GDP. Amino acids promote the binding of Rag A/Rag B to GTP which in turn enables the heterodimer to interact with raptor. This results in movement of mTORC1 from a poorly distinguished cytoplasmic location to the lysosomal surface where the Rag GTPases dock on a multisubunit complex called Ragulator (Sancak et al. 2010). It has been found that this regulator is essential for amino acid-mediated activation of mTOR (Laplante and Sabatini 2012).

## 10.3 mTORC2

mTORC2 comprises of six different proteins, four of which are common to both mTORC1 and mTORC2. This includes a catalytic subunit of mTOR, rictor, deptor and mLST-8, all of which are common in both complexes. Two proteins specific to mTORC2 are mammalian stress-activated protein kinase-interacting protein (mSIN1) and protein observed with Rictor-1 (Protor-1), both of which play a major role in the establishment of mTORC2. In comparison to mTORC1, the function of mTORC2 is still a mystery. However, some recent findings have shown that mTORC2 plays key roles in various biological processes, including cell survival, metabolism, proliferation and cytoskeleton organization (Oh and Jacinto 2011).

## 10.4 mTOR and Aging

mTOR promotes cellular growth by either promoting protein synthesis or by inhibiting autophagy. It has been reported that mTOR activity is required for senescence associated phenotypes. In human cells, it has been found that high levels of TOR activity are required for cellular senescence when the cell cycle is blocked (Demidenko and Blagosklonny 2008). Cells with arrested cell cycle obtain a large morphology on beta galactosidase staining. These cells lose their proliferative competence when the arrest is retracted. Thus, rapamycin, inhibitors of PI3K pathway and serum starvation prevent cellular senescence by inhibiting mTOR. Thus, in conditions where actual growth is not possible, activation of mTOR pathway leads to

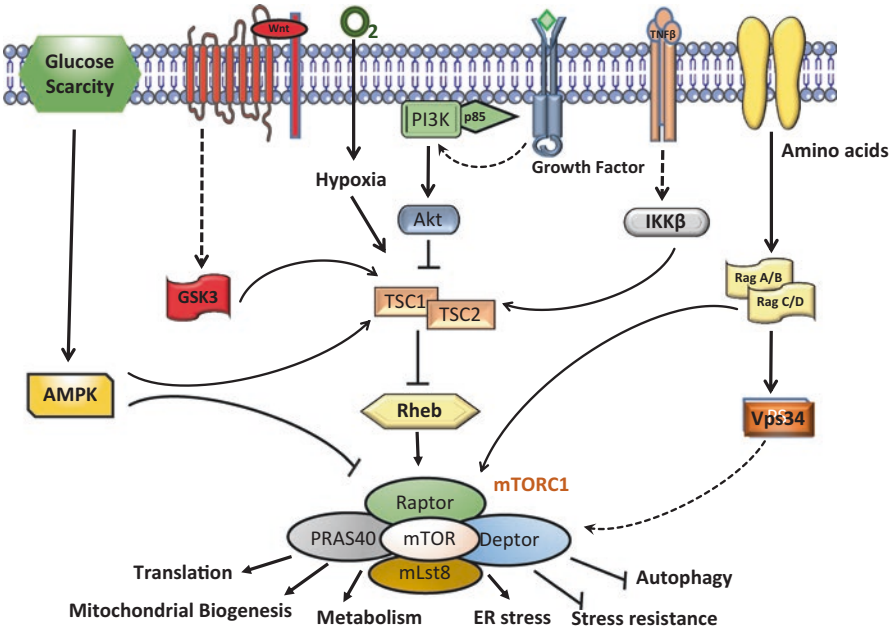


**Fig. 10.1** Schematic representation of mTOR signalling network: mTOR integrates signals for intra- and extracellular signalling molecules and amalgamate them in the central signalling core. Scarcity of amino acids acts as a powerful inhibitor of TORC1 activity. Growth factors like TGFβ, IGF and insulin modulate TORC1 activity through Akt. Wnt signalling has also been demonstrated to regulate TORC1 activity. Wnt-mediated inactivation of Gsk3 alleviates TSC2-driven inhibition of Rheb and results in TORC1 activation.TORC1 also responds to hypoxia. Low oxygen levels inhibit TORC1 activity by a TSC2-dependent mechanism (AMPK, 5′ adenosine monophosphate-activated protein kinase; transcription factors, TSC2/TSC1; kinase: IKKβ, Akt, Vps34, GSK3, PI3K; GTPase, Rheb)

cellular senescence (Blagosklonny 2012). These accumulated senescent cells that cannot be removed from the system by autophagy when mTOR is in active states lead to aging (Fig. 10.1).

### 10.4.1 Autophagy Activation

Activation of autophagy is an mTORC1-mediated cellular degradation process. Although the main function of autophagy is to make the cell free from debris and recycling amino acids during the period of starvation, evidence suggests that it has a major role in longevity (He et al. 2013). It has been found that autophagic flux declines with age; this leads to the accumulation of damaged proteins aggregates and organelles, which give rise to age-related pathologies (Glick et al. 2010). Therefore, an effective strategy would be the activation of autophagy by inhibiting mTORC1 to restore cellular homeostasis. It has also been found that autophagy

plays a crucial role in many age-related diseases including cancer, diabetes, cardiovascular disease and neurodegenerative diseases (Periyasamy-Thandavan et al. 2009). Evidence from yeast and invertebrates studies has suggested that mTORC1-mediated autophagy activation by either dietary restriction or rapamycin indeed extends lifespan (Hansen et al. 2008; Alvers et al. 2009).

### 10.4.2 Mitochondrial Biogenesis

mTORC1 downregulates mitochondrial oxygen consumption by activating HIF-1, which enhances glycolytic flux. In yeast, it has been found that inhibition of mTORC1 results in a metabolic shift towards greater mitochondrial respiration and results in increased chronological lifespan (Ruetenik and Barrientos 2015). It has been shown that yeast strains with reduced target of rapamycin (TOR) signalling have greater overall mitochondrial electron transport chain activity, which provides an adaptive signal during growth by increasing expression of mitochondrial manganese superoxide dismutase (Pan et al. 2011).

### 10.4.3 Stem Cell

Decline in stem cell function is one of the major hallmarks of aging and the onset of age-related diseases. It has been confirmed in various studies that inhibition of mTORC1 preserves or may even revive the stem cells in various tissues. Rapamycin can restore normal self-renewal capacity of haematopoietic stem cells (HSCs) that have high oxidative stress and decreased functional capacity. In mice, inhibition of mTORC1 by rapamycin protects aged mice from influenza by rejuvenating HSCs (Chen et al. 2009). In addition to this, inhibiting mTOR can also enhance somatic cell reprogramming to promote production of induced pluripotent stem cells.

### 10.4.4 Inflammation

Aging has been marked by chronic, low-grade inflammation, and it has been found that dietary restriction extends healthspan and lifespan by ameliorating inflammatory components. Hyperactivated mTOR has been linked to increased inflammation (Johnson et al. 2013).

### 10.4.5 Protein Synthesis

Among the various functions performed by mTOR, regulation of mRNA translation and protein synthesis is most crucial. It has been proposed that a significant reduction in overall protein synthesis could be beneficial during aging and maintains protein homeostasis. According to this view, hyperfunctional biosynthetic and

proliferative processes like protein synthesis result in many age-related pathologies that are essential during growth and development but deleterious during adulthood. Evidence shows that regulation of mRNA translation and protein synthesis may extend lifespan in yeast, worms, fruit flies and mice (Showkat et al. 2014).

## 10.5 Rapamycin: Inhibiting mTOR to Extend Lifespan

Rapamycin and derivatives of this compound have been used clinically as a prescription drug to prevent organ rejection after kidney transplantation. Clinical studies have revealed that rapamycin can be used to treat age-related diseases such as type 2 diabetes, atherosclerosis, heart hypertrophy, osteoarthritis, various neurodegenerative diseases such as Alzheimer's and Parkinson's as well as cancer (Blagosklonny 2010).

In 2009, intervention testing programme of NIA first revealed the life-extending effects of rapamycin on both male and female mice (Harrison et al. 2009). In this study, the treatment was initiated at two different ages (270 days and 600 day). In both the groups, rapamycin extended the median and maximum lifespan; an effect more pronounced in females than in males. This gender difference effect is because of higher blood concentrations of the compound found in females at a given rapamycin concentration in rat chow. However, these gender difference effects on lifespan have not been confirmed in other studies. In another ITP study, rat administration of rapamycin began at the age of 9 months. It was found that rapamycin increased mean lifespan in males and females by 10% and 18%, respectively, and maximum lifespan by 16% and 13% (Miller et al. 2014).

Other studies have also confirmed the beneficial effect of rapamycin on lifespan. It has been found to decrease mortality in aged mice (Lamming et al. 2013). It has also been found to increase lifespan of 129/SV inbred strain mice and also decrease the risk of tumour incidence (Anisimov et al. 2011). All these observations have made rapamycin a suitable candidate for an anti-aging drug. However, the exact mechanism through which these effects of rapamycin occur is yet to be understood completely. In rat model of Alzheimer's disease, rapamycin has been found to provide protection against amyloid-β-induced oxidative stress (Singh et al. 2016). Synergistically with metformin, rapamycin reverses age-dependent oxidative stress in rats (Singh et al. 2017a). It has been found to reverse the aging-induced impaired activities of membrane-bound ATPases and altered levels of redox biomarkers in erythrocyte membranes (Singh et al. 2017b).

## 10.6 Conclusion

The search for an anti-aging intervention is long and elusive. mTOR is a critical mediator of the cellular response, and it has been found to extend lifespan in yeast, invertebrates and mice. However, the complexity of this pathway acts as a stumbling block in understanding how this pathway influences healthspan and longevity. There

are several reports showing the beneficial effects of rapamycin on a diverse array of age-related diseases. However, a long-term study of rapamycin treatment in mice reported increased incidence of cataracts and testicular degeneration (Fischer et al. 2015). In humans, rapamycin supplementation has been found to produce a number of side effects including hyperlipidaemia and hyperglycaemia, anaemia and stomatitis (Kaplan et al. 2014). As rapamycin is an immunomodulatory drug, inhibition of mTORC1 may produce a negative effect on immune system and wound healing.

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# Autophagy Induction: A Promising Antiaging Strategy

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**Abstract**

Aging is a multifactorial biological phenomenon manifested by oxidative damage of biomolecules and cell organelles and their continuous accumulation, resulting in progressive loss of physiological functionality and high risk of mortality. Efforts to develop strategies for extending health span and lifespan are now in spotlight of geroscience. There are several studies suggesting the involvement of autophagy in aging. Autophagy is a conserved and protective intracellular lysosomal degradation process that ensures continuous removal and recycling of accumulated biomolecules and nonfunctional cell organelles to maintain cellular homeostasis and overall functionality of the cells. The age-dependent defective autophagy has also been suggested to further accelerate aging and increase the risk of other aging-related diseases. In addition, autophagy integrates several pro-survival pathway(s) as associated with AMP kinase (AMPK) and mammalian target of rapamycin (mTOR) to regulate growth, division, motility and overall survival of the cells. The pharmacological modulators of autophagy have been found rewarding in case of aging, and thus it is promising to expect autophagy modulators to be the next-generation antiaging drugs. This chapter summarizes the existing advances, perspectives, and challenges in the area of antiaging through induction of autophagy.

**Keywords**

Aging · AMPK · Autophagy · mTOR · Oxidative stress · Sirtuins

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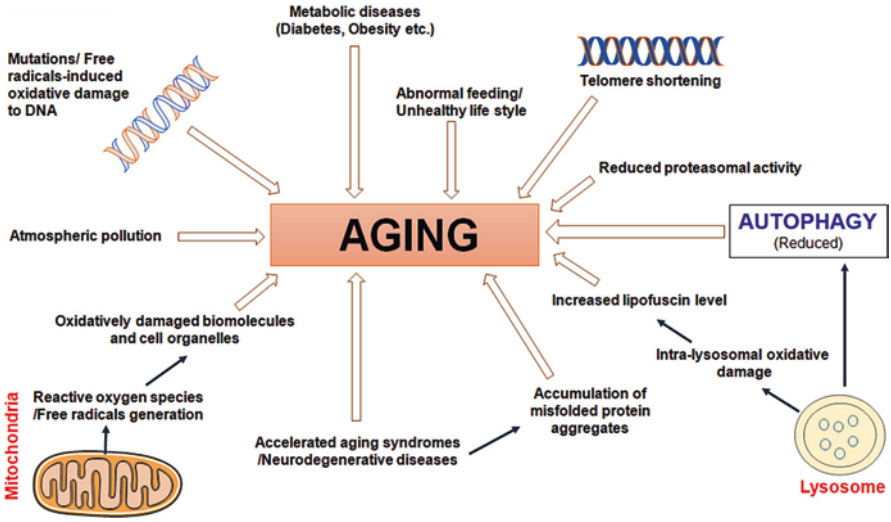


**Fig. 11.1** Different factors that contribute to the progression of aging- and age-related degenerative diseases

Aging is an intricate biological phenomenon which is generally manifested by progressive and persistent decline of physiological performance and increased probability of morbidity and mortality (Finkel and Holbrook 2000). Moreover, aging is also marked by the constant accumulation of oxidized biomolecules and oxidatively damaged cell organelles which increase the susceptibility toward several health risks such as cancer, type 2 diabetes, and neurodegenerative and cardiovascular diseases (Niccoli and Partridge 2012; Korovila et al. 2017). Global efforts of gerontologists have made great strides to enhance our understanding of the aging biology, and several hallmarks of aging have been identified that play a causative role in the pathophysiology of several aging-associated diseases, as shown in Fig. 11.1 (López-Otín et al. 2013, 2016; Kennedy et al. 2014). Although various theories of aging have been proposed, the oxidative stress theory has been extensively explored (Harman 1956). According to Harman, the aging process is mainly attributed to excessive generation of free radicals and continuous accumulation of free radicals mediated oxidized biomolecules in the cells and tissues that lead to increased risk of morbidity and mortality (Harman 1956, 2003). Moreover, aging is also caused at genetic and epigenetic levels mainly influenced by environmental risk factors (Kirkwood 2005).

Oxidative stress is a situation of redox imbalance where free radicals devastate the antioxidant competencies (Sies 2015). ROS are constantly generated endogenously through normal mitochondrial electron transfer reactions. Moreover, exogenous ROS results from sources such as environmental pollutants coming in contact by evading our ecosystem. Thus, the resulting ROS exerts harmful effects by initiating toxic biochemical reactions such as extensive lipid peroxidation and oxidation of nucleic acids and proteins causing accumulation of toxic protein

aggregates (Poli et al. 2004). The oxidized protein aggregates and damaged DNA further trigger either necrotic or apoptotic cell death which lead to impaired physiological functionality and progressive aging (Halliwell 1994). Therefore, the misfolded protein aggregation is the major manifestation of aging as well as aging-related degenerative disorders (Taylor et al. 2002). In addition, free-radical-mediated damaged mitochondrial DNA stimulates mitochondrial shutdown, causing cells to die and organisms to age (Shigenaga et al. 1994). Thus, these time-dependent accumulating damages are collectively believed to be fundamental cause of aging process. Additionally, oxidative stress further induces the progression of several diseases associated with aging (Calabrese et al. 2009, 2010; Texel and Mattson 2011; Rodriguez et al. 2015).

In normal conditions, cells boost up antioxidant defense machinery and other protective systems such as autophagy against oxidative stress (Garg et al. 2017; Singh et al. 2017b). In view of the above, a variety of antioxidants have been ardently sought as possible dietary supplements or therapeutic interventions for the management of aging- and age-dependent degenerative changes (Harman 1981; Floyd and Hensley 2002; Fusco et al. 2007). Recently, various experimental approaches are being developed as potential antiaging strategies. These antiaging approaches include reduction in food intake or caloric restriction, modulation of metabolic signaling pathways using natural and synthetic molecules, rejuvenation of stem cells, and elimination of oxidized biomolecules and damaged cell organelles accumulating during the progression of age through modulation of autophagy process. In the present chapter, attempts have been made to critically review the roles and benefits of autophagy against aging- and age-associated diseases.

## 11.1 Autophagy and Aging

Autophagy is an evolutionary conserved cellular cleanup process that ensures optimal cellular functionality by lysosome-mediated degradation/removal and continuous recycling of protein aggregates and damaged cell organelles (Ohsumi 2014). Autophagy is a multistep cellular process which is demonstrated in Fig. 11.2. The term first used in the 1960s is now proved to be an important adaptive mechanism during nutrient- and oxygen-deprived conditions and contributes to growth, development, and longevity of the organism. Alteration in the physiological process of autophagy has been shown in pathologies of several human diseases (Levine and Kroemer 2008; Jiang and Mizushima 2014). These connections imply that the therapeutic interventions to modulate autophagy process may be a beneficial strategy for the management of such diseases (Rubinsztein et al. 2012). Autophagy has been considered as a wonderful adaptive cellular process and also suggested to manage the aging process of the cells (Yang and Klionsky 2010). Moreover, an age-dependent defective autophagy has been suggested to further contribute to oxidative damage and accumulation of biomolecules, acceleration of aging, and therefore increases the risk of aging-related diseases (Cuervo et al. 2005).

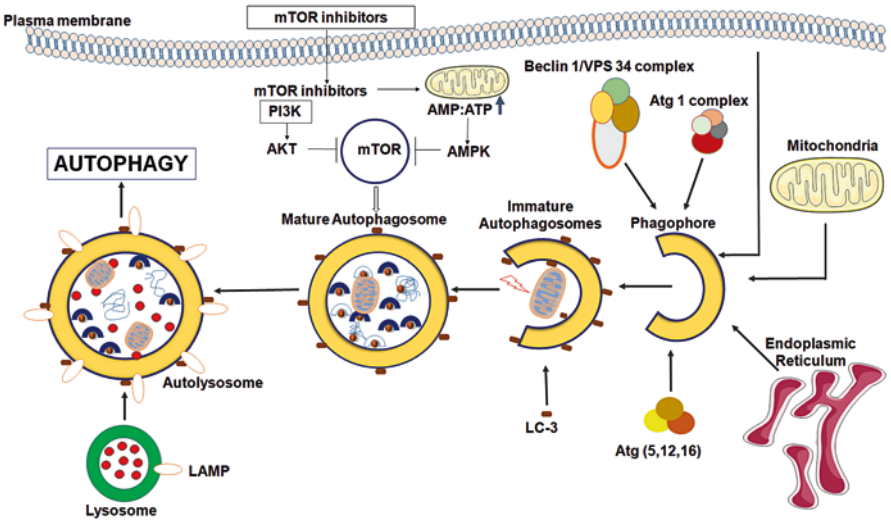


**Fig. 11.2** The diagrammatic representation showing the regulation of different sequential steps of autophagy process by different signaling pathways and a series of autophagy-related proteins. The steps involve the formation of autophagosome which eventually fuse to lysosome to form autophagolysosome, in which the autophagic cargo is enzymatically degraded and recycled to the cytoplasm. Abbreviations: *mTOR* mammalian target of rapamycin, *PI3K* phosphatidylinositol-4,5-bisphosphate 3-kinase, *AKT* protein kinase B, *AMP* adenosine monophosphate, *ATP* adenosine triphosphate, *VPS34* vacuolar protein sorting, *LC3* light chain 3B microtubule-associated *proteins LAMP* lysosomal-associated membrane protein, *Atg* autophagy-related protein

Depending upon the mechanism of cargo transportation to lysosomal lumen, autophagy has been mainly categorized into microautophagy, chaperone-mediated autophagy, and macroautophagy (also known as autophagy) (Kobayashi 2015). In microautophagy, the lysosome directly engulfs cargo through a sequential process of invagination, protrusion, and separation (Mijaljica et al. 2011). However, chaperone-mediated autophagy degrades soluble cytoplasmic proteins that contain a target motif of pentapeptide, KFERQ. Moreover, these cargo proteins along with cytosolic hsp70 and some other co-chaperone of Hsp-70 are targeted to the lysosome through interaction with LAMP2A membrane receptor (Periyasamy-Thandavan et al. 2009), and undergo rapid proteolysis by resident hydrolases (Kiffin 2004). Chaperone-mediated autophagy (CMA) is normally triggered under the influence of oxidative stress and hypoxic conditions, while it also becomes defective with the progression of age (Kiffin 2004; Bejarano and Cuervo 2010). A decreased LAMP-2A receptor has also been associated with defective CMA during aging (Cuervo and Dice 2000). An age-dependent decreased level of LAMP-2A receptor results in compromised interaction and translocation of substrates into lysosome (Cuervo and Dice 2000; Bandyopadhyay and Cuervo 2008). The restoration of CMA leads to improved cellular microenvironment and overall cellular functions during aging (Zhang and Cuervo 2008).

Macroautophagy, the most widely characterized autophagy process, is a sequential process initiated by the sequestering of cytoplasmic constituents to be degraded in a double-membrane-bound structure termed as autophagosome. These autophagosomes are transported to the lysosome where they fuse with it to form structures called autophagolysosomes (Weidberg et al. 2011). Both the inner membrane and material within the autophagolysosomes are degraded and recycled by lysosomal hydrolases (Glick et al. 2010; Axe et al. 2008; Hailey et al. 2010). There is a conserved family of Atg genes (AuTophaGy-related genes), and till now 32 Atg genes are reported in yeast and 14 Atg genes in mammals (Klionsky 2007). Phagophore formation starts with the interaction of Vps34 with a complex protein containing Atg6 (Beclin-1), Atg14, and Vps15. This along with other components such as Atg5, Atg12, Atg16, and FIP200 interacts with Atg1 (ULK1) and Atg13 to initiate early autophagy (Ravikumar et al. 2010).

Plethora of literature suggests that the defective autophagy contributes to the onset and progression of several aging-associated diseases (He et al. 2013; Choi et al. 2012; Lapierre et al. 2013). In addition, autophagy-related genes are found to be important for increased longevity *C. elegans* (Meléndez et al. 2003). Moreover, the inhibition of mTOR was also found to improve the longevity of *C. elegans* (Vellai et al. 2003). In line, the downregulated expression of Atg genes was found in aged human brain in comparison to young counterpart (Lipinski et al. 2010). Furthermore, the upregulated expression of autophagy markers such as LC3B, Atg5, and Atg12 has been shown to maintain the mitochondrial health and energetic homeostasis and increase lifespan (Mai et al. 2012). A group of $NAD^+$-dependent deacetylases, also known as sirtuins, are promising molecules that regulate autophagy process. There are seven mammalian sirtuins which play important role in deacetylation of proteins usually involved in autophagy machinery (Brenmoehl and Hoeflich 2013; Fang et al. 2016). Thus, targeting sirtuins is also an effective approach for improving longevity and healthy aging.

## 11.2 Caloric Restriction-Mediated Autophagy as an Antiaging Strategy

Caloric restriction (CR), one among the best antiaging strategies, improves lifespan and slows down the progression of aging-associated diseases (Anderson and Weindruch 2012). Although the mechanisms of CR is not clearly understood, studies involving rodents, primates, and humans suggest that CR controls multiple signaling pathways to regulate autophagy process that may ultimately extend lifespan and health span (López-Lluch and Navas 2016). CR improves mitochondrial metabolism by increasing mitochondrial content and function. Moreover, CR also affects mitochondria to generate less ROS and optimum ATP; thus CR reduces oxidative damage and progression of aging (Martin-Montalvo and de Cabo 2013).

Aging is mainly associated with dysfunctional autophagy and proteostasis processes which are mainly responsible for continuous removal and recycling of oxidized macromolecules especially aggregated proteins (He et al. 2013; Choi et al. 2012;

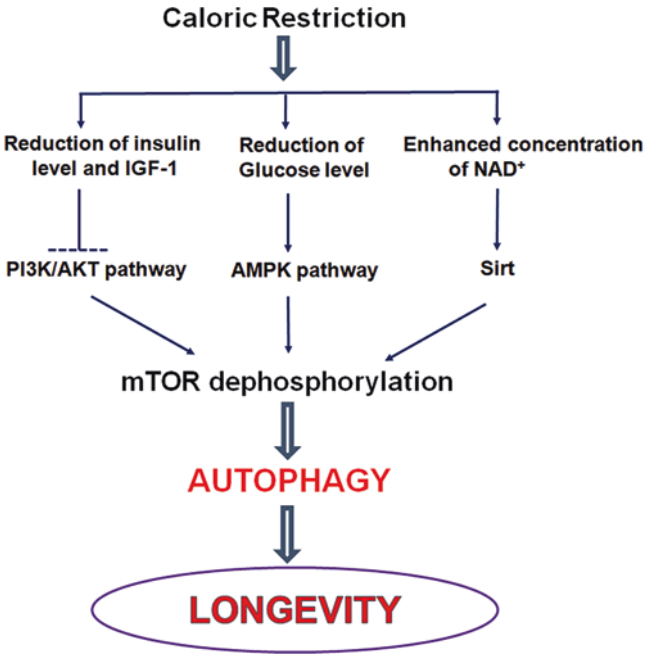


**Fig. 11.3** Schematic representation showing different pro-survival signaling pathways regulated by caloric restriction. *Abbreviations*: *PI3K* phosphatidylinositol-4,5-bisphosphate 3-kinase, *AKT* protein kinase B, *AMPK* adenosine monophosphate-activated protein kinase, *Sirt* sirtuin, *mTOR* mammalian target of rapamycin

Kaushik and Cuervo 2015). However, CR attenuates aging-induced dysfunctional autophagy and proteostasis processes in several species (Morimoto and Cuervo 2014; López-Lluch and Navas 2016). The antiaging effect of CR is regulated by several signaling pathways such as AMPK, mTOR, andIGF-1 etc., which are cardinal integrators of the autophagy process (Testa et al. 2014), as shown in Fig. 11.3. CR also stimulates autophagy process in aging rat heart (Dutta et al. 2014) and liver (Del Roso et al. 2003). Although CR is an effective antiaging strategy, it might be challenging due to its undefined regimen. To overcome the issue, caloric restriction mimetics (CRMs) have emerged as effective molecules for healthy aging. CRMs are either natural or synthetic compounds which mimic the benefits of CR by targeting the similar cellular and molecular events (Ingram et al. 2004; Ingram and Roth 2015).

## 11.3 Caloric Restriction Mimetics-Mediated Autophagy as Antiaging Strategy

CRMs stimulate autophagy process and deacetylate the proteins. The deacetylation is achieved by the compounds that (1) diminish acetyl coenzyme A, (2) inhibit acetyl transferases, and (3) stimulate deacetylases activities (Madeo et al. 2014). CRMs

increase the health span and longevity and reduce the risk diseases associated with aging via activation of autophagy (Pearson et al. 2008; Niu et al. 2013; Kibe et al. 2014). Additionally, several molecules have been identified as potential CRMs that slow down the aging process through activation of autophagy by mTOR inhibitors such as rapamycin (Singh et al. 2017a), glycolytic inhibitors such as 2-deoxy-D-glucose (Handschin 2016), AMPK activators such as metformin, and antioxidants and polyphenols such as resveratrol and fisetin (Mouchiroud et al. 2010; Singh et al. 2017b).

Recent evidence produced by our research group demonstrates that the activation of autophagy slows down aging process and increases longevity (Garg et al. 2017; Singh et al. 2017a, b). We have shown that CRMs such as rapamycin, fisetin, and metformin maintain redox balance and protect rat brain against aging-induced alterations via activation of autophagy. Resveratrol and rapamycin have been shown to delay aging and pathogenesis of associated diseases and increase lifespan in yeast, invertebrate species, and rodents (Kaeberlein 2010; Marchal et al. 2013; Blagosklonny 2013; Tresguerres et al. 2014; Ehninger et al. 2014). Moreover, the supplementation of polyamine spermidine and resveratrol has also been reported to increase the longevity of yeast, nematodes, and fruit flies through activation of autophagy (Morselli et al. 2009; Minois 2014). The antiaging effects of these CRMs become ineffective when autophagy process is inactivated, suggesting that autophagy is an essential process for CRMs-mediated antiaging effects (Mariño et al. 2014).

## 11.4 Mechanistic Cross-talk Between Autophagy and Antiaging Effects

Autophagy integrates several pro-survival signaling pathway(s) including AMPK and mTOR that maintain cellular homeostasis and overall functionality of the cells (Kim et al. 2011; Sarkar 2013). A number of longevity-related genes fall into three nutrient sensing pathways, viz., mTOR, insulin/IGF-1, and sirtuin pathways, which mainly sense cellular glucose level, amino acid, and $NAD^+$/NADH (Mazucanti et al. 2015).

Under the influence of nutrient starvation, growth factor deprivation, and stress signals, autophagy machinery is activated in the cells through inhibition of mTOR, a cardinal integrator of multiple signaling pathways that mainly regulates the autophagy process. Out of the two complexes of mTOR (mTORC1 and mTORC2), mTORC1 mainly modulates autophagy process (Hara et al. 2002; Kim et al. 2003; Jacinto et al. 2004; Vander Haar et al. 2007). Dysregulated mTORC1 activity has been implicated in several diseases associated with defective autophagy during aging; therefore mTOR inhibitors may be a promising strategy for managing these diseases (Santini and Klann 2011; Shafei et al. 2017).

Under glucose deprivation, energy sensor AMPK is activated which further induces alteration in metabolism of the cells by increasing $NAD^+$ level and sirtuin-1 activity to maintain energy homeostasis (Kim et al. 2011). AMPK directly regulates

mTOR and phosphorylates ULK1 (Ser 317 and Ser 777) to activate autophagy process (Kim et al. 2011). AMPK activates sirtuins that deacetylate and regulate the transcription of FoxO to prolong longevity (Burkewitz et al. 2014). Sirt1 and Sirt2 have been demonstrated to regulate autophagy process. Sirt1 directly regulates autophagy via deacetylation of Atg proteins. Upon translocation into nucleus, Sirt1 activates autophagy through induction of FoxO transcription factors (Ng and Tang 2013). Autophagy is also controlled by insulin/IGF-1 that maintains energy balance, growth, development, and differentiation (Renna et al. 2013).

## 11.5 Autophagy in Age-Related Diseases

Adoptive autophagy protects organisms against several aging-associated diseases such as neurodegenerative (Alzheimer's disease and Parkinson's disease), cardiovascular diseases, obesity, diabetes, and cancer. Neurodegenerative diseases are mainly caused by age-dependent accumulation of misfolded protein aggregates: accumulation of amyloid-β (Aβ) and tau proteins in case of Alzheimer's disease (AD) and α-synuclein in case of Parkinson's disease (PD). Moreover, neurodegenerative diseases are also caused by age-dependent autophagy dysfunction (Querfurth and LaFerla 2010; Nixon and Yang 2011). Thus, targeting autophagy may be a newer therapeutic strategy against neurodegenerative diseases. A number of autophagy modulators have been shown to improve Alzheimer's disease condition (Li et al. 2017). Rapamycin-induced autophagy has been shown to reduce Aβ and improve cognitive abilities (Spilman et al. 2010; Cai and Yan 2013). Recently, we have also shown that rapamycin-mediated induction of autophagy activates pro-survival PI3K-Akt-mTOR-CREB signaling pathway(s) that provide neuroprotection during age-related AD pathogenesis (Singh et al. 2017a). Therefore, the inhibition of mTOR and activation of AMPK restore autophagy and promote lysosomal degradation of Aβ (Grotemeier et al. 2010; Eisenberg-Lerner and Kimchi 2012). Similarly, evidence suggests that the activation of autophagy removes α-synuclein and maintains mitochondrial homeostasis in PD (Wang et al. 2016).

Under normal physiological condition, autophagy machinery acts at the basal level in myocardium, and age-dependent defective autophagy leads to cardiac dysfunction (Mei et al. 2015). In addition, autophagy is promptly increased under the influence of stressful conditions and plays a protective role; however excessive autophagy may also induce cell death (Rothermel and Hill 2008). Thus, autophagy is considered as double-edged sword which can either prevent or enhance the pathogenesis of diseases, depending on the situation and amplitude of induction (Rothermel and Hill 2007). The activation of autophagy has been suggested to reduce cardiac pathologies and increase lifespan (Hoshino et al. 2013; Pyo et al. 2013). In line, activation of mTOR and suppression of Sirt1 have been reported to downregulate autophagy in the heart (Hariharan et al. 2010; Sciarretta et al. 2012). Additionally, the pharmacological agents such as trehalose, propranolol, and verapamil were found to activate autophagy in cardiomyocytes (Fleming et al. 2011),

which protects heart tissue by reducing the death of cardiomyocytes in ischemic model (Sciarretta et al. 2012).

The major causes of obesity and type 2 diabetes are oxidative stress, mitochondrial dysfunction, and abnormal inflammatory signaling pathways. The pro-inflammatory cytokines regulate autophagy machinery in obese and type 2 diabetes patients (Harris 2011). In addition, the mutation in autophagy gene Atg7 has been implicated in impairment of glucose tolerance and circulating insulin concentration and reduced pancreatic insulin content, suggesting that autophagy is crucial for efficient functioning of pancreatic beta cells and insulin target tissues (Marsh et al. 2007; Ebato et al. 2008; Jung et al. 2008; Barlow and Thomas 2015). Increased autophagy also leads to apoptosis of subcutaneous adipose tissues in patients with obesity and visceral adipose tissues in patients with type 2 diabetes (Kosacka et al. 2015). The oxidative stress in pancreatic beta cells is regulated by autophagy (Kaniuk et al. 2007), and the defective autophagy in pancreatic beta cells favors the progression of disease from obesity to diabetes (Quan et al. 2012).

## 11.6 Conclusion

In the present chapter, we provided substantial review on the involvement of autophagy process in the biology of aging- and age-related diseases. Defective autophagy contributes to accumulation of oxidized macromolecules and oxidatively damaged cell organelles that eventually lead to aging and onset of age-related degenerative diseases. Therefore, the therapeutic consideration for autophagic modulators would be essential for developing and designing better antiaging strategies.

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# Computational Methods for Developing Novel Antiaging Interventions

12

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**Abstract**

Advances in computational methodologies have ushered in innovations in visualization, calculations, and prediction of factors relating to aging processes and concomitant diseases with novel strategies like comparative genomics, protein interactive networks, and systems biology. Molecular level investigations of antiaging agents like phytochemicals such as curcumin, resveratrol, and quercetin have been carried out by electronic structure calculations by density functional theory and molecular docking studies to cytochrome P450 3A4 protein. It is found that both hydrogen bonding and hydrophobic interactions play a crucial role in the interaction between these phytochemicals and CY3A4 protein, which may provide important insights into modulations of drug metabolism in aging populations.

**Keywords**

Molecular docking · Antiaging · Phytochemicals

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## 12.1 Introduction

Aging is dependent on a web of combined and interdependent interactions between various components of the human system as well as the continuous equilibrium between damage and repair (Suresh et al. 2013). Even revolution of healthcare sector in recent decades has not managed to relieve the elderly of age-related risk factors for the majority of complex diseases, including metabolic and neurodegenerative disorders such as loss of vigor (Christensen et al. 2009; Comfort 1964). As there is neither single mechanism to control aging nor any functional failure of any individual tissue, organ, or system, understanding aging processes can be tricky, but in recent decades, this has been made possible with the rise in scale and accuracy of computational methods.

An example of this can be illustrated through the recent hierarchical multimethod simulation framework adopted to investigate mitochondrial dysfunction prevalent in aging processes, which included monitoring mitochondrial cell populations with several added aging parameters from literature to observe mitochondrial stress responses, damage repair, states of cellular senescence, and mitochondrial count (Hoffman 2017).

Genomic instability, telomere attrition, epigenetic alterations, perturbation of cellular homeostasis, and stem cell exhaustion are some of the various interconnected indications of aging which are common among organisms (Lopez-Otin et al. 2013; Kirkwood 2005), and these have been the target of several computational investigations. Sirtuins, which are related to antiaging pathways, were studied by molecular docking methods, along with resveratrol, which were carried out with HNF-1a structural motif related to type II diabetes mellitus (Kaladhar 2011).

Longevity in different species may be enhanced by replicating energy deprivation conditions through reduced nutrient intake dietary restriction (DR) or genetic/pharmacological interventions (Fontana et al. 2010). Various age-related metabolic, autoimmune, neurodegenerative, cardiovascular diseases may be protected against with the help of DR. By employing the *mummichog* and MetaboAnalyst program, system biology approaches have been directed toward exploring metabolomic explanations of how DR can slow aging rates in *Drosophila* species (Laye et al. 2015). The authors have demonstrated how the methionine metabolism pathway gives rise to intermediates like homocysteine that can lead to aging factors like endothelial cell death and increase in risk of Alzheimer's disease. In recent years, the introduction of autophagy has emerged as a common attribute of several antiaging interventions (Gelino and Hansen 2012) including both DR and reduced target of rapamycin signaling (Alvers et al. 2009). Intermolecular interactions with enzyme active sites of inducers of autophagy like anacardic acids have been explored through density functional theory in earlier computational studies (Marino et al. 2014).

We have highlighted examples of useful in silico methods including algorithms for modeling the complex genetic network of human aging and data mining methods, such as comparative genomics. We have also illustrated how dietary

phytochemicals, which are effective antiaging agents, can be evaluated computationally and may be used further for investigations on binding to specific enzymes important for drug metabolism.

## 12.2 Computational Methods for Understanding and Reversing Aging Processes

### 12.2.1 Modeling Aging Networks

High-performance genomic and proteomic discoveries produced an abundance of openly available data on aging, the retrieval and analysis of which is essential for better comprehension of causes and effects of aging. Databases like GenAge provide information regarding the interactions of antiaging genes in several organisms including humans along with environmental factors. Powerful computational tools can analyze those big data and generate patterns in order to be aware of the genetic network involved in aging processes (Boyd and Crawford 2012) which is evident in case of microarray incorporated databases and analysis platforms like Gene Aging Nexus and AGEMAP (Cevenini et al. 2010).

### 12.2.2 Protein-Protein Interaction Networks in Aging

Protein-protein interaction networks (interactomes) involve proteins as elements which are linked together through physical interactions, and the strength of these interactions is related to the weights of a protein-protein interaction link. However, these links are directionless owing to this probabilistic nature of the network concept. The protein-protein interaction (PPI) networks of aging-associated genes are sub-networks of the interactomes which are composed of links between aging-related genes, which is illustrated in the case of humans in (Fig. 12.1). Schematic diagram of the genetic network study of human aging is depicted in (Fig. 12.2). Datasets like HIPPIE (Human Integrated Protein-Protein Interaction Reference) have evolved for network investigations along with computational topological study of such networks in case of neurodegenerative diseases (Goñi et al. 2008; Schaefer et al. 2012).

### 12.2.3 Comparative Genomics of Aging

Data mining possibilities for comprehending the digital genetic information relevant to aging, which was presented by the human genome sequencing project, form the backbone of comparative genomics which aid in collecting data relating to protein interactions and finding and function allocation to new genes as well as unknown genes, respectively (Brazhnik et al. 2002), along with characterization of the gene

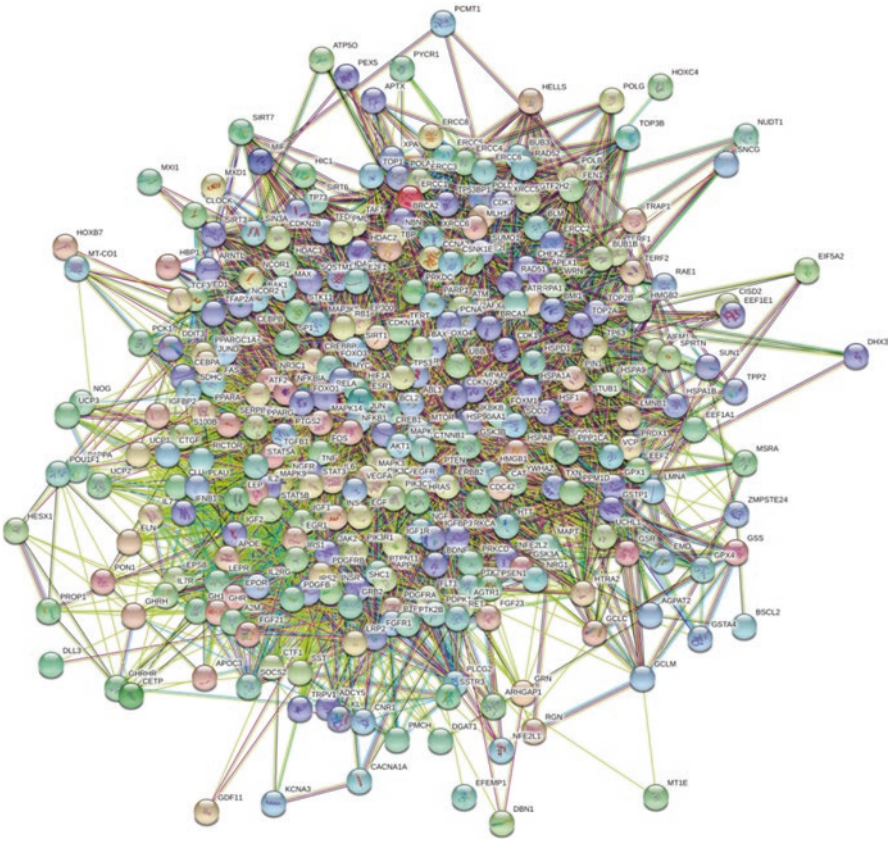

**Fig. 12.1** The human protein-protein interaction network of aging-associated genes. 306 aging-associated genes were assembled using the GenAge Human Database

function (Qin et al. 2010). Comparison of several different genomes in organisms is important due to genomic links to common ancestors correlated with the process of evolution. Algorithms like ClustalW can help in study of domains related to DNA repair and their evolution as in the case of DNA repair proteins of *Escherichia coli* and *Saccharomyces cerevisiae* by several alignments of protein families compared with available genomic sequences. This has also been facilitated with the help of computational tools like the "VISTA" suite which provides visualization of entire genomic sequences and alignment options (using "AVID" alignment tool) to new/user-defined genomic sequences (Frazer et al. 2004). In the long run, we may be capable of evaluating and finding connections in protein families belonging to mammals with contrasting aging rates. The application of comparative genomic methodologies in mammals is limited to requirement for several fully sequenced genomes, which may be fulfilled soon.

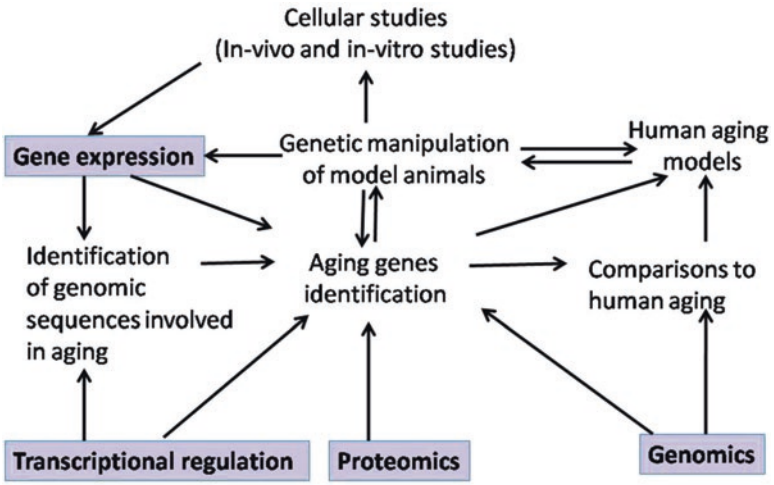


**Fig. 12.2** Schematic diagram of the genetic network study of human aging (De Magalhães and Toussaint 2004)

### 12.2.4 Transcriptional Regulation of Aging

Transcriptional regulation, though a complex process, is also digital in nature as it can be found largely in the form of *cis*-regulatory genetic sequences (noncoding) which are specifically targeted by transcription factors (TFs) that control gene action along with the binding proteins. Bioinformatics tools include phylogenetic footprinting along with several systems biology approaches which have been employed to investigate transcriptional profiling in muscle aging, Alzheimer's, as well as polycystic kidney disease (Song et al. 2009; De Magalhães and Toussaint 2004; Miller et al. 2008; Zahn et al. 2006).

### 12.2.5 Computational Epigenetics

Epigenetics encompass both transmissible changes in gene activity and expression and also durable changes in the cell transcriptional potential that may or may not be passed on. Any dysfunctions in epigenetic regulation due to physiological, pathological, and environmental factors can hasten aging processes (Vincenzo et al. 2009; Fraga and Esteller 2007; Benayoun et al. 2015). Computational epigenetics have evolved as predictive tools of epigenetic variations as well as for selection of biomarkers for related diseases like cancer (Bock and Lengauer 2008). Such statistical analysis is aided by computational software like EpiGRAPH, Galaxy, and R/Bioconductor which facilitate DNA methylation mapping along with genome processing and biomarkers candidate options through microarray development (Bock 2009).

## 12.3 Computational Evaluation of Phytochemicals Related to Antiaging

### 12.3.1 Phytochemicals and Antiaging

Having established the multiple aspects related to aging, the spotlight now turns to certain naturally occurring compounds which can regulate aging pathways and combat age-related diseases and are often a part of daily diet. Majority of phytochemicals, which are mostly secondary metabolite plant products, are known to regulate signaling pathways, cellular metabolism, enzyme activity, and stress resistance as well as possess antioxidant and anti-inflammatory action (Pandey and Rizvi, 2009). Green tea contains polyphenolic chemoprotective agents known as catechins which possess antioxidant properties that can delay age-related decay. Catechins not only prevent cancer but also defend against DNA oxidative damage, cerebral atrophy apart from being neuroprotective. The polyphenolic compound curcumin (found in turmeric) has been used extensively in herbal medicine due to its proposed oxidant-radical scavenging, anti-inflammatory, and anticancer effects.

Quercetin is a polyphenolic flavonoid, which not only possesses free radical scavenging, antithrombotic, anticancer, and antidiabetic activity but also protects against apoptotic neuronal cell death (Pandey and Rizvi 2009). Human CYP1A2 is directly engaged in flavonoid metabolism, and this is responsible for regulation of creation of metabolites with diverse biochemical properties relative to the parent compound, and expression may differ from individual to individual. Resveratrol, a polyphenolic compound, has several antiaging properties like anticarcinogenic and anti-inflammatory activity along with boosting expression of antiaging Klotho genes (Hsu et al. 2014). Quantum chemical calculations show that elongation of conjugated chain in resveratrol, particularly in *s-cis* conformations, may enhance its antioxidant and radical scavenging ability (Lu et al. 2013).

### 12.3.2 Molecular Properties of Phytochemicals

Molecular structure and properties play a crucial role in biochemistry. Gathering of knowledge regarding the arrangement of atoms and molecules is essential in order to be able to study chemical properties and process. Nowadays, computational investigations act as emerging tools for studying a number of molecular parameters (Pattanayak and Chowdhuri 2013a, b, 2014; Chowdhuri and Pattanayak 2013; Chand. et al. 2017). In this chapter, density functional theory calculations were done by using the Gaussian 09, Revision D.01 software package (Frisch et al. 2013) with GaussView as the graphical user interface. The optimized structure was obtained using the HF (Hartree-Fock) theory method. The choice of basis sets for these types of calculations may be chosen to be at the level of either 3-21G or 6-31G(d,p). The polarization ensures adequate description of lone pair electrons. One of the objectives of this work is to investigate the intrinsic electronic properties of phytochemical molecules. Recently, a correlation of electronic properties with antioxidant action of

other phytochemical compounds, which protect DNA against damage from radical oxygen species (ROS), was drawn from DFT calculations (Zerrouki and Farad 2018).

We have calculated the energy of the highest occupied molecular orbital ($E_{HOMO}$), energy of the lowest unoccupied molecular orbital ($E_{LUMO}$), energy gap, Mulliken charges on the different atoms, dipole moment (μ), ionization energy (I), electron affinity, absolute electronegativity (χ), absolute hardness (η), and absolute softness (σ).

### 12.3.3 Mulliken Charges, HOMO-LUMO, and Chemical Reactivity Descriptors Analysis of Curcumin and Resveratrol Phytochemicals

The Mulliken atomic charges of curcumin and resveratrol were calculated by HF level of theory (Fig. 12.3). The most important orbitals in the molecule are the frontier molecular orbitals, called the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO). The HOMO-LUMO of curcumin and resveratrol is shown in Fig. 12.4. Both the ionization potential and the electronegativity are playing an important role for studying the chemical reactivity behavior of atoms and molecules. According to the theorem of Koopmans (Koopmans 1933), all the terms like ionization potential (I), electron affinity (A), the electronegativity (χ), global hardness (η), and softness (S) are often expressed in the energy terms of HOMO and LUMO. Ionization potential (I) is related to the negative value of $E_{HOMO}$. On the other hand, the electron affinity (A) is related to the negative value of $E_{LUMO}$. The HOMO energies of curcumin and resveratrol are found to be −7.82 eV and −7.5 eV, respectively. The LUMO energies of curcumin and resveratrol are found to be 1.3 eV and 2.53 eV, respectively. The total energy (au) of curcumin and resveratrol was found to be −1249.0184 and −761.7424859, respectively. The chemical softness(S) was estimated to be 0.219 and 0.199, respectively, for curcumin and resveratrol phytochemicals.

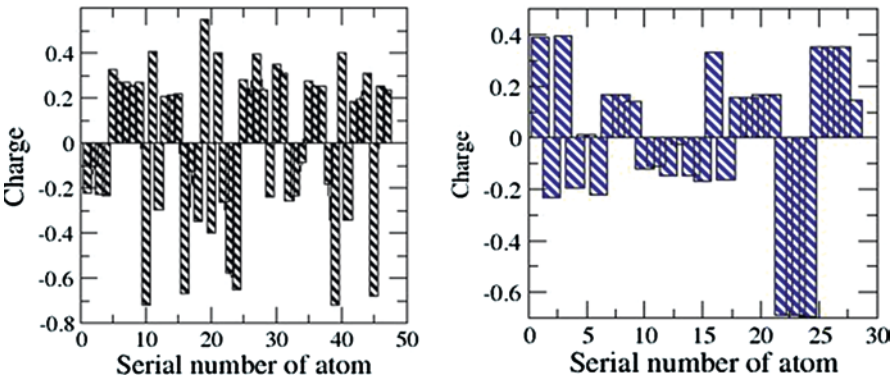


**Fig. 12.3** Mulliken charges of curcumin (left panel, black color) and resveratrol (right panel, blue color) studied phytochemicals by HF level of theory

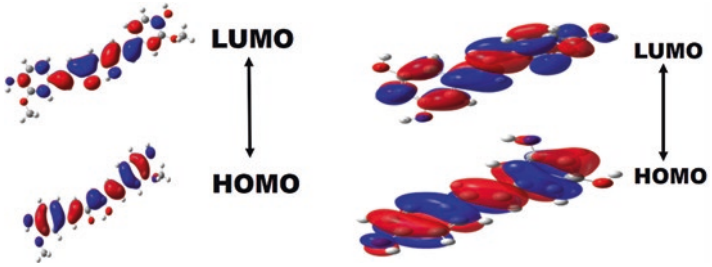


**Fig. 12.4** The frontiers energies of HOMO-LUMO of curcumin (left panel) and resveratrol (right panel) studied phytochemicals

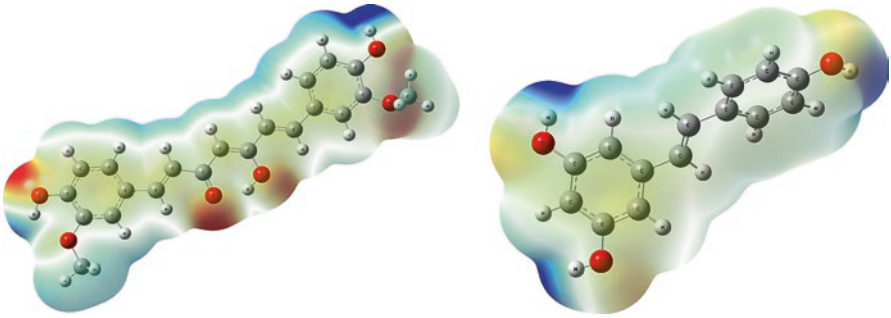

**Fig. 12.5** The molecular electrostatic potentials map of curcumin (left) and resveratrol (right). Red color atom represents the oxygen; gray color atom represents carbon atoms, whereas small white color atoms represent the hydrogen atom. Red color surface represented the regions of the most negative electrostatic potential; blue color surface represents the regions of the most positive electrostatic potential, and green color surface presents the region of zero potential

### 12.3.4 Molecular Electrostatic Potentials (MEPs) of Curcumin and Resveratrol

Molecular electrostatic potential (MEP) simultaneously displays molecular shape, size, and electrostatic potential in terms of color grading. MEP maps are helpful tools in the analysis of the correlation amid molecular structures with its physiochemical property relationship, including biomolecules and drugs (Karabacak et al. 2012). MEP map generated at the optimized geometry of the title molecules using GaussView 5.0 program is shown in Fig. 12.5. It can be seen that the negative regions are mainly over the oxygen atoms. Negative (red color) and positive (blue) regions of electrostatic potential are associated with electrophilic and nucleophilic reactivity. These active sites are found to be clear evidence of biological activity in the title compound.

**Table 12.1** The dipole moment in different direction (μ), polarizability (Δα), and the different components of hyperpolarizability (β) of resveratrol

| Dipole moment in different direction | Value (D) | Polarizability | Value (a.u.) | First-order hyperpolarizability | Value (a.u.) |
|---|---|---|---|---|---|
| $\mu_x$ | 0.6278 | $\alpha_{xx}$ | 199.6446215 | $\beta_{xxx}$ | −63.884163 |
| $\mu_y$ | 0.2162 | $\alpha_{xy}$ | −59.5944327 | $\beta_{xxy}$ | −303.2532889 |
| $\mu_z$ | −1.0578 | $\alpha_{yy}$ | 201.6239407 | $\beta_{xyy}$ | 454.3266286 |
| μ | 1.2489 | $\alpha_{xz}$ | −2.8332854 | $\beta_{yyy}$ | −260.9120348 |
| | | $\alpha_{yz}$ | −0.8779133 | $\beta_{xxz}$ | −11.140439 |
| | | $\alpha_{zz}$ | 67.8875336 | $\beta_{xyz}$ | 6.7196338 |
| | | | | $\beta_{yyz}$ | 7.1483656 |
| | | | | $\beta_{xzz}$ | −28.7739009 |
| | | | | $\beta_{yzz}$ | 28.952763 |
| | | | | $\beta_{zzz}$ | −2.0943099 |

### 12.3.5 Polarizability and First Hyperpolarizability Properties

The static dipole moment, mean polarizability, and first hyperpolarizability properties, which arise of electromagnetic field by different interactions, have been used in various fields. The total dipole moment $\mu_{tot}$ of the studied molecules can be achieved by Taylor expansion. The detailed calculation of molecular dipole moment (μ), the linear polarizability (α), and the first-order hyperpolarizability has been discussed previously (Tanak and Toy 2013). On the basis of the finite-field approach, using HF basis sets, the first hyperpolarizability (β), dipole moment (μ), and polarizability (α) for resveratrol are calculated and given in Table 12.1.

## 12.4 Interaction of Cytochrome P450 3A4 Protein with Phytochemicals Against Aging Disorders

### 12.4.1 Aging Effects on Cytochrome P450 3A4 Protein

CYP3A4 gene is encoded for the protein cytochrome P450 3A4 with sequence length of 503 amino acid residues. This abundantly expressed enzyme is involved in a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent electron transport pathway. Cytochrome P450 3A4 catalyzes a number of metabolic processes including aliphatic oxidation and aromatic hydroxylation and plays an important role in drug metabolism. It has been found that aging processes particularly in patients with liver ailments can cause selective decline in content of CYP3A4 protein from hepatic cells (George et al. 1995; Kinirons and O'Mahony 2004) and may affect functionality of the protein in drug metabolism of erythromycin and cyclosporine among others. Aging also affects expressions of the steroid and xenobiotic receptor (SXR) which targets the CYP450 genes (Miki et al. 2005).

### 12.4.2 Protein-Protein Interaction Network of CYP3A4

Protein-protein interacting network patterns indicate that the CYP3A4 was interacting with resulting over the rest ten genes, which interacts with all the proteins present in the network. By combining all the patterns with aging disorder associated with protein-protein interaction, all the proteins are found responsible for aging disorders, in which all are proved by different sources. The interaction network of CYP3A4 with others is shown in Fig. 12.6. The interaction (in terms of probability score) between CYP3A4 and other proteins is analyzed by STRING database which is given in Table 12.2.

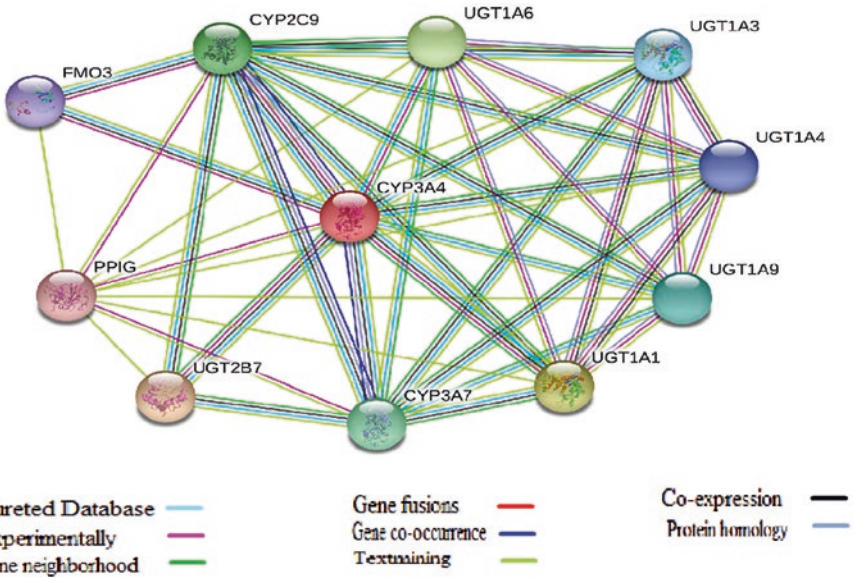


**Fig. 12.6** Interaction of CYP3A4 protein (present in the center) with other ten relevant proteins by STRING software

**Table 12.2** Protein-protein interaction with CYP3A4 analyzed using STRING database

| Node1 | Node2 | Score |
|---|---|---|
| CYP3A4 | UGT2B7 | 0.36 |
| CYP3A4 | UGT1A1 | 0.36 |
| CYP3A4 | UGT1A6 | 0.36 |
| CYP3A4 | CYP2C9 | 0.36 |
| FMO3 | CYP3A4 | 0.181 |
| CYP3A4 | PPIG | 0.042 |

### 12.4.3 Interaction of Protein CYP 3A4 with Different Phytochemicals

Pharmacokinetic interactions between phytochemicals and drugs may proceed through induction/inhibition of CYP enzymes, and these can have radical effects on efficiency of treatment by drugs or enhanced toxicity caused by increased drug concentration in body fluids (Dresser et al. 2000).

Curcumin can pose as enzyme substrate by competitively inhibiting human CYP3A4, which bio-modifies most drugs. In order to explore interactions of various curcumin analogues (CA) with human cytochrome P450 2 C9 (CYP2C9 or 2 C9), molecular docking and molecular dynamics (MD) simulation studies have been previously utilized with focus on binding site conformation. Hydrogen-bonding networks, whether direct or water-bridged between CAs and residues, boost up CAs-2C9 interactions in the binding sites of A0/2 C9 and C0/2 C9, but specifically, hydrophobic interactions predominate in causing binding interactions in the B12 complex (Shi et al. 2012).

Green tea extracts (GTE) and more specifically catechins like (−) epigallocatechin-3-gallate (EGCG) have been also shown to reduce intestinal CYP3A activity, and the hydrogen-bonding potential of catechins may enable them to bind directly to CYP enzymes (Misaka et al. 2013).

Resveratrol irreversibly inhibits cytochrome P450 and suppresses rifampicin-induced expression of CYP450, possibly through modulation of pregnane X receptor (Deng et al. 2014). Muntafiah et al. investigated the antiaging effect of pumpkin seed extract (PSE) on NIH 3T3 fibroblast normal cell induced by doxorubicin. Based on their in vitro test, PSE was not cytotoxic to NIH 3T3, and it was also found that tocopherol (−107,409) has a higher interaction to CYP3A4 compared to doxorubicin (−70, 52). Both of these compounds have a similar binding site in Leu 364, Phe 435, Pro 434, Cys 442, Ile 369, Thr 309, and Ala 305. Tocopherol, which protects against reactive oxygen species responsible for aging, has been computationally analyzed with relation to its stereochemistry and thermodynamic stability in addition to evaluation of its optimized enthalpy (Cho and Richard 2017).

## 12.5 Interaction Between Cytochrome P450 3A4-Protein and Phytochemicals by Molecular Docking

In the field of molecular docking, docking is the method which refers to the computational simulation of a candidate ligand binding to a receptor and is frequently used to predict the preferred binding orientation of small molecule candidate to their protein targets in order to in turn predict the affinity and activity of the small molecule (Bissoyi et al. 2017, 2018). The focus of molecular docking is to computationally simulate the molecular recognition process along with optimization of protein and ligand conformation aiming at overall minimization of free energy of the system. Scoring functions have also been developed to predict the strength of other types of intermolecular interactions, for example, between two proteins or between

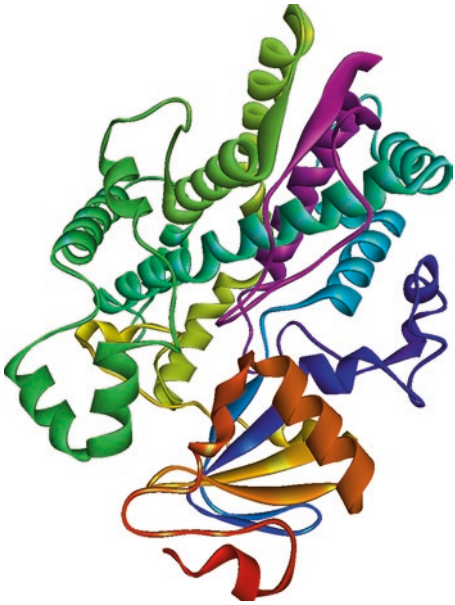

**Fig. 12.7** Overall representation of ribbon structure of human cytochrome P450 3A4 (PDB ID 5VVC) having resolution 1.74 Å. The figure was generated by using visualization software of Discovery Studio suits version 4.5

protein and drug or phytochemical molecules. These configurations are evaluated using scoring functions to distinguish the experimental binding modes from all other modes explored through the searching algorithm. Recently, in silico studies involving molecular docking and simulations were adopted to investigate metabolic pathways by binding of several CYP enzymes including CYP3A4 to pro-carcinogenic agents (Khan et al. 2017). For prediction of interaction of receptor and ligand, various tools have been used, but in this chapter, AutoDock Vina was used to find out the binding affinity and interacting active side residues of protein cytochrome P450 3A4 with phytochemical compounds. After docking job was completed, the visualization and image preparation was performed by using Discovery Studio Visualizer (Visualizer 2005). The intermolecular interaction study of the cytochrome P450 protein with different phytochemicals which shows the inhibitory effect against aging disorders was carried out, and these can be classified by the strength of their geometric constraints. The overall representation of ribbon structure of human cytochrome P450 3A4 (PDB ID 5VVC) is shown in Fig. 12.7. The docking analysis of phytochemicals with protein cytochrome P450 3A4 is given in Table 12.3. The binding energies of catechin, resveratrol, curcumin, and quercetin are found to be −9.8, −10.1, −9.9, and −10.4 kcal/mol, respectively. All the studied phytochemicals are shown to have hydrogen bond interaction with protein cytochrome P450 3A4 except catechin. The result shows that the catechin interacts with ARG106 and GLU374 residues that are involved in electrostatic bonding, and bond lengths vary from 4.43066 to 3.56382 Å, whereas PHE108, PHE215, and ARG106 residues are involved in hydrophobic bond, and hydrophobic bond lengths vary from 5.47019 to 4.44395 Å. The interaction between catechin and titled protein is shown in Fig. 12.8.

**Table 12.3** Docking analysis of phytochemicals with CYP3A4 (cytochrome P450 3A4)

| Phytochemicals | Pub chem Id | Binding energy (Kcal/mol) | Binding residue | Bond name | Bond length (Å) |
|---|---|---|---|---|---|
| Catechin | 9064 | −9.8 | A:ARG106:NH2 – :UNK0 | Electrostatic | 4.39117 |
| | | | A:ARG106:NH2:B – :UNK0 | Electrostatic | 4.43066 |
| | | | A:GLU374:OE1 – :UNK0 | Electrostatic | 3.56382 |
| | | | A:PHE108 – :UNK0 | Hydrophobic | 4.59713 |
| | | | A:PHE215 – :UNK0 | Hydrophobic | 4.44395 |
| | | | UNK0 – A:ARG106 | Hydrophobic | 5.47019 |
| Resveratrol | 445,154 | −10.1 | A:ARG106:NH2 – :UNK0:O | Hydrogen bond | 3.18199 |
| | | | A:ARG106:NH2:B – :UNK0:O | Hydrogen bond | 3.10361 |
| | | | A:ARG106:NH1 – :UNK0 | Electrostatic | 4.35842 |
| | | | A:ARG106:NH1:B – :UNK0 | Electrostatic | 4.46723 |
| | | | :UNK0 – A:PHE215 | Hydrophobic | 4.28371 |
| | | | :UNK0 – A:PHE108 | Hydrophobic | 4.92595 |
| | | | :UNK0 – A:ARG105 | Hydrophobic | 5.06186 |
| Curcumin | 969,516 | −9.9 | A:ARG106:NH1 – :UNK0:O | Hydrogen bond | 3.08077 |
| | | | A:ARG106:NH1:B – :UNK0:O | Hydrogen bond | 3.25095 |
| | | | :UNK0:H – A:ALA370:O | Hydrogen bond | 1.80965 |
| | | | A:ARG106:CD – :UNK0:O | Hydrogen bond | 3.51114 |
| | | | A:ARG106:CD:B – :UNK0:O | Hydrogen bond | 3.28924 |
| | | | :UNK0:C – A:GLU374:OE1 | Hydrogen bond | 3.77756 |
| | | | A:PHE215 – :UNK0 | Hydrophobic | 4.53403 |
| | | | :UNK0 – A:ARG372 | Hydrophobic | 4.77703 |
| Quercetin | 5,280,343 | −10.4 | :UNK0:H – A:SER119:O | Hydrogen bond | 2.43116 |
| | | | A:GLU374:OE1 – :UNK0 | Electrostatic | 3.88186 |
| | | | :UNK0 – A:PHE215 | Hydrophobic | 4.32817 |
| | | | :UNK0 – A:PHE215 | Hydrophobic | 4.30201 |
| | | | :UNK0 – A:PHE108 | Hydrophobic | 4.96197 |
| | | | :UNK0 – A:ARG105 | Hydrophobic | 5.1086 |

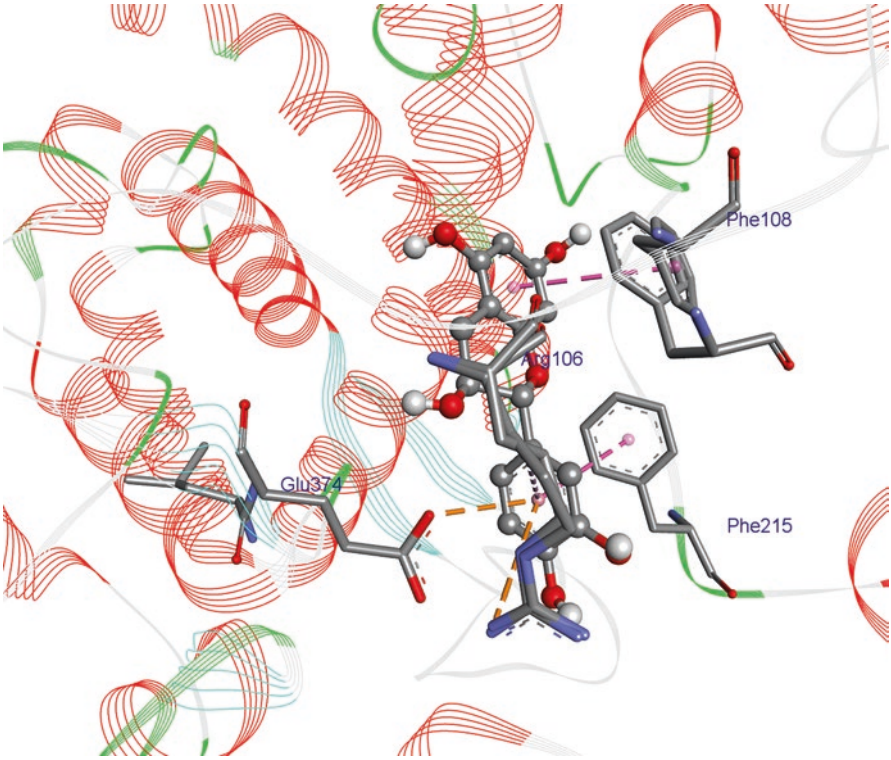


**Fig. 12.8** Schematic representation of interaction between cytochrome P450 3A4 with phytochemical catechin. Here ball and stick model represents the phytochemical. Pink dotted line represents the hydrophobic bonds between binding residues ARG106, PHE108, and PHE215. Deep yellow dotted lines show electrostatic bonds between binding residue ARG 106 and GLU374. The figure was generated by using visualization software of Discovery Studio suits version 4.5

The result shows that resveratrol interacts with ARG106 residue, which is involved in two types of hydrogen bonding with two different atoms of the resveratrol, and bond lengths are 3.18199 and 3.10361 Å, whereas PHE215, PHE108, and ARG105 residues are involved in hydrophobic bond, and bond lengths vary from 5.06186 to 4.28371 Å. The electrostatic bond involving ARG106 shows bond length of 4.35842 Å. The interaction between resveratrol and titled protein is shown in Fig. 12.9. The results show curcumin interaction with ARG106, ALA370, and GLU374 residues that are involved in hydrogen bonding, and bond lengths vary from 3.77756 to 1.80965 Å. However, PHE215 and ARG372 residues are involved in hydrophobic bond, and bond lengths are 4.53403 and 4.77703 Å. The interaction between curcumin and titled protein is shown in Fig. 12.10.

The result illustrates quercetin interaction with SER119 residue that is involved in hydrogen bonding, and bond length is 2.43116 Å. It is to be noted that PHE215, PHE108, and ARG105 residues are involved in hydrophobic bond, and bond lengths

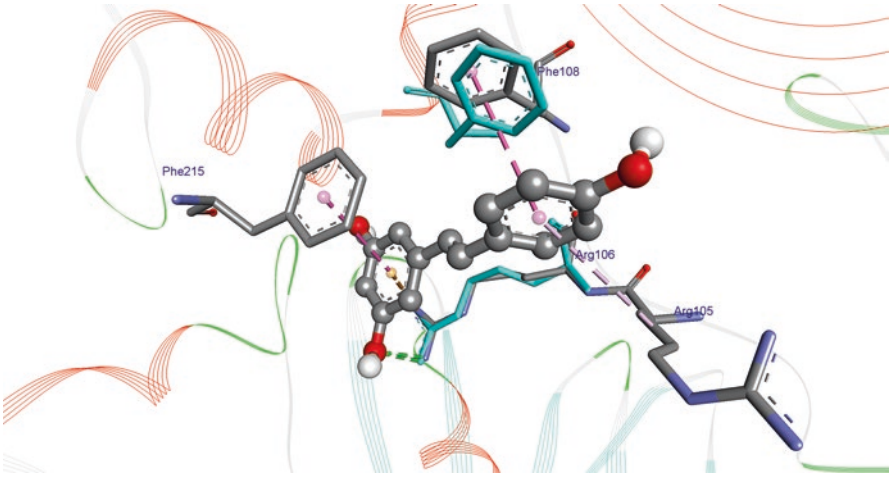


**Fig. 12.9** A diagrammatic representation of hydrogen bond between the resveratrol and residue ARG106 of cytochrome P450 3A4 which is shown in green dotted line. The phytochemical of resveratrol is shown in ball and stick model (gray color). Pink dotted line represents the hydrophobic bonds between binding residues PHE215, ARG106, and PHE108 of cytochrome P450 3A4 and resveratrol. Yellow dotted lines show electrostatic bonds of binding residue ARG 106. The figure was generated by using visualization software of Discovery Studio suits version 4.5

vary from 5.1086 to 4.30201 Å, whereas GLU374 is involved in electrostatic bonding, and bond length is 3.88186 Å. The interaction between quercetin and titled protein is shown in Fig. 12.11. The details of docking analysis of phytochemicals with CYP3A4 (cytochrome P450 3A4) are given in Table 12.3. The interaction between phytochemicals and title proteins is dominated by electrostatic, hydrophobic, and hydrogen bonds. The presence of water molecules in the active site further confirmed the formation of H-bonding.

## 12.6 Conclusions

We have discussed innovations in computational methods which can help us understand aging processes through data mining methods and probe antiaging factors through computational epigenetics and transcriptional profiling. Molecular properties of antiaging have been poised to be integrated into the antiaging framework. The binding energy of catechin, resveratrol, curcumin, and quercetin is found to be −9.8, −10.1, −9.9, and −10.4 kcal/mol, respectively. The result illustrates quercetin interaction with SER119 residue that is involved in hydrogen bonding with bond length of 2.43116 Å. It is to be noted that PHE215, PHE108, and ARG105 residues are involved in hydrophobic bond, and bond lengths vary from 5.1086 to 4.30201 Å, whereas GLU374 is involved in electrostatic bonding, and bond length is 3.88186 Å. Both hydrogen bonding and hydrophobic interactions play a crucial role in the

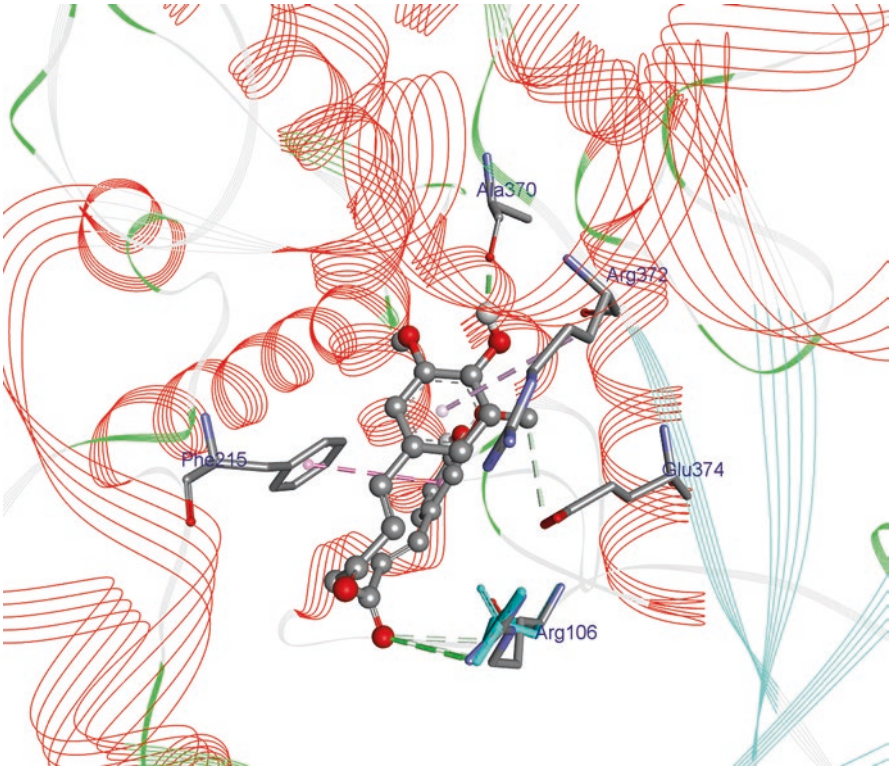


**Fig. 12.10** Details of interaction between the cytochrome P450 3A4 and curcumin. The selected hydrogen bond (which is represented in green color dashed lines) is between residues ARG106 and ALA 307 and curcumin. Violet color dotted line represents the hydrophobic bonds between binding residues AGR 372 and PHE215 and curcumin. The figure was generated by using visualization software of Discovery Studio suits version 4.5

interaction between phytochemicals related to antiaging and cytochrome P450 3A4 protein, which may be relevant to metabolism of antiaging drugs. A critical assessment of the molecular dynamics simulation techniques might require further extension for future predictions.

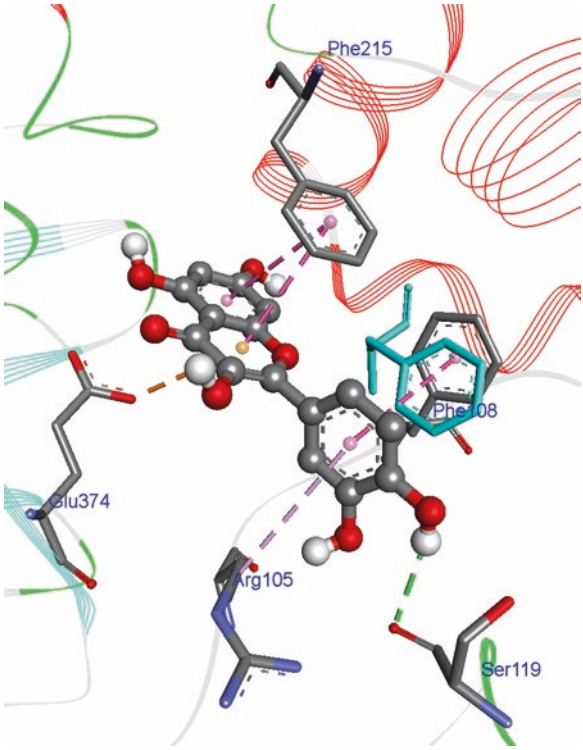


**Fig. 12.11** The binding of compound quercetin with residue SER119 of cytochrome P450 3A4 by hydrogen bonding (shown in green broken line). The phytochemical is shown in ball and stick model (gray color). Pink broken line represents the hydrophobic bonds between binding residues PHE215, ARG105, and PHE108 of cytochrome P450 3A4 and quercetin. Yellow broken lines show electrostatic bonds of binding residue GLU374. The figure was generated by using visualization software of Discovery Studio suits version 4.5

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# Intermittent Fasting-Dietary Restriction as a Geroprotector

13

Gurcharan Kaur, Taranjeet Kaur, Anuradha Sharma, Shaffi Manchanda, Harpal Singh, Shikha Kalotra, and Payal Bajaj

**Abstract**

Old age is one of the major determinants of neurodegenerative diseases. There have been major advancements in understanding the biology of aging along with various interventions that may promote healthy aging. Many nutritional interventions such as caloric restriction, periodic fasting, and alternate day fasting have been proposed that may hamper age-associated cognitive decline. Among the various regimens, intermittent fasting-dietary restriction (IF-DR) seems to be most promising as it has been well documented to provide neuroprotection by enhancing synaptic plasticity and neurogenesis. It is also known to prolong life span and delay the onset of age-associated disorders by reducing inflammation and oxidative stress. IF-DR regimen is known to possibly work by establishing a conditioning response which maintains survival mode in organisms by focusing on energy conservation, thereby causing a metabolic shift from growth to maintenance activities and hence promoting anti-aging effects. IF-DR regimen is also known to improve many physiological indicators such as reduced levels of leptin, insulin, amount of body fat, reduced blood pressure, and increase in resistance to stress. Thus, IF-DR regimen initiated in middle or old age has the ability to impede age-associated neurodegeneration and cognitive decline and may be a potential intervention to abrogate age-related impairment of brain functions.

**Keywords**

Aging · Cognitive decline · Intermittent fasting-dietary restriction · Neuroprotection · Oxidative stress

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## Abbreviations

| | |
|---|---|
| AD | Alzheimer's disease |
| ADCR | Alternate day caloric restriction |
| ADF | Alternate day fed |
| AL | *Ad libitum* |
| AMP | Adenosine monophosphate |
| AMPA | α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid |
| AMPK | Adenosine monophosphate-activated protein kinase |
| ARC | Arcuate nucleus |
| ATP | Adenosine triphosphate |
| BDNF | Brain-derived neurotrophic factor |
| CA | Cornu ammonis |
| CaM | Calmodulin |
| CaN | Calcineurin |
| COX2 | Cyclooxygenase-2 |
| CR | Caloric restriction |
| CREB | Cyclic AMP response element-binding protein |
| DNA | Deoxyribonucleic acid |
| DR | Dietary restriction |
| FOXO | Forkhead box O3 |
| GCs | Glucocorticoids |
| GFAP | Glial fibrillary acidic protein |
| GLP-1 | Glucagon-like peptide 1 |
| GluR2 | Glutamate receptor subunit |
| GnRH | Gonadotropin-releasing hormone |
| GSH | Glutathione |
| HNE | 4-Hydroxynonenal |
| HPA | Hypothalamic-pituitary-adrenal axis |
| Iba1 | Ionized calcium-binding adapter molecule-1 |
| ICAM | Intracellular adhesion molecules |
| IF-DR | Intermittent fasting-dietary restriction |
| IFDRH | Intermittent fasting-dietary restriction plus herbal supplementation |
| IL | Interleukin |
| iNOS | Inducible nitric oxide synthase |
| KA | Kainic acid |
| LPS | Lipopolysaccharide |
| MAL | Middle-aged *ad libitum* fed |
| MAPK | Mitogen-activated protein kinase |
| MDR | Middle-aged dietary restriction |
| MPTP | 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (neurotoxin) |
| mRNA | Messenger ribonucleic acid |
| mTOR | Mammalian target of rapamycin |
| $NAD^+$ | Nicotinamide adenine dinucleotide |
| NADH | Nicotinamide adenine dinucleotide plus hydrogen |

| | |
|---|---|
| NCAM | Neural cell adhesion molecule |
| NF-κB | Nuclear factor kappa B |
| NMDA | N-methyl-D-aspartate |
| NO | Nitric oxide |
| NP | 3-Nitropropionic acid |
| NPY | Neuropeptide-Y |
| NRF | Nuclear respiratory factor |
| NT-3 | Neurotrophic factor-3 |
| PC | Piriform cortex |
| PD | Parkinson's disease |
| PGC-1α | Proliferator-activated receptor gamma coactivator *1*-alpha |
| PI3K | Phosphatidylinositol 3-kinase |
| PM | Plasma membrane |
| PMRS | Plasma membrane redox system |
| PPARγ | Peroxisome proliferator-activated receptor gamma |
| PSA-NCAM | Polysialylated neural cell adhesion molecule |
| PSD95 | Postsynaptic density protein 95 kDa |
| ROS | Reactive oxygen species |
| SIRT-1 | Sirtuin |
| SOCS3 | Suppressor of cytokine signaling 3 |
| TBARS | Thiobarbituric acid reactive substances |
| TFAM | Mitochondrial transcription factor A |
| Trk | Tyrosine kinase |
| VCAM | Vascular cell adhesion molecule |

## 13.1 Introduction

Aging is a naturally occurring, inexorable process which is characterized by progressive loss of physiological integrity, leading to impaired functioning of the body. Aging has been characterized by many hallmark features, such as genomic instability, cellular senescence, attrition of telomeres, mitochondrial dysfunction, epigenetic alterations, and altered intercellular communication (reviewed in López-Otín et al. 2013). Deterioration of physiological functions with aging makes a person prone to many pathological conditions such as diabetes, cancer, cardiovascular diseases, and neurodegenerative disorders. Most age-associated diseases have been closely linked to persistence of chronic inflammatory milieu as evidenced by infiltration of inflammatory mediators such as macrophages and higher circulation levels of adhesion molecules, pro-inflammatory cytokines, and components of complement system (Sarkar and Fischer 2006). Further, various age-related neuropathologies, such as dementia, Alzheimer's disease, and Parkinson's disease along with cognitive decline, have been attributed to enhanced oxidative stress, neuronal degeneration, neuroinflammation, glutamate excitotoxicity, and various other factors (Hamilton et al. 2001).

Several theories have been proposed to understand underlying mechanism of aging including free radical and oxygen stress-mitochondrial theories. Free radical theory of aging explains that damage induced by free radicals to biological macromolecules and inability of cellular endogenous antioxidant mechanisms to counterbalance this stress leads to enhanced oxidative stress, aging, and related pathologies (initially proposed by Harman 1956). On the other hand, mitochondrial theory of aging says that increased oxidative stress induces mutations in mitochondrial DNA resulting in deregulated and disrupted mitochondrial biogenesis and bioenergetics (Loeb et al. 2005). Increased ROS are reported to induce mutations and deterioration of DNA, oxidation, and damage to proteins and lipids. Modification of DNA such as formation of 8-hydroxydeoxyguanosine, protein modifications such as carbonyl formation, nitration, glycation, lipid peroxidation generating 4-hydroxynonenal, and mitochondrial membrane potential are the common parameters which undergo changes during oxidative stress (Sohal and Weindruch 1996; Munch et al. 2000; Lopez-Lluch et al. 2006; Johnson et al. 2007; Mattson 2009; Singh et al. 2015). These modifications of DNA, lipids, proteins, and other biomolecules have been reported to be involved in various neurodegenerative diseases (Martin et al. 2006). Aging is also associated with impairments in learning and memory functions, which occurs due to changes in hippocampal plasticity. Accumulation of oxidative stress in aging hippocampus is the main driving force for these synaptic impairments (Serrano and Klann 2004).

Aging research has witnessed prodigious advancements in recent years, and various interventions have been proposed to encourage healthy aging. These interventions aim to improve physical and psychological well-being and to promote health among aging citizens by delaying the onset of age-associated pathological conditions. Hormesis is an adaptive response of cells and organisms to a moderate, usually intermittent stress (reviewed in Mattson 2008). Hormetins have been characterized as physical hormetins, such as exercise; mental hormetins, such as intense brain activity and meditation; and nutritional hormetins, such as flavonoids and polyphenols (Rattan 2017). Various hormetins have been described which can be used as agents to promote healthy aging and enhance life span. Caloric restriction is one of the nutritional hormetins, which has gained attention of many researchers worldwide.

Caloric restriction (CR) is an extremely popular approach to slow down the degenerative effects of aging. CR has been defined as 20–40% less intake of calories than is normally required by the body (Mattson et al. 2003). Intermittent fasting-dietary restriction (IF-DR) is another variation of CR, which involves alternate day fasting while maintaining complete nutritional intake in the intervening day. IF-DR is also referred to as "every other day feeding" (Martin et al. 2006). In comparison to CR, IF-DR is a better approach as the compliance with IF-DR regimen may be greater than CR regimen. Owing to the periodic nature of fasting, IF-DR regimen mitigates the constant hunger effect experienced by CR practitioners (reviewed in Horne et al. 2015). Moreover, persistent CR may lead to malnutrition, the possibility of which is ruled out in IF-DR regimen due to ad libitum access to food every alternate day. Further, a pilot study comparing the effects of daily CR and IF-DR

regimens on weight loss has reported reversion of weight loss after stopping CR regimen (Catenacci et al. 2016). IF-DR regimen was shown to produce greater energy deficit from weight maintenance requirements than daily CR. Furthermore, IF-DR regimen has been shown to produce similar beneficial effects as CR (Varady 2011; Anton and Leeuwenburgh 2013).

The beneficial effects of IF-DR have been extensively studied in middle age and old age animal model systems by our lab (Singh et al. 2012, 2015) as well as others (Lara-Padilla et al. 2015; Vasconcelos et al. 2015). IF-DR regimen is known to possibly work by establishing a conditioning response which maintains survival mode in organisms by focusing on energy conservation, thereby causing a metabolic shift from growth to maintenance activities and hence promoting antiaging effects (Kaur and Lakhman 2012). IF-DR regimen has been reported to delay the onset of neurodegenerative disorders in experimental models of Alzheimer's disease, Parkinson's disease, and stroke by increasing resistance of hippocampal neurons to degeneration (Mattson 2003). IF-DR regimen is also known to improve many physiological indicators such as reduced amount of body fat (Tinsley and La Bounty 2015), reduction in inflammation (Johnson et al. 2007; Castello et al. 2010), increase in resistance to stress (Vasconcelos et al. 2015), and reduced levels of insulin and leptin (Duan et al. 2003). IF-DR regimen has also been recently reported to exert antitumor effects (reviewed in Mattson et al. 2017).

The architecture of aging brain is prone to modifications by various nutritional and metabolic stimuli. Research in the recent past has tried to unveil various mechanisms of neuroprotection posed by IF-DR. The valuable insights gained from a plethora of studies have helped in better understanding of relationship between energy metabolism and brain functioning. It has also expanded our knowledge regarding various interventions which may be beneficial in improving brain health and providing resistance to age-associated neurological disorders. As already mentioned, brain aging is characterized by reduced synaptic plasticity, cognitive decline, increase in oxidative stress, and inflammatory milieu. Keeping in view these characteristics, we have discussed the effects of IF-DR regimen on age-associated changes in brain architecture and functions in this review.

## 13.2 IF-DR and Brain Plasticity

Over many years, various lines of evidence have suggested that dietary restriction (IF-DR or daily CR) can enhance brain plasticity and cognitive performance in different age group of rats by counteracting molecular and cellular changes that impair cognition (Idrobo et al. 1987; Komatsu et al. 2008; Stranahan et al. 2009). Various preclinical studies have shown the beneficial effects of IF-DR regimen in preventing the cognitive decline associated with aging. The behavioral responses to IF-DR are associated with increased brain plasticity and neurogenesis. For instance, young mice maintained on IF-DR regimen for 11 months performed significantly better on tasks of learning and memory (fear conditioning and Barnes maze), and these behavioral outcomes were associated with increased size of pyramidal neurons of

CA1 region of the hippocampus (Li et al. 2013). In line with this, another study has also demonstrated that IF-DR regimen enhances hippocampal neurogenesis by promoting the survival of newly synthesized neurons (Lee et al. 2002). Studies from our lab have also shown that IF-DR regimen initiated in middle age and old age rats for 3 months exhibited improved motor performance on rotarod and task of spatial learning and memory (morris water maze) than their ad libitum (AL) fed counterparts (Singh et al. 2012, 2015). The studies suggested that these behavioral outcomes could be due to enhanced synaptic plasticity and reduced mitochondrial oxidative stress.

The cellular and molecular mechanisms by which dietary restriction enhances brain plasticity and improves cognitive functions during aging include increase in synaptic activity that causes production of neurotrophic factors (Amigo and Kowaltowski 2014), which in turn, stimulate the formation of new synapses and promote neurogenesis and potentiation (Anton and Leeuwenburgh 2013). Other factors include activation of cellular stress-responsive machinery against oxidative and metabolic stress (Bruce-Keller et al. 1999), and activation of immune and inflammatory mediators (reviewed in Mattson 2015). Brain-derived neurotrophic factor (BDNF) is one of the most notably produced neurotrophic factors in response to fasting in discrete brain regions with most robust production in hippocampus region of the brain (Cotman et al. 2007). BDNF promotes various aspects of synaptogenesis, neurogenesis, migration, and plasticity (Greenberg et al. 2009; Park and Poo 2013). In addition it is critical for synaptic plasticity implicated in optimization of various domains of cognitive functions (Kuipers and Bramham 2006). Highlighting the relevance of this neurotrophic factor in brain function, individuals carrying mutation in this gene exhibited decreased secretion and deficits in memory and increased anxiety and depression (Egan et al. 2003; Hariri et al. 2003). Evidence suggests that BDNF is produced and released at or near to synapses in response to synaptic activity and thus plays a pivotal role in synapse formation and learning and memory (Marosi and Mattson 2014). Mild metabolic stress and increased neuronal activity can induce BDNF production and its downstream signaling to enhance brain plasticity.

BDNF signaling activates the protein translational machinery which is critical for neural transmission and potentiation (Lu et al. 2008). Direct application of BDNF to the hippocampus was observed to upregulate the expression of markers critical in synapse formation and plasticity, viz., postsynaptic density protein 95 (PSD95) and glutamate receptor subunit (GluR2) (Robinet and Pellerin 2011). In addition, BDNF production is known to promote the survival of neurons under conditions of oxidative and metabolic stress (Mattson 2015). IF-DR has shown its beneficial role in protecting hippocampal neurons from seizure-induced excitotoxicity (Bruce-Keller et al. 1999), and another study has speculated that this protection is in part mediated by BDNF signaling (Duan et al. 2001). Further the beneficial effects of IF-DR following excitotoxic stimulus are associated with lower levels of corticosterone, leading to decreased hippocampal cell death, increased plasticity by activation of BDNF and phosphorylated CREB, and reversal of learning deficits (Qiu et al. 2012). Such effects of BDNF signaling likely contribute to the processes by

which IF-DR regimen may enhance cognitive function and prevent brain damage. For instance, in a recent study from our lab, we have shown that IF-DR regimen activated the production of neurotrophic factors BDNF and NT-3 (neurotrophic factor 3) and immature neuronal marker PSA-NCAM (polysialylated neural cell adhesion molecule) in hippocampus, hypothalamus, and piriform cortex (PC) regions of rat brain in response to pilocarpine-induced excitotoxic insult (Kumar et al. 2009). Further the study showed that proliferation rate of neural progenitor cells was also increased in response to dietary restriction as evident from the BrdU immunostaining. These observations highlight the beneficial role of IF-DR as an effective intervention to protect and enhance the resistance of the brain to excitotoxic insult. Studies from our lab have also proposed the potential beneficial role of IF-DR regimen initiated in young and old age rats in attenuating reactive astrogliosis and neuronal plasticity (Sharma and Kaur 2008; Kaur et al. 2008). Young adult rats on IF-DR regimen for 12 weeks demonstrated that kainic acid (KA) excitotoxicity-induced reactive astrogliosis was suppressed and neuronal plasticity was enhanced in response as evident from reduced immunoreactivity of GFAP and enhanced expression of neuronal plasticity markers, PSA-NCAM and NCAM (Sharma and Kaur 2008). The data suggested that IF-DR regimen modulated reactive astrogliosis and prevented age-associated neuronal dysfunction.

Hippocampal neurons play a pivotal role in learning and memory and are vulnerable to neurodegeneration and dysfunction with advancing age. Fasting stimulates the production of BDNF as a result of increased activity of these neurons. BDNF promotes and maintains the growth of dendrites and synapses and also enhances the neurogenesis (Wrann et al. 2013; Longo and Mattson 2014). The newly synthesized cells then integrate into the existing network of neuronal circuits, thus strengthening the synapse and prompting plasticity. After activation, BDNF binds to its high-affinity receptor tyrosine kinase TrkB, resulting in the activation of PI3K/Akt/mitogen-activated protein kinase (MAPK) signaling cascade (Marosi and Mattson 2014). Recent findings have suggested that peripheral signals such as muscle-derived factors can enter the brain and contribute to neuroplasticity and stress resistance (Mattson 2015). For instance, muscle-derived protein FNDC5 is cleaved and secreted as irisin can cross the blood-brain barrier and stimulate BDNF production in the brain which is associated with improved cognitive function following exercise (Wrann et al. 2013).

Apart from the significant contribution of BDNF signaling in maintaining the optimal cognitive functioning and neuronal bioenergetics in the brain in response to IF-DR, glutamate, insulin, and glucagon-like peptide 1 (GLP-1) also contribute to the adaptive responses of the brain to protect it from neurodegeneration (Longo and Mattson 2014). Glutamate activates AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors and NMDA (N-methyl-D-aspartate) receptors resulting in the calcium influx followed by activation of protein kinase (CaM) and phosphatases (CaN) which are calcium-dependent regulators of learning and memory (Longo and Mattson 2014). Their activation in turn leads to activation of transcription factors cyclic AMP response element-binding protein (CREB) and NF-κB (nuclear factor kappa B). Insulin binds to its receptor leading to activation of mTOR

signaling pathway implicated in protein synthesis and cell growth. Finally, GLP-1 activates its receptor, followed by cyclic AMP production in a coupled manner, CREB activation, and BDNF production. All these signaling cascades mediate neuroprotective effects in response to fasting by regulating neuronal bioenergetics.

Both late-onset and early-onset short-term IF-DR regimens have shown beneficial effects in terms of synaptic plasticity. Synaptophysin is a presynaptic marker used as an index of synaptic number and density, and fall in its expression reflects a decrease in neurotransmission affecting spatial memory (Liu et al. 2005). The stabilization in synaptophysin levels in response to short-term IF-DR in discrete brain regions indicates the prevention of decline in synaptic function in both middle and old age Wistar rats (Singh et al. 2012, 2015). Further the studies have shown that hippocampal synaptic potentiation by signaling molecules like CaMKII and CaN was strengthened by IF-DR regimen. Both of these proteins are enriched in postsynaptic density and are involved in calcium signaling and homeostasis, neural transmission, learning, and memory.

Although IF-DR regimen showed neuroprotective role in middle and old age rats, adverse effects were seen on reproductive functions of young adult female rats via estrous cycle disruption and altered levels of estradiol, testosterone, and luteinizing hormone in both male and female rats (Kumar and Kaur 2013). Further, the decrease in gonadotropin-releasing hormone (GnRH) and PSA-NCAM was observed in median eminence region of the hypothalamus. The study suggested that neuroendocrine energy regulators such as leptin, NPY, and kisspeptin target the GnRH neurons on hypothalamus-hypophysial-gonadal axis (HPA) and disrupt the reproductive functions and cause nutritional infertility in the face of energy status of the rats. The findings of this study may suggest that although IF-DR regimen is beneficial in all age groups, but, females in reproductive age may have adverse effects on their reproductive functions.

## 13.3 IF-DR and Oxidative Stress

A steady and notable observation from different model systems reported in various studies is that DR promotes healthy aging by reducing oxidative stress and associated damage (Walsh et al. 2014). In addition to oxidative stress induced within the body due to imbalanced homeostasis, IF-DR has been also reported effective against age-related and LPS-induced oxidative stress in rat hippocampus. LPS-induced increase was observed in lipid peroxidation indicated by TBARS levels, nitric oxide (NO), and protein nitrosylation similar to age-related changes. IF-DR for 30 days (every alternative day feeding) was found to prevent LPS-induced changes in these parameters (Vasconcelos et al. 2015).

Further, a clinical study reported by Johnson evidenced that IF-DR regimen reduced expression of oxidative and inflammatory markers (Johnson et al. 2007). Protein carbonyls are well-reported markers of oxidative stress because of their relative formation in early phase and stability (Dalle-Donne 2003). In 8-week IF-DR study, protein carbonyls and other markers of oxidative stress such as

nitrotyrosine, 8-isoprostane, histidine, and lysine-4-hydroxy nonenal adducts were significantly decreased. Uric acid, the major scavenger of peroxynitrite and hydroxyl radical concentrations, was significantly enhanced in urine of ADCR subjects (Johnson et al. 2007). Age-associated increase in protein carbonyl content was also reported by our lab (Singh et al. 2015). The brain regions of MAL (middle-aged ad libitum fed) rats were found to have higher protein carbonyl content which was significantly reduced in MRD (middle-aged dietary restriction) rats. Reduced protein carbonyl content indicates reduced oxidative stress by IF-DR intervention which may be due to early initiation of repair and maintenance (Singh et al. 2015).

HNE (4-hydroxynonenal), a major aldehydic product from peroxidation-induced breakdown of membrane phospholipids, was found to be progressively increased with age in heart tissue of rat along with protein carbonyl content. Both HNE and protein carbonyl content were found significantly reduced in hearts of alternate day fed (ADF) animals (Castello et al. 2010). In addition to lipid and protein oxidation, ADF dietary regime also improved the reduced glutathione (GSH) concentrations and significantly decreased GSH/GSSG (oxidized glutathione) ratio, thus improving the antioxidant levels within the body.

Plasma membrane redox system (PMRS) enzymes which are important for antioxidant recycling and antioxidant levels such as coenzyme Q and α-tocopherol were also upregulated by calorie restriction (Hyun et al. 2006). Further age-related increase in protein carbonyls, PM lipid peroxidation, and nitrotyrosine were significantly attenuated by CR in cultured neuronal cells (Hyun et al. 2006).

### 13.3.1 Dietary Restriction, Oxidative Stress, and Neurodegenerative Diseases

Dietary restriction is well documented to prolong life span and to render the nervous system resistant to age-associated neurodegenerative diseases (Prolla and Mattson 2001). Dietary restriction is beneficial to vulnerable neurons in PD brain as it is reported to bolster brain and peripheral biogenesis (Longo and Mattson 2014). DR has also been reported to confer resistance to dopaminergic neurons against MPTP-induced Parkinsonism and motor deficit amelioration (Duan and Mattson 1999). Two- to 4-month DR regime was reported to be effective against kainic acid-induced Alzheimer's disease, ameliorating degeneration of pyramidal hippocampal neurons, learning, and memory deficits (Bruce-Keller et al. 1999). Further, DR increased resistance against excitotoxicity and oxidative stress in presenillin-1 and APP-1 mutant mice as compared to ad libitum *fed* animals (Zhu et al. 1999; Mattson et al. 2001). DR was found beneficial against excitotoxic insults caused by 3-nitropropionic acid (NP) to induce Huntington's disease. Striatal neurons in rats maintained on 3-month DR regime developed resistance against 3-NP along with improved motor functioning (Bruce-Keller et al. 1999). Interestingly, DR was also observed to confer protection and extend life span in *Drosophila* model of AD (Kerr et al. 2011). In addition to these conditions, dietary restriction has been reported beneficial in models of ischemic stroke (Yu and Mattson 1999; Fann et al. 2017).

### 13.3.2 Cellular Effectors of Dietary Restriction

Mitochondria is the main focus of aging research since decades. Increased oxidative stress and loss of mitochondrial bioenergetic efficiency are considered as characteristic features of senescence, neurodegeneration, and aging. Dietary restriction imparts reduced workload and wear and tear of mitochondria by promoting mitochondrial biogenesis and induction of autophagy.

#### 13.3.2.1 Mitochondrial Biogenesis

Activation of distinct genetic programs by nuclear and mitochondrial transcription factors allows synthesis of new mitochondria in response to damaged organelles or increased oxidative stress, known as mitochondrial biogenesis. Nuclear respiratory factor (NRF) and mitochondrial transcription factor A (TFAM) are reported downstream transcription factors which coordinate nuclear and mitochondrial gene transcription responsible for mitochondrial bioenergetics (Wu et al. 1999; St-Pierre et al. 2003). PPARγ is an upstream regulator of TFAM and NRF, which acts as nutrient and energy sensor, signals mitochondrial biogenesis, and further transfers utilization of substrate for cellular energy from carbohydrates to fats (Lopez-Lluch et al. 2006). Sirt1 directly deacylates PPARγ, regulates biogenesis and bioenergetics, and prolongs the healthy life span (Rodgers et al. 2005; Nemoto et al. 2005). In a clinical study on human population, an increase in muscular mitochondrial DNA was observed in response to calorie restriction along with reduced oxidative stress and DNA damage, which suggests that calorie restriction exerts positive effects on mitochondrial functioning in young nonobese individuals (Civitarese et al. 2007). Lopez and group reported CR-induced mitochondrial biogenesis and bioenergetics regulation both in vitro and in vivo by studying mitochondrial membrane potential, a bioenergetic parameter (Lopez-Lluch et al. 2006). Calorie restriction is proposed to carry out efficient electron transfer in respiratory chain which meets equivalent ATP production even under reduced oxygen consumption and reduced ROS production. Attenuation of cellular and molecular damage due to oxidative stress and reduced rate of aging is attributed to this change in mitochondrial efficiency in different organisms (Lopez-Lluch et al. 2006).

#### 13.3.2.2 Autophagy

Autophagy is a strictly regulated process of recycling of damaged organelles and damaged and aggregated cellular proteins into biosynthetic and bioenergetic products in order to maintain cellular homeostasis (Morselli et al. 2010; Wohlgemuth et al. 2010; Yang et al. 2014; Ntsapi and Loos 2016; Pani 2015). This cellular mechanism is potentially induced by CR and plays an important role in CR exerted antiaging effects (Ntsapi and Loos 2016). Several studies have revealed that CR-induced autophagy is controlled by Sirtuin 1 expression in in vitro human cells and in *C. elegans* in vivo, whereas knockout of Sirtuin 1 abolished the autophagy induction by nutrient deprivation in cultured human cells as well as autophagy induced in *C. elegans* by dietary restriction (Morselli et al. 2010). Mammalian target of rapamycin (mTOR) pathway negatively regulates autophagy, and its inhibition by CR is

also one of the reported underlying mechanisms of CR-induced autophagy (Yang et al. 2014). Thorough literature studies revealed that dysfunctional autophagy in the brain, muscle, liver, and other organs leads to degeneration and aging, and dietary restriction mends the dysfunctional autophagy and delays aging by regulating different pathways (Kume et al. 2010; Morselli et al. 2010; Wohlgemuth et al. 2010; Yang et al. 2014; Ntsapi and Loos 2016; Pani 2015).

### 13.3.3 Molecular Effectors of Dietary Restriction

Dietary restriction targets the pathways and molecules responsible for energy metabolism, maintaining homeostasis and synthesis.

#### 13.3.3.1 Sirtuins

Sirtuins are family of $NAD^+$-dependent mitochondrial deacetylases, which monitor oxidative and energy metabolism along with mitochondrial dynamics within mitochondrial matrix and maintain cellular homeostasis (Tang et al. 2017; Su et al. 2017). Dietary restriction has been reported to enhance SIRT1 expression and mitochondrial biogenesis through SIRT-1-mediated deacetylation of PGC-1α, a major regulator of biogenesis (Amigo and Kowaltowski 2014; Cohen et al. 2004; Nemoto et al. 2005). SIRT1 orchestrates oxidation of fatty acids in the muscle and liver and mobilization of lipid in adipose tissue, thus suggesting that its activation in dietary restriction may induce metabolic reprogramming (Rodgers et al. 2005; Fiskum et al. 2008; Amigo and Kowaltowski 2014). In yeast, protein Sir2, a gene product of SIR2, catalyzes histone deacetylation and $NAD^+$ cleavage. Since $NAD^+$ and NADH are metabolic cofactors in various key reactions, Sir2 may be used as metabolic sensor which could regulate gene expression according to cellular metabolic state (Guarente 2000; Tanner et al. 2000). Based on this hypothesis, several studies proposed that Sir2/SIR2 mediate cytoprotective effects of dietary restriction in yeast (Canto and Auwerx 2009). Beneficial effects of CR in mammals have been attributed to sirtuins and their interconnections with other cell circuitries such as AMPK1, CREB, and PGC1, which are reported to be activated by fasting (Schulz et al. 2007; Canto and Auwerx 2009; Price et al. 2012; Pani 2015). In several cellular and animal studies, Sirt1 activity has been linked to neuronal plasticity, rendering protection against misfolded protein excitotoxicity in Parkinson's, Alzheimer's, and Huntington disease (Parker et al. 2005; Gao et al. 2011; Donmez et al. 2012). Clinically the neuroprotective effects of Sirt1/SIRT1 can be mimicked and enhanced experimentally by resveratrol which is known to mimic effects of dietary restriction (Amigo and Kowaltowski 2014; Pani 2015).

#### 13.3.3.2 AMPK and Cross Talk with mTOR, SIRT1, and FOXO Encoded Proteins

Adenosine 5′ monophosphate-activated protein kinase (AMPK) pathway has been reported to play an important role in preventing aging and senescence (Ido et al. 2015). It is regulated by intracellular ATP/AMP ratio and serves as cellular nutrient

and energy sensor with ability to modulate whole body metabolism (Xu et al. 2012). AMPK might arbitrate the beneficial effects of dietary restriction via regulating mitochondrial metabolism and biogenesis. AMPK has been reported to prevent oxidative stress-mediated senescence and aging by inducing autophagy via suppression of mTOR pathway (Canto and Auwerx 2011). Mammalian target of rapamycin (mTOR) is another important pathway to regulate energy balance which responds to hormonal and nutritional cues (Powell et al. 2012; Xu et al. 2012). The role of mTOR as important longevity pathway suggests that inhibition of mTOR complex 1 (mTORC1) activity is sufficient to increase life span. In mammals calorie restriction has been shown to reduce mTOR signaling (Stanfel et al. 2009). So it is hypothesized that in need of energy/fasting/calorie restriction, ATP demand increases which suppresses mTOR pathway, thus leading to autophagic destruction of damaged cells resulting in longevity and increased life span.

In a recent review by Pani (2015), it has been proposed that neurodegenerative diseases, mTOR promotes tau and amyloid β aggregation by promoting protein synthesis and inhibiting onset of autophagy. CR inhibits mTOR thus inducing autophagy, protects from cognitive decline, and ameliorates disease-related pathology in AD (Pani 2015). Further, AMPK phosphoregulates PGC-1α and enhances $NAD^+$ levels which acts as rate-limiting step in SIRT1 deacetylation (Canto and Auwerx 2009). Thus AMPK allows specific and higher activity of SIRT1 and promotes neuroprotective effects of sirtuins (Canto and Auwerx 2011). The other family of transcription factors, FOXO, provides another evidence of CR and AMPK and increased life span correlation. Genetic evidences suggest that FOXO proteins have the ability to enhance longevity by providing resistance to oxidative stress, protein structure protection, promotion of autophagy, and lipid metabolism (Fontana et al. 2010; Gross et al. 2008). AMPK directly phosphorylates different members of FOXO, which act as mediators of AMPK-induced autophagy (Nakashima and Yakabe 2007).

#### 13.3.3.3 CREB and CREB-Sirt1 Cross Talk

Neurotrophins, the neurotrophic factors, promote neuronal heath by modulating genetic factors majorly via cAMP-responsive element-binding (CREB) factor (Riccio et al. 1999; Finkbeiner 2000). The beneficial neuroprotective effects of calorie restriction in the forebrain of mice lacking CREB were reported to be abolished, thus suggesting the important role of CREB in CR-mediated neuroprotection (Fusco et al. 2012). Further, reports have evidenced significantly reduced levels of CREB expression in aged and neurodegenerative disease associated brains. The CREB-mediated neuroprotection is also dependent on CREB-sirtuin cross talk (Cui et al. 2006; Caccamo et al. 2010). Increased neurotrophins in response to CR and DR increase the CREB expression which further induce Sirt1 expression and its mediated pathway. Sirt1 expression is reported to be highly reduced in absence of CREB, and nutrient availability regulates expression of CREB and CREB-related genes in the brain (Fusco et al. 2012). CREB has been also reported to transactivate neurotrophin BDNF and TrkB encoding gene expression in the brain (Deogracias et al. 2004). Furthermore, deletion or mutation of CREB leads to neurodegeneration

and neuronal damage induced by huntingtin mutant (Cui et al. 2006). In *C. elegans* CREB has been reported to be essential for memory, and differential regulation of CREB is one important factor underlying age-related decline in memory. In mammalian brains also CREB is referred to as memory regulator, and overexpression of CREB in the hippocampus enhanced the performance of aged animals in long-term memory experiments (Kauffman et al. 2010).

## 13.4 IF-DR and Neuroinflammation

CR is known to inhibit immunosenescence (Koubova and Guarente 2003) which refers to the age-associated decline of immune functions (Solana et al. 2006). Food restriction inhibits the pro-inflammatory pathways and enhances anti-inflammatory pathways in various tissues including the brain. In the hypothalamus, increased mRNA expression of anti-inflammatory signaling molecules including suppressor of cytokine signaling 3 (SOCS3), interleukin-10 (IL-10), and neuropeptide-Y (NPY) was observed in CR animals (MacDonald et al. 2011). CR suppressed LPS-induced release of IL-1β, IL-6, and TNF-α and enhanced anti-inflammatory corticosterone (MacDonald et al. 2014). Increase in glucocorticoids (GCs) after CR is one of the possible mechanisms by which caloric restriction exerts its anti-inflammatory effect (Levay et al. 2010). GCs show dual effects on regulation of inflammation under stressful conditions. Mild stress results in anti-inflammatory effects of GCs as they reduce pro-inflammatory cytokine production and increase the expression of anti-inflammatory proteins. On the other hand, pathological stressful stimuli lead to chronically elevated GCs and also promote pro-inflammatory cytokines and microglia activation (Vasconcelos et al. 2016). CR suppresses the activation of microglial cells, which are primary immune cells in the brain (Jochen Gehrmann et al. 1995). Microglial cells possess receptors for hormones such as leptin and ghrelin, which are known to be altered by CR. Leptin is an appetite hormone and also has pro-inflammatory effects (Luheshi et al. 1999) which is reduced by CR (Govic et al. 2008).

CR reduces the ionized calcium-binding adapter molecule-1(Iba1) expression in LPS-induced animals. LPS induction causes upregulation of Iba1, protein specifically expressed by microglia, in activated microglia (Imai and Kohsaka 2002). In AL fed animals, LPS increases the mean intensity of Iba1, but this increase in Iba1 expression was not observed in animals exposed to CR which may suggest that CR inhibits microglial activation. Significant decline of Iba1 expression was also observed in both hippocampus and piriform cortex (PC) in animals put on IF-DR with herbal supplementation which indicates that CR is effective to prevent inflammation during aging (Singh et al. 2017). CR also attenuated LPS-stimulated microglial activation in hypothalamus arcuate nucleus (ARC) (Radler et al. 2014) and inhibited NF-κB activation and NF-κB-driven inflammatory gene expression in aged rats (Grosjean et al. 2006). NF-κB is considered as a master regulator of innate immunity, and NF-κB in cytosol fraction is in inactive state, complexed with inhibitory IKβα protein. The activation of NF-κB occurs by phosphorylation of IKβα at

serine residue 32 and 36 by IKKα complex (Grosjean et al. 2006). The phosphorylation of IKβα causes degradation of inhibitory IKβα, thus allowing translocation of NF-κB to the nucleus, and permits the binding of NF-κB to regulatory elements in DNA promoters subsequent to genes involved in inflammatory response. NF-κB regulates expression of pro-inflammatory molecules such as tumor necrosis factors (TNF-α and TNF-β), interleukins (IL-1β, IL-2, and IL-6), chemokines (IL-8 and CCL5), adhesion molecules (ICAM-1, VCAM, and E-selectin), and enzymes like iNOS and COX2 (Chung et al. 2002).

Hunger is an adaptive response to fasting that involves neuroendocrine signals in addition to sensory and cognitive changes that activate food seeking behavior. Several studies have reported that hunger-related neuropeptides and hormones play a pivotal role in mediating the beneficial effects of IF-DR on aging and its associated diseases. A recent study from our lab has reported that NPY which is a "hunger peptide" and energy regulator was activated in the hypothalamus of middle-aged male Wistar rats in response to IF-DR regimen as compared to their AL counterparts (Singh et al. 2015). The study further showed that rats maintained on IF-DR exhibited reduced expression of leptin receptor in the hypothalamus. NPY expression is influenced by peripherally produced hormone signals, including appetite suppressor leptin and appetite stimulant ghrelin. Leptin regulates NPY release through leptin-NPY-GnRH pathway (Fernandez-Fernandez et al. 2006). In addition, leptin also regulates energy homeostasis via central activation of the nervous system through its receptor, Ob-Rb. Low leptin levels are known to induce orexigenic signals in hypothalamus and suppressing energy expenditure (Valassi et al. 2008). A study by Shi et al. (2012) showed that caloric restriction was unable to elevate the levels of circulating adiponectin in NPY deficient mice thus suggesting the central role of this neuropeptide in peripheral adaptation to energy restriction. In addition, NPY activation is linked to anti-inflammatory response following CR by suppression of microglial activation (Sonti et al. 1996; Sousa-Ferreira et al. 2011). Several studies have shown that CR exerts anti-inflammatory role by altering the level of circulating leptin and ghrelin resulting in enhanced production of NPY (Felies et al. 2004; Sousa-Ferreira et al.. 2011; Radler et al. 2015).

Recently, our lab has reported the effect of IF-DR along with supplementation of herbal extracts *Withania somnifera* and *Tinospora cordifolia.* This regimen suppressed inflammation induced due to aging in middle-aged female rats by reducing and normalizing the expression of inflammatory molecules such as NF-κB, Iba1, TNFα, IL-1β, and IL-6 in both hippocampus and PC regions of the brain. This was observed in both IF-DR and IF-DR + Herbal (IFDRH) supplementation groups (Singh et al. 2017). This study also demonstrated that IFDRH regimen reduced anxiety-like behavior in middle-aged rats, which was mediated by anti-inflammatory effect posed by this dietary intervention.

## 13.5 Conclusion

As discussed in the preceding sections, IF-DR regimen provides neuroprotection by various mechanisms (Fig. 13.1), thereby raising the possibility of extended health span and delayed onset of age-related disorders. The effects of IF-DR regimen have shown evolutionary conservation ranging from simple organisms such as *Saccharomyces cerevisiae* (yeast), *Caenorhabditis elegans* (nematode), and *Drosophila melanogaster* (fruit fly) to mammals (Longo and Mattson 2014). The lifestyle in present-day society, which has seen technological advances in food processing, agriculture, as well as transportation, has given rise to sedentary work

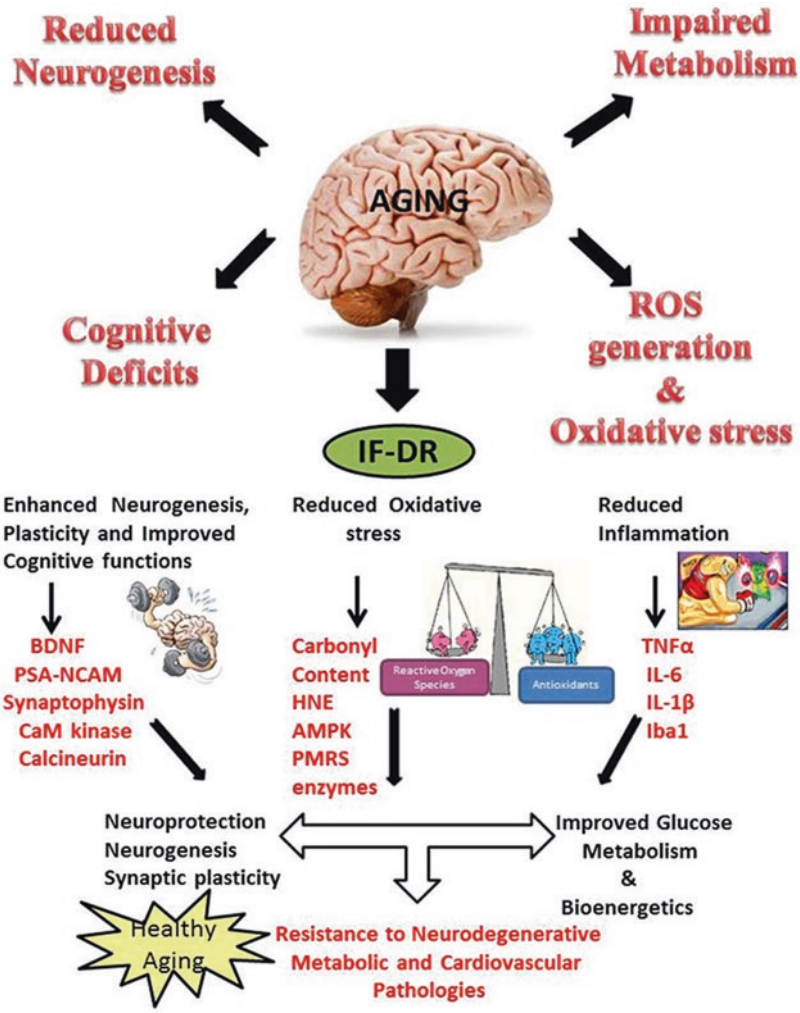


**Fig. 13.1** Beneficial effects of IF-DR and its possible mechanisms

culture in many fields. Besides consuming energy dense food, people are also less exposed to vigorous exercise schedules. This has resulted in impaired physical and mental health and early onset of diseases. Further, medical practitioners and pharmaceutical industry also aim to treat diseases with chemically synthesized drugs and/or surgery; instead the focus should be on the prevention of diseases. This can be achieved by proper education of children as well as parents regarding the importance of intermittent challenges to the brain for sustaining optimal brain health. IF-DR regimen can prove to be a boon in this scenario. Different cultures and religious groups have been practicing fasting since times immemorial. These include Hindus, Buddhists, Christians, Jews, as well as Muslims, who fast during the month of Ramadan. IF-DR regimen, thus, provides a scientific validation to the traditional practice of fasting. Though the scientific literature provides immense evidence for the potential beneficial effects of IF-DR, yet its translation to human subjects on a regular basis is still a challenge.

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# Melatonin and Its Antiaging Activity: New Approaches and Strategies for Age-Related Disorders

14

Sibel Suzen

**Abstract**

Melatonin (N-acetyl-5-methoxy tryptamine, MLT) is a hormone that is produced by the pineal gland. It is synthesized regularly with high levels at night. Age-related decline in MLT contributes to an increased susceptibility to a number of pathophysiological disorders like neurodegenerative diseases, cancer, and aging. There are strong evidences that both Alzheimer's disease and Parkinson's disease are associated with low levels of MLT. Because of its wide-ranging antioxidant and radical scavenger effects, MLT may act as a protective agent against many age-related illnesses. MLT's protection may be possible for both protein and fat tissues in the body by crossing all cell membrane. Currently available data make us to determine that MLT is beneficial for the aging process. Administration of MLT is able to increase the life span of several animals including some rodents. Although, to preserve health in old age becomes a primary goal for biomedicine, there is a necessity for extensive studies on the administration of MLT in order to increase the quality of life in advanced age. In this chapter experimental approaches to antiaging activity of MLT as well as its possible therapeutic significance are reviewed and discussed.

**Keywords**

Antiaging · Antioxidant · Free radical · Melatonin · Oxidative stress

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## Abbreviations

| | |
|---|---|
| AD | Alzheimer's disease |
| CR | Caloric restriction |
| GSH | Glutathione |
| HD | Huntington disease |
| HNE | 4-hydroxy-2-nonenal |
| MDA | Malondialdehyde |
| miRNA | MicroRNA |
| MLT | Melatonin |
| mtDNA | Mitochondrial DNA |
| NAS | *N*-acetylserotonin |
| OS | Oxidative stress |
| PUFA | Fatty acid |
| ROS | Reactive oxygen species |
| SIRT | Sirtuin |

## 14.1 Introduction

Life expectation has been getting improved across the world and the number of elderly is growing rapidly. As a consequence of the rise in the old age people, the occurrence of age-associated diseases has also increased. Therefore the policies and approaching to discover new and effective antiaging molecules are important (Rizvi and Jha 2011). Various molecules are candidate to stop aging, cancer, and degenerative disorders. It has been realized that the decline of different physiologically important molecules such as melatonin (MLT) over the life span is strictly connected to the aging process. Replacement of these molecules is a common approach against aging (Heutling and Lehnert 2008). MLT absence is related to suppressed immunocompetence, and treatment of MLT increased life span and delays aging. Many of its activities are helpful for the prevention of aging (Karasek and Reiter 2002; Karasek 2004). There are strong evidences that MLT is a geroprotective agent which reduces the aging process. As MLT may delay aging process with its versatility of actions, it may also reduce and/or delay the occurrence of age-related diseases such as of Alzheimer's disease (AD) (Rosales-Corral et al. 2012; Gurer-Orhan et al. 2016).

## 14.2 Oxidative Stress in Disease and Aging

The simplest description of oxidative stress (OS) is basically an imbalance between the creation of free radicals and the capacity of the organism to prevent their damaging effects. OS may cause many pathophysiological disorders in the body. The most important ones are neurodegenerative diseases like Parkinson's disease and AD, gene mutations and cancers, atherosclerosis, heart failure, heart attack,

inflammatory diseases, and aging. Considering this connection, the goal of numerous ongoing studies is to reveal the underlying mechanisms and role of OS in disease onset and development. Specifically, there is significant importance on finding new therapeutic strategies for decreasing OS (Tekiner-Gulbas et al. 2013; Shirinzadeh et al. 2016).

Although, there are several endogenous and exogenous antioxidant molecules that can offer protection, aging is still not possible to prevent or stop. Among antioxidants MLT and related compounds have been confirmed to be significantly effective (Suzen et al. 2006; Gurkok et al. 2009; Yilmaz et al. 2012). In addition to this increased MLT levels revealed very promising results against some other diseases like age-related macular degeneration (Chakravarty and Rizvi 2011), acute respiratory distress syndrome (Ochoa et al. 2003), glaucoma (Yi et al. 2005), and sepsis (Gitto et al. 2004). Recent findings showed that antioxidant properties of MLT help protect against heart muscle injury caused by heart attack (Chen et al. 2003; Reiter et al. 2010a, b).

The MLT level differs throughout the life span. During fetal period, the fetus has easy access to maternal MLT via the placenta (Waddell et al. 2012). The levels of MLT then peak during puberty, and then decrease starts in middle-aged. This decline may be reflected many vital changes in the elderly (Savaskan et al. 2005). Induced OS and neurodegeneration experiments display that MLT can protect and defend neurons during aging process (Kaewsuk et al. 2009).

It was proved that MLT has a great capacity to inhibit cell proliferation in some cancer types (Pawlikowski et al. 2002; Srinivasan et al. 2008). It has been used as an adjuvant therapy in cancer patients with solid tumors undergoing chemotherapy or radiation therapy. The use of 20 mg of MLT once daily versus conventional treatment alone increased the 1-year survival rate by 45% (Wang et al. 2012).

Animal studies showed that MLT has a protective effect against the onset of diabetes in diabetes-prone rats. Treatment of MLT improved the animals' cholesterol and triglyceride levels (Sartori et al. 2009). McMullan et al. (2013) showed that poor MLT secretion or lack of MLT secretion might lead to type 2 diabetes or related with a higher risk of having type 2 diabetes. Additional distinctive and powerful property of MLT is its capability to cross the blood-brain barrier. Preclinical studies discovered that MLT shows neuroprotective effects against *beta-amyloid plaque*, in AD patients in the early stages (Pappolla et al. 1999). MLT secretion declines in AD, and replacement of MLT helps protection from Aβ toxicity at the mitochondrial level (Cardinali et al. 2010).

Reactive oxygen species (ROS) produced mainly by mitochondria and that create damage to mitochondrial constituents then eventually cause degradative processes. These harmful reactions to cells and cell components associated with the aging process (Bonomini et al. 2015). Under physiological circumstances oxidative harm to mitochondrial DNA (mtDNA) with age can lead to DNA strand breaks and somatic mtDNA mutations (Richter 1995). Accumulation of excess mtDNA may cause to impairment of the respiratory chain complexes. This never-ending cycle is the reason of increase in mitochondrial ROS and buildup of additional mitochondrial DNA mutations during aging (Sohal and Weindruch 1996).

Since OS plays an essential role in the aging process and chronic diseases linked with senescence, the use of a potent antioxidant compound like MLT may develop a hopeful, safe, and effective approach to slow aging and age-related diseases (Poeggeler 2005). There is strong suggestion that MLT diminishes cancer at the initiation, progression, and metastasis phases and two theories related to action of MLT on cancer. These are either membrane receptor-mediated action or membrane receptor-independent action. Research have shown that MLT's co-administration significantly helps to conventional drugs to inhibit cancer and metastasis by preventing the entrance of cancer cells into the vascular system. MLT reduced the toxic effects of anticancer medication, increasing their effectiveness (Reiter et al. 2017; Gurer-Orhan et al. 2017).

## 14.3 The Mechanism of Aging

Aging involves multifunctional process and many accompany theories. Therefore, there are still speculations about the biological mechanism of aging. Free radicals that cause OS are the main suspect of this occurrence. It is known that free radicals, ROS, and reactive nitrogen species (RNS) are generated by our body by various endogenous systems that can damage cell membrane as well as cause the accumulation of damaged proteins. ROS can affect proteostasis, causing the accumulation of damaged proteins in cells which lead additional protein misfolding or aggregation (Powers et al. 2009). These radicals can be removed by endogenous reducing agents, like glutathione, but excess free radical attacks on collagen can cause cross-linking of protein molecules. As a result due to the lack of proper cell division, injured cells cannot be changed by new cells, and cellular senescence starts by DNA damage, oncogenesis. The immune system is able to remove senescent cells in young people, but this process is not very easily happen in the elderly (Rodier and Campisi 2011). It can be concluded that aging could be due to accumulation of somatic mutations, telomere shortening, protein damage, or mitochondrial dysfunction (Kriete et al. 2011).

In a different theory of the mechanism of aging increase of unrepaired molecular damage which ultimately cause to cellular defects and age-related diseases (Kirkwood 2005). Regarding aging, exposure to sources of damage will definitely affect life span. Additional factors consist of genetics, epigenetics, diet, physical activity, and chance (Jansen-Dürr and Osiewacz 2002).

Life span could be dignified by the equilibrium between cellular damage of metabolic procedures happening within the cell and molecular reactions that can repair the injury. Oxygen might produce oxygen radicals at the mitochondrial and peroxisomal level, which may attack DNA, protein, cell membranes, and organelles. In elderly buildup of altered macromolecules and membranes may damage cell functioning and accelerate the aging process (Bergamini et al. 2004). Autophagy plays very crucial part in the degradation of damaged organelles including mitochondria (Jin 2006) and effective antiaging cell repair mechanism responsible for the antiaging activity of caloric restriction (CR). CR, or energy restriction, is a dietary regimen that reduces calorie intake without incurring malnutrition or a reduction in

essential nutrients. Period and level of CR have significant results on the antiaging effects. Research supports that autophagy has a chief role in the delay of the aging process by antiaging interventions like CR. Antioxidant drugs for clinical use are promising for retardation of aging and age-associated diseases. Geriatric studies which involve aging mechanisms at the molecular and cellular levels are opening important new windows into understanding aging (Cavallini et al. 2008).

## 14.4 Aging and the Brain

Aging brain causes many neurodegenerative diseases. It was shown that the antioxidant enzymes SOD, catalase, glutathione peroxidase, and glutathione reductase are not present in high quantities in the brains of Alzheimer's patients (Pappolla et al. 1992). ROS of mitochondrial basis are responsible of the major mtDNA injury. This may be due to a lack of mtDNA mending mechanisms and the absence of the defense by histone proteins (Barja 2004). Due to the high mutation rate of mtDNA, as the amount of mutant mtDNA growths, the cellular energy declines (Yoneda et al. 1995). Data have shown that oxidative-induced mutations in mtDNA increased with age and stored in the brain (Chomyn and Attardi 2003). Many age-related diseases such as Alzheimer's and Parkinson's diseases have been shown to be related with excess quantity of mtDNA mutations. OS is one of the important reasons to Aβ accumulation and has a crucial role in the pathogenesis of AD (Bekris et al. 2010; Chen and Zhong 2014) and similarly in Parkinson's diseases (Hwang 2013).

Numerous clinical complications in the elderly are associated with nervous system aging, including loss of cognition, awareness, memory, some gastrointestinal problems, and balance impairment. There are some aging theories linked to signaling pathways have been developed. The most considerable is that of free radicals, which describes the important role of antioxidants such as MLT. In aging membrane fatty acid structure is affected such as reduction in the levels of polyunsaturated fatty acids (PUFAs) and an escalation in monounsaturated fatty acids. PUFAs, such as arachidonic acid, are decreased in the hippocampus of aged rats and forms malondialdehyde (MDA), which causes DNA damage (Head et al. 2002). In the aged brain, high amount of MDA is present. 4-Hydroxy-2-nonenal (HNE), the peroxidation of linoleic acid, is more stable than free radicals and causes more injury in the brain (Papaioannou et al. 2001). One of the significant indicators of aged brain is increased levels of HNE which have also been found in Alzheimer's and Parkinson's disease (Zarkovic 2003).

Data shows that aging brains have high amount of oxidized mitochondrial proteins which have increased levels of protein carbonyl groups. Protein oxidation is one of the main reasons of weakening in physiological functioning that goes along with aging (Cakatay et al. 2001; Nicolle et al. 2001). An important number of expressed microRNAs (miRNAs) are differentially regulated during aging, associating miRNAs as regulators of brain aging. miRNA-mediated, brain functional changes are effective on life span.

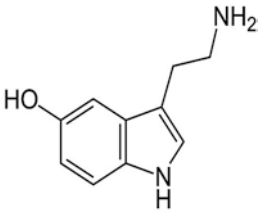


**Fig. 14.1** Chemical formula of melatonin

## 14.5 Antioxidant Properties of Melatonin

N-Acetyl-5-methoxytryptamine is a hormone known as MLT (Fig. 14.1). In a healthy circadian cycle, MLT is released by the pineal gland in the brain as well as many other organs including the retina, skin, cerebellum, liver, kidneys, ovary, and pancreas when it starts to get dark (Reiter et al. 2003; Vriend and Reiter 2015). MLT is known to be produced in the plant kingdom as well (Reiter et al. 2015). The research has been shown that it is an amazing molecule. Besides its regulatory role in circadian rhythms, there are many other functions that MLT shows such as anti-inflammatory properties (Carrillo-Vico et al. 2005; Chahbouni et al. 2010), homeostatic effects in the mitochondria (Paradies et al. 2010), and inhibition of cancer progression (Jung-Hynes et al. 2010). Several of these activities are facilitated by G-protein-coupled MLT receptors in cellular membranes; other activities appear to contain its interface with orphan nuclear receptors and with molecules. MLT's capacity to scavenge ROS is receptor-independent. This ability makes it challenging to define precisely how MLT functions to exert its actions. We know that MLT contribute to improve cellular and organismal physiology (Suzen 2007; Reiter et al. 2010a, b).

MLT is synthesized using a four main steps starting from the precursor tryptophan (Fig. 14.2). It is hydroxylated by tryptophan hydroxylase to 5-hydroxytryptophan which then decarboxylated by aromatic-L-amino acid decarboxylase to give serotonin. Serotonin carry on to either MLT synthesis or go through different metabolic pathways. For MLT synthesis, serotonin is acetylated with serotonin *N*-acetyltransferase on its free amine and then O-methylated on the hydroxyl group by hydroxyindole-*O*-methyltransferase to form MLT (Hardeland 2010; Tan et al. 2015). Approximately 90% of MLT is cleared in a single passage through the liver. A small quantity of unmetabolized MLT is excreted in the urine (Vijayalaxmi et al. 2002).

MLT was recognized as a potent free radical scavenger (Tan et al. 1993, 2002) and effective antioxidant (Reiter et al. 2000; Rodriquez et al. 2004). This necrohormone is able to scavenge many different types of reactive oxygen and nitrogen species mainly hydroxyl radical, hydrogen peroxide, singlet oxygen, nitric oxide, and peroxynitrite anion (Reiter et al. 2003). The structure-activity relationships show that the indole ring of the MLT molecule is the reactive center dealings with oxidant species with the contribution of the methoxy and amide side chains (Karaaslan and Suzen 2015; Suzen 2015).

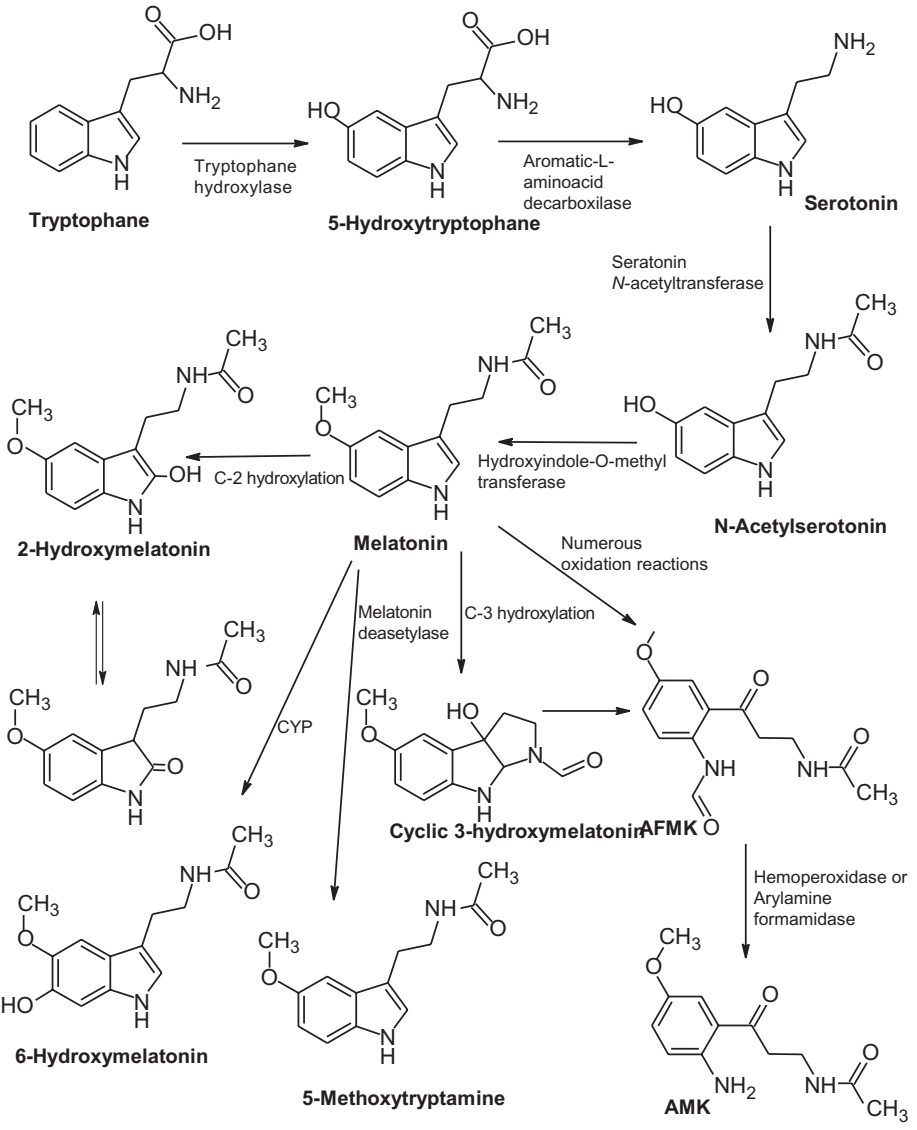


**Fig. 14.2** Biosynthesis and catabolism of melatonin

In vivo efficiency of MLT could be due to the cascade of its antioxidant metabolites (Hardeland et al. 2009). It is interesting that MLT does not redox-cycle in contrast with biological antioxidants like vitamin C, α-tocopherol, lipoic acid, etc. It behaves like "suicidal antioxidant" by performing molecular rearrangement and removing the free electron from the system (Johns and Platts 2014) (Fig. 14.3).

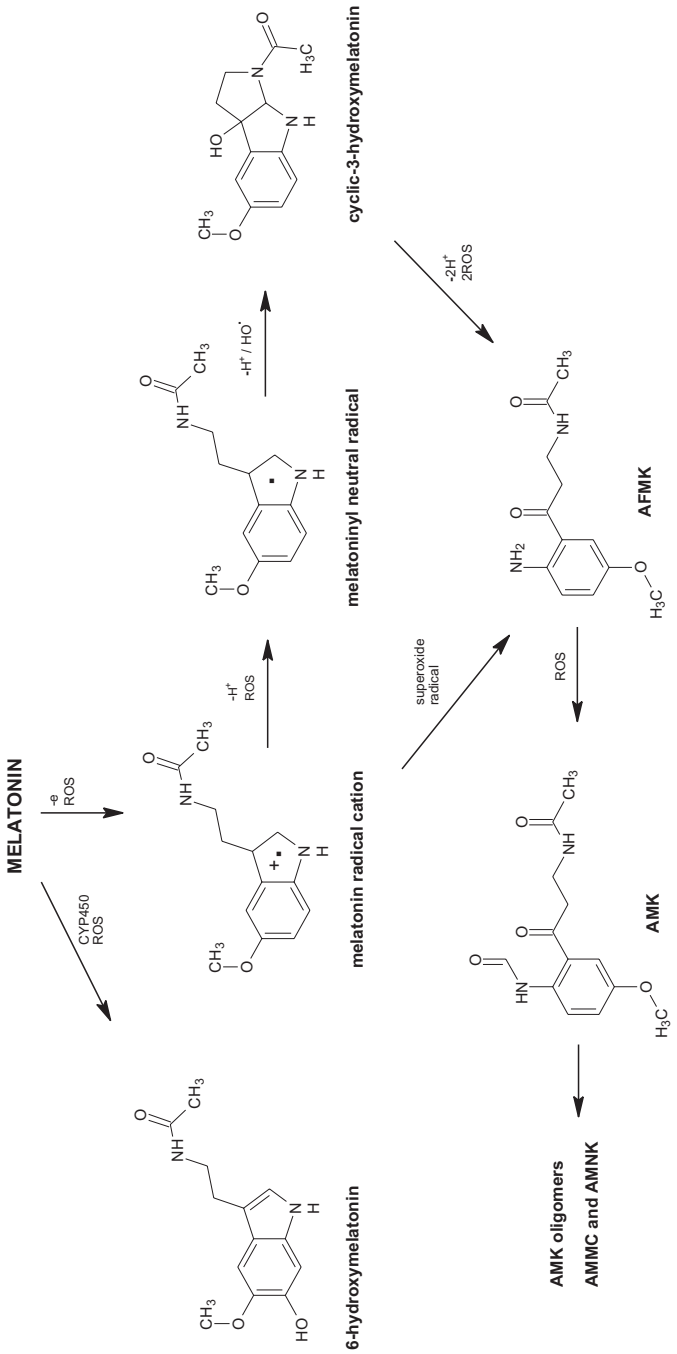


**Fig. 14.3** Antioxidant mechanism of MLT

MLT is very active as an antioxidant at the mitochondrial level when compared with synthetic antioxidants and accomplished better in decreasing damage to this organelle. After administration MLT is absorbed in very good quantity and accumulate in the matrix of mitochondria. MLT can be categorized as a naturally synthesizing mitochondrial-targeted antioxidant due to its easy access to mitochondria either via production in mitochondria or consumed in the diet (Reiter et al. 2014).

MLT is a powerful antioxidative agent also by secondary effects by enhancing activity of antioxidative enzymes (Fischer et al. 2008) such as manganese superoxide dismutase (MnSOD), copper-zinc superoxide dismutase (Cu/Zn-SOD), GPx and gamma-glutamylcysteine synthetase (γ-GCS), and glutathione (GSH) (Martín et al. 2002). This effect may be possible through a connection of membrane and/or nuclear MLT receptor activation (Kleszczynski and Fischer 2012).

## 14.6 Melatonin and Aging

Aging is characterized by a progressive decline of physiological functions and metabolic processes. Even though not much is known about the exact physiological mechanisms of age-related changes in the body, it can be said that mainly the changes happen in the suprachiasmatic nucleus (Wu and Swaab 2005).

MLT due to its known powerful ROS combating properties has attracted many attentions. MLT has also a definite immunomodulatory action both in mammals. The age-related weakening of the immune system occurs with the decrease of plasma MLT concentration (Esquifino et al. 2004). Furthermore, MLT shows valued antiaging effects in rats and protects cells from lipid peroxidation and other damaging progressions associated with OS (Paradies et al. 2010). The age-related decrease in serum MLT levels may play an important part in the raised oxidative damage in the elderly (Reiter et al. 2002). Low-dose and long-term treatment of MLT against age-induced OS in mice tissues, namely, the brain, liver, spleen, and kidney, exhibited significant drop in the level of GSH, GSH-Px, and alkaline phosphatase activity (Manda and Bhatia 2003).

Mitochondria are responsible for definite serious process such as the generation of ATP. During this process mitochondria also are a main location for the production of ROS which needs to be scavenge before they damage organelles (Murphy 2009). The mitochondrial theory is one of the main aging theories. Oxidative damage of mitochondria could be responsible to many serious pathologies and especially to aging. There are some evidences related to use of antioxidants to prevent aging, but this is sparse concerning the actual application of regularly used antioxidants to influence the progression of the diseases or aging (Reiter et al. 2016).

Existence of MLT in different compartments in the body showed that very high amount is present in the skin (Fischer et al. 2006). This amount is may be 10- to 1000-fold higher than in the plasma (Reiter and Tan 2003; Reiter et al. 2005). It was found that melatoninergic antioxidative system (MAS) in the skin is modifying skin homeostasis and takes an important part to prevent the harmful UV solar skin injury

(Slominski et al. 2005). It is important to apply MLT before the UV irradiation (Dreher et al. 1998).

MLT has many attractive features for pharmaceutical use. MLT has many advantages like very low toxicity (Jahnke et al. 1999) and easily crossing physiologic barriers (Ceraulo et al. 1999; Bonnefont-Rousselot and Collin 2010). In addition to these advantages, MLT antioxidant capacity does not decline after being metabolized (Tan et al. 2001; Galano et al. 2013; Gurer-Orhan and Suzen 2015). Therefore, it is not unexpected that there are many research-related MLT and synthetic derivatives for several purposes (Suzen 2013; Galano 2016). The decline of MLT secretion in older ages depends on the degeneration of the serotonergic and noradrenergic neuron systems. This follows by the demolition of ovarian cyclicity which is related to MLT or by 5-hydroxytryptophan administration (Rúzsás and Mess 2000). Studies are demonstrating that dietary MLT supplementation has useful effects against age-related bone loss in old rats. Rats administrated with MLT had bigger bone volume and improved microstructure of aged bones against the control group (Tresguerres et al. 2014).

Sirtuins (SIRTs1–7) are a class of proteins that possess either mono-ADP-ribosyltransferase or deacylase activity and regulate the cell cycle, DNA repair, cell survival, and apoptosis. Therefore recently they are favorable in many pharmacological procedures and research in antiaging, cancer, or neurodegenerative diseases (Mayo et al. 2017). It was found that in yeasts there are some cellular factors called "silent information regulator" (Sir2 or sirtuin) which is present in human in seven forms (Blander and Guarente 2004). Many studies show the increase in activity, particularly on SIRT1, after MLT administration. A study showed that administration of MLT for dentate gyrus of rats reduced the OS as well as significantly increased SIRT1 levels (Kireev et al. 2013, 2014). SIRT1 have significant consideration as mediators of life span extension in some model organisms. Induction of SIRT1 expression also reduces neuronal degeneration in AD and HD. Tajes et al. (2009) observed that MLT is able to act like SIRT1 inducer and increases the level of young neurons.

Obviously SIRT1 is associated with the aging process. Since there is natural decrease in nighttime MLT levels in older age, it was suggested that this situation may increase the development of aging-related diseases such as cancer risk (Jung-Hynes and Ahmad 2009). Studies showed that SIRT1 is overexpressed in prostate cancer cells and MLT administration (20 mg/L) in drinking water reduced tumor growth (Jung-Hynes et al. 2011). Inhibition of SIRT1 activity by sirtinol improves the antitumor activity of MLT in a human osteosarcoma (Cheng et al. 2013). Chen et al. (2015) proved that SIRT3 facilitates the antioxidant activity of the MLT in hepatocytes. SIRT1, p53, and eNOS are some of the key indicators of progressive vascular dysfunctions associated with aging. MLT stimulates SIRT1 in primary neurons of young animals, as well as in aged neurons. It evidently improves the endothelial injury and reduced loss of SIRT1 by decreasing p53 expression (Rodella et al. 2013).

Aging is related with many physiological processes. One of them is immunosenescence which is a decline of immune function characterized by a decrease in the

functional activity of natural killer cells, granulocytes, and macrophages. MLT has been confirmed to have an immunoenhancing influence in mammals (Nelson 2004; Espino et al. 2012). Knowing that MLT is synthesized also by human lymphocytes supports the MLTs action in the regulation of the immune system (Carrillo-Vico et al. 2004). It induces production of interleukin-2 (IL-2), interleukin-6 (IL-6), and interleukin-12 (IL-12) and helps to reduction of CD8+ cells (Srinivasan et al. 2005).

Due to the biphasic chemical structure of MLT, it is able to diffuse straightforwardly in every skin and cell compartment. UV-exposed skin assists MLT metabolism to produce antioxidant MLT metabolites which can protect the skin cells from ROS (Tan et al. 2000, 2007). Molpeceres et al. (2007) investigated the daily MLT supplementation on liver apoptosis induced by aging in rats. Results revealed that liver apoptotic cell death which is increased by ROS was prevented by antioxidant properties of MLT.

One of the sources of hydroxyl radical is UV irradiation. When hydroxyl radical produced in the skin reacts directly with MLT (Berneburg et al. 1999). The products of the reaction of MLT and hydroxyl radical are 2-OH-MLT and 4-OH-MLT which are then metabolized to AFMK and AMK (Hardeland et al. 1993). MLT and antioxidant metabolites are able to scavenge hydroxyl radicals occurring under UV solar radiation and play an important role to reduce lipid peroxidation, protein oxidation, mitochondrial damage, and DNA damage. MLT is a promising molecules to protect the skin from aging. There is a strong indication that MLT may prevent UV injury if it is administrated before UV irradiation in applicable concentrations right at the irradiation site (Fischer et al. 2002; Lee et al. 2003).

After pinealectomy it is possible to observe morphometric and biochemical changes such as thickness of epidermis and dermis on skin architecture due to the lack of MLT secretion. Exogenous MLT administration to pinealectomized rats expressively improved these changes in all body areas and increased the levels of antioxidant enzymes, catalase and glutathione peroxidase (Eşrefoğlu et al. 2005). There is evidence that MLT administration to pinealectomized rats protects the cells. There is no nuclear irregularity and heterochromatin condensation present in the cell, and most importantly mitochondrion which is the main related factor to skin aging is undamaged. Data show that MLT has powerful therapeutic effects in healing age-related damage (Eşrefoğlu et al. 2006). Indeed MLT effect on the myocardial mitochondria of aged rats is found significant. In the MLT administrated group, ATP levels, cyt-c levels, and Bcl2 and Bax ratios were found significantly higher compared with the control group. Data propose that MLT has a protecting effect on mitochondrial function in aged rat (Guo et al. 2017). MLT precursor and metabolite *N*-acetylserotonin (NAS) might display antiaging activity like MLT on mice. Both NAS and MLT after 4 weeks of treatment significantly improved the antioxidant ability of the brain (Oxenkrug et al. 2001).

Ability of MLT interacts with many forms of free radicals such as $H_2O_2$, $^{\bullet}OH$, singlet oxygen ($^1O_2$), superoxide anion ($^{\bullet}O_2^-$), peroxynitrite anion ($ONOO^-$), and peroxyl radical ($LOO^{\bullet}$), and antioxidant activity indicates a free radical scavenger cascade in case of UV irradiation (Fischer et al. 2006; Tan et al. 2007). Evidence proposes that both aging and cancer are related due to DNA damage caused by ROS

(Longo et al. 2005). Anisimov et al. (2003, 2006) showed that lower doses of MLT treatment did decrease the body weight of mice and increased life span of the last 10% of the survivors as well as significantly decreased tumor incidence. This data reveals that MLT act as a dose-dependent geroprotector.

The effect of MLT on life span was very significant on fruit flies. The maximum life span was determined 61.2 days in controls and 81.5 days in the MLT administrated group. The results also revealed that MLT administration made fruit flies stronger against superoxide-type free radicals. A similar study showed that MLT, addition daily to the nutrition medium at a concentration of 100 μg/ml, meaningfully increased the life span of the *D. melanogaster* Oregon wild strain (Bonilla et al. 2002).

After having MLT in the night drinking water (10 mg/l) since the age of 3 months until natural death, survival of LIO rats was found expressively higher against control group (80% and 57%, respectively, $p < 0.05$) (Vinogradova et al. 2005). Both MLT and its metabolite NAS administered in C3H mice with drinking water prolonged life span in male animals by about 20% versus control animals ($p < 0.01$) (Oxenkrug et al. 2001). Studies showed that life span-promoting activity of MLT may be due to activation of SIRT1 (Ramis et al. 2015). SIRT1-mediated mitochondrial biogenesis may reduce the manufacture of ROS, a potential source of aging (Guarente 2007).

Female fertility is adversely affected by aging, with a decline in oocyte quality being the major contributing factor for female infertility with ovarian aging. Female fertility is adversely affected by aging, with a decline in oocyte quality being the major contributing factor for female infertility with ovarian aging. At both physiological and pharmacological concentrations, MLT reduces OS and adjusts cellular metabolism. Research of reproductive physiology proves the role of MLT in reproduction system. MLT has cytoprotective effects due to it has antioxidant properties. Long-term MLT administration prevents ovarian aging in mice. MLT shows various functions at different phases of follicle growth. Data shows that delay in ovarian aging take places via several mechanisms mainly related to MLT's antioxidant properties (Cruz et al. 2014; Tamura et al. 2014, 2017). Neurodegenerative diseases such as Huntington disease (HD) and AD are chronic, progressive diseases and described by the damage of mental functions and loss of memory. Studies proved that in AD patients MLT inhibits the amyloidogenic action of β-amyloid protein. Evaluation of the neuroprotective activity of MLT in AD is promising and competent to be an anti-AD therapy (Shukla et al. 2017). Due to MLT's antioxidant potential and anti-amyloid activity, it is not unexpected that MLT is defensive in many age-related diseases. Long-term MLT treatment produces anti-amyloid and antioxidant effects before the amyloid formation in the brain (Zhang et al. 2004). MLT treatment has been proposed to increase circadian rhythmicity by reducing agitated behavior and confusion and produce valuable effects on memory in AD patients (Cohen-Mansfield et al. 2000). This may be one of the potential approaches for symptomatic treatment.

## 14.7 Conclusions

Aging of the skin is a complicated progression involving many endogenous factors and numerous environmental factors. Dermatological research in the area of dermato-endocrinology is hopeful to develop effective antiaging agents from extremely promising MLT and related MLT derivatives (Kleszczynski and Fischer 2012). MLT has attracted a great deal of exploratory consideration. Research shows that there are numerous MLT-related compounds as a result of its promising pharmacological activities (Galano et al. 2017).

MLT seems to have substantial antiaging properties (Reiter et al. 2000). Search for a pharmaceutical agent that can increase the quality of life in the elderly suggests that the agent definitely have antioxidant and immunoenhancing properties. In this perspective, the role for MLT in our lives is undiscussable.

Data underlined that some of the harmful effects of OS in elderly may be prevented. So, therapies focused on reducing OS are beneficial. Use of MLT could prevent or delay the weakening of the immune system which is closely related with aging (Yoo et al. 2012). Similarly, dietary intake of MLT showed a reduction in proinflammatory markers and increase in anti-inflammatory markers in serum (Delgado et al. 2012). Depending on these findings and with lack of toxicity, high lipophilicity, and antioxidant properties, MLT is one of the most attractive molecules to be inspected in relation to age-associated diseases and should be supposed as a promising molecule to increase the quality of life in aging population.

Mitochondria are responsible for definite serious process such as the generation of ATP. During this process mitochondria also are a main location for the production of ROS which needs to be scavenge before they damage organelles (Murphy 2009). The mitochondrial theory is one of the main aging theories. Oxidative damage of mitochondria could be responsible to many serious pathologies and especially to aging. There are some evidences related to use of antioxidants to prevent aging, but this is sparse concerning the actual application of regularly used antioxidants to influence the progression of the diseases or aging (Hardeland 2012; Reiter et al. 2016).

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# 15 Antiaging and Neuroprotective Properties of Mediterranean Diet Components in Humans

Akhlaq A. Farooqui and Tahira Farooqui

**Abstract**

Mediterranean diet consists of fresh fruits, vegetables, legumes, whole grains, fish, olive oil, garlic, and red wine. Levels of saturated fats are very low in Mediterranean diet. Among Mediterranean diet components, fresh fruits and vegetables provide various vitamins, carotenoids, flavonoids, fiber, and metal ions (potassium, magnesium, and calcium). Fish provides eicosapentaenoic and docosahexaenoic acids; olive oil is enriched in polyphenols (tyrosol, hydroxytyrosol, and oleuropein); red wine contains resveratrol; and garlic is enriched in sulfur compounds (alliin, allicin, S-allyl cysteine, and diallyl trisulfide). High levels of free radicals and neuroinflammation play an important role in cardiovascular diseases, type 2 diabetes, and neurological disorders. Mediterranean diet-derived metabolites are known to block free radical damage and retard neuroinflammation in above pathological conditions. Collectively, these studies indicate that the consumption of Mediterranean diet from the childhood to the old age not only leads to decrease in cardiovascular diseases, type 2 diabetes, and many types of cancers but also slows the onset of neurological disorders.

**Keywords**

Mediterranean diet · Vegetables and fruits · Olive oil · Resveratrol · Docosahexaenoic acid · S-Allyl-L-cysteine · Allicin · Oxidative stress · Neuroinflammation

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## 15.1 Introduction

Aging is a complex and multifactorial process, which is driven by the induction of oxidative stress, onset of persistent low-grade inflammation, energy failure, and mitochondrial dysfunction along with shortening of telomeres. These processes contribute to various structural, functional, and metabolic changes in the brain, leading to cognitive decline and increase in the vulnerability to chronic visceral and brain diseases (diabetes, metabolic syndrome, cancer, and neurodegenerative diseases)". The onset of these diseases produces cognitive decline, eventually resulting in cell death. The cognitive decline in old age not only slows processing and encoding of new information into episodic memory, but markedly affects the ability to recall semantic knowledge, which remain relatively stable in the adult life span (Park and Payer 2006). Cognitive decline is modulated by several factors (Fig. 15.1). Collective evidence suggests that age-related decline in cognitive function is caused by (a) changes in gray matter and white matter volumes and deterioration of neuronal and mitochondrial membranes (Giorgio et al. 2010; Thambisetty et al. 2010), (b) alterations in cerebral blood flow (CBF) to the brain regions (Lu et al. 2011), and (c) decrease in functional connectivity in these regions (Geerligs et al. 2015). In addition to abovementioned changes in the brain, age-related cognitive decline is also affected by the decrease in the monoaminergic neurotransmission (Bäckman et al. 2006, 2010), insulin resistance, alterations in endothelial

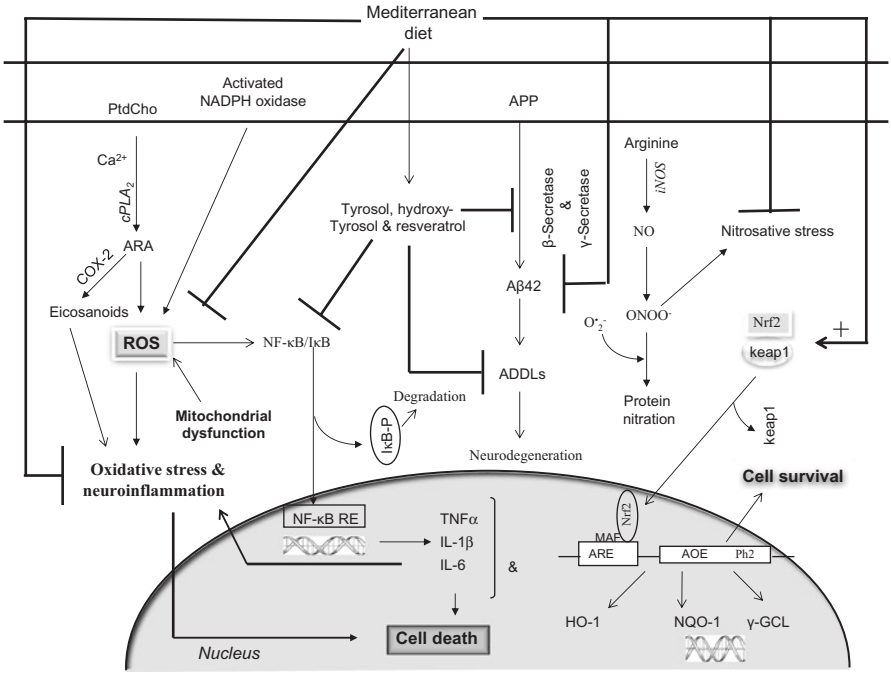


**Fig. 15.1** Factors which regulate cognitive decline in the aging brain

function, decrease in hormone, and changes in the brain-derived neurotrophic factor (BDNF) levels. In addition, aging is also accompanied by decrease in p-CREB (phosphorylated cAMP response element-binding protein) and reduction in neuropeptide Y (NPY) (Hattiangady et al. 2005) and lack of social network. These alterations in neurotransmitter and growth factor signaling produce reductions in synaptic density and plasticity (adaptability) (Sametsky et al. 2010) and produce 50% reduction in the length of myelinated axons (Rabbitt et al. 2001), making the brain networking increasingly less efficient with aging. Furthermore, in the frontal cortex after age 40, there is reduction in expression of genes related to synaptic plasticity, vesicular transport, and mitochondrial function (Lu et al. 2004). These processes modulate the age-dependent cognitive decline. The most important biological marker of aging is the shortening of telomere. It not only affects life expectancy but also increases the individual susceptibility to the development of chronic visceral and neurodegenerative diseases (Paul 2011; Rafie et al. 2017). Lifestyle (diet and exercise) and metabolic factors (particularly an increased visceral adipose tissue and circulating glucose levels) promote rapid shortening of telomeres and decrease in telomerase activity, suggesting the key role of the environmental factors in the cellular senescence (Epel et al. 2006). It is hypothesized that successful cognitive aging requires interactions between neural plasticity and cognitive plasticity (Greenwood and Parasuraman 2010). Age-related decline in cognitive function can be improved by the healthy lifestyle (long-term consumption of Mediterranean diet and exercise) to increase insulin sensitivity with the addition of cognitive training protocols (Chapman and Mudar 2014; Chapman et al. 2015) as well as physical exercise regimens (Chapman et al. 2013; Farooqui 2014) to maintain cognition. Consumption of Mediterranean diet and exercise are known to promote and maintain cognitive gain, which is linked with increases in cerebral blood flow, functional connectivity, and signal transduction processes associated with neural cell survival (Farooqui 2014). The main objective of this chapter is to present readers with the beneficial effects of components of Mediterranean diet on signal transduction processes related to healthy brain function.

## 15.2 Components of Mediterranean Diet

Mediterranean diet contains many nutrients, vitamins, and minerals (Farooqui 2012). Regular intake of Mediterranean diet slows the age process by retarding free radical damage, reducing neuroinflammation, and supporting, maintaining mitochondrial function, and increasing longevity (Farooqui 2012). Flavonoids induce their effects by modulating and maintaining cognitive functions through the increase in cerebral blood flow, inhibiting oxidative stress-mediated neuronal damage, reducing neuroinflammation, and stimulating neuronal signaling pathways that involve serine/threonine-specific protein kinase (Akt), extracellular signal-regulated kinase (ERK), and elevation in the expression of brain-derived neurotrophic factor (BDNF) (Miller and Shukitt-Hale 2012; Rendeiro et al. 2013). Flavonoids also contribute to the regulation of carbohydrate digestion, insulin secretion, insulin signaling, and

glucose uptake in insulin-sensitive tissues through various intracellular signaling pathways (Hanhineva et al. 2010).

Olive oil constituents (tyrosol, hydroxytyrosols, oleocanthal, and oleuropein) and garlic components (allicin, alliin, diallyl sulfide, diallyl disulfide, diallyl trisulfide, S-allyl cysteine, ajoene) induce antioxidants and anti-inflammatory effects (Fig. 15.2). Similarly, fish is enriched in omega-3 fatty acids (EPA; 20:5n3) and docosahexaenoic acid (DHA; 22:6n3) (Fig. 15.2). These fatty acids are transformed by 15-lipoxygenases into docosanoids (resolvins, neuroprotectins, and maresins) (Serhan 2008; Farooqui 2012). Docosanoids induce antioxidant, anti-inflammatory, and antiapoptotic properties. Red wine contains a polyphenol called resveratrol (Fig. 15.2). Resveratrol promotes antiaging, cardioprotective, and cerebroprotective effects.

Regular consumption of Mediterranean diet components slows cognitive decline by preventing free radical damage (oxidative stress) to neural cell components and inhibiting neuroinflammation (Farooqui 2012). Furthermore, regular consumption of Mediterranean diet also retards the onset of type 2 diabetes and metabolic syndrome. These pathological conditions may contribute to the development of stroke, Alzheimer's disease, and depression. Long-term intake of Mediterranean diet increases longevity not only by protecting the telomere length (Lopez-Miranda et al. 2007, 2012; Boccardi et al. 2013) but also by producing antiproliferative, antiviral, and hypocholesterolemic effects (Joseph et al. 2007).

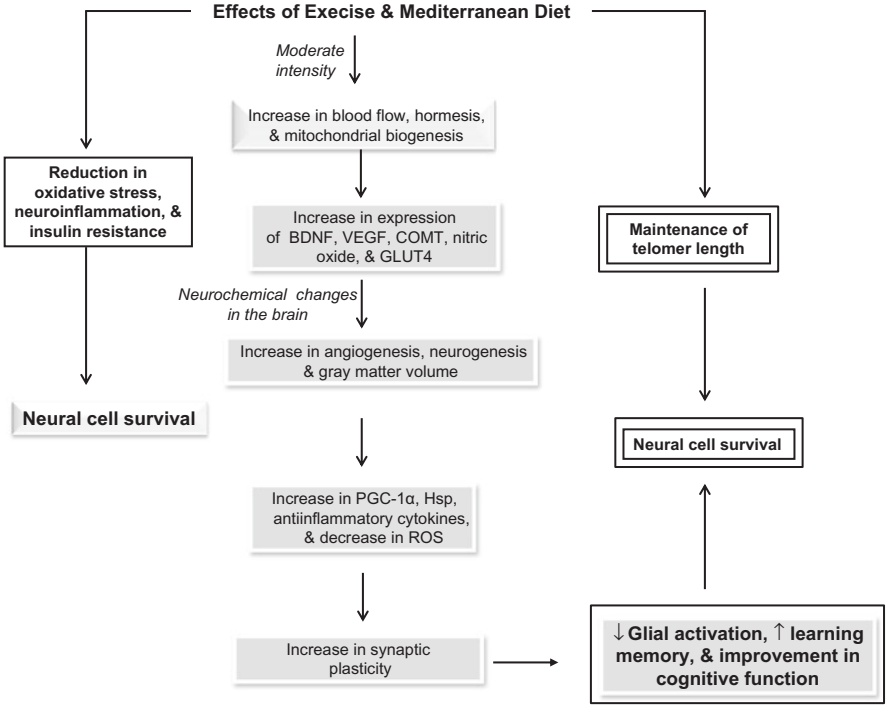


**Fig. 15.2** Chemical structures of Mediterranean diet components

## 15.3 Effects of Mediterranean Diet-Derived Mediators on Aging, Type 2 Diabetes, and Neurological Disorders

Aging is accompanied by the loss of several functions such as thinking, remembering, and reasoning that interfere with daily activities. Individuals with cognitive dysfunction lose ability to learn, recall, concentrate, and problem-solve. Cognitive function is regulated not only by neurochemical and intricate synaptic changes but also by neuronal and glial interactions (Morrison and Baxter 2012). Decline in cognitive function predisposes individuals to dementia and neurological and psychiatric disorders, eventually affecting the quality of life. The regular consumption of Mediterranean diet slows the progression of cognitive decline. The Alzheimer's Society recommends the consumption of Mediterranean diet. This approach not only slows aging but also improves memory by decreasing cognitive dysfunction (Alzheimer's Society Mediterranean Diet 2016; Aridi et al. 2017). Investigators have developed various cognitive tests to measure cognitive dysfunction. These tests include patient's responses to questionnaires, determination of blood components, brain scans, personal history, and a specific cognitive test. Cognitive testing can range from a few minutes to more than 2 h (Alzheimer's Australia Tests 2016). Many lifestyle-related factors, such as heart disease, diabetes, depression, excessive consumption of alcohol, smoking, lack of exercise, and poor dietary habits (Alzheimer's Association 2016), play an important role in the maintenance of our health. These factors are reversible. However, age and family history are irreversible factors, which are not affected by the lifestyle-mediated changes. In a study of 2258 community-dwelling, non-demented New Yorkers, intake of Mediterranean diet decreases the onset of Alzheimer's disease over an approximately 4-year period compared to individuals who poorly consumed Mediterranean diet. These individuals have an approximately 40% greater risk of onset of AD (Scarmeas et al. 2006). The Mediterranean diet consumption reduces cognitive impairment during aging. However, this controversial issue is still under investigation. At least two studies on the basis of MRI study has demonstrated that regular intake of Mediterranean diet not only preserves cortical thickness (Mosconi et al. 2014) References of Mosconi et al have been repeated. Please give one reference and remove a and b. Results based on meta-analysis of several studies have indicated that regular consumption of the Mediterranean diet decreases mild cognitive impairment (MCI) by 27% and chances of developing AD by 36% among cognitively normal adults (Singh et al. 2014). Aging process substantially decreases hippocampal neurogenesis, a process by which stem cells in the hippocampus transform themselves into new mature neurons that may integrate into the local circuitry. These observations suggest that neurogenesis is not only closely associated with neural plasticity and brain homeostasis but also plays an important role in preserving the cognitive function and repairing the damaged brain cells in the aging brain (Kuhn et al. 1996; Rao et al. 2005, 2006; Drapeau and Abrous 2008).

Type 2 diabetes, a lifestyle pathological condition, is linked with heart disease, peripheral vascular disease, nephropathy, blindness, as well as stroke, Alzheimer's disease, and depression (Farooqui 2013). Regular consumption of Mediterranean diet not only reduces the risk of onset of type 2 diabetes but also

delays the induction of neurological disorders by decreasing the oxidative stress, neuroinflammation, and insulin resistance and stimulating the immune system. The reduction in above parameters preserves cognitive function, reduces platelet aggregation, and regulates hormonal metabolism. In contrast, regular intake of Western diet, which is enriched in simple sugars; high in saturated fat, protein, and salt; and low in fiber, increases the risk of type 2 diabetes, stroke, AD, and depression (Farooqui 2013, 2015). Oleuropein, which is a constituent of olive oil (Fig. 15.2), increases the total antioxidant capacity in plasma of healthy elderly people (Oliveras-López et al. 2013). Oleuropein also increases catalase activity in erythrocytes and decreases superoxide dismutase and glutathione peroxidase activities (Oliveras-López et al. 2013). By binding to Aβ peptide, oleocanthal induces the morphological and functional changes in neurons, which contributes to the pathogenesis of AD. Oleocanthal nonselectively inhibits cyclooxygenase (COX) activity (Abuznait et al. 2013). The treatment of mice with oleocanthal for 1 month significantly reduces levels of Aβ not only in the hippocampus but also in cerebral microvessels (Abuznait et al. 2013), suggesting that this component of olive oil may slow the development of AD by blocking the production of Aβ (Lopez-Miranda et al. 2007; Sofi et al. 2008) and reducing the activation of astrocytes and microglia. Activation of glial cells contributes to increase in neuroinflammation through the upregulation and elevated secretion of proinflammatory cytokines.

Organosulfur compounds of garlic are metabolized by the humans. Thus, γ-glutamyl-cysteine is converted to alliin (+*S*-allyl-L-cysteine sulfoxide), which is then transformed into allicin (thio-2-propene-1-sulfinic acid *S*-allyl ester). This metabolite acts as antibiotic. It is a stronger antibiotic than penicillin or tetracycline. It stimulates humoral and cell responses of the immune system. Upon heating in the presence of oxygen, allicin is transformed into ajoenes [(*E*, *Z*)-4,5,9-trithiadodeca-1,6,11-triene 9-oxides], which possess many pharmacological activities, including antithrombotic, lipoxygenase inhibitory, fibrinolysis enhancing, and platelet activation inhibiting effects along with antimicrobial, anticancer, and cholesterol-lowering effects (Powolny and Singh 2008). In aqueous solutions, allicin quickly decomposes to several other small sulfur-containing metabolites (Amagase et al. 2001). These metabolites produce antioxidant, anti-inflammatory, antidiabetic, anti-atherosclerosis, antimicrobial, anticancer, and immune-modulatory effects. Abovementioned effects are produced by the modulation of NF-κB and Nrf2. Thus, downregulation of NF-κB by organosulfur compounds of garlic retards the production and secretion of TNF-α, IL-1β, IL-6, and chemokines (Ho and Su 2014; Xiao et al. 2012). Organosulfur compounds of garlic produce antioxidant effects by stimulating Nrf2 pathway. Under physiological conditions, Keap1 and Nrf2 complex is present in the cytoplasm (Suzuki and Yamamoto 2015). In the presence of organosulfur compounds of garlic, Nrf2 translocated from cytoplasm to the nucleus, where in the presence of Maf, it interacts with ARE and modulates the induction of antioxidant enzymes, which plays an important role in neuroprotection.

Fish contains EPA and DHA. EPA is metabolized by 15-lypoxygenase (15-LOX) into three-series prostaglandins and thromboxanes, five-series leukotrienes, and E-series resolvins (resolvin $E_1$ or $RvE_1$). The oxidized metabolites of EPA induce

anti-inflammatory and antiproliferative effects**.** Moreover, EPA is also oxidized by 5-cyclooxygenase (5-COX) and 5-lipoxygenase (5-LOX). These enzymes produce three-series prostaglandins and thromboxanes and five-series leukotrienes, respectively. The biological effects of these metabolites are different from the corresponding metabolites of arachidonic acid (ARA). Thus, EPA-derived $PGE_3$, $LTB_5$, and $TXA_3$ produce less efficient aggregation of blood platelet than ARA-derived $PGE_2$, $LTB_4$, and $TXA_2$. Similarly, $PGE_3$, $LTB_5$, and $TXA_3$ produce less vasoconstrictive effect on blood vessels than $PGE_2$, $LTB_4$, and $TXA_2$ (Calder 2009). 15-LOX transforms EPA into resolvins of the E series (Arita et al. 2006, 2007), including resolvin $E_1$ and resolvin $E_2$. $RvE_1$ and $RvE_2$ produce strong anti-inflammatory and pro-resolution effects in vivo (Arita et al. 2006). $RvE_1$ and $RvE_2$ interact with their G protein-coupled receptors (see below). $RvE_1$ suppresses the activation of NF-κB and reduces the expression of tumor necrosis factor-α (TNF-α) through binding with PMN (Arita et al. 2007).

The 15-LOX-like enzyme transforms DHA into resolvins $D_1$–$D_6$ ($RvD_1$, $RvD_2$, $RvD_3$, $RvD_4$, $RvD_5$, and $RvD_6$). DHA is also metabolized by COX-2 in the presence of aspirin. This leads to the production of D-series resolvins (AT-Rv). In humans and mouse, these metabolites produce their anti-inflammatory and pro-resolutionary effects by interacting with neutrophils and glial cells. In microglial cells, both 17*S* and 17*R* D-series resolvins inhibit the expression of proinflammatory cytokines (Serhan 2008; Serhan et al. 2008). Resolvins act through specific receptors called resolvin D receptors ($resoDR_1$) (Serhan 2008; Serhan et al. 2008). Interactions between D-series resolvins and $resoDR_1$ regulate neuroinflammation and immunoregulatory function in the brain. In the brain, D-series resolvins not only inhibit the expression of cytokines (TNF-α, IL-1β, and IL-6) but also regulate the trafficking of leukocytes at the injury site (Serhan et al. 2008). 15-LOX also converts DHA into protectin $D_1$ ($PD_1$). In the brain this metabolite is known as neuroprotectin $D_1$ (Hong et al. 2003). $NPD_1$ reduces stroke-mediated neuronal injury (Marcheselli et al. 2010) and induces neural cell survival by stimulating neuroprotective gene-expression programs and initiating its antiapoptotic effects. These processes also suppress Aβ42-mediated neuronal cell death in AD (Lukiw et al. 2005). Furthermore, DHA and $NPD_1$ promote neuroprotective effects by maintaining the integrity of synapses and decreasing the number of activated microglia in the hippocampus (Pomponi et al. 2008). In macrophages, DHA is also oxidized by a 14-LOX. The action of this enzyme results in the formation of new metabolite called as maresin (MaR1) (Fig. 15.4). This metabolite is involved in the termination of PMN infiltration, as well as stimulation of macrophage phagocytosis. 7S,14S-diHDHA, an isomer of MaR1, is also produced by macrophages. This isomer is less active than MaR1 in terminating PMN infiltration. This observation suggests that DHA produces several MaRs, which may stereoselectively regulate catabasis and facilitate arrival of tissues to homeostasis (Serhan et al. 2009).

Resveratrol is a polyphenolic trans-stilbene (Fig. 15.2), which has the ability to cross blood-brain barrier (BBB), inhibits oxidative stress, and attenuates neuroinflammation. The bioavailability of resveratrol is low because it is metabolized into glucuronide and sulfate derivatives. Resveratrol acts by stimulating Sirtuin (SIRT1).

This enzyme regulates transcription, metabolism, and cellular stress response (Alcain and Villalba 2009). Sirtuin also blocks the activation of microbial cells by inhibiting the activation of NF-κB and retarding the expression of proinflammatory cytokines (Capiralla et al. 2012). In addition, intake of resveratrol produces conditions similar to caloric restriction (Baur and Sinclair 2006). Antioxidant and free radical scavenging properties of resveratrol are due to its ability to donate hydrogen atoms or electrons to the free radicals generated by the oxidative stress according to the following reaction (Hussein 2011) (Fig. 15.3):

$$\text{Resveratrol} + {}^{\bullet}\text{OH} \rightarrow \text{Resveratrol}^{+} + \text{OH}^{-} \leftrightarrow \text{RV}\left(-\text{H}\right)^{\bullet} + \text{H}_2\text{O}$$

Resveratrol-mediated activation of heme oxygenase produces antiaging effects. Resveratrol produces inhibition of neuroinflammation by blocking the activation of microglial cells, inhibiting NF-κB activation, and decreasing the expression of proinflammatory cytokines. Treatment of animals with resveratrol not only stimulates neurogenesis and angiogenesis in the microvasculature but also diminishes astrocyte hypertrophy in the hippocampus (Maruszak et al. 2014). Furthermore,

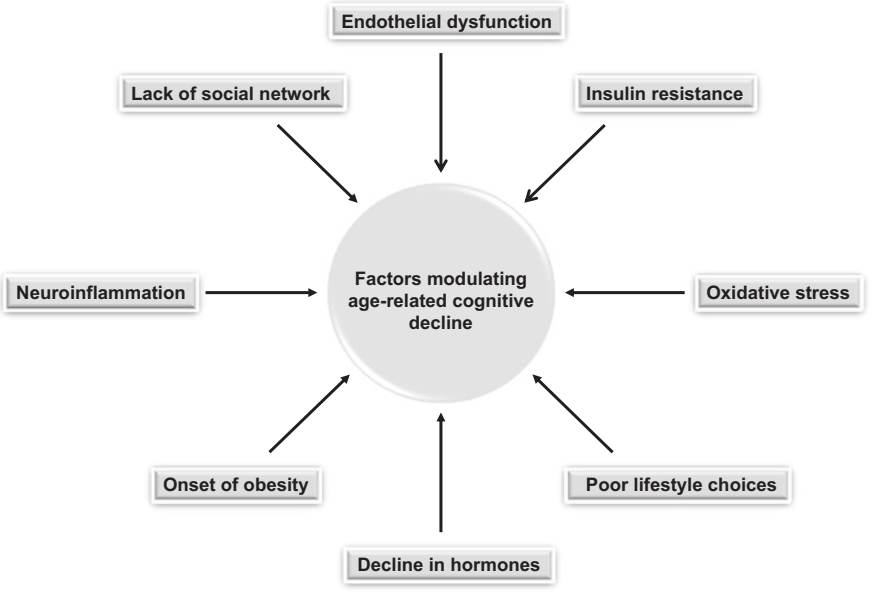


**Fig. 15.3** Effects of Mediterranean diet on neuronal cell death and survival. Phosphatidylcholine (PtdCho); arachidonic acid (ARA); cytosolic phospholipase $A_2$ ($cPLA_2$); cyclooxygenase-2 (COX-2); reactive oxygen species (ROS); nuclear factor kappaB (NF-κB); nuclear factor κB-response element (NF-κB-RE); inhibitory subunit of NF-κB (IκB); phosphorylated IκB (IκB-P); tumor necrosis factor-α (TNF-α); interleukin-1β (IL-1β); interleukin-6 (IL-6); amyloid precursor protein (APP); beta- amyloid (Aβ42); inducible nitric oxide synthase (iNOS); superoxide ($O_2^-$); and NFE2-related factor 2 (Nrf2); heme oxygenase 1 (HO-1); NADH quinine oxidoreductase, γ-glutamylcystein ligase (γ-GCL), inducible nitric oxide synthase (iNOS); nitric oxide (NO); superoxide ($O_2^-$); peroxynitrite (ONOO-)

intraperitoneal injections of resveratrol also produce neuroprotective effects through the upregulation of superoxide dismutase and catalase (Mokni et al. 2007). Resveratrol also protects against the colchicine-mediated cognitive impairment, reduces levels of MDA and nitric oxide, and normalizes levels of reduced glutathione (Kumar et al. 2007). Several studies have indicated that in late middle age, intake of resveratrol not only improves memory but also induces better mood function. Collective evidence suggests that resveratrol acts by modulating hippocampal plasticity and suppressing chronic low-level inflammation (Kodali et al. 2015). Resveratrol increases the cerebral blood flow and decreases levels of secreted intracellular Aβ peptides and α-synuclein in animal models of AD and PD (Wang et al. 2008). Resveratrol also stimulates the degradation of intracellular Aβ. This process involves proteasomal enzymes (Marambaud et al. 2005). In the Tg2576 mouse model of AD, resveratrol stimulates α-secretase-mediated degradation of APP. This process generates AICD. This metabolite improves memory and induces neurite extension leading to neuroprotection. α-Secretase-mediated degradation of APP prevents the generation of Aß42, a neurotoxic peptide, which contributes to the pathogenesis of AD (Wang et al. 2006a). Resveratrol also blocks the aggregation of the Aβ peptide (Riviere et al. 2007). Resveratrol has no effect on degradation of APP by β- and γ-secretases. Furthermore, it does not stimulate Aβ degradation by proteasomes (Marambaud et al. 2005). Resveratrol reduces the concentration of 8-iso-prostaglandin F2$\alpha$ and prevents free radical generation (Candelario-Jalil et al. 2007). Resveratrol also downregulates genes that control the expression of iNOS and production of prostaglandin $E_2$ ($PGE_2$), decreases the activation of cathepsin, and reduces the availability of NO (Kim et al. 2006).

Fresh fruits are important components of Mediterranean diet. They contain flavonols, anthocyanins, procyanidins, vitamins, and minerals, which produce beneficial effects in the vascular system associated with heart disease and neurological disorders. The beneficial effects of flavonoids in heart disease and neurological disorders are due to antithrombotic, anti-ischemic, antioxidant, and vasorelaxant activities (Fraga et al. 2010). Regular consumption of flavonoids also produces antitumoral, antiviral, and antibacterial effects in our bodies. Converging evidence suggests that flavonoids decrease the risk of heart disease and neurological disorders by improving vasodilatation and reducing blood clotting, as well as by preventing the oxidation of low-density lipoproteins (LDLs) (Atmani et al. 2009). Neurovascular unit, which comprises neural cells, pericytes, and endothelial cells, controls local brain perfusion. Neurovascular units are niches for neural stem/progenitor cells in the adult brain. Neurovascular units have the ability to undergo neurogenesis and angiogenesis, processes, which are linked with the formation of neurons and remodeling of blood vessels. The generation of new blood vessels promotes neurorestorative processes, such as synaptogenesis, which in turn lead to enhancement in neuroplasticity and neuronal function (Gomez-Pinilla et al. 2008; Farooqui 2014).

The consumption of fresh fruit-derived flavonoids promotes the formation of healthy neurons and astrocytes, which contribute to the neurovascular unit. The consumption of flavonoids also maintains health of endothelial cells at the BBB. In the cerebrovascular system, endothelial cells regulate cerebral blood flow by

modulating the generation of NO. In the brain, release of NO maintains microvascular pressure by regulating the dilation of the larger upstream arteries not only via endothelial cell-dependent mechanisms but also through vasomotor responses (Cipolla 2009). Thus, it is possible that flavonoid-mediated enhancement in neurovascular unit health and NO-mediated dilation in peripheral vessels may increase cerebral perfusion. This process may promote cognition in the stroke patients. Anti-inflammatory effects of the flavonoids are due to their ability to inhibit cyclooxygenase, lipoxygenases, and iNOS, which contribute to the generation and release of prostaglandins $E_2$, $F_2$, and thromboxane $A_2$ and peroxynitrite, respectively (Wang et al. 2006b). Converging evidence suggests that flavonoids play a key role in neuroprotection against acute injury (stroke) and AD and PD. Specific receptors through which flavonoids produce their effects have not been characterized. However, it is reported that flavonoids may produce their effects by binding with the γ-aminobutyric acid type A (GABAA) receptors in the brain, insulin-like growth factor 1 (IGF-1) receptor in hippocampal neurons (Marder and Paladini 2002), 5-HT1A serotonin receptor (Bodesheim and Holzl 1997), glutamatergic AMPA receptor, and adenosine (type I) receptors (Marder et al. 2003). These receptors are linked with protein kinases (protein kinase C, tyrosine kinases, serine/threonine kinases, and mitogen-activated protein kinase (MAPK)) through different signal transduction mechanisms (Schroeter et al. 2002). These observations suggest that flavonoids exert their beneficial health effects through multiple signal transduction mechanisms.

Above mentioned studies indicate that components of Mediterranean diet may reduce the detrimental effects of oxidative stress and neuroinflammation not only by normalizing mitochondrial dysfunction, decreasing insulin resistance, and improving endothelial function but also by improving signal transduction processes associated with cognitive function. In addition, regular consumption of Mediterranean diet also reduces homocysteine levels (Seshadri et al. 2002; Elias et al. 2005). Therefore, the consumption of Mediterranean diet increases longevity by maintaining the length of the telomeres (Boccardi et al. 2013). Decrease in telomere length produces cognitive impairment not only in poststroke patients (Martin-Ruiz et al. 2006) but also in older community-dwelling women (Yaffe et al. 2011). Furthermore, telomere degradation is linked with chronic inflammation (Kaszubowska 2008; Carrero et al. 2008) and cognitive decline (Gorelick 2010). This suggests that regular intake of Mediterranean diet increases longevity by maintaining the length of telomeres and retarding chronic oxidative stress and inflammation in visceral organs and brain.

Regular consumption of Mediterranean diet blocks the risk of cognitive decline in heart disease, cancer, diabetes, and metabolic syndrome along with neurological disorders (stroke, AD, and PD) (Farooqui 2012). This is because intake of Mediterranean diet inhibits oxidative stress and retards inflammation through Mediterranean diet-derived metabolites. In addition, these metabolites also induce insulin sensitivity and produce antiapoptotic effects, leading to the increased neural cell survival. The consumption (regular intake) of Mediterranean diet and regular exercise (45–60 min/day). (F2-isoprostane and 9-hydroxyoctadecadienoic acid for oxidative stress. Moreover, the consumption of Mediterranean diet increases levels of reduced glutathione and plasma ascorbic acid (Dai et al. 2008; Gaskins et al. 2010).

## 15.4 Effects of Mediterranean Diet and Exercise on the Brain Aging

The consumption of Mediterranean diet and regular exercise (45–60 min/day) produce beneficial effects on aging process (Fig. 15.4). Molecular mechanisms contributing to beneficial effects of Mediterranean diet consumption and exercise involve changes in the brain structural integrity by enhancing neurogenesis and angiogenesis with more secretions of growth factors, promoting formation of dendritic connections among neurons (Gomez-Pinilla et al. 2008; Farooqui 2014). Mediterranean diet-derived metabolites and regular exercise improve cognitive function by increasing the gray matter volume (Hillman et al. 2008) and initiating the differentiation of stem cells into neurons in the dentate gyrus. This increase in neurogenesis and angiogenesis is linked with exercise-mediated increase in cerebral blood flood flow and elevation in cerebral blood volume (van Praag et al. 1999). Thus, regular intake of Mediterranean diet and exercise promote the formation of new neurons and blood vessels, which is reflected in increase in cerebral blood flow and cognitive processing. Exercise also induces an increase in levels of VEGF, BDNF, catechol-O-methyltransferase (COMT), endorphins, and NO (Neeper et al. 1996; Stroth et al. 2010; Carmargo et al. 2013). In addition, consumption of Mediterranean diet and regular exercise also modulate the expression of genes, which promote insulin-like signaling, energy metabolism, neurogenesis, and synaptic plasticity (Reagan 2007; van Praag et al. 2005). These processes contribute to neuronal survival in the aging brain.

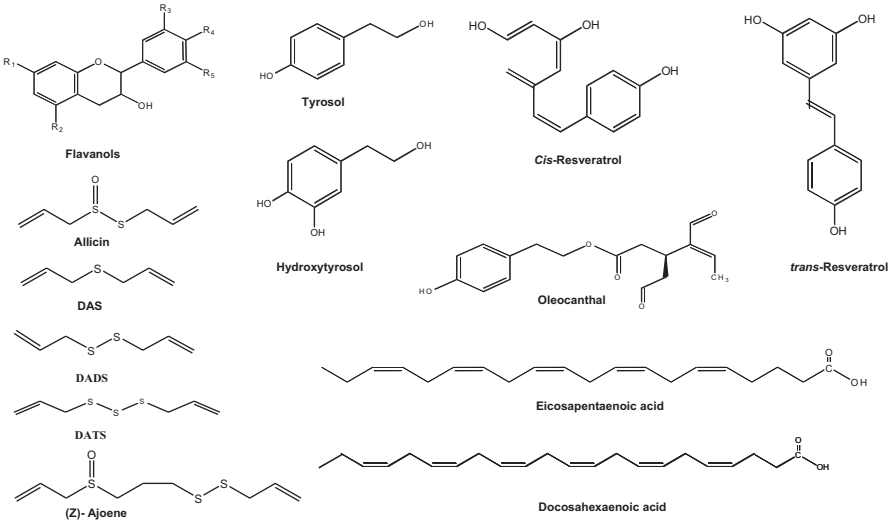


**Fig. 15.4** Beneficial effects of exercise and Mediterranean diet consumption on the brain. Vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1), catechol-O-methyltransferase (COMT), reactive oxygen species (ROS), and glucose transporter 4 (GLUT4)

## 15.5 Conclusion

Aging is multifactorial process, which slows metabolic processes and ultimately results in increased vulnerability to cellular degeneration and death. Aging also involves alterations in genomic stability, defects in nuclear architecture, decrease in telomere length, epigenetic alterations, and chromatin remodeling leading to alterations in neural cell signaling and intercellular communication. In addition, aging is also accompanied by mitochondrial dysfunction, increase in ROS, elevation in cytokines, and deregulation of autophagy. The deregulation of these processes contributes to the pathogenesis of age-related diseases. As mentioned earlier, the consumption of Mediterranean diet provides mediators, which slow aging, retard increase in ROS, inhibit cytokines, and improve cognitive decline in elderly population. Moreover, long-term consumption of Mediterranean diet also decreases risk of diabetes, metabolic syndrome, dementia, and AD.

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# Epigenetic Changes in Aging and Modulation by Dietary Nutrients

16

Shambhoo Sharan Tripathi

**Abstract**

Aging is a complex natural process, involving various factors exhibited by all living organisms. It is visible in a consistent deterioration of regular physiological functions in a time-dependent fashion. Various scientific studies in different model organisms indicate that epigenetic alterations play a huge role in the aging process. Such types of epigenetic change occur in the genes responsible for aging and also affect their function. These types of epigenetic changes occur at different levels that include DNA methylation, change in levels of the core histones, post-translational modifications of histone and replacement of canonical histones with another form of histone, and altered noncoding RNA expression, during both individual's aging and replicative senescence. Dietary nutrients can significantly affect the epigenetic medications. This chapter will discuss how these changes affect the functioning of the genes and are targeted by the dietary nutrients.

**Keywords**

Aging · Dietary nutrients · DNA methylation · Epigenetics · Histone modifications · ncRNA

## 16.1 Introduction

Aging is an irreversible and intricate biological process of growing older. After reproductive maturity, during the organism's life, the efficiency of various physiological processes declines with time (Kirkwood 2005; Hayflick 2007).The incompetence and malfunction of maintenance repair and turnover pathways are the major

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causes of aging and age-related diseases. Scientists are working on the genetic and epigenetic regulations of molecular mechanism(s) of aging and interactions among various gene products. The damage in molecular structures is associated with an accumulation of molecular oxidative environment. Concomitantly, numerous strategies are being tried and tested to slow down the aging process and prevent age-related pathologies. The final aim of such studies is to get better quality of human life during old age and lengthen the overall health span and life span.

There are different experimentally proposed models of epigenetic alterations especially in aging such as DNA methylation, heterochromatin loss model, and histone modification (Goll and Bestor 2005; Smith and Peterson 2005; Kouzarides 2007). In addition, noncoding RNAs in some species affect the expression of the genes linked to aging, which is also considered as a mechanism of epigenetic changes (Bernstein and Allis 2005). Some of them are discussed below.

### 16.1.1 DNA Methylation

DNA methylation is the basic characteristic of epigenetic changes; the reaction is catalyzed by DNA methyltransferases (DNMT) enzymes that transfer a methyl group from S-adenosyl-methionine (SAM) to the fifth carbon of a cytosine. DNA methylation is understood as a method of gene silencing, and it works differently. The shortest way is interfering with transcription factors or other transcriptional machinery that interact with cytosine in the major groove of DNA double helices. Since most of the transcription factors have DNA recognition motif-containing CpG-rich and GC-rich binding sites. Alternatively, transcriptional machinery can be directly excluded from methylated promoter DNA by altering nucleosome stability or position (Bird and Wolffe 1999). There are groups of methyl-CpG-binding domain proteins (MBDs) that affect the DNA methylation of genes. So far, five major mammalian MBD proteins have been identified: methyl-CpG-binding protein 2 (MeCP2), MBD1, MBD2, MBD3, and MBD4. Except for MBD3, the rest of proteins exhibits a higher binding affinity for methylated than unmethylated DNA (Fraga et al. 2003). Although each MBD augments transcriptional repression through direct interaction with co-repressors, viz., Sin3, c-Ski, and N-CoR, and makes a complex structure. This structure further binds to the histone deacetylases and employing them to the sites of methylation for the establishment of silent chromatin (Jones et al. 1998; Kokura et al. 2001; Bogdanovic and Veenstra 2009).

Several lines of evidence prove that DNA methylation is also associated with aging. DNA methylations at multiple CpG sites in human genome have been identified as candidate for age prediction (Zbiec-Piekarska et al. 2015). Methylation of CpGs in the EDARADD, TOM1L1, and NPTX2 genes was especially correlated with age, and by building a regression model using two cytosines from these loci, the authors were able to predict the age of an individual with an average error of 5.2 years (Bocklandt et al. 2011).

Lifestyle factors can positively or negatively have an effect on life span and are also identified to affect the DNA methylation. For instance, smoking may have

pro-aging effects by inducing methylation of DNA and changes of genes function involved in age-associated diseases such as cardiovascular pathologies and cancer (Breitling et al. 2011; Lee and Pausova 2013; Besingi and Johansson 2014). On the other hand, proper antioxidant intake, caloric restriction, and physical activity may exert antiaging action by neutralizing injurious DNA methylation alterations (Miyamura et al. 1993; Fang et al. 2007; Li and Tollefsbol 2010; Qi et al. 2010). All these aforementioned genes have a role in human pathologies. For example, EDARADD mutations are related to slow wound healing (Langton et al. 2008) and cause loss of teeth, hair, and sweat glands (Yan et al. 2002). In Parkinson's disease (PD), NPTX2 protein expression is high (Moran et al. 2008) and in pancreatic cancer (YB 2007), whereas TOM1L1 downregulation is reported in esophageal squamous cell carcinoma (Qi et al. 2010). On the basis of these findings, it has been hypothesized that epigenetic drift with aging and DNA methylation is associated with longevity (Jones et al. 2015; Mendelsohn and Larrick 2017). In several in vitro experiments, it has been proved that DNA methylation plays an important role in the regulation of life span. In one experiment with the model of premature senescence induced by hydrogen peroxide ($H_2O_2$) in human embryonic lung fibroblasts, a global hypomethylation was investigated during both stress-induced and replicative senescence (Zhang et al. 2008). In another study, when normal diploid fibroblasts from mice, hamsters, and humans were grown in culture, the 5-methylcytosine (5mC) content of DNA markedly decreased. The greatest rate of loss of 5-methylcytosine residues was observed in mouse cells, which survived the least number of divisions. Immortal mouse cell lines had more stable rates of methylation (Wilson and Jones 1983). Additionally, azacytidine (5-aza-CR) and azadeoxycytidine (5-aza-CdR) are known to inhibit the methylation of cytosine (5-mC) in DNA of human diploid fibroblasts and significantly reduce the doubling potential of cells in vitro (Holliday 1986). Inhibition of DNMTs with 5-azacytidine (5-AzaC) or with specific small interfering RNA (siRNA) against DNMT1 and 3b induced the cellular senescence of human umbilical cord blood-derived multipotent stem cells (hUCB-MSCs) and increased p16 (INK4A) and p21 (CIP1/WAF1) expression (So et al. 2011). In such a way, DNMTs play a role in maintenance of plasticity potential and self-renewal of stem cell that are related to aging and regeneration. In fact, in hematopoietic stem cells of the mouse which have reduced Dnmt1 activity cannot suppress myeloerythroid regulators as well as disallowing them from differentiating into lymphoid progeny. The above evidence leads to the conclusion that constitutive methylation is crucial for the renewal of hematopoietic stem cells (Broske et al. 2009).

Demethylation is also thought to play a prominent role in hematopoietic differentiation (Calvanese et al. 2012). Notable, it is possible to generate pluripotent stem cells from adult somatic cells through the induction of defined factors (Takahashi and Yamanaka 2006). These induced pluripotent stem cells (iPS) are similar to embryonic stem cells (ES) in morphology, proliferation and teratoma formation; however they are different with regard to gene expression and DNA methylation patterns and fail to produce adult chimeras. Thus, epigenetic reprogramming is required for the development of viable stem cell therapies (Okita et al. 2007). The

significance of DNA methylation in aging is also established by a large number of in vivo experiments, e.g., hypomethylation of whole genome is reported in the various organs of mice and rats with advancing of age (Vanyushin et al. 1973; Singhal et al. 1987; Mays-Hoopes 1989). A population-based investigation was performed to understand the fundamental dynamics of individual normal epigenomes. In this study making use of pyrosequencing probed 217 nonpathologic human tissues from 10 anatomic sites and analyzed DNA methylation of 1413 autosomal CpG loci associated with 773 genes in both young and old subjects. A strikingly significant CpG island-dependent connection between methylation and aging was observed. Loci outside of CpG islands lost methylation with age, whereas loci in CpG islands gained methylation with age (Christensen et al. 2009). In normal human prostate tissue procured from 45 organ donors, hypermethylation was observed as a function of age for CpG islands in RARb2, RASSF1A, GSTP1, NKX2-5, and ESR1 genes. Upon mutations in these genes, the chances of cancer pathogenesis and progression are increased (Kwabi-Addo et al. 2007). In an age-related study, in which the peripheral blood DNA from 318 humans of middle and advanced age were analyzed, it was found that the global DNA methylation levels were correlated to the frailty status in middle/advanced-aged subjects. A 7-year follow-up disclosed that a significant decrease in 5mC content was associated with a worsening in health status (Bellizzi et al. 2012).

Werner syndrome (WS) is characterized by growth retardation and premature aging, the gene known as WRN is mutated in patients. This gene plays roles in telomere maintenance, DNA repair, and transcription (Lutomska et al. 2008). It is reported that WRN aberrantly methylated in advanced oral squamous cell carcinoma (Supic et al. 2009); nevertheless, no study is available to show the methylome of WS patients. It would be interesting to know if these patients display atypical DNMT expression as well as atypical DNA methylation patterns compared to healthy controls.

### 16.1.2 The Role Heterochromatin in Aging

Heterochromatin is one of the first models of aging. According to this model, heterochromatin domains established early in embryogenesis are broken down during the aging process, contributing to the derepression of silenced genes and leading to aberrant gene expression patterns (Villeponteau 1997; Tsurumi and Li 2012). The loss of heterochromatin also leads to changes in global nuclear architecture. As a consequence, the expression of genes, located in those regions, gets affected directly or indirectly causing aging and cellular senescence. Like another model of aging, the heterochromatin loss model is proved by lots of experimental data, but there are also mystifying observations. Decay of the heterochromatin resulting in failure of transcriptional silencing happens during aging in all eukaryotes examined from yeast to humans (Smeal et al. 1996; Villeponteau 1997; Haithcock et al. 2005; Larson et al. 2012; Tsurumi and Li 2012), and accelerating or reversing this process can either shorten or lengthen the life span. Histone acetylation in heterochromatin

area is indispensable for gene silencing. Therefore, treatment with histone deacetylase (HDAC) inhibitors or deletion of genes encoding HDACs in yeast sirtuin-2 (SIRT2) or its counterparts SIRT1 in metazoan species shortens life span, whereas chemical activation or overexpression of SIRT2 extends life span (Haigis and Sinclair 2010; Guarente 2011). Yeast SIRT2 was first recognized as an H4K16Ac deacetylase, whereas mammalian SIRT1 is an H3K9Ac or H4K16Ac deacetylase (Haigis and Sinclair 2010). Sirtuins play a role in aging including genome maintenance by deacetylating histone and other transcriptional proteins (Oberdoerffer et al. 2008). Global heterochromatin loss is a mode of epigenetic variations and possibly associated with the origin of various molecular events of aging. It may also link with the various models of aging like the free radical theory, genetically programmed senescence, telomere shortening, genomic instability, nutritional intake, and growth signaling.

### 16.1.3 Histone and Protein Modifications

Histone modifications are occurring in both conditions either in gene activation or gene repression. Modifications for active transcription that includes acetylation of histone 3 (H3) and histone 4 (H4), and dimethylation (Me2) or trimethylation (Me3) of H3K4 and H3K36, are also known as euchromatic modifications. H3K9, H3K27, and H4K20 sites of methylation are located in heterochromatic regions (Ruthenburg et al. 2007). ATP-dependent chromatin remodeling and incorporation of specialized histone variants in chromatin structure are known as an epigenetic control mechanism (Wang et al. 2007a, b).

Methylation pattern of histones is associated with specific proteins that distinguish these marks and thus convey their silencing or activating effects. Aged animals have a different pattern of gene expression, and it is correlated with epigenetic modification of genes. An increase in H4K20 methylation has been observed in old age rat tissue (Sarg et al. 2002). With age, human cells have also reduced histone synthesis. Moreover, H4K20Me3 has also been reported in high amount in cells from patients with Hutchinson-Gilford progeria syndrome (HGPS) (Sarg et al. 2002). HGPS is a rare aging disorder that is distinguished by the early and rapid onset of some devastating phenotypes, for example, severe growth retardation, loss of subcutaneous fat, alopecia, loss of bone density, and poor muscle development (Kudlow et al. 2007). Along with, H4K20Me3 modification was in concert with another histone modification such as decrease of H3K27Me3 and H3K9Me3. The enzyme responsible for methylation of H3K27, i.e., methyltransferase, was also shown to be downregulated in HGPS cells (Shumaker et al. 2006). In another experiment, after several passages in culture condition of cells derived from normal individuals (more than 80 years), showing a similar pattern of chromatin modification like HGPS patients (Scaffidi and Misteli 2006).

In yeast, SIRT2 (member of NADP-dependent HDACs family) maintains chromatin modification (Sengupta and Seto 2004). It silences gene by deacetylating histones H3 and H4 and with the help of other silencing proteins specifically to

heterochromatic regions located at ribosomal DNA, telomeres, and silenced mating-type loci. SIRT2 shortens the life span after inactivation, whereas its overexpression lengthens life span (Kennedy et al. 1995; Haigis and Guarente 2006; Longo and Kennedy 2006). Therefore, nuclear distribution of SIRT2 complex regulates yeast aging. This modulates the chromatin structure of the specific region of the genome. Apart from this, the second protein is histone chaperones, also called as the histone binding protein (HBP), that directly control the chromatin structure together with the histone (Vaquero et al. 2003). It is reported in *Drosophila* that the dynamic exchange of histones by chaperones might function in epigenetic regulation (Ahmad and Henikoff 2002a, b).

### 16.1.4 ncRNA Model of Aging

ncRNAs represent a functionally and structurally diverse class of RNA species that participate in a wide range of basic cellular processes including protein translation, mRNA splicing, chromatin organization, and the regulation of gene expression (Esteller 2011). Several classes of ncRNAs (e.g., miRNAs, rRNAs, tRNAs, and many lncRNAs) fulfill discrete functions within the cells. RNA genes are sequences that transcribe ncRNAs and play a direct role in RNA processing and degradation as well as indirectly control gene expression (Ying et al. 2008).

It has been shown in many studies that the status of miRNAs level changes throughout the life span in several species and can be associated with age-related disorders (Wang et al. 2008). Knocking out the miRNA of gene lin-4 in the nematode decreased the life span and accelerated tissue aging, whereas overexpression of this miRNA extended it (Boehm and Slack 2005). Evidence in mammals proposes that miRNAs might play a role in age-associated conditions such as neurodegeneration (Nelson and Keller 2007; Wang et al. 2008).

## 16.2 Epigenetic Modulation by Dietary Nutrients

There is enough supporting data that advocates the role of the nutrients in the epigenetic modification, either at the global scale or at locus-specific sites (Bogdarina et al. 2010; Vucetic et al. 2010; Dudley et al. 2011; Jousse et al. 2011; Altmann et al. 2012). For DNA methylation, there are three ways by which nutrition affects its pattern, i.e., (1) by providing substrates for proper DNA methylation, (2) by being a cofactor it modulates the DNMT enzyme activity, and (3) by regulating one-carbon cycle they influence the activity of the enzymes regulating the one-carbon cycle. Importantly, all three mechanisms are mutually compatible with each other and may operate together in a time. Aging is the essential risk factor for developing cancer. It is well established by an investigation that epigenetics play a role in both cancer and aging. Dietary nutrients (Tables 16.1 and 16.2) such as resveratrol are an important mediator of aging and act on SIRT1 (a kind of HDAC) that leads to increased

**Table 16.1** Dietary nutrient targeting DNA methylation and histone modification

| Nutrients | Sources | Molecular targets | References |
|---|---|---|---|
| Epigallocatechin-3-gallate (EGCG) | Green tea | DNMT, HDAC, H3 and H4 acetylation, HMT, H3K27m3, NF-kB, IL-6, BMI-1, SUZ12/HAT, EZH2, | Tsang and Kwok (2010) |
| Resveratrol | Mulberries, blueberries cranberries, grapes, and peanuts; red wine is most abundant dietary source | RBP/SIRT1, TNFα, IL-8 | Tili et al. (2010a, b) |
| Curcumin | Turmeric | EOMES/HAT, HDAC, H3 and H4 acetylation, p53, GATA4, GZMB, PRF1 | Mudduluru et al. (2011) |
| Diindolylmethane (DIM) and indole-3-carbinol (I3C) | Brussels sprouts, cabbage, broccoli, and kale | COX-2/HDAC | Wang et al. (2010) |
| Phenethyl isothiocyanate (PEITC) | Cruciferous vegetables | H3 and H4 acetylation, p21, GSTP1/HDAC | Shankar et al. (2013) |
| Ellagitannins | Pomegranates, raspberries, walnuts | HDAC and HAT | Wen et al. (2009) |
| Butyrate | Cheese, butter colonic fermentation of dietary fiber | H3 and H4 acetylation HDAC, NRF2 | Shankar et al. (2013) |
| Sulforaphane | Broccoli, cabbage, and kale | H3 and H4 acetylation, H3K9ac, H3K9me3 | Meeran et al. (2010b) |
| | | HBD-2, H3K27me3, RARβ, HBD-2, p21, BAX/HDAC | |
| Quercetin | Buckwheat and citrus fruits | SIRT1,IP-10, MIP-2/HAT | Manikandan et al. (2011) |
| Genistein | Soybeans and soy products | DNMT, H3, H4, H2A and H2B acetylation, H3K4me2, H3K9me3, p21, p16, PTEN, p53, FOXA3, BTG3, RARβ, hTERT, CCLD/HAT, HDAC, SIRT1, p16 | Fang et al. (2005, King-Batoon et al. (2008), and Majid et al. (2009) |
| Organosulfur compounds | Garlic and onion | H3 and H4 acetylation, p21/HDAC | Druesne et al. (2004) |
| Phenolic compounds; hesperetin, naringin, apigenin, and luteolin, | Fruits and vegetables | DNA methylation by indirectly regulate DNMT activity | Lee et al. (2005, Fang et al. (2007), and Mukherjee et al. (2015) |
| Vitamins B2, B6, and B12 | | Serine hydroxymethyltransferase (SHMT), methyltetrahydrofolate-homocysteine methyltransferase (MTR) | Zhang (2015) |

**Table 16.2** Dietary nutrients targeting ncRNA

| Nutrient | miRNA | Gene | Function | Reference |
|---|---|---|---|---|
| Genistein | miR-574-3p | RAC1, EGFR, EP300 | Tumor suppressor miRNA, inhibiting cell proliferation, migration, and invasion | Chiyomaru et al. (2013a) |
| | miR-34a | HOX | Tumor suppressor miRNA, apoptosis, low invasiveness, decreased cell proliferation | Chiyomaru et al. (2013b) |
| | miR-27a | Sprouty2 | Oncogenic miRNA, promoting tumor growth and migration | Taylor et al. (2009) and Xu et al. (2013) |
| | miR-155 | OXO3, PTEN, casein kinase, and p27 | Oncogenic miRNA, promoting tumor growth and migration | de la Parra et al. (2016) |
| | miR-221, miR-222 | ARH1 | Regulates the expression of ARH1 gene, determining decreased proliferation and invasiveness | Chen et al. (2011) |
| | miR-23b-3p | PTEN | Induction of apoptosis in the moment of downregulation | Zaman et al. (2012) |
| Epigallocatechin-3-gallate (EGCG) | miRNA-330 | AR | Antagonizes androgen receptor function | Siddiqui et al. (2011) |
| | miR-210 | HRE | Disable cell proliferation and suppress cell growth | Wang et al. (2011) |
| | miR-let7b | 67LR | Inhibits melanoma cells growth via inhibition of HMGA2 | Yamada et al. (2016) |
| | miR-16 | Bcl-2 | Apoptosis induction | Tsang and Kwok (2010) |
| Kaempferol | miR-200 | ZEB1, ZEB2 | Inhibitory activity regarding the epithelial-to-mesenchymal transition and migration | Jo et al. (2015) |

longevity both in vitro and in vivo (Wood et al. 2004; Bass et al. 2007, Barger et al. 2008; Subramanian et al. 2010). Moreover, many other phytochemicals, for instance, epigallocatechin-3-gallate (EGCG), have also beneficial effects on the aging process (Queen and Tollefsbol 2010). Other dietary components such as phenethyl isothiocyanate (PEITC) as well as genistein present in cruciferous vegetables and soybeans, respectively, may also have advantageous effects on life span through their cancer preventive properties (Li et al. 2009; Meeran et al. 2010a; Li et al. 2011).

## 16.3 Conclusion and Future Perspective

Aging phenotype includes the accumulation of cell divisions and macromolecular damage during the life span of the organism. These phenotypes are the consequences of epigenetic modifications of the genome. Epigenetic modifications include, but are not limited to, DNA methylation, histone modification, and ncRNA. Young and healthy cells have comparatively different epigenetic patterns than the old one that influence the functioning of cells. Pathways involved in cellular senescence, which has been shown to contribute to the aging phenotype, can be regulated by epigenetic modifications. Epigenetic changes are essential for many aspects of aging, and dietary nutritions are important resources to improve various adverse effects of these biological processes. The quality and quantity of the diet are vital in healthy aging. The diet quality has been explained very well; however the diet which consists of phytochemicals (e.g., genistein, EGCG, etc.) modulates epigenetic processes such as DNA methylation, histone modifications, and noncoding RNA. Extensive results clearly represent that the dietary nutrition has considerable potential in reducing the incidence of age-related diseases. Many questions are unanswered regarding the role of epigenetics in aging and their targets, but one thing is now clear that epigenetic changes are a basic abnormality during aging. More research is required in the direction of new dietary molecules which affect health span and life span while controlling the epigenetic changes.

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# Role of Phytochemicals in Eliciting Longevity Genes

17

Kalaiselvi Periandavan and Prema Velusamy

**Abstract**

Phytochemicals are diverse secondary metabolites derived from plants, and it has been proven that phytochemicals can extend longevity by evolutionarily conserved mechanisms. The positive impact of dietary phytochemicals on overall health and longevity has been studied extensively over the past decade. The emerging role of phytochemicals as an effector of metabolic and longevity signals offers new therapeutic perspectives. In this regard, we will discuss the role of phytochemicals in eliciting the longevity genes and also the various mechanisms involved. This review will give a broad outline of how different phytochemicals modulate signaling pathways that modulate the expression of specific set of genes. This review will also highlight the most exciting perspective for research in the future in this rapidly developing field of signaling pathways which include the genes encoding heat shock protein, genes responsible for the antioxidant response, genes involved in metabolism, etc. and are crucial for the phytochemicals to elicit longevity.

Despite various beneficial biological functions, phytochemicals might have adverse side effects like carcinogenicity and genotoxicity at high doses or concentrations. Hence, the future research challenge is to determine the optimal dose range and to perform intervention studies in order to improve longevity.

**Keywords**

Aging · Phytochemicals · Longevity · Oxidative stress

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## 17.1 Introduction

Life expectancy is defined as the average total number of years that a species is expected to live. Differently, life span is the duration of time that one individual lives from birth till death. Maximum life span is an inherent characteristic of each and every species and remains relatively unaltered through generations, while it may be increased or decreased over generations that needs a number of biological pathways to be altered, rewired, or reprogrammed (Ma et al. 2015). Even though human life span has been unaltered for the past million years at approximately 125 years, life expectancy has gradually increased (~27 years during the last century), especially in Western countries (Hayflick 2000). This increase might be mainly due to the control of many communicable diseases by the invention of antibiotics and preventive measures like vaccination that had ultimately resulted in population aging (Elavarasan et al. 2012), which reflects a human success story of increased longevity. In the current global scenario, living up to the age of 70 or 80 is common in many parts of the world. Human population in the world had reached 7 billion in 2012, among which 562 million (or 8.0%) were aged 65 and above. In 2015, 3 years later, the elderly population rose by 55 million, and it reached 8.5% of the total population of the world (World Population Aging 2015). Among the various continents, Asia is referred to as the population giant, in terms of size of its older population (617.1 million in 2015), which is more than half of the world's aging population. By the year 2050, nearly 1000 million older people are projected to live in Asia, accounting for about two-thirds (62.3%) of the world's total older population even though the estimated speed of aging for Asia and Latin America remain the same (World Population Aging 2015).

Aging or senescence is the decline in the ability of the organism to withstand stress of any kind resulting in the increased risk for mortality and morbidity. "Senescence" is derived from the Latin word *senex*, meaning "old age." The analysis that many animals living in a natural environment generally die earlier because of natural causes like disease, predation, starvation, or drought (Holliday 2006)] suggests that aging is a unique phenomenon applicable to the highly evolved human species (Hayflick 2000).

Many gerontologists accept that aging is an adaptive process, caused by factors of multiple etiologies, and these factors tend to get modulated by the genetic and environmental factors (Holliday 1995). The detrimental effects of aging are best observed in postmitotic tissues, where cells that are irreversibly damaged or lost cannot be replaced by mitosis of intact ones (Murali et al. 2008).

Increasing life span without proper health is deleterious. Survival with good health and physiological functions has been termed as "successful aging," "healthy aging," or "exceptional aging." Research with supercentenarians have revealed that people with longevity have the onset of disease with disability and decline in physical and cognitive function at older ages; thereby their health span approaches life span (Andersen et al. 2012). Nearly 30% of centenarians and 70% of supercentenarians escape many of the major age-related diseases including dementia, or many of the centenarians and supercentenarians have exhibited a delay on the onset of major age-related disease until age ≥80 years. Even though women have the greater

probability of survival, male centenarians have better cognitive and physical functional status (Newman and Murabito 2013).

Many different molecular players have been identified to be responsible for longevity: Apo E (Schächter et al. 1994), cholesteryl ester transfer protein (CETP) (Koropatnick et al. 2008), Forkhead box protein O3 (FOXO3A) (Willcox et al. 2008), insulin-like growth factor 1 (IGF-1), mammalian target of rapamycin (mTOR) (Kenyon 2010), RAC-alpha serine/threonine-protein kinase (AKT1) (Pawlikowska et al. 2009), sirtuins (Lin et al. 2000), and mitochondrial DNA haplotypes 9 (Alexe et al. 2007).

The exact mechanisms by which these longevity genes are shown to modulate the aging process are still unknown except for few genes. However, it is clear that these genes are involved in pathways of lipid metabolism and DNA repair which delay the onset of age-associated diseases like cardiovascular disease, dementia, and Alzheimer's disease. Meta-analysis of all compiled human genome-wide association study (GWAS) conducted to broadly examine the genetics of resistance to age-associated disease by Jeck et al. (2012) identified ten different locations across the genome which are shown to be associated with the susceptibility to multiple age-related diseases. These locations have genes associated with cellular senescence or inflammation pathways, portraying the significance of these pathways in influencing the human health span. Even though a number of genes have been identified, it is undeniable that many genetic variants combine to influence human life span: no single gene variant is found to be responsible.

Healthy longevity has been an unrelenting quest of human from ancient times. Exceptional longevity is a complex trait which is not only determined by genetic factors but also by external and environmental factors (Christensen et al. 2006). The major external factors that affect longevity include dietary patterns, stress, and sedentary lifestyle. The environmental factors include exposure to toxicants and pollutants that interfere with normal metabolic and physiological processes leading to mutations or decline of organ functions.

Even though the complex relationship among dietary habits/intervention and aging has not been fully explored, research from animals and human data suggest that dietary intervention can retard aging process, preventing or protecting them from various age-associated diseases and their pro-inflammatory status, the inflammaging (Fontana and Partridge 2015). Healthy diets with less concentrations of refined sugars and proteins from animal sources can substantially decrease the risk of age-related diseases, thereby favoring successful aging and longevity (Longo et al. 2015). On the contrary, a bad dietary lifestyle is shown to accelerate the aging process by modulating the pathways and mitogenic stimuli, finally accelerating aging phenotype (Verburgh 2015).

Moreover, effective interventions have to be developed to sustain or enhance longevity as the younger generations are having a lifestyle that leads to obesity, which makes them less healthy, and even they tend to have shorter lives than their parents. Dietary and pharmacological interventions such as restriction of food or methionine (Flurkey et al. 2010) and administration of rapamycin, an inhibitor of mTORC1 (Miller et al. 2014), can retard aging by comprehensive interactions of multiple targets.

Phytochemicals called as nutraceuticals are defined as naturally derived bioactive compounds that are found in plant kingdom and have health benefits. Nutraceutical is a conjunction among nutrition and pharmaceutics, and it was coined in 1989 by Stephen De Felice (Gupta et al. 2010). However, many nutraceuticals have been referred to as agents that delay aging or age-related diseases; the translational gap between basic and clinical research has yet to be filled. Many in vitro and in vivo experimental evidences suggest that phytochemicals can influence the expression of numerous longevity genes, but the molecular interactions between phytochemicals and signaling pathways that modulate aging and age-related diseases are obscure. These dietary phytochemicals trigger a condition called hormesis (Verburgh 2015), which states their ability to induce the stress-protective gene expression and resist aging.

Many cellular proteins and signaling pathways have been identified as candidates that are indispensable for life span prolonging. We will discuss about the major cellular proteins that influence aging and few phytochemicals that modulate the expression of longevity genes.

## 17.2 Signaling Proteins and Aging

### 17.2.1 Proteins That Boost Antioxidant Status

The free radical theory of aging proposes that aging occurs as a consequence of the deleterious effects of free radicals produced during cellular metabolism (Harman 1981). Oxidative stress is caused due to the loss of balance between ROS production and antioxidant defenses affecting all the vital organs resulting in aging. Hence, circumventing oxidative stress is one of the key processes involved in delaying aging and age-associated diseases.

### 17.2.2 Key Proteins That Regulate Life Span

The key proteins and pathways that regulate life span directly or indirectly by reducing oxidative stress include Nrf2 (Kensler et al. 2007), insulin/IGF1 signal transduction pathway (Kenyon 2010), DAF 16/FOXO (Kwon et al. 2010), sirtuins (Imai et al. 2000), and heat shock factor 1 (HSF-1) (Shemesh et al. 2017).

## 17.3 Phytochemicals and Antiaging

The dream of longevity is not new, and a multitude of reviews have been written to address the process of aging in an elaborate fashion (Murphy and Partridge 2008). Identification and isolation of long-lived *C. elegans* mutants have triggered an array of research activities to identify many life span-modulating genes, and *C. elegans* mutant has been the organism for studying anti-aging strategies.

### 17.3.1 Garlic

*Allium* vegetables including garlic and onion have been reported to have health benefits from ancient times (Rivlin 2001). Epidemiological studies have identified that diets rich in *Allium* vegetables are associated with lowered risk of cancer (Tanaka et al. 2004 and other age-associated diseases like diabetes, neurological diseases (Powolny and Singh 2008), and cardiovascular disease (Ried et al. 2008).

Different forms of garlic including raw garlic, garlic oil, garlic powder, oil-extracted garlic macerates, aged garlic extract (AGE), and individual garlic-derived compounds such as ajoene, *S*-allyl cysteine, diallyl thiosulfinate (allicin), diallyl disulfide (DADS), and diallyl trisulfide (DATS) have been tested (Charron et al. 2016) for cardiovascular benefits and antiaging potential. S-Allyl-L-cysteine (SAC) is the primary thio-allyl compound in aged garlic extract (AGE), and the antiaging effects of SAC were extensively studied by Moriguchi et al. (1997). His studies have shown that chronic intake of a low dosage of SAC in the diet improved the deficit in learning performance in SAMP8 mice and memory consolidation in SAMP10 mice. These findings have substantiated that SAC helps in reducing age-related learning disabilities and cognitive disorders.

Chronic administration of S-allyl cysteine is also shown to activate Nrf2 factor, one of the longevity genes, and enhance the activity of antioxidant enzymes in the striatum, frontal cortex, and hippocampus in Wistar rat (Franco-Enzástiga et al. 2017). The protective effect of garlic extract on ROS formation, MMP-1 protein and mRNA expressions, cytokines such as interleukin (IL)-1β and IL-6, senescence-associated β-galactosidase activity, and SIRT1 activity in UVB-irradiated HaCaT cells is an added evidence for garlic to act as a potent antiaging agent (Kim 2016).

### 17.3.2 Coffee

Coffee use dates back to the Stone Age and is one of the three most-popular beverages in the world (alongside water and tea) that is rich in antioxidants and caffeine. In the recent past, coffee has been recognized as a potent beverage for healthful aging, with special emphasis by its ability to protect from cardiovascular diseases (Ding et al. 2014) and mild cognitive impairment (Takahashi and Ishigami 2017). Caffeine, a secondary metabolite with pesticide activity, which paralyzes and kills certain insects is a xanthine alkaloid compound. It acts as a stimulant that fends off drowsiness in humans and is mostly distributed through drinks including tea, coffee, soft drinks, and chocolate.

Park et al. (2017) have studied the association of coffee consumption with total and cause-specific mortality among nonwhite populations and found that increased coffee consumption was associated with a significantly low risk for death in Latinos, Japanese Americans, African Americans, and whites. Similarly Ding et al. (2014) have identified that regular coffee drinking in moderate amounts is associated with a decreased incidence of death from cardiovascular disease, neurological diseases, and

suicide. Moreover, habitual coffee drinking following acute myocardial infarction was shown to be associated with a reduced risk of mortality (Brown et al. 2016).

Sutphin et al. (2012) have reported that caffeine is capable of extending life span and improving health span in *C. elegans*. Life span extension by caffeine might be due to its epistatic interaction with dietary restriction and reduced insulin signaling. Studies by Lublin et al. (2011) have shown that caffeine significantly decreased the age-dependent acceleration of mortality rate which was dependent on DAF- 16.

Studies with worms have been carried out to study the influences from caffeine and non-caffeine sources of coffee with respect to longevity. Dostal et al. (2010) identified SKN-1 as a major downstream signaling molecule involved in the caffeine-independent delay in amyloid beta toxicity using coffee extract. Lublin et al. (2011) identified IIS as an important player in life span extension by caffeine. Moreover, the polyphenol chlorogenic acid present in coffee has lipid-lowering effect in diet-induced obese mice by downregulating sterol regulatory element-binding protein 1 (Takahashi and Ishigami 2017). Studies by Sutphin et al. (2012) have clearly shown that caffeine appears to act, at least in part, by activating the FOXO transcription factor DAF-16 as it could not extend longevity in Daf 16 mutants, but it extended the life span of Sir-2, Hif-1, and Cep-1 mutants to some extent, although the magnitude of effect of caffeine was comparatively lesser in wild type. Hence, it is evident that the life span-extending effects of caffeine may be mediated by several genetic pathways.

### 17.3.3 Curcumin

A myriad of health benefits have been attributed to curcumin, which was first isolated as "yellow coloring matter" from *Curcuma longa* by Vogel and Pelletier in 1815 (Bandyopadhyay 2014). Curcumin, a known powerful antioxidant, has the capacity to mitigate age-associated cellular damage induced by the production of reactive oxygen species (ROS) (Queen and Tollefsbol 2010). Lee et al. (2010) have reported that curcumin extends life span of different strains of *Drosophila melanogaster* and attributed this effect to its ability to afford protection against improvement in locomotion, oxidative stress, and chemopreventive effects. Extension of life span was also found to be gender as well as genotype specific. Curcumin also is shown to modulate the expression of a plethora of aging-related genes, including the insulin, JNK, and methuselah signaling pathways.

Motterlini et al. (2000) reported that curcumin reduced oxidative stress by upregulating the expression of HO-1 in bovine aortic endothelial cells. In addition, curcumin is also shown to inhibit NF-κB, which is the main mediator of inflammation, to activate the expression of many pro-inflammatory cytokines. Furthermore, curcumin decreases or blocks the mTOR, which integrates the input from multiple signaling pathways and acts as a sensor of cellular nutrient and energy levels and redox status in cells (Sikora et al. 2010). Shen et al. (2013) have shown that curcumin-enriched diets increase antioxidant enzyme activity and mean life span in

*Drosophila*. A 75% improved life span and activity for curcumin-fed flies in Ab1–42-expressing transgenic *Drosophila* was observed by Caeser et al. (2012).

### 17.3.4 Quercetin

Quercetin, the major flavonol found in several fruits and vegetables including broccoli, apples, onions, cherries, blueberries, and red grapes, is a natural antioxidant with potential anticancer and antiaging activities. Quercetin is shown to have putative health beneficial effects with special reference to its ability to boost antioxidant status (Belinha et al. 2007). Many studies have identified that quercetin increases the ability of the organism to resist stress and extend life span. Mev-1 mutant, which is characterized by an increased accumulation of endogenous ROS (provides a special test system to prove the antioxidative capacity), exhibited a significant quercetin-mediated gain in life span (Ishii et al. 1998). It is thereby conceivable that the antioxidative property of quercetin may have impacted life span extension in mev-1 mutants. On the other hand, Saul et al. (2008) established that quercetin-mediated longevity is observed in a daf-16(mgDf50) loss-of-function mutant. These finding dictates that the reduction of internal oxidative stress is not the exclusive role of quercetin. Quercetin-induced longevity and stress resistance have been described in three different studies that identified the antioxidant properties and the UNC-43/SEK-1 pathway as the major mechanism behind its life span extension (Pietsch et al. 2009). The other genes that might be tentatively involved in quercetin's ability to increase life span might be age-1 and daf-2, which are central players in IIS to inhibit DAF-16 activity.

### 17.3.5 Resveratrol

Resveratrol (3,5,4′-trihydroxy-trans-stilbene), a polyphenolic phytoalexin which is found in red wine and the skin and seeds of grapes, has been reported to possess a wide range of biological and pharmacological activities including antiaging effects. It increases longevity in the short-lived invertebrates *C. elegans* and *Drosophila* (Howitz et al. 2003) and prolongs life span and retards the onset of age-related markers in a short-lived vertebrate fish *Nothobranchius furzeri* (Valenzano et al. 2006). Resveratrol is shown to affect gustatory responsiveness to a significant extent and is shown to prolong life span in honey bees (wild type) under normal oxygen conditions. Moreover, resveratrol is shown to have a satiety effect on honey bees and further reduce food intake (Rascón et al. 2012). Cidea, a gene which regulates energy balance in brown fat, was upregulated on high-cholesterol diet feeding, and it was downregulated by resveratrol supplementation. This clearly indicates the ability of resveratrol to prevent the deleterious effects of excess caloric intake and modulate known longevity pathways. The life-prolonging ability of resveratrol is caused by influencing the insulin sensitivity, PGC 1 alpha, and sirtuins similar to that of calorie restriction in honey bees (Baur et al. 2006). The knockdown or

knockout of Sirt-1 prevented the autophagy induction by resveratrol in human cells, and it is suggested that autophagy is required for the life span-prolonging ability of resveratrol, similar to that of calorie restriction (Morselli et al. 2010). These studies clearly indicate the ability of resveratrol to prevent the deleterious effect of excess calorie intake and modulate known longevity pathways by mimicking calorie restriction.

### 17.3.6 Green Tea Catechins

Green tea is obtained from the leaves of the plant *Camellia sinensis*, consumed primarily in China, Japan, and a few countries in North Africa and the Middle East, and is reported to contain 4000 biologically active compounds, one-third of which are polyphenols (Weisburger 2002). Tea and tea flavonoid consumption has been linked to lower incidences of chronic diseases such as cardiovascular disease and cancer (Pandey and Rizvi 2009). The health benefits associated with tea consumption have been attributed in part to the antioxidant and free radical scavenging activity (Rice-Evans 1999). Green tea and its catechins, namely, gallocatechin, (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), (-)-epigallocatechin-3-gallate (EGCG), and catechin and (-)-epicatechin (EC), are best known for their antioxidant properties (Yang et al. 1999).

EGCG treatment increases the mean life span of *C. elegans* and reduces its susceptibility to lethal oxidative stress. Studies by Abbas and Wink (2009) show that EGCG pretreatment suppresses hsp-16.2 expression under oxidative stress and increases the life span.

EGCG is shown to safeguard the aged rats when challenged with hypercholesterolemic diet (Senthil Kumaran et al. 2009). EGCG brought about an augmentation in the activities of enzymic antioxidants like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase and improved the nonenzymic antioxidants like tocopherol, ascorbic acid, and glutathione. EGCG ameliorated the malondialdehyde and protein carbonyl levels and emerged out as a good antioxidant neutraceutical and a neuroprotective agent in alleviating the age-associated oxidative damage in aged rat brain (Srividhya et al. 2009). EGCG is shown to mediate the downregulation of NF-AT and thereby macrophage infiltration in experimental hepatic steatosis (Krishnan et al. 2014). These findings suggest the multifaceted role of EGCG in mitigating age-associated derangements.

### 17.3.7 Other Phytochemicals Reported as Longevity Agents

The other phytochemicals that have been reported to promote longevity in model organisms are glaucarubinone (Zarse et al. 2011), icariin and its derivative icariside II (Cai et al. 2011), arachidonic acid 5-lipoxygenase inhibitor nordihydroguaiaretic acid (West et al. 2004), aspirin (Strong et al. 2008), phloridzin (Xiang et al. 2011),

butein (Howitz et al. 2003), celastrol (Kiaei et al. 2005), crocin (Bakshi et al. 2009), ellagic acid (Saul et al. 2011), gallic acid (Saul et al. 2011), myricetin (Grünz et al. 2012), oleuropein (Katsiki et al. 2007), tocopherol (Sattler et al. 2004), coenzyme Q10 (Strachecka et al. 2014), tocotrienol (Aan et al. 2013), blueberry extract (Wilson et al. 2006), and tannic acid (Saul et al. 2010).

These studies indicate that even though genetics play a major role in determining the life span, dietary intervention by small molecules can influence many longevity. However, the mechanisms by which they influence the life span extension being still not absolutely identified. From the experimental evidences, it cannot be denied that these small molecular interventions have beneficial effect on healthy aging.

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# Antiaging Interventions: An Insight into Polyphenols and Brain Aging

18

S. Asha Devi and S. Raja Sekhar

**Abstract**

Neurodegenerative diseases are progressively increasing globally and most often are associated with the aging process. Time and again, neuroscientists and clinicians have tried many approaches to maintain a healthy brain with normal aging. Irrespective of the approaches, oxidative stress is the marker of several age-related disorders of the brain, and the primary consideration of nutrigerontologists is toward lessening the burden of reactive oxygen species through dietary interventions that can positively trigger numerous genes encoding many antioxidant enzymes and pro-apoptotic and anti-inflammatory factors and finally maintain a redox balance. Among the various approaches, naturally derived bioactive compounds have attracted the attention of scientists, and what is more is that polyphenols have gained popularity because of the various benefits derived from them either on their own or in combination with nonpharmacological means such as physical exercise. Human and animal experiments using flavonoids, a class of polyphenols, have suggested a positive relation between flavonoids such as catechin and preservation of cognitive function with age. This review is, firstly, an assembly of recent findings on nutrient signaling pathways of polyphenols, commonly found in fruits and vegetables, and, secondly, their impact on the brain as natural medicaments in promoting mental health with successful aging and longevity.

**Keywords**

Aging · Apoptosis · Flavonoids · Neurodegeneration · Oxidative stress

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## 18.1 Introduction

Aging is accompanied by a decline in several physical and mental faculties. Astonishingly, aging progresses in a geometrical manner past middle age in humans and animals as well. However, as the world moves ahead with increased life expectancy, the normally aging elderly population is facing cognitive decline encompassing lowered learning skills and spatial and episodic memory leading to greater incidences of neuroinflammatory neurological degeneration. Knowing these would open up a wide array of options to deter the onset of not only cognitive loss during normal aging but, more importantly, the possibilities of delaying the onset of disorders apart from mild dementia and mild cognitive impairment (MCI) that may be a challenging situation for the senescent individuals. Hence identifying the important regulators and molecular mechanisms of brain aging, in particular cognitive aging, will pave suitable pathways toward interventions that could be effective for attaining healthy brain during normal aging. Understanding the biological mechanisms of cognitive aging has provided an array of options to deter the onset of not only cognitive loss during normal aging but, more importantly, the possibilities of delaying the onset of mild dementia and mild cognitive impairment (MCI) that may be a challenging situation for the senescent individual. Hence identifying the important regulators and molecular mechanisms of brain aging, in particular cognitive aging, will pave suitable pathways toward interventions that could be effective for attaining healthy brain during normal aging. Presently, therapies are targeted toward lowering physical complications that are unable to eliminate the pathology leading to an unimaginable burden to the mankind (Deak et al. 2016). Interestingly, resorting to fruits and vegetables is beneficial to humans (Nooyens et al. 2011; Kumar and Khanum 2012). Nutritional supplements derived from fruits and vegetables and those that optimize cognitive well-being and function include those essential for the synthesis and preservation of acetylcholine (ACh), a neurotransmitter for learning and memory function and those required as an anti-inflammatory and anticoagulant agents. Loss of brain function during aging is inevitable. Middle-aged adults who still haven't experienced but are on the border of forgetfulness or MCI should somehow be benefited by certain nutritional interventions. Equally important is that in those who already have these symptoms, such interventions should be toward prevention of the onset of full-blown dementia (Burns and Zaudig 2002). Hence, it is important to target nutritional interventions to suppress the onset of cognitive impairment. This review is focused on the importance of polyphenols, especially from grape seeds in preserving cognitive capacity and evidence that support the role of major polyphenols, with emphasis on grape seed products and their bioavailability and potential neuroprotective functions as an antiaging, antioxidant, and anti-inflammatory compounds. Finally, the review attempts to highlight their possible benefits for brain health and prevention of dementia that is projected to triple globally by the year 2040.

## 18.2 Phenolic Compounds in Grape Seeds

Grapes are rich in phenolic compounds and are mainly distributed in skin, stem, leaf, and seed, but not in the juicy pulp preferred by all. Constituting the largest category/group of grape polyphenols, flavonoids deemed to be the main molecules that have biological properties. In grapes, flavonoids are located primarily in the berry skin and the seeds (Waterhouse 2002; Lepiniec et al. 2006; Bogs et al. 2007).

The above phenolic compounds are usually extracted from grapes using solvents such as ethanol, formic acid, acetone, and methanol in various proportions. Although solvent extraction is popular, several other methods are used like microwave-assisted extraction (Hong et al. 2001), supercritical fluid extraction (Fiori. 2007; Vatai et al. 2009), and ultrasound-related extraction (Novak et al. 2008; Ghafoor et al. 2009). Polyphenols in grape seed are flavonoids comprising gallic acid and monomeric flavan-3-ols, catechin and epicatechin (Shi et al. 2003), and proanthocyanidins (Table 18.1).

### 18.2.1 Antioxidant Activities of Grape and Its Products

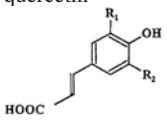
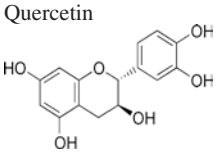
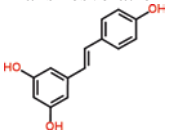
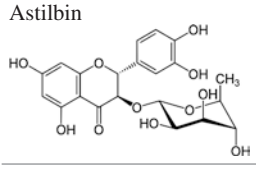
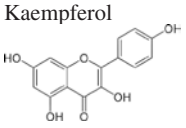
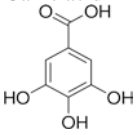
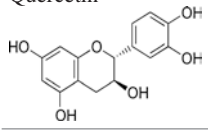
The phenolic constituents of grape seed extract are of two groups: phenolic acids and flavonoids. Grape and its products are identified for their antioxidant activities that are health promoters. For instance, grape seeds decrease the levels of low-density lipoproteins in the plasma (Sano et al. 2007) and grape wine protects against hypercholesterolemia and fatty streak storage in experimental hamsters (Auger et al. 2005) and defends against hyperglycemia (Asha Devi et al. 2006).

Grape seed proanthocyanidin extract (GSPE) are antioxidants made of polyphenolic acids, such as gallic acid, and are known to scavenge reactive oxygen species-mediated ischemic-reperfusion (I-R) injury and apoptosis of cardiac cells (Sato et al. 2001; Georgiev et al. 2014). Its antioxidant properties are largely related to their free radical scavenging activity and metal-chelating action. One of our review articles (Asha Devi and Abhijit 2017) reported the outcome measures of utilizing different types of learning modules and the brain regions involved in the behavior. Further, experimental evidences were enlisted regarding the role of GSPE supplementation in alleviating the extent of deposition of lipofuscin, a product of lipid peroxidation in the hippocampus of aging rats. Polyphenols are now recognized for their efficient cell signaling pathways and expression of genes (Dell et al. 2005; Soobrattee et al. 2005).

### 18.2.2 Bioavailability of Polyphenols from Grape Seeds

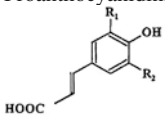
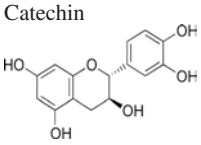
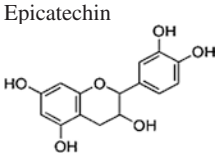
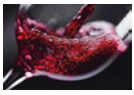
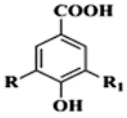
Studies have indicated that, following ingestion of polyphenol-containing berries, active intestinal absorption occurs and some metabolites can exert affects in vivo (Archivo et al. 2010). Although polyphenols are water-soluble, the exact mechanisms of how they cross the blood-brain barrier (BBB) have not yet been fully

**Table 18.1** Grape and grape products

| Major phenolics | Sample/region | Benefits | References |
|---|---|---|---|
| Proanthocyanidins, quercetin  | Grape skin  | Accumulation of proanthocyanidins in Shiraz grape skin is independent of that in seeds. However, in both skin and seeds, synthesis is seen in berry development and attains maximum levels around veraison | Downey et al. (2003) |
| Quercetin  | Grape stem  | Have high amounts of phenolics and antioxidant potency. Identified flavanols were rutin and quercetin 3-O-glucuronide, stilbenes (trans-resveratrol and resveratrol dehydrodimer), and astilbin (a dihydroflavonol glycoside) | Makris et al. (2008) |
| Trans-resveratrol  | | | |
| Astilbin  | | | |
| Rutin | | | |
| Kaempferol  | Grape leaf  | Phenolic content and antioxidant capacity in terms of μM TEAC/g muscadine leaf skin is 12.8 (Trolox equivalent antioxidant capacity) | Pastrana-Bonilla et al. (2003) |
| Gallic acid  | | | |
| Quercetin  | | | |

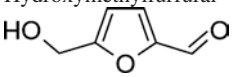
(continued)

**Table 18.1** (continued)

| Major phenolics | Sample/region | Benefits | References |
|---|---|---|---|
| Proanthocyanidins  | Grape seeds  | GA(+)-catechin and epicatechin are major polyphenolics in the seeds | Pastrana-Bonilla et al. (2003), Yilmaz and Toledo (2004) |
| Gallic acid | | Proanthocyanidin concentration in some crosses (Monastrell x Syrah grape (*Vitis vinifera*) exceeds that in either parent | Hernandez-Jimenez et al. (2009) |
| Catechin  | | Plasma catechin concentrations are insignificantly different after single moderate servings of dealcoholized red wine (DRW) or water and alcohol (ARW) | Bell et al. (2000) |
| Epicatechin  | | | |
| Resveratrol, catechin, quercetin | Red wine  | Resveratrols are bioactive components of wine and improves TAC, hydroxyl and superoxide activity, HRSA scavenger activity (hydroxyl radical), super oxide radical scavenger activity (SRSA) | Rivero-Perez et al. (2008) |
| Hydroxycinnamic acid  | | | |
| | | Lyophilized extract of wine (JW-E) from Jacquez grapes (*Vitis aestivalis-cinerea x Vitis vinifera* grapes) against harmful effects of IL-beta. Protects cartilage | Panico et al. (2006) |
| | | | Auger et al. (2005), Monagas et al.(2005) |
| | | Catechin, quercetin, and resveratrol prevent atherosclerosis (AS) in experimental hamsters | |

(continued)

**Table 18.1** (continued)

| Major phenolics | Sample/region | Benefits | References |
|---|---|---|---|
| Hydroxycinnamic acid<br>Hydroxymethylfurfural<br>  | Raisins<br>  | Studies on phenolic content in sun-dried, dipped, and golden raisins are in the order of 10% compared to fresh grapes. Formation of hydroxymethylfurfural in the sun-dried is because of Maillard browning reactions | Karadeniz et al. (2000) |

elucidated. Studies on animals and humans have shown an age-related increase of BBB permeability in healthy individuals (Toornvliet et al. 2006; Farrall and Wardlaw 2009; Blau et al. 2012). Advanced magnetic resonance imaging (MRI) techniques have shown increased BBB permeability in one of the important areas of the brain for cognitive functioning – the hippocampus – of healthy older people aged between 55 and 90 years who had no cognitive decline (Montagne et al. 2015). In studies on mice, Elahy et al. (2015) have shown inflammation-related BBB dysfunction and decreased tight junctions in the aged compared to the young.

Furthermore, although studies on the mechanisms of permeation of polyphenols across the BBB have been elucidated by Youdim et al. (2003, 2004), it is still speculative as to whether the metabolites of polyphenols cross by diffusion or through specific carrier-facilitated mechanism. It is said that the BBB is an interface that can regulate molecular alterations and exchanges between the blood and different regions of the brain.

Among several selected interventions to improve cognition, there are reports of a positive correlation between flavonoid consumption and cognitive ability. Experimental evidences from animals as well as humans are suggestive of polyphenols as protectants not only against the development of neurodegenerative diseases but also in ameliorating cognitive function in suggesting that polyphenols may potentially have a protective effect on the development of neurodegenerative diseases (Macready et al. 2009) and may improve cognitive function in patients with established neurodegenerative diseases (Weichselbaum and Buttriss 2010). In fact, the studies were evidenced from a total of 55 different cognitive tests encompassing a broad range of cognitive domains, and most studies incorporated at least one measure of executive function/working memory, with nine reporting significant improvements in performance as a function of flavonoid supplementation compared to a control group (Jagla et al. 2010). However, equally essential is to characterize the essential biologically active constituent that influences cognition.

## 18.3 Polyphenols and Neuropreventive Potential Against Cognitive Aging

Experimental evidences demonstrate that polyphenols presented in foods might be beneficial in reversing neuronal and behavioral aging. Experimental evidences have indicated that repeated dosing of supplemental GSPE is more effective in increasing the bioavailable concentrations of major constituents of the extract rather than single dosing in rat models (Ferruzzi et al. 2009). Generally, polyphenols are widely known notably for their antioxidant capabilities compared to certain other antioxidants such as vitamins E and C (Barros et al. 2006). Because the brain is vulnerable to age-related oxidative damage and other insults including inflammation, studies on proanthocyanidins from grape seeds are largely based on the free radical-generated oxidative stress hypothesis. In a study by Deshane et al. (2004), rats ingesting grape seed extract (GSE) experienced changes in expression or modifications of specific brain proteins that might protect against pathologic events. Due to their antioxidant activity, in scavenging free radical, grape seeds prevent organs and tissues from oxidative stress-induced damage while modifying the body's negative mechanism of redox status. Further evidences have been obtained from behaviors of rats aged 19–21 months, wherein consumption of a 10% grape juice improved the release of dopamine from striatal slices, as well as in their cognitive performance in the Morris water maze, while the 50% grape juice improved their antioxidant capacity (Joseph et al. 2009). While supplementing rats with GSE at 100 mg/kg b.wt. for 30 days has been shown to reduce the accumulation of age-related oxidative DNA damages in neural tissue (Balu et al. 2005, 2006), a dose of GSE at 60 mg/kg b.wt has been shown to effectively inhibit DNA damage in the rat hippocampus (Hwang et al. 2004) and improve behavior. In addition, GSE has been proved to alleviate hypoxic ischemic brain injury in neonatal rat with 50 mg/kg b.wt (Feng et al. 2005).

Furthermore, in addition, the alleviation of several indices of oxidative stress such as lipid peroxidation and protein carbonylation and upregulation of antioxidant enzymes in the aging hippocampus and cerebral cortical regions (the regions concerned with spatial learning) have been reported in rats on a daily supplement of 75–100 mg/kg b.wt grape seed extract for 30 days (Asha Devi et al. 2011), and the reduction in age-mediated oxidant injury to brain cells elevates a vital neurotransmitter, acetylcholine (Asha Devi et al. 2006). In fact, grape seed extract, a natural product, induces neuroprotective action in composition of brain proteins and their expression, thus impacting the many actions of psychoactive drugs by maintaining a healthy brain (Kim et al. 2006). Our research has shown that GSPE treatment in aging rat model results in an attenuation of age-associated changes in the hippocampus and medial prefrontal cortex including cognitive impairments (Abhijit et al. 2017). Additionally, GSPE treatment elevates number of neurons in the CA1 subfield of the hippocampus of middle-aged rats compared with their controls (Abhijit et al. 2018).

Brain-derived neurotropic factor (BDNF), in the control of synaptic plasticity and long-term memory (Pruunsild et al. 2011) and of flavonoids activating specific signaling proteins such as extracellular signal-regulated kinases (ERK) in modulating the activation of cAMP response element-binding protein (CREB) and increased BDNF expression in the hippocampus in middle-aged animals, suggests pathways for GSPE flavanols (De Nicoló et al. 2013). Bensalem and his coresearchers (2016) have shown that an 8-week polyphenol-enriched diet consisting of polyphenol-rich extract from grape and blueberry (PEGB) in middle-aged mice could improve spatial memory and br related to the observed increase in hippocampal calmodulin kinase II *(CaMKII)* mRNA levels along with nerve growth neurotrophic factor (NGF) mRNA levels and was similar to supplemented adult mice. The existence of specific polyphenol binding sites at the plasma membrane in the rat brain has been suggested by Han et al. (2006). These binding sites support observations by Abhijit et al. (2018) on the bioavailability of GSPE constituents, catechin, epicatechin, and gallic acid, in the medial prefrontal cortex and hippocampus. Further, we found that age-related oxidative stress in the hippocampus of middle-aged rats was effectively alleviated in terms of altered glutathione (GSH) level and of glutathione reductase (GR) and glutathione peroxidase (GPx) activities by a 120-day GSPE supplementation, thereby suggesting an efficient coordination between the endogenous and exogenously supplemented natural antioxidant. Interestingly, in these middle-aged rats, the observed age-related decrease in CA1 neurons and volume was restored by the grape seed polyphenols, catechin, epicatechin, and gallic acid, in the extract (Abhijit et al. 2018).

Studies have also shown that cognitive decline in humans may be prevented (Kang et al. 2005; Macready et al. 2009) or improved (Jagla et al. 2010) with the consumption of polyphenol-containing fruits and of vegetable consumption (Krikorian et al. 2010; Weichselbaum and Buttriss 2010; Cimrova et al. 2011; Huhn et al. 2015). Figure 18.1 is a diagrammatic representation of our findings on GSPE as an effective intervention against brain aging.

## 18.4 Polyphenols and Neurodegenerative Diseases

A recent estimate put forth by the World Health Organization (WHO) (2006) projects that a figure of 24 million people globally is affected by dementia. Therefore, maintaining healthy cognitive function is necessary for quality aging especially during the transition into older age. However, successful strategies for prolonging the longevity have concomitantly increased the number of older adults, which is alarmingly expected to grow to approximately 81 million by the year 2040 (WHO 2006). As an alternative to drug-based treatments, several animal and human studies have signified the importance of natural products, for instance, the regular consumption of flavonoids extracted from grape seeds in preventing dementia with age. Human subjects with a high consumption of polyphenols from grapes and berries have a lower risk of neurodegenerative disorders including Alzheimer's disease (Ramirez et al. 2005). Moreover interesting is that polyphenols from grape seeds referred to as proanthocyanidins have antiaging effects on cardiovascular function and protective effects against

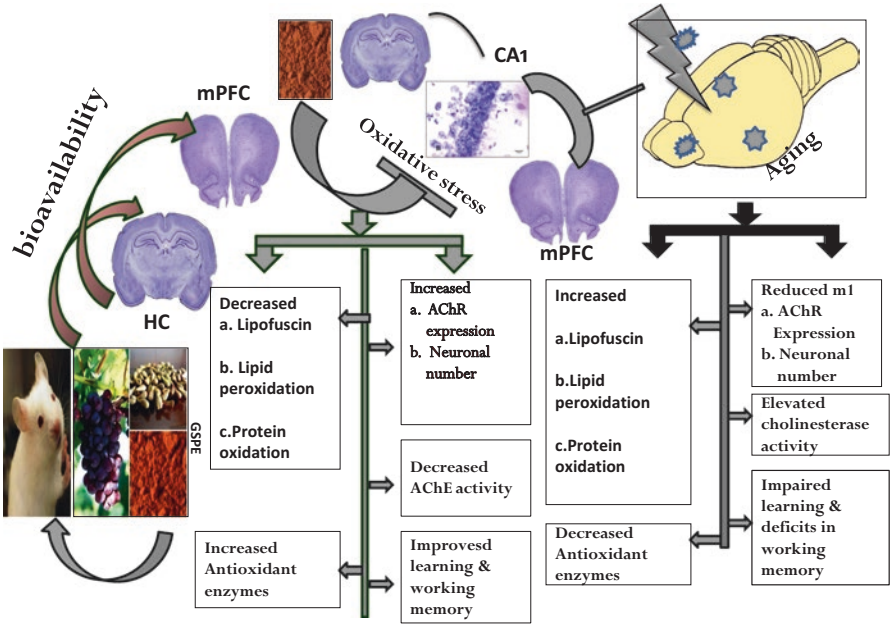



**Fig. 18.1** GSPE intervention on oxidative stress and cognitive aging. Schematic representation of the effects of free radicals and oxidative stress on brain aging. Aging decreases the m1ACh receptors and increases AChE activity in the hippocampus and prefrontal cortex. Increased lipid peroxidation and protein oxidation are accompanied with deficit in antioxidant defense. Grape seed proanthocyanidin extract (GSPE), however, attenuates the age-related effects on oxidative stress and cognition

cardiovascular disease, an important risk factor initiating dementia (Fillet et al. 2008). An even more interesting finding is that cognitive reserve (CR) in Alzheimer's patients on red wine consumption is enhanced (Ono et al. 2008). When referring to age-related neurodegenerative diseases, a frequently used term is CR which defines the ability to maintain cognition despite disease-free normal aging or disease-related characteristic changes to the brain such as storage of amyloid beta in Alzheimer's disease (Stern 2009; Rentz et al. 2010) compared to normal disease-free aging. However, CR deviates from brain reserve wherein brain volume and the extent of the neuronal network can impact cognitive ability (Tucker and Stern 2011). In many AD patients, ACh levels are the best measures of judging memory of the spatial type in rats, and the key enzyme is acetylcholine esterase (AChE). In AD, studies suggest GSE as an efficient anti-AChE natural product that increases acetylcholine levels and increases ACh release in the hippocampus (Rhodes et al. 1996). Concomitant studies in animal models of Alzheimer's disease have demonstrated that mice supplemented with grape seed extract have reduced amyloid-beta protein deposition and this was accompanied by a reduction in cognitive decline (Wang et al. 2008). Liu et al. (2011) have demonstrated in AD patients an effective reduction in amyloid-β (Aβ) oligomers in the brain. These Aβ-oligomers result in impaired memory due to synaptotoxicity. They observed that GSPE supplementation for 5 months could inhibit Aβ-oligomerization in vitro and

deter AD-associated pathology in the brains of transgenic mice, Tg2576. GSPE is shown to block Aβ-fibril synthesis and tau protein besides destabilizing preformed Aβ and tau-promoted pathology (Ono et al. 2008). A closely related study is that of Ho et al. (2009) on the abnormal misfoldings of the microtubule-associated tau that are finally observed as neurofibrillary tangles (NFTs) in tau-related neurodegenerative diseases and of the NFTs being attenuated by grape seed polyphenol extract. Further, the cognitive decline experienced in experimentally induced epilepsy in rats is reported to be reversed by GSE through a reduction in oxidative stress and mitochondrial injury (Zhen et al. 2014). GSPE has in common with other polyphenols a neuroprotective property that enables the scavenging of free radicals and upregulating antioxidant defenses, for instance, through the upregulation of the transcription factor nuclear (erythroid-derived 2)-related factor 2 (Nrf2) pathway followed by the modulation of signal transduction cascades (Kelsey et al. 2010).

Despite the fact that AD and Parkinson's disease (PD) have varied pathological symptoms, the underlying cellular and molecular mechanisms seem to overlap quite significantly. A major share of these include amyloidogenesis and tau in AD and αS (amorphous α-synuclein) in PD. Polyphenols from red wine have been projected time and again as being neuroprotective to explain how these are essential as an intervention therapy for AD, and PD is a great task not only for basic neuroscientists but also for clinicians. This statement needs greater concern since despite the observation on a 5-year follow-up study of 1357 human subjects aged 65 years and over, who exhibited a significant negative correlation between grape flavonoids and occurrence of dementia, this does not convincingly answer whether subjects with a mild form of cognitive impairment or dementia are benefited by grape consumption (Commanges et al. 2000). Evidences from a study by Lee et al. (2017) on human subjects with MCI emphasize the benefits of grape as an effective strategy for slowing the progression of dementia as a "positive outcome." Importantly, the molecular mechanisms include anti-inflammatory activities, free radical scavengers, antioxidant capacity, metal chelators, and antiamyloid action (Basli et al. 2012; Wang et al. 2014).

## 18.5 Conclusion

During normal aging, the decline in cognition is not inevitable. Biochemical, molecular, and pharmacological evidences suggest that interventions of naturally occurring polyphenols can combat not only the normal age-associated cognitive deficits but also those of pathological conditions.

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# Activation of Plasma Membrane Redox System: A Novel Antiaging Strategy

19

Kanti Bhooshan Pandey and Syed Ibrahim Rizvi

**Abstract**

The possibility to manipulate the aging process and extend healthy life has always fascinated humans. Scientific studies during the past few decades have dissected molecular mechanisms which play important roles in determining longevity. There is increasing evidence that the hypothesis based on structural and functional damage during aging involves a change in cellular redox states. Recent studies document that the plasma membrane redox system (PMRS) of eukaryotic cells acts as redox balancer by transferring reducing equivalents that are used to maintain homeostasis. As a compensatory mechanism, overexpression of PMRS has been noted during aging. It is hypothesized that activation of PMRS may provide a strategy to counteract redox shift and oxidative stress during aging. The present chapter deals with activation of PMRS and its role in antiaging intervention.

**Keywords**

Aging · Ascorbate · Life span · Oxidative stress · PMRS

## 19.1 Introduction

Inevitable decline in functional ability of physiological systems in the body in post-reproductive phase of life is conventionally referred to as aging. The deteriorative alterations cumulatively lower the fitness level and the ability to preserve redox state, a vital condition for successful operation of various biochemical activities

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inside the cell (Sohal and Orr 2012; Zheng et al. 2018). Among the various mechanisms proposed to explain the aging and age-associated impairments, an altered redox state or impaired homeostasis in tissues is the most accepted phenomenon.

Endogenously generated reactive oxygen radicals (ROR) and mild oxidative stress are essential for various signaling activities in cellular reactions; however, in context to aging process, oxidative stress implies a condition of cellular state with inadequate antioxidant defenses to combat excessive ROR, thereby resulting in accrual of molecular and structural damage (Harman 1956; Pandey and Rizvi 2010). In the last decades, advanced studies have been performed in this area, and it is realized that the role of redox state in aging process needs to be incorporated with structural damage hypothesis (Sohal and Orr 2012).

Comprehensive studies on antiaging interventions suggest that maintaining/restoring the homeostasis or redox state may be an effective strategy for survival and promoting healthy life span (Schafer and Buettner 2001; Chiurchiù et al. 2016; Saraswat and Rizvi 2017). It has been documented that the determination of redox balance or cellular homeostasis is done by measuring the reduction potentials of redox duplets like GSH/GSSG, NADPH/$NADP^+$, and thioredoxine reduced/oxidized. These redox couples restore the cellular homeostasis via different pathways and mechanisms (Schafer and Buettner 2001; Forman et al. 2009; Sohal and Orr 2012). Keeping this rationale in mind, it has been hypothesized that all the pathways/mechanisms entailed to withstand varied kind of stressors and to conserve redox homeostasis may be paramount in promoting healthy longevity. In the present chapter, we have described a plasma membrane-based novel redox system that operates in the body to counteract redox imbalance. Its activation may act as a compensatory mechanism to intervene aging and associated impairments.

## 19.2 Plasma Membrane Redox System

Plasma membrane regulates numerous facets of cellular physiology including nutritional transport and signal transduction in addition to protecting cell against external stressors (Wang et al. 1999; Hyun et al. 2006b; Hyun and Lee 2015). Due to proteins and lipids which are vulnerable to oxidative insult due to their inherent composition, plasma membrane is always menaced in all eukaryotes. The oxidative injury to the proteins and lipids present in the membrane transporters disturbs the activity, fluidity, and deformability of these transporters, which significantly results in aging and related cellular impairments (Pandey and Rizvi 2011a, b). Compromised plasma membrane state results in redox imbalance and may directly induce cell death (Circu and Aw 2010). In eukaryotic cells, it has been found that a group of oxidoreductase enzymes incorporating quinone reductases such as cytochrome b5 reductase and NADH-quinone oxidoreductase associated with plasma membrane and are involved in conserving redox homeostasis of the cell, thus collectively known as plasma membrane redox system (PMRS) (Adlard and Bush 2011; Hyun and Lee 2015). Later studies have confirmed that besides maintaining redox shift, this system plays many other vital roles (Rizvi et al. 2006; Hyun et al. 2006b; Adlard and Bush 2011). PMRS

transfers reducing equivalent from the intracellular donors like reduced nicotinamide adenine dinucleotide (NADH) and ascorbate (ASC) or both to extracellular acceptor which is utilized to restore oxidized environment to reduced form. It has been suggested that PMRS functions in maintenance of redox state of sulfhydryl residues in membrane-associated proteins, reducing the ROR, cell growth stimulation, recycling of α-tocopherol, prevention of peroxidative modification of lipids, and reducing of ferric ion before its uptake by iron in a transferring-independent pathway (VanDuijn et al. 1998; Rizvi et al. 2009; Adlard and Bush 2011).

PMRS also plays a critical role in modulation of the cellular NAD+/NADH ratio to counter shifts in energy requirement (Hun et al. 2012). In addition, it also has the ability to compensate mitochondria dysfunction which is reflected by increased activity of PMRS enzymes in mitochondria deficient cells (Hyun et al. 2006b). Under stress condition, a transcription factor Nrf-2 induces a NAD(P)H-dependent reductase in the inner surface of the plasma membrane, NAD(P)H-quinone oxidoreductase1 (NQO1) (Rushmore et al. 1991). Higher NQO1 levels have been proposed to enhance resistance during cellular energy deprivation and proteotoxicity (Hyun et al. 2012).

Ascorbate free radical (AFR) reductase is another important component of PMRS that is involved in maintaining the extracellular ASC concentration by using electrons derived from intracellular ASC or other donors (de Grey 2005; Rizvi et al. 2009). The combined action of PMRS and AFR reductase in regeneration of ASC seems very useful since ASC is an important antioxidant in cellular system and involved in primary defense machinery to various stressors. ASC also functions as an enzyme cofactor which plays an important role in reactions involved in catecholamines and peptide hormone synthesis (Harrison and May 2009). It is interesting that despite involvement in vital functions of the cell, humans and guinea pigs cannot synthesize ASC in the body because of the lack of enzyme L-gulonolactone oxidase, which is required for ASC biosynthesis in mammals (Nishikimi et al. 1994). Encounter of an oxidant triggers the oxidation of ASC to AFR, which forms an unstable intermediate dehydroascorbate (DHA) that undergoes irreversible hydrolysis to form 2,3-diketo-L-gulonic acid, in consequence to which the level of the vitamin decreases in the cell. The reaction of two AFR molecules results in the formation of one ASC and one DHA molecule (Fig. 19.1).

Higher PMRS enzymes and AFR reductase activities have been reported during aging and associated complexities (Lenaz et al. 2002; Rizvi et al. 2009; Pandey and Rizvi 2013). Further studies have provided evidence that activation of PMRS and AFR reductase systems acts as compensatory mechanism that minimizes aging-induced oxidative stress and restores the plasma ASC level (de Grey 2005; Rizvi et al. 2009; Pandey and Rizvi 2010, 2013). Hyun and co-workers have documented that during aging, PMRS activation in brain cells helps to counteract dysfunction in mitochondria and oxidative insult (Hyun et al. 2006a). Reports suggest that another important component of PMRS is coenzyme Q (CoQ), which, in its reduced form, acts as an antioxidant and provides protection to lipids from oxidative injury. This protection may be either direct or through conserving the reduced state of both α-tocopherol and ascorbate (Kagan et al. 1990; Hyun et al. 2006a). These studies

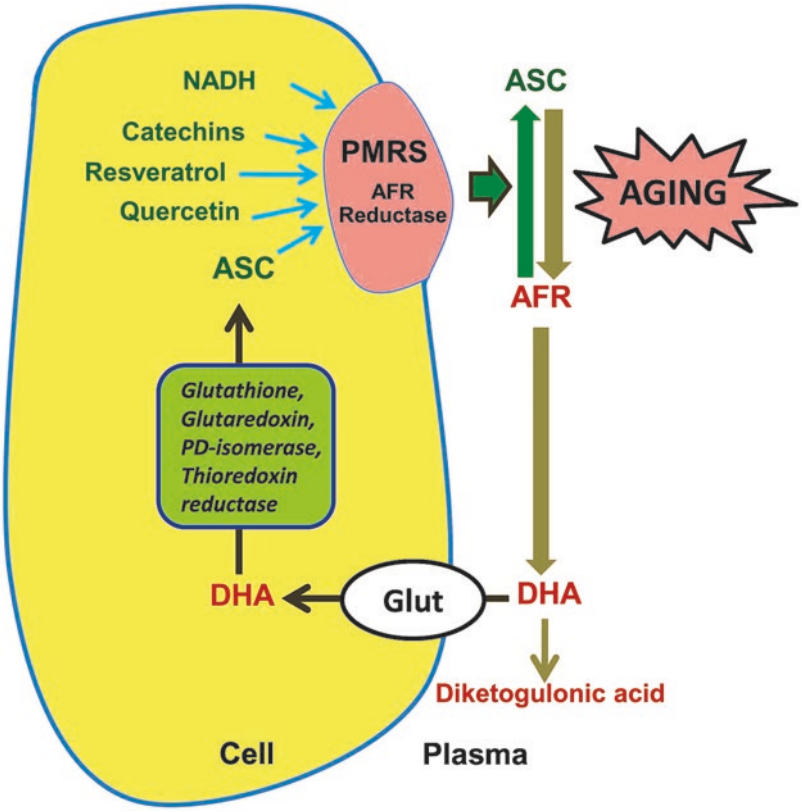


**Fig. 19.1** Schematic representation of involvement of plant polyphenols and other compounds inactivation of PMRS. Activated PMRS transfers reducing equivalents to AFR and reduces it into ASC thus helps in increased ASC recycling during aging. *ASC* ascorbate, *AFR* ascorbate free radical, *DHA* dehydroascorbate, *NADH* nicotinamide adenine dinucleotide, *Glut* glucose transporter, *PD* protein disulfide. (Pandey and Rizvi 2013)

potentiate the notion that activation of PMRS has significant action in maintaining the cellular redox state and can serve as novel biomarker of aging.

### 19.2.1 Activation of PMRS

Overexpressed PMRS in old individuals in comparison to the young and its activation during adverse cellular conditions have provided a clue that its activation/upregulation may be a potent strategy to intervene aging and associated consequences thereby promoting the healthy life span (Pandey and Rizvi 2010; Hyun et al. 2012). Thus, a compound with the ability to upregulate PMRS may act as antiaging agent. Recent studies on the health-promoting activities of polyphenols have reported that some polyphenols possess the ability to enter inside the cell.

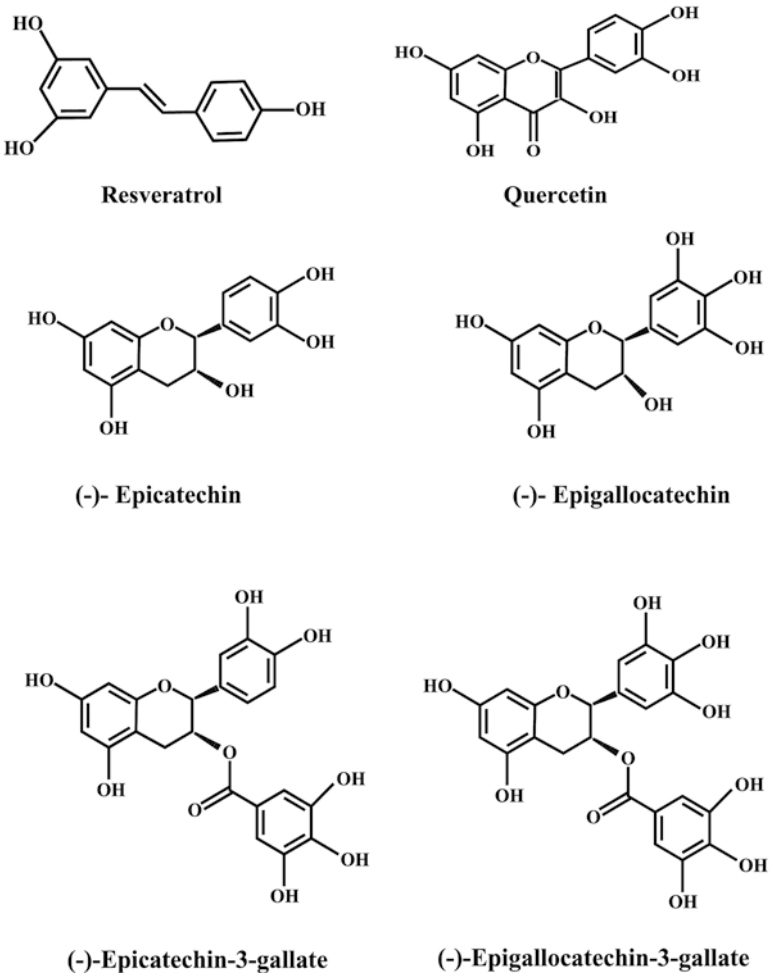


**Fig. 19.2** Molecular structures of plant-derived polyphenols having the ability to activate PMRS

Ones inside the cell, these polyphenols accumulate in higher concentration and activate PMRS by donating the reducing equivalent which is utilized to recycle the AFR back into ASC (Fiorani and Accorsi 2005; Rizvi and Pandey 2010; Pandey and Rizvi 2012, 2013) (Figs. 19.1 and 19.2).

Interestingly most of the polyphenols, which activate PMRS, have already been documented as beneficial during aging. It has been proposed that activation of PMRS may be classified among the mechanisms by which these polyphenols exert antiaging benefits. Polyphenols are naturally occurring components in plants, synthesized during adverse conditions such as exposure to stressors or during attack by pathogens (Pandey and Rizvi 2009). During the last decade, polyphenols have been broadly studied for their pleiotropic biological effects which are beneficial in

promotion of human health (Scalbert et al. 2006; Pandey and Rizvi 2009, 2011a, b; Moreira et al. 2014; Mohammed et al. 2017).

Later studies have reported that resveratrol, green tea catechins, and quercetin exhibit the ability to activate the PMRS in human cells. A study performed on human red blood cells of both males and females between the age 18 and 82 has reported that resveratrol significantly upregulated the PMRS thereby promoting enhanced ASC recycling during aging (Pandey and Rizvi 2013). Likewise in other studies, epicatechin, epigallocatechin, epicatechin-3-gallate, epigallocatechin-3-gallate, quercetin, ASC, and NADH have also been documented to activate the PMRS (Hyun et al. 2006b; Pandey and Rizvi 2009, 2010, 2012).

Caloric restriction (CR) which may be defined as reduction in the intake of calories without malnutrition is the most described strategy for healthy aging and to extend longevity. The molecular mechanisms of CR are not fully understood, but it has been reported that CR upregulates the PMRS and reduces oxidative stress during aging (Hyun et al. 2006a). A study performed on brain cells reports that CR increased the PMRS enzyme activity including AFR reductase, NQO1, NADH-ferrocyanide reductase, COQ10 reductase, NADH-cytochrome c reductase, and level of α-tocopherol (Hyun et al. 2006a, 2012).

## 19.3 Conclusion

Based upon available reports, activation of PMRS is one of the most promising approaches for a successful antiaging strategy since PMRS has the ability to act in response to adverse conditions and safeguard the cells during aging. Future research efforts on PMRS enzyme activities and agents which can upregulate its activity may provide a better understanding toward healthy life extension.

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**Conflict of Interest** Nil

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# Impact of Sarcopenia in Healthy Aging and Suggested Interventions

20

Tuğba Erdoğan, Gülistan Bahat, and Mehmet Akif Karan

**Abstract**

Sarcopenia is a condition characterized by loss of skeletal muscle mass and function with aging and is associated with frailty, physical disability, falls, and higher mortality.

Many factors play role in the pathophysiology of sarcopenia. These include genetic factors, mitochondrial defects, decreased anabolic hormones, inflammatory cytokines, insulin resistance, decreased protein intake and activity, poor blood flow to muscle, and growth-derived factor-11 deficiency. The molecular mechanisms underlying and/or associated with sarcopenia have significant role in aging. Hence, interventions targeting sarcopenia shall have an impact on healthy aging. At present, the best-demonstrated and practically recommended approaches to prevent/treat sarcopenia are resistance exercises and intake of adequate protein and vitamin D. Adequate intake of protein and resistance exercises are mandatory to preserve muscle mass. Many other promising treatment modalities are currently being investigated listed on the way.

**Keywords**

Aging · Intervention · Molecular mechanisms · Sarcopenia

## 20.1 Definition and Epidemiology of Sarcopenia

The term "sarcopenia" was first proposed by Rosenberg and Roubenoff in 1995 to define age-related muscle loss (Rosenberg and Roubenoff 1995). Sarcopenia is a condition characterized by progressive and generalized loss of skeletal muscle mass

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and function with aging. The European Working Group on Sarcopenia in Older People (EWGSOP) provided a working definition of sarcopenia as "a syndrome characterized by progressive and generalised loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life and death" (Cruz-Jentoft et al. 2010). A similar approach was taken by the International Working Group on Sarcopenia (IWGS) in 2009 which provided a consensus definition of sarcopenia as "age-associated loss of skeletal muscle function and mass" (Fielding et al. 2011). EWGSOP suggested three categories of sarcopenia: pre-sarcopenia, sarcopenia, and severe sarcopenia. The pre-sarcopenia is characterized by low muscle mass without reduced muscle strength or physical performance. The sarcopenia stage is characterized by low muscle mass, accompanied with low muscle strength or low physical performance. Severe sarcopenia is characterized by low muscle mass, low muscle strength, and low physical performance.

The prevalence of sarcopenia in the literature varies widely and is likely to be affected by the population studied and the different methods used to assess muscle mass, muscle strength, physical performance, and different muscle mass adjustment methods. Moreover, significant differences between cutoff values lead to a difference in prevalence. In the systemic review reported by Cruz-Jentoft et al., the EWGSOP-defined sarcopenia prevalence was reported as 1–29% in community-dwelling elderly, 14–33% in long-term care residents, and 10% in acute hospital care (Cruz-Jentoft et al. 2014).

## 20.2 Effects of Sarcopenia on Healthy Aging

Sarcopenia is related with reduced physical capability (Tanimoto et al. 2012), mobility impairment, disability, deterioration of respiratory function, impaired cardiopulmonary performance, unfavorable metabolic effects (Karakelides et al. 2005), immune deprivation, decreased quality of life (Visser and Schaap 2011), frailty, difficulties in instrumental and basic activities of daily living, osteoporosis, falls (Landi et al. 2012), increased length of hospitalization and readmission (Gariballa and Alessa 2013), and death (Landi et al. 2013).

## 20.3 Diagnosis of Sarcopenia

There is substantial work performed to yield consensus definitions organized by different groups, and presence of both low muscle mass and low muscle function is required for diagnosis of sarcopenia as the consensus of all (Cruz-Jentoft et al. 2010; Muscaritoli et al. 2010; Fielding et al. 2011; Morley et al. 2011; Dam et al. 2014). For muscle mass, recommended measurement techniques include computed tomography (CT) and magnetic resonance imaging (MRI), dual X-ray absorptiometry (DXA) scan, and bioelectrical impedance (BIA). MRI and CT are considered to be accurate imaging techniques that can separate fat from other soft tissues which

makes these methods gold standards for measuring muscle mass in research. But for routine clinical practice, they are not appropriate because of limited access, high cost, or radiation exposure. For research and clinical use, DXA is the suggested alternative method because DXA can differentiate fat, muscle, and bone mineral tissue and at the same time radiation exposure is minimal. Another method that can be used for muscle mass measurement is BIA. BIA is a portable, widely available, rapid, noninvasive, inexpensive, readily reproducible technique appropriate for both ambulatory and bedridden patients and operator friendly not requiring high-level training. Low muscle mass cutoff values show differences between populations. EWGSOP recommends use of normative data of the study population if available instead of other predictive reference populations, with cutoff points of muscle mass at two standard deviations below the mean reference value (Cruz-Jentoft et al. 2010). Population-specific cutoff values have been reported in a few number of population including the Turkish population (Bahat et al. 2016).

There are three different approaches to adjust lean mass to body size, which include muscle mass indexes adjusted for height squared (Baumgartner et al. 1998), for total body mass (Janssen et al. 2002), or for BMI (Cawthon et al. 2014). There is no consensus on which correction method is better. Low muscle mass/height$^2$ was present almost exclusively only among normal or underweight patients, whereas indexing to body weight and BMI classified more overweight and obese patients as having low muscle mass. Kittiskulnam et al. reported that the degree of correlation between muscle strength and muscle mass was highest when muscle mass was adjusted by BMI and lowest for by height$^2$ (Kittiskulnam et al. 2017). The muscle mass adjusted by BMI may be better for association of low muscle mass with functionality than the other methods.

There are fewer well-validated techniques to measure muscle strength. Although for gait and physical function lower limbs are more relevant than upper limbs, handgrip strength has been widely used and is well correlated with most relevant outcomes (Cruz-Jentoft et al. 2010). Low handgrip strength is a clinical marker of poor mobility and a better predictor of clinical outcomes than low muscle mass (Laurentani et al. 2003). Low muscle strength cutoff values should better be determined separately for each population. Again, the population-specific handgrip strength cutoff values have been reported in few populations including Turkish population (Bahat et al. 2016; Fried et al. 2001).

There are comprehensive tests to measure physical performance such as 6-min walk test, usual gait speed, short physical performance battery (SPPB), and the stair climb power test. The SPPB evaluates gait, balance, strength, and endurance. Usual gait speed is part of the SPPB, but it can also be used as a single parameter for research and clinical settings. Timed get-up-and-go (TGUG) test is an important test, especially when evaluating the dynamic balance. It can serve as a performance measurement.

Most commonly, sarcopenia diagnosis is recommended to be made by evaluation of handgrip strength and usual gait speed as measures of muscle function. According to the algorithm published by the EWGSOP in 2010, the first step to evaluate sarcopenia is the walking speed. If the walking speed is >0.8 m/s, evaluation of handgrip strength is required. If any of the assessments (i.e., handgrip strength or walking

speed) is identified low, muscle mass should be measured to evaluate sarcopenia. The approach recommended by the IWGS in 2011 is similar.

As detailed above, as sarcopenia is associated with many adverse outcomes (i.e., mobility disability, falls, fracture, cognitive impairment, metabolic syndrome, cardiovascular diseases, and mortality) accompanying the unhealthy aging, recognition of factors causing sarcopenia is important. Accordingly, interventions that aim to prevent or improve these etiologic factors/mechanisms may facilitate healthy aging.

## 20.4 The Pathophysiology of Sarcopenia

Multiple, interrelated factors contribute to the development and progression of sarcopenia. These factors contribute in varying degrees to the age-related losses of muscle mass, strength, muscle quality, and physical reserve in older adults. Increased levels of pro-inflammatory cytokines (e.g., IL-6, TNF), altered endocrine function (testosterone, insulin, estrogen, growth hormone), cellular apoptosis, mitochondrial dysfunction, and inadequate nutrition (particularly dietary protein) have all been implicated as potential contributing factors to loss of muscle mass, strength, and contractile quality. Endothelium-dependent vasodilation decreases with aging, due to decreased nitric oxide bioavailability. These changes lead to decreased microvascular oxygenation. Another contributor to the development of sarcopenia is the decrease in blood flow to muscle with aging.

In 2016, Morley extensively reviewed the pathophysiological causes of sarcopenia (Morley 2016). When muscle contracts mechanoreceptors such as titin and dystroglycan have been activated. The activity of muscle growth factors is increased by the mechanoreceptors. The synthesis of muscle is increased by these factors and recruits satellite cells and motor units. So, muscle function is regenerated and muscle function increases. Muscle contraction leads to muscle injury. With aging muscle injury increases and also muscle regeneration and function decrease.

Because of the decrease in muscle growth factors, the protein synthesis/degradation ratio, the satellite cell, and the motor unit activation decrease. There is type II fiber atrophy with aging, and this results in diminished muscle mass, power, and strength (Purves-Smith et al. 2014). In sarcopenic patients the motor unit number index (munix) reduces. The 25% loss of motor neurons with aging leads to germinating of small motor neurons that innervates type II fibers which leads to an ultimate loss of type II fibers. Also with aging ciliary neurotropic factor (CNTF), which stimulate motor unit formation, declines.

### 20.4.1 Myokines

Myokines which are produced by skeletal muscle can affect muscle growth and repair (Demontis et al. 2013). Interleukin-6 is produced predominantly by adipose tissue that infiltrates the muscle. Intramuscular lipid infiltration is related with decreased muscle mass, decreased muscle strength, and increased levels of

inflammatory markers. IGF-1, IGF-binding proteins, musclin, myostatin, CXCL-1, and leukemia inhibitory factor have direct effects on muscle. Myostatin is a regulatory factor primarily exerting its effect in the skeletal muscle. It is a member of the TGF-β family and inhibits muscle growth. Growth hormone inhibits myostatin levels. Decrease in growth hormone with aging may cause an increase in myostatin levels. VEGF-B, IL-8, and follistatin-like1 increase angiogenesis in muscles. Contractile protein accumulation is increased by IL-5 and this induces myotube hypertrophy (Pistilli and Quinn 2013).

### 20.4.2 Genetics

In older persons genes play a role in 50–80% of muscle strength and 65% of muscle mass (Garatachea and Lucia 2013). In muscle contraction angiotensin-converting enzyme alleles play an important role. In men ACTN3 gene deficiency is associated with reduced strength and endurance activity. CNTF, IL-5, myostatin, insulin growth factor, collagen type II, the vitamin D receptor, and the androgen CAG receptor genes are also related with muscle strength. Older persons have higher perilipin 2 levels, and it is related with lipid droplets. It causes a decrease in muscle strength and the proteins associated with muscle atrophy, i.e., atrogin and MURF1.

### 20.4.3 Mitochondria

Mitochondrial dysfunction plays a role in the pathogenesis of aging (Marzetti et al. 2013). The production of cellular energy, free radical signaling is controlled by mitochondria, and it can activate apoptotic pathways. Along with aging, there is an increased fusion, and it leads to a giant mitochondria. Removing giant mitochondria from cell is difficult, and because of that, cells function poorly. Older mitochondria lose its outer membrane; this leads to increase their tendency to apoptosis. This is interrelated to a reduction in CiSD2 gene expression.

The peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) regulates mitochondrial biogenesis and function. Muscle fiber adaptation to exercise is regulated by PGC-1α (Amold et al. 2011). In old animals the functional loss of mitochondrial enzymes is reduced by the activity of PGC-1α, and this protects muscle from damage. PGC-1α gene expression reduces in older persons (Garatachea and Lucia 2013). With aging PGC-1α levels decrease, and this causes translocation of BAX to mitochondrial membrane with activation of the mitochondrial membrane pore and loss of cytochrome C. This causes mitochondrial apoptosis. Also this reduction causes low-grade inflammatory reaction with increased levels of IL-6 and TNFα. Excessive release of PGC-1α harms the heart and muscle. Therefore increasing PGC-1α to physiological levels in sarcopenic tissue may be a fundamental therapeutic approach to treat muscle wasting. Muscle mitochondria-centered approaches

can be a reasonable option for the treatment of sarcopenia. However, all approaches need to be further explored.

### 20.4.4 Protein Synthesis and Degradation

IGF-1 receptor or insulin activation controls protein synthesis and/or degradation. This stimulates the phosphoinositide 3-kinase (PI3K)-AKT – mammalian target of rapamycin (mTOR) signaling pathway. Increased mTOR leads to an increase in protein synthesis. AKT and PGC1a stop FOXO activity; this leads to reducing the transcription of atrogenes. These atrogenes are MURF-1 or TRIM63 and atrogin1. Atrogin1 deteriorates proteins that increase protein synthesis. Myofibril breakdown is directly controlled by MURF-1 and ubiquitin tripartite motif containing protein 32 (TRIM32). MURF-1 leads to devastation of the thick myosin filament by attacking the myosin light chain and the myosin binding protein. TRIM32 devastates desmin and then the Z-band and finally the thin actin filament. Therewithal TRIM32 directly limits PI3 K-AKT activity which leads to increased proteolysis. Myofibrils form the vast majority of muscle protein. Destruction of these myofibrils causes loss of muscle function (Cohen et al. 2012). In sarcopenia protein destruction and ubiquitination increase. TRIM32 and/or MURF1 inhibitors represent attractive therapeutic targets in the treatment of sarcopenia.

## 20.5 Treatment of Sarcopenia

Sarcopenia treatment components that have convincing evidence-based data include exercise, nutritional support, and hormonal treatment options. There are also new emerging treatment alternatives.

### 20.5.1 Exercise

The primary treatment of sarcopenia is exercise. With exercise, both muscle strength and muscle mass can be increased. In aerobic exercise, large muscle groups move in a rhythmic pattern for a certain period of time. Resistance exercises are performed against a force or weight applied (i.e., weight lifting). Both resistance and aerobic exercises reduce muscle mass and strength decline that occurs with aging. Resistance exercise among different types of exercise to struggle with sarcopenia is the safest and most effective method to improve both muscle mass and muscle function. It seems to be an important tool in the treatment of sarcopenia by promoting positive functional (strength and power) and structural (hypertrophy and phenotypic changes) adaptive responses. In a meta-analysis assessing 121 randomized controlled studies, it was found that physical function, walking speed, timed up-and-go test, ladder climbing power, and muscle strength were improved in older people who had progressive resistance training twice or three times a week. Aerobic

exercise such as walking, running, swimming, or biking are known to have benefits on cardiovascular fitness, flexibility, and endurance capacity (Koopman et al. 2010). It is also known that aerobic exercise is less likely to contribute to muscle hypertrophy, but this exercise may increase the cross-sectional area of muscle fibers. Aerobic exercise training affects skeletal muscle by enhancing mitochondrial bioenergetics, protein synthesis, and insulin sensitivity, reducing inflammation and oxidative stress, (Short et al. 2004). In addition to the beneficial effects of aerobic exercises on cardiovascular health, resistance and endurance exercises performed three times a week have been found to be effective in sarcopenia (Phu et al. 2015).

### 20.5.2 Nutritional Support

Nutrition intervention is considered to be one of the anchors of intervention in sarcopenia, but most of the evidence is based on short-term protein synthesis studies. Older people are at risk for inadequate protein intake. Health, Aging, and Body Composition Study (Houston et al. 2008) showed that higher protein intake was associated with less appendicular lean muscle mass loss over a period of 3 years. It has been suggested that the daily intake of 0.8 g/kg protein recommended in healthy adults is inadequate to prevent the occurrence of sarcopenia in the older adults. Increased muscle wasting in the elderly, comorbid diseases, and their exacerbation periods increase the protein requirement of the patients. It is suggested that protein intake of 1.2–1.5 g/kg/day is needed in the older adult arranged by consideration of compulsory inactivity periods and stress factors. with adequate energy intake (Deutz et al. 2014). Comorbid diseases in the advanced age and immobility process increase muscle loss. Increased loss in the process of recruitment makes protein support more important in this period. The quality of the protein is also important. Essential amino acids are important stimulants of protein synthesis. Particularly, leucine has anabolic effect on muscle through stimulation of motor pathway. Essential amino acids, especially leucine and beta-hydroxy-methylbutyrate (HMB) supplement, improve the parameters related to muscle mass and function (Cruz-Jentoft et al. 2014). A systematic review published by Cruz-Jentoft et al. in 2014 assessed the exercise and nutritional support underlying sarcopenia. Nutritional support treatments have focused on the effect of protein supplementation (sufficient calories can be provided with other nutrients in general), amino acid supplementation, and beta-hydroxy-methylbutyric acid supplementation (HMB: leucine bioactive metabolite) (with arginine or alone) on muscle mass and/or muscle function in 8–24 weeks. It has been stated that the activity of protein supplementation in muscle mass and function is unclear in studies in which the quality level is moderate to good according to their own evaluations. It was noted that the EAA (essential amino acid) supplementation had partial positive effects and the HMB had partial positive effects, but the samples were with a small number of patients, and fatty acid supplementation had no effect (Cruz-Jentoft et al. 2014). In addition, the daily protein intake needs to be distributed throughout the day in proportion to the meals to have optimal benefit from the ingested protein (Layman 2009). Vitamin D plays an important role

in muscle and bone metabolism. When vitamin D binds to the skeletal muscle receptor, muscle protein synthesis increases and calcium intake from the cell membrane increases (Bischoff et al. 2001). Vitamin D deficiency is associated with atrophy especially in type II muscle fibers and sarcopenia (Ziambaras et al. 1997). Older patients with low vitamin D levels have reported difficulties in stair climbing-standing up, proximal muscle weakness, and balance problems (Mowe et al. 1999). In postmenopausal women vitamin D supplementation alone has a significant protective effect against the formation of sarcopenia and provides a significant improvement in muscle strength (Stratos et al. 2013). The maximum blood level for the healthiest effect of vitamin D has been reported as 50 ng/ml (Shuler et al. 2012). Maintaining adequate and optimal level of vitamin D is one of the mainstays of the evidence-based sarcopenia treatment.

In summary, balanced protein, energy, and vitamin D intake, particularly as part of a multimodal therapeutic approach to treating and preventing sarcopenia in the elderly, may be useful, especially when combined with regular exercise.

### 20.5.3 Pharmacological Treatment

#### 20.5.3.1 Testosterone

From 30 years of age, testosterone levels decrease at the rate of 1% per year (Morley 2011; Morley et al. 1997). This decrease in testosterone is related with a decline in muscle mass and strength (Baumgartner et al. 1999). Numerous studies have revealed that testosterone increases muscle mass and decreases fat mass at low doses (Wittert et al. 2003) and increases muscle mass and muscle power at higher doses (Bhasin et al. 2005). In lower doses, testosterone enhances protein synthesis, and this increases muscle mass (Ferrando et al. 2003). In high doses, testosterone activates satellite cell recruitment and reduces adipose stem cells (Kovacheva et al. 2010). In frail older people and people with heart failure, testosterone increases both strength and walking distance. In frail older people, testosterone with protein supplementation reduced the hospitalization rate (Chapman et al. 2009). A study by Stephanie et al. has shown that testosterone alone or with drugs such as finasteride, 5-alpha reductase inhibitor, corrects body compositions. This study, consisting of patients with a mean age of 71 years, showed improvement of metabolic function and muscle mass at lower extremity with appropriate testosterone levels at the end of 36 months (Stephanie et al. 2005). Results from recently published individual trials showed that testosterone has beneficial effect on bone strength and bone mineral density (Nieschlag 2015; Irwig 2014). Osteoporosis and sarcopenia frequently coincide (osteosarcopenia).

In his 2016 review, Morley suggests that the most effective and safest drug developed for sarcopenia is the testosterone (Morley 2016). The role of testosterone replacement to treat the decline in serum testosterone concentration that occurs in aging men was addressed in the multicenter Testosterone Trials in 2017, an integrated set of seven trials in nearly 800 men over age 65 years with low testosterone and sexual dysfunction, and reduced vitality, who were randomly assigned to

testosterone gel or placebo for 12 months. Initial results suggested that testosterone had a beneficial effect on sexual function, depressive symptoms, and mood and possibly physical function (walking distance) (Resnick et al. 2016). But it is known that there are some side effects, and because of these side effects, its use is restricted in the clinic. Testosterone replacement can lead to adverse outcomes such as prostate enlargement, gynecomastia, polycythemia, fluid retention, and sleep apnea. It remains controversial whether or not testosterone has an effect on cardiovascular events, especially in the first 3 months after administration (Mogentaler et al. 2015; Borst et al. 2014). A meta-analysis of the controlled studies of testosterone in older males did not show an increase in mortality (Carona et al. 2014). Very recently, in their study Cheetham et al. reported that testosterone usage was related with lower risk of cardiovascular outcomes (Cheetham et al. 2017). However, in this analysis, most of the men were relatively healthy and young, and it may be older men at higher cardiovascular risk that are most vulnerable to side effects of testosterone therapy. In their study Budoff et al. reported that treatment with testosterone gel for 1 year, compared with placebo, was associated with a significantly greater increase in coronary artery noncalcified plaque volume (Budoff et al. 2017). Therefore, it is noted that at the current time, clinicians should remain aware that the cardiovascular risks and benefits of testosterone replacement in older hypogonadal men have not been adequately resolved (Orwoll 2017). More attention should be paid to the potential role of testosterone in the treatment of sarcopenia.

#### 20.5.3.2 Anabolic Steroids/Selective Androgen Receptor Modulators (SARMs)

**Anabolic Steroids**

Nandrolone is an injectable anabolic steroid. It enhances muscle mass and fiber area, but there is no data for increased strength (Macdonald et al. 2007). In three studies of persons with hip fracture, it has been shown that there is nonstatistical improvement in functional status (Farooqi et al. 2014). In hemodialysis patients, ingesting oxymetholone was related with a raise in handgrip strength, fat-free mass, and muscle mRNA levels for several growth factors and a decrease in fat mass, but it also induced liver injury (Supasyndh et al. 2013). MK0773 (TFM-4AS-1) is a 4-aza steroidal drug. It has androgen gene selectivity. It has been shown that it increased insulin growth factor-1 (IGF-1) and also stair climbing capacity and gait speed in females. But due to increased signal for cardiac failure, this study was terminated (Papanicolaou et al. 2013).

**Selective Androgen Receptor Modulators (SARMs)**

Fear of adverse side effects from the testosterone has led to the search for selective androgen receptor modulators (SARMs) which could be theoretically safer. SARMs act by binding to the androgen receptor. SARMs show different sensitivities when compared to testosterone (Mohler et al. 2009). LGD-4033 is a nonsteroidal, orally active SARM. The phase I trial showed an increase in muscle mass but did not show any effect on fat mass at 21 days of the trial (Basario et al. 2013). In a 12-week study

with female cancer patients, an another SARM, "enobosarm," increased stair climb performance and total lean mass (Dalton et al. 2011). In two phase III trials, it maintained body mass and enhanced stair climbing power in patients with cancer in one of two trials (Steiner 2013). Overall, these studies of SARMs have shown no superiority to the testosterone. Currently it is not recommended because there is not enough supportive study to use it.

#### 20.5.3.3 Growth Hormone/Insulin Growth Factor-1

Growth hormone (GH) is one of the effective hormones in the maintenance of muscle and bone mass. GH produces its anabolic effect by release of liver-derived IGF-1. There is a decrease in the pulsatile frequency and amplitude of both growth hormone and IGF-1 with aging. Because of this decline, central obesity, deterioration in mental functions, and fragility with loss of muscle mass and physical function occur. In a study conducted, growth hormone supplementation showed a 2 kg increase in lean muscle mass and a 2 kg decrease in fat mass. Many studies have shown that although there is an increase in muscle mass with growth hormone replacement in the elderly who are healthy and without growth hormone deficiency, there is no significant effect on muscle strength (Burton and Sumukadas 2010; Papadakis et al. 1996). Both high and low levels of IGF-1 increase risk of cardiovascular disease. A study of IGF-1 found an increase in side effects such as edema, myositis, orthostatic hypotension, and gynecomastia. Both GH and IGF-1 are not currently recommended in the treatment of sarcopenia (Sullivan et al. 1998).

#### 20.5.3.4 Myostatin and Activin 2 Receptor Inhibitors

Growth differentiation factor-8 or myostatin which prevents muscle protein synthesis and satellite cell production and promotes fibrosis is produced in skeletal muscle. Myostatin inhibition causes muscle hypertrophy. Hence, several agents which are mechanistic myostatin antagonists, including hormones such as soluble activin type IIB receptors (myostatin binds to activin type I, IIA, and IIB receptors for its action), follistatin (a natural myostatin-binding protein), and recombinant myostatin antibodies, are all in development. In people with muscular dystrophy, myostatin antibody (MYO-029) has increased muscle mass (Wagner et al. 2008). Muscle fiber diameter increased in 10 mg/kg dose. Side effects in high doses include urticaria and aseptic meningitis. In patients with androgen deprivation therapy for prostate cancer, another myostatin antibody (AMG 745) causes decreased fat and increased lean body mass after 28 days (Padhi et al. 2014). In the persons receiving active drug, confusion, fatigue, and diarrhea were more common. In persons with advanced cancer, LY2495655 increased handgrip strength and muscle volume (www.clinicaltrials.gov). In monkeys an activin II receptor ligand trap, ACE-011, increased bone strength and mass (Fajardo et al. 2010). In 48 postmenopausal women, after a single dose of ACE-031, lean body mass and thigh muscle volume had been increased (Attie et al. 2013). Another ligand trap, ACE-083, is in development phase. Due to side effects such as epistaxis, telangiectasia, and changes in gonadotropin levels, the company stopped the development of these compounds. Studies are going on, but

there is not currently enough evidence to use myostatin and activin receptor inhibitors in the treatment of sarcopenia.

*Bimagrumab* A human monoclonal antibody directed against type II activin receptors. In a 24-week randomized, double-blind, placebo-controlled study in older adults with sarcopenia, it is shown that treatment with bimagrumab increased muscle strength and mass, and in those with slow walking speed, it enhanced mobility (Rooks et al. 2017).

#### 20.5.3.5 Angiotensin-Converting Enzyme Inhibitors (ACEIs)

Angiotensin-converting enzyme inhibitors have a positive effect on skeletal muscle function with various mechanisms, such as regulation of endothelial function, anti-inflammatory effect, and regulation of angiogenesis which leads to regulation of skeletal blood flow (Sumukadas et al. 2008). ACE inhibitors may also increase the number of mitochondria and IGF-1 levels (Papadakis et al. 1996). It has been shown that perindopril increases walking distance in older people with left ventricular systolic dysfunction (Hutcheon et al. 2002). In older persons with functional impairment, perindopril also improved 6-min walking distance (Sumukadas et al. 2007). In HYVET study perindopril decreased hip fracture (Peters et al. 2010). Although there are positive effects in a small number of prospective studies, large studies are needed to investigate the effects of ACE inhibitors on sarcopenia.

#### 20.5.3.6 Fast Skeletal Troponin Activators (Tirasemtiv)

Tirasemtiv, a selective fast skeletal muscle troponin activator that synthesizes the sarcomere to calcium and amplifies the response of muscle to neuromuscular input in humans, has been reported to improve muscle power and muscle fatigability in humans (Hansen et al. 2014). Tirasemtiv slowed the rate of decrease in muscle strength (Malik et al. 2014). There is not currently enough evidence to suggest tirasemtiv; further studies are needed.

#### Drugs Targeting Systemic Inflammations

They modulate both energy balance and muscle protein balance.

**Omega-3-Suplements** Omega-3-polyunsaturated fatty acids (n3-PUFA) especially *eicosapentaenoic acid (EPA)* reduce mitochondrial oxidant emissions, increase postabsorptive muscle protein synthesis, and enhance anabolic responses to exercise in older adults. And also it has anti-inflammatory effect (Calder 2013). It is synthesized from ingested alpha-linolenic acid or is consumed in fish and fish oil such as cod liver, sardine, and salmon oil. The administration of omega-3 fatty acids and EPA capsules or supplements with EPA has been shown to be associated with weight stabilization, gains in lean body mass, and improvements in quality of life markers in weight-losing patients.

**OHR/AVR118** This is a broad-spectrum peptide immunomodulator drug, which modulates cytokine action. The drug mitigates the deleterious effects of various pro-inflammatory cytokines that are implicated in the etiology of cachexia, whose activation has a direct effect on muscle metabolism. A phase II study involving patients with advanced cancer and cachexia showed an improvement in dyspepsia, anorexia, depression, and strength (Chasen et al. 2011).

This agent has worked in cachexia treatment until now. However anti-inflammatory activity is likely to have positive effects on the underlying sarcopenia. Further studies are needed to assess the safety and adequacy of these agents in patients with sarcopenia.

**VT122** This is a fixed-dose combination of propranolol and etodolac, a COX-2 inhibitor. In a randomized phase II study in 59 weight-losing non-small cell lung cancer patients, treatment with the combination of propranolol and etodolac resulted in an increase in lean body mass (Bhattacharyya et al. 2013). Further studies are needed to investigate the effects of this treatment on sarcopenia.

### Drugs Targeting Muscle Homeostasis

Maintenance of skeletal muscle mass is mainly determined by the balance between muscle protein synthesis and proteolysis. Different signaling cascades involved in muscle protein turnover are targeted by the new drugs.

**Ghrelin and Its Analogs** Ghrelin, an endogenous GH secretagogue (GHS), is produced from the fundus of the stomach and increase appetite, food intake, and weight gain. A randomized, blinded, placebo-controlled trial using the oral ghrelin mimetic MK-677, which activates the ghrelin receptor to increase growth hormone, resulted in increased fat-free mass by 1.6 kg with no significant change in strength or function (Nass et al. 2008). Another study using the capromorelin, a ghrelin receptor agonist, demonstrated increases in body weight and fat-free mass and also improved tandem gait, and at the end of treatment for a year, stair climbing also improved in sarcopenic individuals (White et al. 2009). More trials are needed to detect the effectiveness and safety of these agents in the long-term treatment of sarcopenia.

### Potential Future Targets for Drug Development to Treat Sarcopenia

These agents are the other potential agents that are still in the research phase (Morley 2016).

**The Anabolic Catabolic Transforming Agent (ACTA) Espindolol (Mixed Agonist/Antagonist B1, B2, B3 Activity)** Espindolol is the S-enantiomer of pindolol. It decreases fat mass and increases muscle mass in older animals (Potsch et al. 2014). A phase II trial showed a decrease in fat mass and an increase

in muscle mass (Steward Coats et al. 2011). It also increased handgrip strength. More trials are needed to investigate the effects of this treatment on sarcopenia.

**Ruxolitinib** An orally bioavailable drug that selectively inhibits Janus kinase 1 (JAK1) and Janus kinase 2 (JAK2), correlated with decreased levels of phosphorylated JAK and of signal transducer and activator of transcription (STAT). A phase I–II study of patients with myelofibrosis showed that ruxolitinib was associated with weight gain (Verstovsek et al. 2010). A randomized phase III trial comparing ruxolitinib with the best available therapy in patients with primary myelofibrosis showed that the ruxolitinib group had a mean gain in body weight of 4.43 kg by week 48 (Harrison et al. 2012). Whether weight gain through implementation of ruxolitinib may also improve sarcopenia needs to be further explored.

**PPAR-δ Agonists and AICAR (5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranoside)** Studies have recommended a role for both the peroxisome proliferator-activated receptor-δ (PPAR-δ) and AMP-activated protein kinase in regulating the metabolic and contractile characteristics of myofibers. In 2008, Narker et al. conducted studies investigating the effect of modulating these receptors in mice. The PPAR-δ agonist GW1516 significantly increases exercise capacity when combined with exercise but does not increase this in sedentary mice. However, AICAR (5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranoside), the activator of AMP-activated protein kinase, increases exercise performance by 44% even in sedentary mice (Narkar et al. 2008). It should be proved whether these drugs are suitable for humans, especially elderly people.

*Biguanide*: It inhibits BAX translation to mitochondrial membrane and enhances nitric oxide function.

*TRIM 32 Inhibitor*: It inhibits destruction of thin actin filaments, desmin, and the Z-band proteolysis.

*Growth Differentiation Factor (GDF11)*: Satellite cell rejuvenation

*Ciliary Neurotrophic Factor Agonist*: It increases function of motor neuron endplate.

*Myokine Activator and Inhibitor*: It modulates function of muscle.

*CisD Protein Replacement*: It increases permeability of outer mitochondrial membrane.

*Sirtuin/Resveratrol/Polyphenol*: It enhances the interaction of nuclear/mitochondrial protein.

*Nitric Oxide (Isosorbide Dinitrate)*: It increases blood flow of muscle.

*MicroRNA (miR-1, miR-29, miR208, and miR486) Modulator*: It modulates satellite cell quiescence.

*RNA Antisense*: It modulates RNA function.
*PGCI-α Agonist*: It plays a role in mitochondrial biogenesis.
*Serum and Glucocorticoid Inducible Kinase 1 (SGK1)*: It decreases autophagy and proteolysis and improves protein synthesis.

## 20.6 Conclusion

Sarcopenia seem to heavily impact aging. At present, the best-demonstrated and practically recommended approaches to prevent/treat sarcopenia are resistance exercises and intake of adequate protein and vitamin D. Many other promising treatment modalities are currently being investigated listed on the way.

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# Antioxidants for Health and Longevity

# 21

Ramiah Sivakanesan

**Abstract**

Aging is an inevitable process due to functional and structural loss in the body accrued over a period of time owing to harmful effects of free radical generated by a variety of events. Aging is associated with changes in cell metabolism which leads to decrease in cell size, number, and atrophy of organs. Cell loss is most evident in the brain and heart, in which regeneration of lost cells does not occur. Many theories explain the process of aging, but the free radical theory provides plausible evidence for its occurrence. Endogenous metabolic events and exogenous factors are responsible for the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Reactive oxygen species is collectively used in a broad sense to free radicals like superoxide ($O_{2^{\cdot}}^{-}$), hydroxyl ($OH^{\bullet}$), and lipid peroxyl ($LOO^{\bullet}$) radicals and non-free radicals such as hydrogen peroxide ($H_2O_2$), ozone ($O_3$), singlet oxygen ($^1O_2$), and lipid peroxide (LOOH). Uncontrolled increase in ROS concentration enhances free radical-mediated chain reactions which generally target proteins, lipids, polysaccharides, and DNA. Human body has the capability to counteract the ROS by enzymatic antioxidants superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx); nonenzymatic nutrient antioxidants β-carotene, α-tocopherol, ascorbic acid; and metabolic antioxidants, bilirubin, uric acid, ceruloplasmin, ferritin, transferrin, albumin, and glutathione.

**Keywords**

Dietary antioxidants · Longevity · Reactive oxygen species

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## 21.1 Introduction

Aging is a natural physiological process associated with loss in cellular integrity and cell mass guided by programmed cell death or apoptosis. Many believe that this process could be slowed down if not completely halted. Human internal environment is maintained by a number of metabolic events which depend on oxidation of nutrients by the cell and elimination of metabolic end products which are considered to be waste materials.

## 21.2 Cellular Events in Aging Process and Theories of Aging

### 21.2.1 General Changes in the Cell

Changes associated with aging are inevitable.

#### 21.2.1.1 Cell Loss and Body Composition Changes

Aging is associated with changes in cell metabolism which leads to decrease in cell size, number, and atrophy of organs. Cell loss is most evident in the brain and heart, in which regeneration of lost cells does not occur. Cell loss in the brain is selective, with the greatest loss occurring in the (a) basal ganglia (b), substantia nigra, and (c) hippocampus.

Body composition changes continuously throughout life. After sixth decade body weight (BW) decreases significantly, around 7 kg per decade in males and 6 kg per decade in females. There is decrease in fatness also. Lean body mass (LBM) decreases by about 6% for each decade of age, the loss accelerating in later life and greater in males. Between 70 and 75 years, about 1 kg LBM is lost.

Body cell mass decreases with age due to decreasing number of cells in organs and increased disuse of skeletal muscle with age. By 70 years of age, skeletal muscle has lost 40% of its maximal weight in early adult life compared with 18% for the liver, 9% for the kidney, and 11% for the lungs. Muscle thus contributes most to the loss of cell mass. Loss of cells in organs such as the liver and kidney will lead to a loss of reserve tissue available to cope with disease conditions.

Loss in BW in the eighth decade of life corresponds more to loss of body cell mass in males and more to loss of body fat in females. Bone density decreases by 12% in males and 25% in females by the ninth decade. Changes in bone density begin at the age of 40 years. Estrogen withdrawal accounts for much of the bone loss among women between 40 and 60 years.

#### 21.2.1.2 Organelle Changes

The endoplasmic reticulum of aged cells is disordered, and its usual association with ribosomes is lost. Hence free ribosomes are present in greater numbers than normal. This results in abnormalities in synthesis of protein to be exported out of the cell. Hence quantitative and qualitative activity of many enzymes decreases (Digital World Medical School 2016).

Abnormalities in size, shape, and cristae in the mitochondria occur with aging. These, together with reduced levels of cytochrome C reductase, impair energy production. An increased rate of organelle breakdown in aged cells is associated with the presence of increased numbers of (a) phagolysosomal vacuoles in the cells and (b) deposition of lipofuscin – a brown pigment believed to be derived from degraded organelle membranes, particularly evident in the heart, brain, and liver (Chandrasoma and Taylor 2001; Digital World Medical School 2016).

Abnormalities develop in some of the cytoplasmic structures in aged cell. The contractility of myofibrils in muscle cells is decreased. The ability of the nerve cells to synthesize acetylcholine declines with aging. The phagocytic efficiency of macrophages is reduced. Cell surface hormone receptors become abnormal, resulting in disturbances in binding of ligands such as insulin (Digital World Medical School 2016). As such the vital activities that sustain the life process is greatly reduced, and the cells lose their viability and physiological capability.

#### 21.2.1.3 DNA Abnormalities

DNA abnormalities are mainly the result of a progressive failure of cellular DNA repair mechanisms. Failure of DNA repair can potentially affect any cellular function and frequently leads to cell death (Digital World Medical School 2016).

### 21.2.2 Apoptosis

Apoptosis is alternatively referred to as programmed cell death. Apoptosis literally means “a falling away from”. It is a pathway of cellular suicide and is responsible for programmed cell death in several important physiological as well as pathological processes listed below:

(a) Programmed destruction of cells during embryogenesis, as occuring in implantation, organogenesis, and developmental involution
(b) Hormone-dependent physiologic involution, such as involution of the endometrium during the menstrual cycle or the lactating breast after weaning, or pathologic atrophy as in the prostrate after castration
(c) Cell deletions in proliferating populations such as in the intestinal crypt epithelium or cell death in tumors
(d) A variety of mild injurious stimuli (heat, radiation, cytotoxic cancer drugs) that cause irreparable DNA damage that in turn triggers cell suicide pathway (e.g., via the tumor suppressor protein TP53) (Anonymous 2009)

Apoptosis usually involves single cells or clusters that appear on H &E stained sections as round or oval masses with intensely eosinophilic cytoplasm. The nuclear chromatin is condensed, and it aggregates peripherally, under the nuclear membrane, into well-delimited masses of various shapes and sizes. Ultimately karyorrhexis occurs, at a molecular level, reflected in fragmentation of DNA into nucleosome-sized pieces, presumably through activation of endonucleases. The cells rapidly

shrink, form cytoplasmic buds, and fragment into apoptotic bodies composed of membrane-bound vesicles of cytosol and organelles. Fragments are quickly extruded and phagocytosed or degraded.

### 21.2.3 Theories of Aging

Many theories have been proposed to explain the basis of aging. Hayflick (1985) in his review article on "theories of biological aging" classified them as follows:

(a) Organ theories (immune or neuroendocrine)
(b) Physiological theories (free radical, cross-linking, and waste-product accumulation)
(c) Genome-based theories (somatic mutation, error theory, and program theory)

#### 21.2.3.1 Organ Theories

**Immunological Theory**
According to the immunological theory, with aging there is decline in normal immune response accompanied by increased autoimmune manifestation.

**Neuroendocrine Theory**
The neuroendocrine theory of aging is based on the decline in neurons and endocrine cells which are vital to coordinate the activities. Ten percent decrease occurs in total brain weight with age (Brody 1980).

#### 21.2.3.2 Physiological Theories

**The Free Radical Theory**
Since free radicals are highly unstable reactive molecules, they tend to propagate chain reactions during which many stable molecules are converted to free radicals through a process of oxidation. Uncontrolled increases in oxidant concentrations tend to enhance free radical-mediated chain reactions which generally target proteins (Stadtman and Levine 2000), lipids (Rubbo et al. 1994), polysaccharides (Kaur and Halliwell 1994), and DNA (Richter et al. 1988; LeDoux et al. 1999). The indiscriminate damages lead to loss in cellular architecture which culminates in the death of the cells (Harman 1981).

**The Cross-Linkage Theory of Aging**
Bjorksten (1974) claims that free radicals are effective cross-linkers. With aging macromolecules like proteins, DNA, and RNA are linked covalently or by a hydrogen bond between them. Cross-linking of collagen increases viscosity in the extracellular compartment, thereby impairing the flow of nutrients and waste products into and out of cells. Cross-linking of DNA affects its usual function leading to mutation or cell death (Hayflick 1985).

**Waste-Product Accumulation**

Lipofuscin found in lysosomes indicates cellular wear and tear and may impair the cellular function (Hayflick 1985). Lipofuscin is an intralysosomal undegradable polymeric substance, and aged lipofuscin-rich cardiac myocytes become overloaded with damaged mitochondria, leading to increased oxidative stress, apoptotic cell death, and the gradual development of heart failure (Terman et al. 2008).

### 21.2.4 Genome-Based Theory

Genes may be instrumental in determining longevity. The genetic material is constantly exposed to endogenous and exogenous materials that could bring about mutational changes in DNA. The somatic mutation theory is built on the concept that accumulation of a sufficient level of mutations in somatic cells will produce physiological decrements characteristic of aging (Hayflick 1985).

## 21.3 Generation of Free Radicals and Reactive Oxygen and Reactive Nitrogen Species

Living organisms are endowed with the inherent potential to generate energy using molecular oxygen. This inevitably results in the generation of reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) owing to the reactive nature of oxygen. Atoms are most stable in the ground state. An atom is considered to be "ground" when every electron in the outermost shell has a complimentary electron that spins in the opposite direction. The oxygen atom contains unpaired electrons in both the outer and inner shells; however the total number of electrons is even. Molecular oxygen ($O_2$) is a diatomic molecule containing two unpaired electrons in the outer shell. The Lewis structure for oxygen molecule is :Ö::Ö:. Therefore double bond is necessary to satisfy the octet rule for both oxygen atoms, and hence molecular oxygen is not very reactive with the two electrons involved in a chemical bond.

Reactive oxygen species (ROS) refers to a variety of molecules and free radicals (chemical species with one unpaired electron) derived from molecular oxygen. Reactive oxygen species is collectively used in a broad sense to free radicals ($O_2^{\cdot -}$,$OH^{\cdot}$) and non-free radicals ($H_2O_2$, $^1O_2$) of the biological system. Free radicals are thus atoms, molecules, or ions with unpaired electrons. Free radicals are formed from molecules via the breakage of a chemical bond such that each fragment keeps one electron (Halliwell and Gutteridge 2007; Bahorun et al. 2006). These are formed in our body during various physiological and pathological processes.

Oxygen-derived free radicals include hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet -}$), peroxyl ($ROO^{\bullet}$), and lipid peroxyl ($LOO^{\bullet}$) and non-radicals like hydrogen peroxide ($H_2O_2$), ozone ($O_3$), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), and lipid peroxide (LOOH). The non-radical derivatives are generally referred to as oxidants but can easily lead to free radical reactions in living organisms (Genestra 2007). The

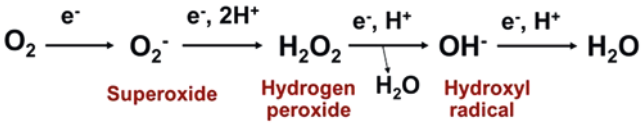


**Fig. 21.1** Generation of free radicals from oxygen

nitrogen-derived free radicals are nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$), and peroxynitrite ($ONOO^{-}$) (Koppenol et al. 1992). Sequential reduction of molecular oxygen leads to the formation of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, and hydroxyl radical as part of normal aerobic process (Fig. 21.1). Free radicals, ROS, and RNS do play a role in physiological function. They are unstable, highly reactive species which possess the ability to oxidize other molecules in an attempt to attain a stable state. In such a process the oxidized molecules become unstable and reactive and continue the oxidation process causing damage to the oxidized molecules.

Free radicals are formed during normal biochemical reaction involving oxygen. Metals containing proteins, as well as other sources of metals, are potent electron-transferring agents. Endogenous free radicals are generated in the biological system during normal cellular metabolism such as mitochondrial electron transport and endoplasmic reticulum oxidation; enzymatic activity including NADPH oxidase, xanthine oxidase, monoamine oxidase, tyrosine hydroxylase, L-amino oxidase, diamine oxidase, glycolate oxidase, alpha-hydroxy acid oxidase, nitric oxide synthase, and L-gulonolactone oxidase; and events like prostaglandin synthesis, autoxidation of adrenaline, activated phagocytic cells, and cytochrome $P_{450}$ activity (Tandon et al. 2005; Bandyopadhyay et al. 1999; Babior 1978; Slater 1984; Sinclair et al. 1991). Energy required for the cellular activities is generated principally in mitochondria by aerobic oxidation whereby molecular oxygen is completely reduced to water. Nearly 3–5% of the daily oxygen utilized is converted to superoxide, hydrogen peroxide, and hydroxyl radicals.

Endogenous free radicals are generated from immune cell activation, inflammation, mental stress, excessive exercise, ischemia, infection, cancer, and aging. Exogenous ROS/RNS results from air and water pollution, cigarette smoke, alcohol, heavy or transition metals (Cd, Hg, Pb, Fe, As), certain drugs (cyclosporine, tacrolimus [an immunosuppressive drug], gentamycin, bleomycin [an antitumor antibiotic]), industrial solvents, cooking (smoked meat, used oil, fat), and radiation (Pham-Huy et al. 2008).

Perez-Campo et al. (1998) have reviewed the relationship between oxidative stress and maximum life span (MLSP) in different vertebrate species. They are of the view that MLSP correlates negatively with the antioxidant status in animals and human beings show the minimum levels of antioxidants.

## 21.4 Harmful Effects of Free Radicals

A free radical is easily formed when a covalent bond between entities is broken and one electron remains with each newly formed atom. When free radicals steal an electron from a surrounding compound or molecule, a new free radical is formed in its place. Newly formed radical then looks to return to its ground state by stealing electrons with antiparallel spins from cellular structures or molecules. Thus the chain reaction continues and can be "thousands of events long" (Karlsson 1997).

### 21.4.1 Peroxidation

Lipid peroxidation refers to the oxidative degradation of lipids. Polyunsaturated fatty acids (PUFAs) are abundant in cell membranes and low-density lipoproteins (LDL). The PUFAs are responsible for the fluidity of cellular membranes which governs its semi-permeability. Free radicals seize electrons often from PUFAs in cell membranes, which results in cell damage via a free radical chain reaction mechanism (Fig. 21.2). In addition, end products of lipid peroxidation may be mutagenic and carcinogenic. The effect of ROS on the carbon-carbon double bond of PUFAs weakens the carbon-hydrogen bond ($CH_2$), letting a hydrogen atom by dissociation and leave behind an unpaired electron on the carbon atom (•CH). The resultant carbon radical is stabilized by molecular rearrangement to produce a conjugated diene, which then can react with an oxygen molecule to produce lipid

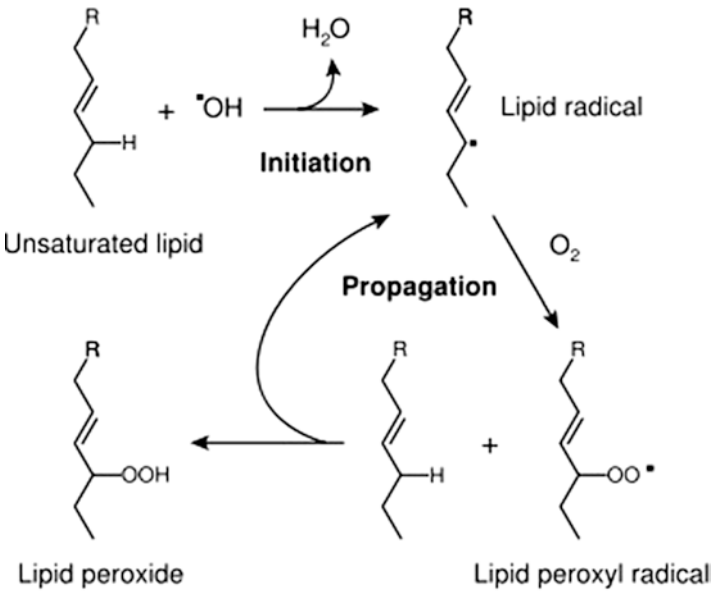


**Fig. 21.2** Lipid peroxidation

hydroperoxide (LOOH) and at the same time propagate lipid peroxidation further. A free radical stealing the single electron from the hydrogen associated with the carbon at the double bond leaves the carbon with an unpaired electron and hence becomes a free radical as well (Halliwell and Gutteridge 1985). When lipid peroxidation occurs in biological membranes, their structure and function are in disarray causing highly damaging consequences to the cell. One of the products of lipid peroxidation, malondialdehyde (MDA), has been extensively measured in a variety of conditions. The MDA concentration is significantly higher in normal elderly people (396.39 ± 43.58 nanomoles/dL), elderly hypertensive (551.16 ± 199.52 nanomoles/dL), elderly diabetic (555.87 ± 88.39 nanomoles/dL), and elderly diabetic hypertensive (749.42 ± 260.6 nanomoles/dL) patients compared to normal young subjects (352.26 ± 67.59 nanomoles/dL) (Akila et al. 2007). Diabetes is usually accompanied by increased oxidative stress which result from overproduction of precursors to reactive oxygen radicals and decreased efficiency of inhibitory and scavenger systems. There is evidence that both free radical production and antioxidant defenses are disturbed in diabetes (Lyons 1991; Ceriello 2000). Evidence has been generated also from our own laboratory that there is significant increase in MDA levels among diabetic patients (2.22 ± 1.58 μmol/L) compared to the controls (1.21 ± 0.6 μmol/L) (Udayangani et al. 2015) and proteinuric diabetic patients had even higher MDA levels of 5.2 ± 3.4 μmol/L (Udayangani 2015).

Richard (1985) studied the peroxide-producing potential of tissues in vitro and observed that human brain and kidney tissue homogenates are found to be most resistant to autoxidation, in agreement with humans having the longest MLSP. Based on his observation, he concluded that longevity of different mammalian species is determined in part by intrinsic differences in tissue peroxidation potential due possibly to unusually high concentrations of antioxidants and other defenses against peroxidation reactions.

### 21.4.2 Oxidation of Nucleic Acid/DNA by ROS

The hydroxyl radical ($OH^{\bullet}$) is the mediator of much of the DNA damage causing strand breaks, which are initiated by abstraction of a deoxyribose hydrogen atom by the hydroxyl radical. ROS forms DNA adduct which is characterized by deletion and mutation and causes genetic effects. The oxidation leads to degradation of bases, single- or double-stranded DNA breaks, purine, pyrimidine or sugar-bound modifications, mutations, deletions or translocations, and cross-linking with proteins. Sugars and base moieties are degraded by ROS and cause oxidation of bases and cross-linking to protein (Sies 1985). The hydroxyl radical ($OH^{\bullet}$) oxidizes guanine to 8-hydroxy-2-deoxyguanosine (8-OHdG), which eventually leads to GC→ TA transversions during subsequent DNA replication (Floyd 1990). DNA alteration has been suggested to be responsible in part in the processes of aging (Fraga et al. 1990).

### 21.4.3 Oxidation of Proteins

The ROS cause oxidation of sulfhydryl groups and modification of amino acids. The proteins may undergo fragmentation, resulting in the loss of their biological activity. ROS can react with several amino acid residues in vitro, generating a wide range of products from modified and less active enzymes to denatured, nonfunctioning proteins (Butterfield et al. 1998; Said and Aiman 2014).

Free radicals are implicated in a number of diseases like diabetes mellitus, cardiovascular diseases, hypertension, atherosclerosis, cancer, and neurodegenerative diseases besides its involvement in aging as summarized by Velavan (2011).

## 21.5 Antioxidants and Their Role in Preserving Cellular Integrity

A biological antioxidant has been defined as any substance that is present at low concentrations compared to an oxidizable substrate and significantly delays or prevents the oxidation of that substrate (Halliwell and Gutteridge 2007). The beneficial effect of antioxidant depends on their ability to work in aqueous and nonaqueous environment in the body. Antioxidants play a vital role in eliminating or keeping the ROS and/or RNS under check. An imbalance between the excessive formation of ROS and/or RNS and limited antioxidant defenses results in "oxidative stress" leading to various deleterious processes. Based on their location, antioxidants could be grouped as:

(a) Plasma antioxidants: ascorbic acid (vitamin C), bilirubin, uric acid, transferrin, ceruloplasmin, β-carotene
(b) Cell membrane antioxidants: α-tocopherol (vitamin E)
(c) Intracellular antioxidants: superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx)

However according to their nature and action, they are grouped into:

(a) Enzymatic antioxidants: SOD, catalase, GPx, glutathione reductase
(b) Nonenzymatic antioxidants:

Nutrient antioxidants: β-carotene, α-tocopherol, ascorbic acid
Metabolic antioxidants: bilirubin, uric acid, ceruloplasmin, ferritin, transferrin, albumin, glutathione

### 21.5.1 Antioxidant Protection by Nutrient Antioxidants

Plants are important contributors of various dietary constituents and nutrients. Among them vitamin E, vitamin C, and carotenoids are considered as natural antioxidants. They are very useful to the body and supplement the action of various endogenous antioxidants to combat the deleterious effects of free radicals. All the dietary nutrients are quite specific in its structure and function.

#### 21.5.1.1 Vitamin E

Vitamin E occurs in the diet as a mixture of several closely related compounds, called tocopherols of which α-tocopherol is the most potent form of vitamin E. Tocopherols are considered as nature's best antioxidants. Because of their lipophilic nature, they are present in circulating lipoproteins, cell membranes, and fat deposits. Vitamin E donates extra electrons to needed unpaired electrons in order to stop the damaging potential of the free radical. As a result, vitamin E is converted to tocopheryl radical which is no longer active. Although free radical damage can't be stopped all together, it can be minimized.

Vitamin E is a fat-soluble antioxidant and is the primary defender against effects of free radicals in the body. It protects components of the cell and their membrane from destruction. It is stored in the liver and fat cells. Vitamin E acting as an electron sink is an efficient lipid-soluble antioxidant that functions as "chain breaker" during lipid peroxidation in cell membranes and various lipid particles including LDL (Packer 1998; Kagan et al. 2002).

#### 21.5.1.2 Vitamin C

Vitamin C alternatively termed as ascorbic acid is a water-soluble and versatile free radical scavenger which gives up electrons very easily when they are needed. It helps to regenerate vitamin E from tocopheryl radical. It has the ability to recycle over and over again. Ascorbic acid upon oxidation is converted to dehydroascorbic acid which could be regenerated by glutathione (Fig. 21.3). Vitamin C is a powerful reducing agent which can directly scavenge superoxide, hydroxyl radicals, singlet oxygen, and $H_2O_2$.

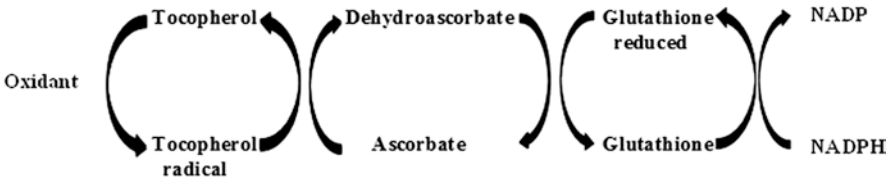


**Fig. 21.3** Interaction of antioxidants in biological system

#### 21.5.1.3 Carotenoids

Carotenoids such as α-, β-, and γ-carotene, lycopene, and lutein function as important antioxidants, and they quench $O_2^{-\cdot}$ and $ROO^{\cdot}$ radicals. Among carotenoids the most potent one is β-carotene.

Synergistic interaction by vitamin C, vitamin E, and carotenoids has shown to prevent lipid peroxidation (Niki et al. 1995).

### 21.5.2 Antioxidant Protection by Enzymatic Antioxidant

#### 21.5.2.1 Superoxide Dismutase

Superoxide dismutase (SOS) is a metalloenzyme present in most of the cells. It converts superoxide radical to $H_2O_2$.

$$2O_2 \bullet^- + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

The three SOD forms are:

(a) Cu-Zn SOD present in the cytoplasm
(b) Mn-SOD found in the mitochondria
(c) Cu-SOD in extracellular SOD

#### 21.5.2.2 Catalase

Catalase is responsible for converting the $H_2O_2$ to harmless water and oxygen. It is found in high concentration in the liver and erythrocytes. The brain, heart, and skeletal muscle contain only low amounts.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

#### 21.5.2.3 Glutathione Peroxidase

Glutathione peroxidase (GPx) is a selenium-dependent enzyme which detoxifies hydrogen peroxide using glutathione. Glutathione (GSH), a tripeptide found in most cells, is oxidized (GSSG) in the reaction catalyzed by glutathione peroxidase and prevents the damage to biomolecules by $H_2O_2$. Glutathione reductase (GR) keeps the glutathione pool in cell in a reduced state using NADPH derived from hexose monophosphate shunt (Fig. 21.3).

$$H_2O_2 + GSH \xrightarrow{\text{GPX}} H_2O + GSSG$$

$$GSSG + NADPH^+ \xrightarrow{\text{GR}} GSH + NADP$$

### 21.5.3 Other Dietary Antioxidants

Zinc, copper, and selenium protect against oxidative stress indirectly serving as cofactors for antioxidant enzymes superoxide dismutase and glutathione peroxidase.

### 21.5.4 Antioxidant Protection by Non-nutrient Antioxidants

Phytochemicals are non-nutrient compounds found in plant-derived foods that have biological activity in the body. They contribute taste, aromas, colors, and other characteristics to food. Polyphenols are the important phytochemicals that can work either indirectly or directly by stopping free radicals from propagating.

The most common group of plant phenolics are flavonoids and nearly 4000 have been identified in plants. They are sometimes referred to as "super antioxidants." In addition to the antioxidant potential, they show antiviral, antiallergic, anti-inflammatory, antithrombogenic, and anticarcinogenic effects. Flavonoids share a common structure (two benzene rings and a central pyran ring) which determines their antioxidant functioning. They are grouped into flavonols, flavanols, flavonones, anthocyanins, isoflavones, and flavones.

Our recent study on soy-incorporated traditional breakfast food items in diabetic individuals revealed that the serum antioxidant capacity measured as the ferric reducing ability of plasma (FRAP) significantly increased from 817.80 ± 176.5 to 1059.75 ± 200.6 μmol/l over an experimental period of 120 days. In the control group of diabetics, the FRAP value was 820.61 ± 147.1 μmol/l at the commencement and decreased to 723.64 ± 101.3 μmol/l during the same experimental period. Soybean is a relatively good source of vitamin E (7.15 μg of tocopherol/g of seeds) (Vasantharuba et al. 2007) and ascorbic acid (37.84 mg/100 g) (Okuwa and Orji 2007). Further the flavonoid content is 3.84 mg/100 g and is relatively higher compared to tannins (0.46 mg/100 g), phenols (0.04 mg/100 g), and saponins (0.17 mg/100 g) (Okuwa and Orji 2007). These antioxidants would have enhanced the total antioxidant capacity.

### 21.5.5 Human and Animal Studies on the Role of Antioxidants in Longevity

With the current understanding from human and animal studies, evidence supports that vitamins A and E may only provide life span benefits when started early in life (Chong-Han 2010).

#### 21.5.5.1 Thai Traditional Formula

A Thai traditional formula has been claimed to prevent and/or cure disease. Based on this assertion, Luanchoy et al. (2014) carried out phytochemical screening tests in the six herbs included in the formula for longevity and documented the presence of phenolic compounds, tannins, and flavonoids in *Cyperus rotundus* and *Albizia*

*procera*; phenolic compounds and flavonoids in *Piper nigrum*, *Diospyros rhodocalyx*, and *Streblus asper*; and phenolic compounds, but neither flavonoids nor tannins, in *Tinospora crispa* extract. Further their investigations revealed only *Albizia procera* possessed highly potent antioxidant activity, although its potency was lower than that of vitamin C and Trolox.

#### 21.5.5.2 Blue Mountains Eye Study

Ava Grace Tan et al. (2008) through a prospective population-based cohort study provided evidence of long-term beneficial association between antioxidants, mainly vitamin C (either alone or in combination with other antioxidants), and nuclear cataract development, a well-known biological marker of aging. Their findings that subjects in the highest quintile of total intake of vitamin C contributed by diet and supplements had a reduced risk of incident nuclear cataract is a convincing evidence for the protective role of vitamin C. Furthermore, intake of vitamins C and E, β-carotene, and zinc in combination above median value was also associated with a reduced risk of incident nuclear cataract. The reason for the damage to crystalline proteins, lens fiber membranes, and lipids resulting in lens opacities is due to the oxidative consequence of superoxides and hydroperoxides (Boscia et al. 2000).

#### 21.5.5.3 Oyster Mushroom Supplements

Sánchez et al. (2015) studied the effect of selected oyster mushroom supplements on the longevity of the Mexican fruit fly, *Anastrepha ludens*. They reported that *Pleurotus djamor* ECS-0142 strains with the highest antioxidant capacity when supplemented at 1% level showed slightly but significantly greater survival than those on the control diet. However 5–20% concentrations of mushrooms in the diet resulted in a decrease in life expectancy.

#### 21.5.5.4 Mediterranean Diet

The traditional Mediterranean diet is long known for its health-preserving ability. The traditional Mediterranean diet is built on health-promoting characteristic such as high consumption of legumes, cereals, fruits, and vegetables, moderate consumption of milk and dairy products and ethanol and intake of fat with high monounsaturated to saturated ratio, as well as low consumption of meat and meat products (Trichopoulou et al. 1995). Based on the fact that the Mediterranean diet contributes a significant amount of flavonoids through fruits, vegetables, and beverages and polyphenolic compounds from olive oil, Trichopoulou and Vasilopoulou (2000) concluded that the diet has considerable antioxidant properties. Even though they claimed that there is no direct evidence that the antioxidants are central to the benefits of the Mediterranean diet, based on epidemiological data they suggested that there is indirect evidence to indicate antioxidants may play a major role.

#### 21.5.5.5 Antioxidant Profiles in Italian Centenarians

Plasma levels of vitamin C, uric acid, vitamin A, and vitamin E and activities of SOD and GPx were estimated in healthy subjects of different age groups. In subjects

≤60 years, 61–80 years, 81–99 years, and ≥100 years, the vitamin C (μM) concentrations were, respectively, 49.5 ± 14.5, 49.8 ± 15.8, 35.7 ± 9.8, and 29.6 ± 4.5. Subjects who were 81 years or more had significantly lower vitamin C concentration than the subjects in other age groups (Polidori et al. 2007). Similarly the uric acid concentrations were significantly lower in 81–99 years (243.2 ± 64.2 μM) and ≥100 years (218.6 ± 57.4 μM) old subjects than subjects in the age categories ≤60 years (324.1 ± 88.0 μM) and 61–80 years (293.7 ± 79.9 μM). However, plasma levels of the fat-soluble vitamins A (3.5 ± 1.8 μM in centenarians higher than other age groups) and E (49.9 ± 8.3 μM in centenarians higher than other age groups) were significantly higher in centenarians compared to younger groups. This study concluded that in Italian population, elevated levels of plasma vitamins A and E seem to be important for longevity.

#### 21.5.5.6 Green Tea

Consumption of green tea has caught the attention of many. The tea plant *Camellia sinensis* upon processing yields a variety of white, green, and black tea. *C. sinensis* is grown in many Asian countries. The health benefits of green tea is due to flavonoids, mainly catechins, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). Epigallocatechin gallate is an efficient antioxidant, and its concentration in green tea is higher than black tea (Cheng 2000). The concentration of total polyphenols in dried green tea leaves vary from 8% to 12% (Min and Peigen 1991).

Apart from its antioxidant effect, green tea lowers total cholesterol level as well as improves the ratio of LDL cholesterol to HDL cholesterol (Cheng 2006).

Maurya and Rizvi (2008) reported that the tea catechins have strong antiaging activity; hence consumption of green tea, which is rich in catechins, may delay the onset of aging.

#### 21.5.5.7 Resveratrol

Resveratrol, a polyphenol found in grape, is claimed as an antiaging agent. Resveratrol is a calorie-restriction mimetic agent (Barger et al. 2008). Sirtuins are $NAD^+$ dependent histone/protein deacetylases which are target for resveratrol. Seven sirtuins (SIRT) have been reported in mammals, of which SIRT-1 via its deacetylase activity mediates the beneficial effects on health and longevity of resveratrol (Markus and Morris 2008). Resveratrol, abundantly present in wine, scavenges $O_2^-$ and $OH^\bullet$ in vitro, as well as lipid hydroperoxyl free radicals.

## 21.6 Natural Sources of Nutrients and Phytochemicals with Antioxidant Capacity

### 21.6.1 Vitamin C

Vitamin C is widely distributed in fresh fruits and vegetables. Some rich sources of it are presented in Table 21.1.

**Table 21.1** Dietary sources of vitamin C

| Sources | Amount of vitamin C (mg/100 g food) |
|---|---|
| Rosehip | 2000 |
| Kiwifruit | 90 |
| Broccoli | 90 |
| Orange juice (3/4 cup) | 75 |
| Strawberry | 50 |
| Orange | 50 |
| Lemon | 40 |
| 1 potato | 25 |
| Banana | 9 |
| Apple | 6 |

### 21.6.2 Vitamin E

Vitamin E in nature is found in dietary articles that are rich in polyunsaturated fat. Hence vegetable oils like sunflower, soybean, and safflower oils are among the best sources of vitamin E. Tocols is the name designated to tocopherols and tocotrienols. Tocopherols exist as four homolog forms, alpha, beta, delta, and gamma, and alpha-tocopherol is the major tocopherol in many edible oils. By far the richest source of vitamin E is wheat germ oil. Oils extracted from cereals like rice bran and corn also provide some vitamin E. Almonds among the nuts are considered as a good source of vitamin E, and peanuts, hazelnuts, and sunflower seeds contain considerable amount of vitamin E. The daily vitamin E requirement is 15 mg, and one tablespoon of wheat germ oil provides 20.3 mg, which is more than the recommended daily allowance. The US Department of Agriculture's nutrient database indicates that wheat germ contains 4.53 mg of vitamin E in 28 g. Germination of soybean seed for 48 h increases the vitamin E content from $7.15 \pm 0.34$ μg/g of seed to $12.63 \pm 0.54$ μg/g of seed (Vasantharuba et al. 2007).

### 21.6.3 Carotenoids

The plant kingdom contributes to our carotenoid requirements. The carotenoid family has many types, but the most common ones are α-carotene, β-carotene, beta-cryptoxanthin, lutein, zeaxanthin, and lycopene. The color of the fruits and vegetables is due to their carotenoid content, and hence they are considered as good sources. Dark leafy green vegetables like spinach, broccoli, and leaf cabbage (kale) are good sources of carotenoids, and those grown in Sri Lanka such as *Alternanthera sessilis*, *Sesbania grandiflora*, and *Centella asiatica* are also good sources. Values for α-carotene, β-carotene, lutein + zeaxanthin, lycopene, and β-cryptoxanthin from approximately 200 references were evaluated and reported by Holden et al. (1999).

### 21.6.4 Phytochemicals

The naturally occurring chemical compounds found in plants which provide health benefits for humans further than those attributed to macronutrients and micronutrients are grouped as phytochemicals (Hasler and Blumberg 1999). The foods containing considerable amount of such phytochemicals are also referred to as functional foods and are being extensively studied. Among the phytochemicals, polyphenols are recognized as having health-promoting roles. Based on the structure, polyphenols are divided into (a) simple phenolic acids, e.g., ferulic, caffeic, p-coumaric, vanillic, gallic, ellagic, p-hydroxybenzoic, and chlorogenic acids; (b) stilbenes, e.g., resveratrol; (c) curcuminoids, e.g., curcumin; (d) chalcones, e.g., phlorizin and naringenin chalcone; (e) lignans, e.g., matairesinol and secoisolariciresinol; and (f) flavonoids (Bravo 1998; Harborne and Baxter 1999; Williams et al. 2004).

The flavonoids are composed of seven subclasses, namely:

(a) Flavonols, e.g., quercetin [in apples and onions]
(b) Flavanols as monomeric, e.g., catechin [in red wine, grapes, and black tea] and epicatechin [in cocoa and chocolate], oligomeric, and polymeric compounds, e.g., proanthocyanidins, also called condensed tannins [in apple, grape seed, and cocoa]
(c) Anthocyanins, e.g., cyanidin [black berries]
(d) Flavones, e.g., luteolin [in tea, fruits, and vegetables] and apigenin
(e) Flavanones, e.g., naringenin [citrus fruits, tomato] and hesperidin [in orange juice]
(f) Flavanonols, e.g., taxifolin [in red onion]
(g) Isoflavones, e.g., genistein [in soya] and daidzein [in soymilk]

Dietary phytochemicals have been known to possess anti-obesity potential, and their mechanisms of action have been reviewed (González-Castejón and Rodriguez-Casado 2011).

The phytochemicals present in foods have been reported to possess some possible health benefits. Isoflavones can reduce blood pressure and increase blood vessel dilation; anthocyanins also cause blood vessel dilation and improve insulin sensitivity; proanthocyanidins inhibit LDL oxidation and inflammation; catechins and epicatechins through vasodilation improve blood flow to the brain, in addition to improving insulin sensitivity (Heneman and Zidenberg-Cherr 2008).

## 21.7 Conclusion

Aging, an inevitable process, is the result of free radical-mediated damage to cellular fabric. Antioxidants provide relief from oxidant stress. Natural antioxidants present in the diet that we consume contribute to the body's antioxidant defense system along with the endogenous antioxidants. As age advances, the antioxidant protection diminishes when dietary intake dwindles. There are convincing

evidences from a number of studies to indicate that aging could be slowed down by antioxidants giving the hope that life can be prolonged if adequate antioxidant capacity of the body is maintained. Thus dietary antioxidants could prove its value for an aging population. Fruits, vegetables, and other plant-based foods are rich in bioactive phytochemicals with antioxidant potential, especially the phenolics, that may provide desirable health benefits beyond basic nutrition to reduce the risk of the development of chronic diseases due to oxidant stress. There are many more effects of antioxidants which are not covered in this chapter.

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# Interventions to Prevent Premature Aging After Traumatic Brain Injury

22

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**Abstract**

Worldwide, more than 10 million people suffer from traumatic brain injury (TBI). A TBI is a time-dependent series of events starting with a traumatic insult damaging the tissues and then followed by secondary damage to the tissue including neuronal excitotoxicity, neural inflammatory changes, and apoptosis. Long-term physiologic effects of TBI include accelerated aging through volume decrease and variability in synapsis plasticity. There are several immune- and receptor-modulating therapies hypothesized to prevent the aging effects of TBI. This chapter will focus on the aging effects of TBI and therapies to prevent this acceleration of aging.

**Keywords**

Aging · Brain · Cognitive decline · Concussion · Traumatic brain injury

## 22.1 Traumatic Brain Injury

This chapter will introduce brain injury and discuss the role TBI has in aging and the antiaging therapies available to treat TBI-associated aging.

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Different types and severities of TBI accelerate aging through different physiologic pathways. Rapid acceleration and deceleration of the brain may lead to a complex cascade of neuronal damage, in which neuronal cell membranes are disrupted due to mechanoporation of lipid membranes and axons are compressed or stretched in a relatively short period of time (Barkhoudarian et al. 2011). As a result, ion channels lose their ability to regulate ion flow, and a nonspecific flux of ions into and out of the neuron is common, causing rampant depolarization and subsequent action potential throughout the brain. This leads to an inefficient and indiscriminate release of neurotransmitters and excitatory amino acids (Giza and Hovda 2014). After the primary TBI (closed head or penetrating) has occurred, a secondary phase of injury can occur indirectly hours to days afterward. The secondary injuries often cause the majority of structural and functional damage. Cerebral edema, altered metabolism, inflammation, oxidative stress, excitotoxicity, and altered cerebral blood flow occur and can trigger inflammation, necrosis, and apoptosis (Winkler et al. 2016).

## 22.2 Physiology of Cerebral Aging

Advancing age is associated with multiple changes in the brain that can be observed at the macroscopic and microscopic levels. White matter (WM) volume loss and myelin integrity deterioration begin after the fourth decade of life and are more pronounced in prefrontal areas of the brain (Harada et al. 2013). The WM volume loss is likely due to multiple mechanisms including demyelination and shrinkage of neurons (Bennett et al. 2017). Gray matter (GM) volume, in contrast, follows a linear pattern of decline starting at approximately at age twenty (Salat 2011).

Neurotransmitter system changes also occur as part of normal aging. The dopaminergic system activity declines in older adults, while total dopamine synthesis does not. The serotonergic system degrades with normal aging. The level of serotonin, serotonin transporters, and serotonin receptor binding declines. The decline in serotonergic signaling capability may be related to a decrease in neuronal plasticity and likely predisposes the elderly to depression, neurodegenerative disease, and other pathologies (Peters 2006).

## 22.3 Effects of Traumatic Brain Injury on Aging

The negative effects of TBI are closely related to normal cerebral age-related changes. Loss of brain volume and myelin density occurs at accelerated rates in TBI victims. Studies have linked worsening severity of injuries with worse rates of atrophy and volume loss. Loss of WM was found by Klein et al. (1996) to be the most observed impact of TBI on aging through changes in information processing and fluid intelligence, which significantly impact higher executive functions as well, demonstrating accelerated aging. TBI accelerates aging through loss of effective remyelination as well (Franklin et al. 2002). TBI also significantly alters the normal aging trajectory of neuroendocrine dysfunction through chronic hormonal and

immunologic changes (Lieberman et al. 2001). TBI-related microglial upregulation leads to oxidative stress and free radical production. Decreased serotonin and dopamine receptor binding are also examples of neuromediators linked to cognitive decline in aging associated with TBI (Volkow et al. 2000).

## 22.4 Therapies to Prevent the Aging Effect of Traumatic Brain Injury

Although there exist many guidelines for the management of TBI, there is very little evidence to support any one standard therapy to alleviate the detrimental premature aging effects of TBI that may occur in the years following injury (Margulies and Hicks 2009). The short- to intermediate-term treatment of TBI depends primarily on the development of secondary injury. Rehabilitative care and physical therapy can be used to help restore altered functional ability such as decreased movement, balance, and postural control, as well as aid in the reduction of post-concussive symptoms like headache and confusion (Hugentobler et al. 2015).

In the long term, TBI has been shown to induce many different age-related cognitive effects on the injured, such as diminished memory, attentional capacity, concentration, and decision-making skills, as well general disorientation (Moretti et al. 2012). Prevention and treatment of these long-term cognitive aging effects of TBI will vary based on the individual injury, with traditional therapies focusing on increasing cognitive reserve. Cognitive training programs have been shown to increase neuroplasticity in the areas of the brain that are affected after TBI. Another strategy aimed at improving long-term cognitive function after TBI is minimizing the factors that often worsen cognitive decline such as alcohol, drug abuse, and psychiatric disorders including depression and social isolation.

While pharmacological treatments have been shown to have some success in animal models, at this time there are no pharmacological treatment options available that have demonstrated clear clinical efficacy in treating TBI patients. The difficulty in translating lab-based results to preventing TBI-related premature aging effects most likely varies from experiment to experiment. Most injury models utilized in the laboratory setting induce homogenous injury patterns and severity when in reality TBI is quite heterogeneous. There is also a lack of understanding of pharmacokinetics and pharmacodynamics of the compounds tested. Dose and regiments vary from trial to trial and often are administered close to the time of injury which often is impossible in a clinical scenario. Trial results often focus on a specific outcome marker which can represent only a small part of the injury cascade in TBI injuries thus not performing as well in the complicated, multifactorial TBI progression in a patient (Wei and Xiao 2013). Clinical outcomes are often insufficiently sensitive endpoints to detect clinical significant effects (Howard et al. 2017).

To this end, most research indicates that no single pharmacological medication will provide the needed treatment to counteract the aging effects of TBI but that instead different combinations of therapies should be used to address multiple aspects of neuroprotection, neuro-inflammation, and regeneration (Marklund and Hillered 2011).

Numerous compounds, hormones, and growth factors have been shown to have neuroprotective effects with potential value in the treatment of TBI-associated premature cognitive decline and dementia. A brief discussion of the most likely candidates for success in TBI is included below. These include medications, growth factors, and some non-pharmacological therapies.

## 22.5 Progesterone

Progesterone is an important steroid that plays a role in the female monthly menstrual cycle as well as conception and early pregnancy. However, progesterone also has a role in both genders as a neurosteroid. The original interest in this agent being studied for potential treatment in TBI was the noted gender-specific outcomes for patients with TBI. Recovery and long-term outcomes were significantly better in females. Researchers have demonstrated that the hormone improves functional outcome after blunt TBI by mitigating cerebral parenchyma damage. It inhibits the Acetylcholine receptor and stimulates synthesis of myelin proteins. Rat studies showed that systemic application of the neurosteroid still resulted in mitigation of the meningeal plasma extravasation (Limmroth et al. 1996). Progesterone is thought to promote blood-brain barrier regrowth and repair, decrease the deregulated inflammatory cycle, and decrease apoptotic cell death (Guo et al. 2006). The benefits of progesterone treatment have been noted to be decreased in the setting of vitamin D deficiency (Wei and Xiao 2013).

While progesterone is thought to have more chance at success than other more targeted medications because of its pleiotropic effects and observed neuroprotective effects in a multitude of injury models, most Phase III trials have demonstrated failure of progesterone to be neuroprotective (Stein 2011). ProTECT III trial was a blinded study where patients with TBI were randomized to receive progesterone or placebo, enrolling patients from 49 different TBI centers. The primary outcome was a favorable outcome as per the Glasgow Outcome Scale-Extended and was stopped early for futility after 882 patients were enrolled (Howard et al. 2017). The SyNAPSe trial was also a blinded and randomized Phase III trial which found no change in Glasgow Outcome Scale or mortality (Skolnick et al. 2014).

While some scientists have determined progesterone to be a failed therapy for antiaging related to TBI, there are others who believe that the recent clinical trials were faulty and cannot truly determine the efficacy of the drug. Specifically, Howard et al. (2017) discuss design weaknesses of the recent Phase III trials including suboptimal dosing and treatment durations and recommend returning to Phase IIB testing. A Cochrane review in 2016 only included 5 randomized clinical trials with a total of 2392 subjects. Only one of the trials was considered to be at low risk of bias. The conclusion of the review was that there was no evidence that supported progesterone as improving mortality or neurologic function. The review also concluded that the patient populations were extremely homogeneous and this could affect their ability to apply the results to such a general population (Ma et al. 2016).

## 22.6 Vitamin D

Vitamin D can be considered a neural steroid as it is both synthesized and acts on the central nervous system. Patients with deficiency in vitamin D show elevated levels of inflammatory cytokines such as IL-6 and TNFa. Vitamin D deficiency is associated with many age-related systemic disorders such as hypertension, atherosclerosis, and cancer. There is also a strong association with functional declines in the elderly. Minimizing these effects after TBI is hypothesized to decrease inflammatory cytokines and therefore inflammation in the brain after injury (Cekic and Stein 2010).

A review of the literature revealed that vitamin D deficiencies are associated with worse inflammatory responses and combination therapy with progesterone looks promising, but evidence does not support a recommendation to supplement TBI patients to definitively prevent the aging effects of TBI (Lawrence and Sharma 2016). Some scientists suggest that vitamin D supplementation should be a part of a holistic approach including a cadre of nutritional supplements and this is associated with lower mortality rates. They speculate that a well-rounded nutritional panel including vitamin D supplementation would lessen the cognitive decline associated with TBI.

Hua et al. (2012) showed that vitamin D combined with progesterone enhanced memory sparing in rats following TBI. Elderly patients who experience TBI may have an even more pronounced acceleration of aging and have been identified as a population potentially benefiting from vitamin D treatment more than others. Elderly patients treated with both progesterone and vitamin D had faster and more significant recovery of cognitive function with a stalling of the accelerated aging findings associated with TBI (Stein and Cekic 2011).

## 22.7 N-Acetyl Cysteine (NAC)

NAC is another medication that is well-known to be safe and well tolerated by patients. It is used for cystic fibrosis, acetaminophen toxicity, and renal protection prior to high-contrast dye loads during complicated CT scans. NAC has both neuroprotective and antioxidant properties. This is partly due to increased levels of brain glutathione. Glutathione is a critical intracellular free radical scavenger. NAC has been demonstrated in a double-blind placebo-controlled study to reduce the effects of mild TBI after blast injury in the military (Hoffer et al. 2017). Eakin et al. (2014) used two different injury models and two different species to demonstrate that early NAC administration reversed behavioral deficits thought to be related to cognitive decline. Servicemen and women exposed to significant ordnance blast and met criteria for mTBI received either NAC or placebo for 7 days. Eighty-one participants were evaluated for multiple symptoms including neurocognitive dysfunction and were three times more likely to be symptom-free at 7 days if they had gotten NAC (Hoffer et al. 2013). Hicdonmez et al. (2006)

showed through autopsy of closed head injury of rats that a single dose of NAC showed significantly less trauma-induced oxidative brain damage.

## 22.8 Phenserine

Phenserine was developed initially as potential treatment for Alzheimer's dementia (AD). It was discovered that phenserine and three active metabolites readily cross the BBB and produce many benefits that may mitigate the early aging process in both TBI and AD. The benefits appear to be multifactorial. Phenserine has anti-inflammatory properties stimulating immune activation of mononuclear cells which causes them to produce anti-inflammatory cytokines. Phenserine also suppresses glutamate-induced excitotoxicity. Glutamate is an excitatory neurotransmitter that ultimately allows influx of Ca2+ and water into cells which then leads to cytotoxicity. Phenserine has also been shown to improve outcome when cells are exposed to oxidative stress and also shown anti-apoptotic properties in cells. Phenserine protects against oxidative stress by upregulating endogenous antioxidant proteins. Phenserine has also demonstrated its ability to lower production of amyloid precursor protein in cells which is high in damaged neurons post-TBI. Suppression of the production of amyloid precursor protein has been shown to improve recovery of cells from TBI by reducing neuronal cell loss, reducing astrocyte production which leads to reducing the size of the area affected by TBI, and reducing behavioral impairments. Another way in which phenserine may protect against premature aging and recovery from TBI is through stimulation of neurogenesis. Neurogenesis, or the development of new neural stem cells, is enhanced by the presence of phenserine. It has also been shown to increase neural cell viability and encourage the development of neural stem cells into the neuronal cell type as opposed to the glial cell type. Phenserine combats the action of amyloid precursor protein on this differentiation (Hoffer et al. 2017).

Treatment of mTBI in animal models reversed injury-induced AD pathways. Researchers cannot suggest the use of this medication as a treatment at this time, but positive animal research is encouraging (Lian et al. 2012).

## 22.9 Erythropoietin (EPO)

Erythropoietin (EPO) is a growth factor for hematopoietic cells that is produced in the kidney. EPO stimulates the maturation of red blood cells and is approved for treatment of anemia. EPO is also produced in the brain in response to injury and has shown neuroprotective effects in animal models. Zhou et al. (2017) showed decreased edema in EPO-treated rats compared to saline-treated rats after induced TBI. In humans, it has been shown to decrease mortality from TBI, but there was a concurrent increase in the risk of venous thromboembolism. EPO reduces the inflammatory response of cells and decreases cytokine production. It also increases cerebral blood flow and decreases cerebral vasospasm (Margulies and Hicks 2009;

Diaz-Arrastia et al. 2014). In contrast, Hellewell et al. (2018) showed no reduction in inflammatory brain markers in moderate-to-severe TBI patients with EPO administration within 24 h of injury.

## 22.10 Minocycline

Minocycline is in the tetracycline family of antibiotic agents. It has been shown to readily cross the BBB. It has anti-inflammatory, anti-apoptotic, and antioxidant activity. Although these effects require dosing higher than standard antibiotic therapy doses, it also has the benefit of preventing infection in patients post-TBI (Diaz-Arrastia et al. 2014). Kovesdi et al. (2012) conducted a comprehensive animal study testing multiple neurogenic outcomes after mTBI induction and found that minocycline-mitigated neurobehavioral abnormalities are associated with premature aging. More research is needed to study potential uses of this antibiotic in reversal of TBI-related aging.

## 22.11 Cyclosporine

Cyclosporine's mechanism of action includes maintaining the mitochondrial membrane and preserving mitochondrial function. This allows the mitochondria to continue to function in cell metabolism including processing free radicals. It also exhibits improvement in axonal injury as well as learning and memory. Its immunosuppressive effects may also be beneficial in traumatic brain injury. Cognitive performance has been shown to be improved after cyclosporine administration in both TBI and stroke patients (Margulies and Hicks 2009; Diaz-Arrastia et al. 2014). While some researchers continue to explore cyclosporine, Dixon et al. (2016) report findings of the operation brain trauma therapy trial and found no benefit, as well as a difficult narrow therapeutic window to translate into successful clinical treatments.

## 22.12 Statins

Statins are prescription medications used to decrease serum cholesterol but have been shown to have potential benefits with brain-injured patients. The mechanism appears to be decrease in brain edema secondary to helping maintain the integrity of the BBB as well as increasing cerebral blood flow while decreasing inflammation and cell death (Diaz-Arrastia et al. 2014; Margulies and Hicks 2009). Specifically statins have been associated with preservation of the hippocampus by several studies which mediate premature aging (Lim et al. 2017). Clinical researchers have had difficulty translating this success into practice.

Sanchez-Aguilar et al. (2013) enrolled 36 patients with moderate-to-severe TBI to receive rosuvastatin or placebo for 10 days. The treatment arm was associated

with better functional outcomes at 3 and 6 months and inflammatory markers. Although this is a small number of patients, results suggested antiaging benefits of statins in the subacute injury phase of moderate-to-severe TBI.

In a retrospective review, researchers compared mortality and morbidity of TBI patients getting chronic statin therapy who either had therapy continued within 48 h or had their statin's discontinued. Patients who had their statin therapy discontinued had four times higher mortality rate. This study demonstrates significant bias as it was not randomized and had a small sample size. While this study cannot imply statins as neuroprotective and able to prevent aging, it was hypothesis forming and warranted further investigation (Orlando et al. 2013). Following this study, a retrospective observational cohort study published in 2017 was conducted where 397 older adult patients were studied. The patients who had statins discontinued in the hospital tended to have more severe head injuries by GCA. After adjusting for these score differences, there was no difference in cognitive decline or other markers. A study randomizing mTBI patients to receive atorvastatin or placebo was unable to demonstrate a difference in neurologic protection or recovery (Robertson et al. 2017).

## 22.13 Glibenclamide

Glibenclamide is a second-generation sulfonylurea marketed under the brand name glyburide that is used for the treatment of type II diabetes. It was noted that stroke patients who were on glibenclamide for type II diabetes at the time of their stroke exhibited better recovery, even if the initial stroke symptoms were similar. Glibenclamide's primary action is at the rather newly discovered Sur1-Trpm4 ion channel and via brain Katp channels. It appears to act as a neuroprotective agent by decreasing vascular permeability and therefore cerebral edema (Kurland et al. 2013). It also has been shown to decrease hemorrhagic conversion of ischemic strokes. Glibenclamide reduced the effects of hippocampal injury and showed improved outcomes on cognitive testing in both ischemic and hemorrhagic strokes and as well in TBI. Glibenclamide also acts by promoting neurogenesis and has anti-inflammatory as well as anti-apoptotic properties all mediated by its inhibition of Sur1 (Diaz-Arrastia et al. 2014).

## 22.14 Hypertonic Saline

Hypertonic saline increases plasma volume and mobilizes water across the BBB by dehydration of the vascular endothelial cells. In the setting of intracranial hemorrhage, this can serve to decrease intracranial pressure and increase cerebral perfusion pressure. Hypertonic saline when given in bolus treatment can reduce ICP, but there will be a rebound increase when the effects wane (Asehnoune et al. 2017). There is now evidence to support hypertonic saline infusion, termed continuous hyperosmolar therapy, in the setting of ICH and TBI in general. Increasing blood plasma volume can also increase organ blood flow and create a positive inotropic

effect. It should be considered as an adjunct therapy in the prevention of TBI-related morbidities (Margulies and Hicks 2009).

## 22.15 Hypothermia

Hypothermia can reduce the neurologic sequelae of TBI. It is currently used as standard protocol after cardiac arrest to prevent neurologic sequelae. These effects are mediated by decreasing brain metabolism after injury as well as reducing axonal loss and microvascular dysfunction which often leads to the development of cerebral edema. Therapeutic hypothermia may prolong the window for subsequent therapies to be administered. When used in combination with other therapies, such as free radical scavengers, it shows promise as an additional treatment for TBI (Margulies and Hicks 2009).

## 22.16 Conclusion

TBI encompasses a varied set of injuries and leads to accelerated cerebral aging through structural, neurotransmitter, and cognitive alterations. While several therapies to prevent aging related to TBI have shown promise in Phase II trials, translation to the clinical use has been difficult.

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# Current Approaches of Anti-inflammatory-Dependent Antiaging Strategies

23

Hafize Uzun

**Abstract**

Inflammation plays a role in the etiology of neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases, which have the highest mortality rates. It has been argued that the lifetime of macromolecular damage caused by chronic inflammation can be the cause of aging process. There are studies indicating that aging is also closely related with low-grade chronic inflammation, defined as sterile inflammation. Another definition of sterile inflammation is pathogen-free inflammation resulting from various environmental conditions such as mechanical trauma, ischemia, stress, or ultraviolet radiation. The combined effects of inflammation on genome, epigenome, mitochondria, a variety of intracellular structures, and cellular membranes need to be defied for diverse areas of eventual antiaging interventions. These interventions include specific exercise programs, caloric restriction, anti-inflammatory diets and nutritional supplements, and specific cell-based therapies including platelet-rich plasma (PRP) and stem cells as both mitigating agents in the aging process. Inflammation suppression is one of the most important factors for healthy longevity. This chapter will focus on the current approaches of anti-inflammatory-dependent antiaging strategies.

**Keywords**

Antiaging strategies · Low-grade inflammation · Healthy longevity · Anti-inflammatory diet · Stem cells

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## 23.1 Aging

Aging is known as a complicated process, affected by both genetic and epigenetic modifications (epigenetics/epigenomics), mitochondrial damage, immunosenescence, endocrinosenescence, microbiota, oxidative stress, stochasticity, and environmental factors. Aging affects whole-body systems such as cells, tissues, and organs. It may eventually cause severe tissue deterioration. It is unfortunately not possible to explain aging with a single mechanism or theory, because aging involves multiple processes. These processes are interpenetrating with inflammatory responses. Currently, there is a question that must be resolved: Is inflammation a cause of aging or an effect or both?

## 23.2 Inflammation

Under normal circumstances, while a small amount of inflammation protects the body against various diseases and impasses, chronic inflammation can be the cause of various diseases in the body. Figure 23.1 shows the effects of inflammation in aging and some targets for increased longevity.

## 23.3 Mechanisms of Inflammation in Aging

Aging is related with modifications in the immune system which are collectively designated as "immunosenescence" (Fulop et al. 2013). Long-term inflammation is both a result and a cause of immunosenescence. There is a wide knowledge about the impact of aging on cellular components in innate immune system (macrophages, polymorphonuclear leukocytes (PNL), natural killer cells (NK), and dendritic cells) and extracellular components composed of recognition molecules (CRP, serum amyloid protein, mannose-binding protein). Thymic involution, increased serum pro-inflammatory markers, blunted T cell proliferation, and shorter telomere lengths are related with chronic stress. These factors are involved in inflammation associated with aging or in particular in a number of age-related and high-mortality etiologies and, so, related to "inflammaging" (Bauer et al. 2015).

Inflammaging is associated with metabolic pathways. Integration of inflammation and metabolism happens at various times and space scales. If low-grade inflammation arises as a result of metabolic dysfunction due to overnutrition, this is termed "metaflammation." Metaflammation is associated with reduced metabolic rate.

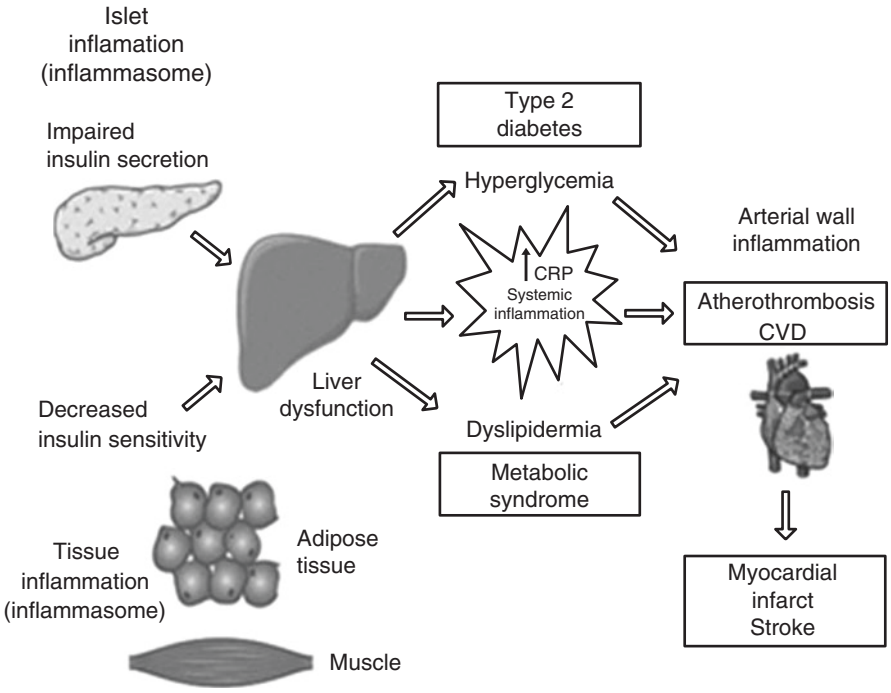


**Fig. 23.1** Role of inflammation in various target organs playing a key role in the pathophysiology of type 2 diabetes and its associated metabolic abnormalities, leading to an increased risk of cardiovascular disease. *CRP* C-reactive protein, *CVD* cardiovascular diseases

### 23.3.1 DAMPs

Sterile inflammation is a type of inflammation without pathogen and with a large number of sterile stimuli, including environmental conditions such as mechanical trauma, ischemia, stress, or ultraviolet radiation. Aging is also related with sterile inflammation (Rock et al. 2010). As a result of such damage-related stimuli, molecular agents called collective damage-associated molecular patterns (DAMPs) are secreted. These DAMPs promote to the formation of this kind of inflammatory response by stimulating innate immune effectors. DMAPs can be found intracellularly or extracellularly. They recognize and function by both pattern recognition receptors (PRRs) and non-PRRs on both immune and nonimmune cells. Among the factors strongly contributing to the aging process are free reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are generated by oxidative stressed cells that cause oxidative damage to biomolecules. These particular oxidative processes stimulate sterile inflammation by releasing DAMPs and interact these with PRRs like TLRs and NLRP3.

### 23.3.2 Genetic and Epigenetic Modifications

It is known that genes participated in the regulation of inflammation contribute to the genetic basis of aging. Several etiological factors are probably able to contribute to elevated low-grade inflammatory activity in tissues such as adipose, muscle, skin tissues and organs such as the brain, liver, lung, pancreas, eye, sexual organs, immune system, and gut microbiota (Cevenini et al. 2010).

Epigenetics is associated with the phenotype of gene expression control without modification of DNA sequences. DNA methylation, chromatin modifications (acetylation, methylation, and phosphorylation of histone proteins), and posttranscriptional modifications have been reported as the key epigenetic modifications in the regulation of inflammatory genes (Medzhitov and Horng 2009). This dynamic epigenetic layer regulates with environmental factors the expression of genes associated with disease states. Exposure to long-term toxins, infections, or environmental factors causes epigenetic changes leading to the formation of age-related diseases. Inflammation accompanied by metabolic dysfunction in aging may also have a share in these changes.

### 23.3.3 MicroRNA

The single-chain, noncoding functional short RNA (21–23 nucleotide) molecules, responsible for the regulation of many biological activities, are called microRNA (miRNA). Depending on age, it is known that the changes which occur in the transcription are controlled by miRNA. A model of a group of miRNAs called geromiRNAs has been demonstrated in organisms that govern the longevity. Studies in the human genome have specially focused on miR-146, miR155, miR-126, miR-21, and miR-181a-5p, associated with inflammation and senescence. While miR-181a exhibits the same relationship with the anti-inflammatory cytokines, it has an opposite relationship with the pro-inflammatory cytokines.

Such miRNAs play a key function in the interaction between DNA damage response (DDR), cell senescence, and inflammation. It is thought that the determination of miRNA functions is promising in the aging process and the way to delay the condition of ARD (Olivieri et al. 2015).

### 23.3.4 Redox Stress

Oxidative stress describes the corruption in favor of prooxidants of prooxidant-antioxidant balance in tissues and the body. ROS, which occurs in the aging process and increases, is a cause and a consequence of "redox stress." Severe oxidative stress or damage associated with a highly elevated level of ROS/RNS production will inevitably impair the cells' self-repair ability and thus can lead to cell death. Moderate levels of oxidative stress, i.e., positive oxidative stress, induced by a moderate level of ROS/RNS, can be triggered by several stressors to protect

against further lethal challenges that otherwise would cause cell death and tissue injury (Sohal and Orr 2012).

### 23.3.5 Mitochondrial Damage

In recent years, it has been reported that mitochondrial DNA mutations increase with age (Bua et al. 2006). The organs whose function is affected by age-related mtDNA mutations are the ovary, testis, and adrenal organs (Wei and Lee 2002). In addition, apoptosis or programmed cell death is also defined as a highly regulated process leading to the induction of an inflammatory response and cell death without harming the surrounding tissue. Mismanagement of apoptosis due to mitochondrial dysfunction leads to a number of harmful consequences, such as increased inflammation and tissue injury, especially when it develops with age (Green et al. 2011). Mitochondrial dysfunction may modulate inflammatory processes and mitochondrial protection attenuates inflammation (Lopez-Armada et al. 2013). Circulating levels of mtDNA increase with aging. Furthermore, one study revealed that persons that have elevated mtDNA levels have also elevated cytokine (TNF-α, IL-6, RANTES, IL-1ra) levels.

### 23.3.6 Frailty

Frailty is an extension of the physiological aging process, being at the crossroads of biological age, and the manifestation of chronic age-related diseases (Fulop et al. 2010). The inflammatory process of frailty is characterized by increases in the levels of pro-inflammatory cytokines, such as IL-6, as well as the CRP level and WBC count (Fulop et al. 2015).

### 23.3.7 Telomere Attrition and Cellular Senescence

Telomeres are the heterochromatic regions consisting of specialized DNA repeat sequences (nucleotides of TTAGGG repeats) located at the end of linear chromosomes. Telomeres play an essential function in maintaining chromosome stability. They protect chromosomes from damage during DNA replication. Telomeric DNA sequences differ in structure and function from other DNA sequences, besides their biological function. One of the underlying molecular mechanisms of biological and cellular aging is thought to be “telomeric attrition (shortening)” (Bernadotte et al. 2016). This phenomenon results from aging (repeated cellular divisions) due to mechanism of replication (telomeres shorten with every cell division) and also from stress, infection, and chronic diseases (Effros 2011). It is therefore emphasized that telomeric shortening may be a good biomarker for cellular aging. But, the mechanisms behind these are still not understood (Bernadotte et al. 2016).

### 23.3.8 Hormesis

The definition of hormesis is a process in which exposure to a lower dose of a chemical substance that damages higher doses, or environmental factors, has a healthy effect on the cell or organism. Various studies have identified many hormetic effects caused by oxidant compounds and inflammation. Three hormetic scenarios have been suggested in this case: (1) parallel response pathways, (2) nuclear factor erythroid-derived 2-like 2 (Nrf2) inductors and their effects, and (3) obesity paradox.

### 23.3.9 Nuclear Factor-κB (NF-κB) Pathway

It has been shown that pro-inflammatory conditions may cause age-related cellular and subcellular dysfunctions. The nuclear factor-κB (NF-κB), a transcription factor and an intracellular signaling pathway, plays a key role in inflammation by regulating the expression of several genes involved in inflammation.

### 23.3.10 Endoplasmic Reticulum (ER)

The function of ER in aging is closely related with inflammation. Recently one study have revealed that aged mice have increased ER stress markers (GRP78/BiP, C/EBP homologous protein, and X-box binding protein-1) and elevated inflammatory response according to young mice (Ghosh et al. 2014).

## 23.4 Biomarkers of Aging

### 23.4.1 Biomarkers of Inflammaging

Advancing age is related with elevated concentrations of all of the inflammation biomarkers except IL-1β. There are numerous studies in the literature to describe the relationship between inflammation and longevity. Advancing age and body mass index (BMI) are associated with higher C-reactive protein (CRP) levels (Ansar and Ghosh 2013). Inflammatory mediators, their levels and genetic variants, have been related to longevity; so the target inflammatory mechanisms need to be identified to develop an effective strategy (Table 23.1).

### 23.4.2 Biomarker Panel for Aging

The development of sensitive and specific biomarkers to distinguish and measure accurately healthy aging still remains an important goal. However, it is more

**Table 23.1** Inflammatory mediators in longevity

| | |
|---|---|
| 1. *Cytokines* | |
| *Pro-inflammatory cytokines* | |
| *Cytokine* | *Actions* |
| *IL-1* | Decreased levels with longevity (Bruunsgaard et al. 1999) |
| *IL-6* | Increased levels with longevity (Palmeri et al. 2012) |
| *IL-12* | Elevated levels related with longevity (Palmeri et al. 2012) |
| *TNF* | Elevated levels correlate with mortality (Roubenoff et al. 2003) |
| *Anti-inflammatory cytokines* | |
| *Cytokine* | *Actions* |
| *IL-4* | Decreased levels with longevity (Palmeri et al. 2012) |
| *TGF-β* | High levels related with longevity (Forsey et al. 2003) |
| *Soluble TNF receptors (sTNFR)* | Correlates with mortality (Varadhan et al. 2013) |
| 2. *Acute phase proteins* | |
| *Protein* | *Actions* |
| *C-reactive protein* | Associated with exceptional longevity and mortality (Wassel et al. 2010) |
| 3. *Transcriptional factors* | |
| *Factor* | *Actions* |
| *NF-κB* | Induce aging (Salminen and Kaarniranta 2009) |
| *FOXOs* | As prolongevity factors (Webb and Brunet 2014) |
| 4. *Adipokines* | |
| *Adipokine* | *Actions* |
| *Leptin* | Transgenic leptin expression is associated with reduced mortality and prolonged survival time (Naito et al. 2011) |
| *Adiponectin* | Higher levels and genetic variants in *ADIPOQ* contribute to longevity (Atzmon et al. 2008) |
| 5. *Danger-associated molecular patterns (DAMPs)* | |
| *HMGB1*, *histone*, *S100*, *HSPs* | Loss of intracellular DAMPs increases genomic instability, epigenetic alteration, telomerase attrition, and reprogrammed metabolism, reducing longevity (Huang et al. 2015) |
| 6. *Toll-like receptors (TLRs)* | |
| *TLRs* | Higher TLR expression in long-lived individuals (Arranz et al. 2010) |
| 7. *Oxidative stress parameters* | |
| *Parameters* | *Actions* |
| Reactive oxygen species (particularly $O_2^{\cdot-}$) | Primary cause of aging (Beckman and Ames 1998) |
| Protein carbonylation (PCO) | Decreased PCO is related with increased longevity (Bhattacharya et al. 2011) |
| Lipid peroxidation | Determine distinctive longevities of different animals (Hulbert et al.) |
| DNA oxidation | Related to aging rate (Barja and Herrero 2000) |

accurate to define a panel for aging instead of a single biomarker. This panel can be classified as:

1. Physical function and anthropometry (walking speed, chair stand, standing balance, grip strength, body mass index, waist circumference, muscle mass)
2. Blood-based candidate markers (IL-6, TNF-α, CRP, network analysis of inflammatory markers, HbA1c, plasma glucose, adipokines, thyroid hormones, vitamin D, NT-proBNP, troponin)
3. Molecular-/DNA-based markers (DNA/chromosomal damage, telomere length, DNA repair)
4. Novel markers (bilirubin, advanced glycation end products, metallothioneins, DNA methylation, microRNAs)

The practicability of measurements and outcome predictions of each of them show variability. The biological and molecular mechanisms of aging are quite complex. For this reason, it is doubtful that a single biomarker can have a valid measure of healthy aging, and further research is needed (Wagner et al. 2016).

## 23.5 Anti-inflammatory-Dependent Antiaging Strategies

Chronically increased circulating inflammatory markers are common in older persons, but mechanisms are completely unclear. Aging is commonly related to an elevated predisposition to illness and death. For this reason, preventive therapeutic interventions, antiaging strategies, and successful aging achievement to slow aging are urgently needed.

Current knowledge suggests that inflammation biomarkers such as CRP, TNF-α, and sTNFR-1 exaggerate initial increase with aging. That being the case, various strategies associated with inflammation and aging have been suggested. These strategies are divided into two groups as pharmacological interventions, nutritional interventions, and lifestyle regulations (Table 23.2). Aging is a complicated biological process that is induced by both intrinsic and extrinsic factors. Although the exact descriptive and mechanistic model of aging has yet to be elucidated, researchers have focused on developing practical and effective antiaging strategies. Human alpha-1 antitrypsin (hAAT) is an anti-inflammatory mediator and plays a major role in direct or controlled inflammation, especially with its immunoregulatory, anti-inflammatory, and cytoprotective properties. One of the study suggests that hAAT functions has potent anti-inflammatory and cytoprotective effects and may be considered as therapeutic choice for gene therapy (Yuan et al. 2018). In the future, hAAT may be a novel therapeutic potential promising candidate targets to aging and aging-related disabilities.

**Table 23.2** Antiaging strategies

| | |
|---|---|
| *Diet* | |
| *The Mediterranean diet (MeDi)* | Correlated with a low morbidity and mortality (Gu et al. 2010) |
| *Calorie restriction* | Impacts various cellular pathways, protects against cellular damage, improves longevity (Picca et al. 2017) |
| *Isoflavone intake* | With exercise, have positive effect on antiaging in postmenopausal women (Park et al. 2014) |
| *Antioxidant-rich supplementation* (vitamin C, vitamin E, tocopherols, carotenoids, melatonin, etc.) | May be useful to prevent age-related pathophysiological conditions (Fusco et al. 2007) and delay oxidative stress-associated aging |
| *Drugs* | |
| *Activation of telomerase* | |
| *TA-65®* (a telomerase activator) | Reduce short telomeres in percent, have no effects on mean telomere length (Harley et al. 2011) |
| *Ashwagandha root extract* | Increase telomerase activity (Raguraman and Subramaniam 2016) |
| *Autophagy inducers* | |
| *Rapamycin* | Extends chronological life-span by activating autophagy in *Saccharomyces cerevisiae* (Alvers et al. 2009) |
| *Senolytic therapeutics* | |
| *Dasatinib and quercetin combination* | Effective in eliminating senescent mouse embryonic fibroblasts and delaying age-related symptoms (Zhu et al. 2015) |
| *Navitoclax* (Bcl-2 family inhibitor) | Senolytic in different types of human senescent cells (Zhu et al. 2016) |
| *Epigenetic modulators* | |
| *Histone deacetylase (HDAC) inhibitors* | Minimization of the initiation and progression of age-related diseases, with inflammatory roots such as Alzheimer's disease,are neccesary to ensure health and extended life span (Yoon and Eom 2016) |
| (4-Phenylbutyrate, trichostatin A (TSA), sodium butyrate, suberoylanilide hydroxamic acid) | |
| *Anti-inflammatory drugs* | |
| Nonsteroidal anti-inflammatory drugs | Lengthen life-span and increase resistance to stress in *Drosophila* (Danilov et al. 2015) |
| *Regular exercise* | Has multi-system- and cellular-level antiaging effects (Garatachea et al. 2015) |

### 23.5.1 Pharmacological Intervention

Old age should be assessed in terms of biological, psychological, and social aging as well as chronological aging. The basic measure of chronological aging is the age of the person, while the basic measure of biological aging is the age of the person, i.e., cardiovascular aging. It is also known that there is a significant relationship with chronic silent inflammation and CVD. Thus, people with chronic inflammatory

diseases have higher risk for CVD (Esser et al. 2015b). It is important to further investigate these promising observations that medical strategies that diminish inflammation may be helpful in the treatment of obesity, T2DM, and related CVD (Esser et al. 2015a). The prevention and management of cardiometabolic diseases require a multifactorial approach in which targeting inflammation may represent a valuable therapy. The remarkable complexity of the immune system makes it difficult to target an individual pathway for the prevention of cardiometabolic diseases. Anti-inflammatory therapies raise hope for the prevention of CVD. However, caution is required. A summary of antiaging strategies that discussed anti-inflammatory therapies in the setting of chronic disorders such as T2DM and CVD here is provided in Table 23.3. Also Fig. 23.2 summarizes impacts of aging and suggested drugs associated with CVD and aging.

#### 23.5.1.1 Salicylates

Salicylates are nonsteroidal and anti-inflammatory drugs. Mechanism of salicylates on longevity is not clear yet; studies on antithrombotic and antioxidant effects by modulating inflammatory molecules are still in progress (Redondo et al. 2003). The particular mechanism behind the effect of aspirin on longevity is uncertain. The effect on longevity may be due to these functions (Strong et al. 2008), or it might be as a result of an alleviation of insulin/IGF-1 signaling by the DAF-16/FOXO transcription factor (Ayyadevara et al. 2013). In one study, *C. elegans* was shown to increase glutathione-S-transferase activity when aspirin therapy was administered, which is considered to be an effective factor in long-lived survival (Ayyadevara et al. 2013). In addition to its potential longevity-promoting effect (Agüero-Torres et al. 2001), salicylates prevent numerous diseases.

Low-dose salicylate intake is related with decreased PGE-M. Salicylate acetylates irreversibly COX-1 and modifies the activity of COX-2, thereby inhibiting enzymes involved in the conversion of arachidonic acid to pro-inflammatory prostaglandins (Abbatecola et al. 2004). Salicylates are involved in enhanced conversion of omega-3 fatty acids derived from mediators such as resolvins, protectins, and

**Table 23.3** Anti-inflammatory therapies in the setting of chronic disorders such as T2DM and CVD

| Drugs | Action mechanism |
|---|---|
| Salicylates | Targeting IKK-ß-NF-κB |
| Etanercept (the TNF receptor: Fc fusion protein); infliximab, adalimumab (specific monoclonal antibodies) | Targeting TNF-α |
| Anakinra, canakinumab, gevokizumab, LY2189102 | Targeting IL-1 |
| Tocilizumab | Targeting IL-6 |
| Metformin, salicylates, CNX-012-570, ZLN024 | AMPK activators |
| Resveratrol, SRT2104 | Sirtuin activators |
| Rapamycin (everolimus) | Mammalian target of rapamycin (mTOR) inhibitors |
| CCX140-B, JNJ-41443532 | C-C motif chemokine receptor 2 antagonists |

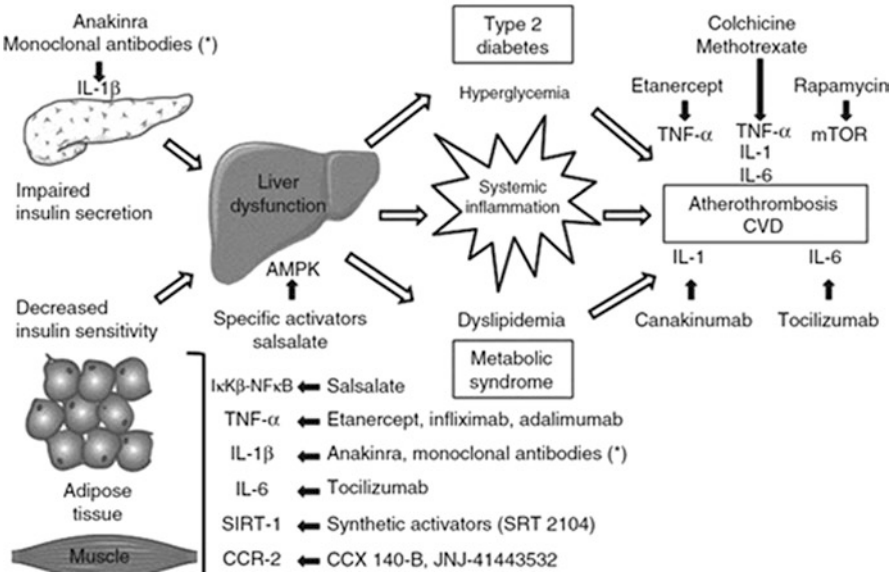


**Fig. 23.2** Most important actors of inflammation used as potential targets for pharmacotherapy in type 2 diabetes, associated with metabolic abnormalities, and CVD. Note that the precise sites of action of the various compounds remain poorly known and to a large extent only speculative. (*) Monoclonal antibodies: canakinumab, gevokizumab, LY2189102; *AMPK* AMP-activated protein kinase, *CCR2* C-C motif chemokine receptor 2, *CVD* cardiovascular disease, *IKKb-NFb* I(kappa) B kinase-b nuclear factor-kappaB, *mTOR* mammalian target of rapamycin, *SIRT-1* sirtuin-1

other proresolutions. These mediators inhibit production and trafficking of pro-inflammatory factors.

#### 23.5.1.2 Rapamycin

Rapamycin is generally used in post-renal transplantation to prevent organ rejection (Kahan 2004). It specifically inhibits the mTOR that controls cell proliferation, growth, and survival. Rapamycin also can be used as an anti-inflammatory drug for various diseases such as chronic kidney disease, atherosclerosis, and lung infection. It has also been reported that rapamycin prevents tumors and osteoporosis, furthers hyperlipidemia, and prevents atherosclerosis via increasing adipose tissue lipase activity (Yoon et al. 2015).

#### 23.5.1.3 Sirtuin Activators

SIRT1 is a gene that plays a role in DNA repair, inflammation, apoptosis, and cell senescence. These activators manage age-associated disease as obesity, lung inflammation, and T2DM. Their development may be useful for improving longevity and also in alleviating age-related diseases. Melatonin which is found in low levels in Alzheimer's disease has a protective effect against neurotoxic amyloid beta protein and reduces the acetylation of SIRT1 substrates (Cheng et al. 2006; Tajes et al. 2009). Caloric reduction in some experiments shows that it increases melatonin

production in the gastrointestinal tract (Bubenik and Konturek 2011). Treating with melatonin of elderly rats decreases intra-abdominal fat, diminishes plasma insulin and leptin levels, develops immunocompetence, enhances thymus weight, and increases blood concentration of testosterone and thyroid hormones. The other effect of melatonin is upregulating the expression of run-related transcription factor 2 (Runx2) and bone morphogenetic proteins, increasing bone density and volume in older rats. The mechanisms behind the aging effect of melatonin are uncertain: melatonin can disrupt bodily rhythms, reduce free radical damage, or reduce age-related mitochondrial dysfunction. Melatonin may also increase the incidence and duration of tumors. Due to this serious potential side effect, more research is needed before melatonin is used as antiaging (Yoon et al. 2015).

### 23.5.2 Topical Peptides

Natural peptides have several basic roles that result in physiological processes such as defense, immune, stress, growth, homeostasis, and reproduction. On the other hand, synthetic peptides, less than 500 Da, play a role in extracellular matrix synthesis, pigmentation, innate immunity, and inflammation. These peptides have antioxidative, antimicrobial (Rahnamaeian and Vilcinskas 2015), and whitening effects and can be divided into five groups as matricins peptides, carrier peptides, peptide mimetics or neurotransmitter-inhibiting peptides, enzyme inhibitor peptides, and structural protein digestion. The functions of synthetic peptides are (1) to increase skin penetration, (2) to bind specific receptors, (3) stability, and (4) resolution. Also, the main function of signal peptides is to initiate a signaling cascade. Carrier peptides carry or stabilize trace elements that are essential for wound healing and enzymatic reactions like copper and manganese. Another strategy is contraction of the muscle to decrease common aging signs like fine lines and wrinkles. They are undertaken by the neurotransmitter released such as acetylcholine. Local synthetic peptides act as specific inhibitors of the neurosecretion which mimic the synaptic protein SNAP-25 that are known as neurotransmitter inhibitor peptides and pass through the skin and loosen muscles, leading to the reduction and softening of aging signs. Enzyme inhibitor peptides such as soy oligopeptides, silk fibroin peptide, and rice peptides inhibit various enzymes such as tTAT-superoxide dismutase and induce hyaluronan synthase and proteinases, direct or indirect pathways.

### 23.5.3 miRNA-Based Therapy

It can be considered that miRNAs released by cells that activate DDR/SASP also signal to non-aging cells that do not spread at the same time and increase the inflammation. The usage of miRNA mimics (miRNA replacement therapy) and miRNA inhibitors (antagomiR therapy) has been successful in treating a broad range of

diseases including skin diseases like aberrant pigmentation (e.g., miR-434-5p, miR-145, miR-25), skin aging (e.g., miR-29, miR-155), UV damage to the skin (e.g., miR-141), acne (e.g., miR-143), psoriasis (e.g., miR-203, miR-146a, miR-99), acute dermatitis (e.g., miR-146a), and cardiovascular diseases like atherosclerosis (e.g., miR-21, miR-145, miR-122), arrhythmogenesis (e.g., miR-328), vascularization (e.g., miR-92a, miR-503, miR-126), cardiac hypertrophy, and fibrosis (e.g., miR-199b, miR-98, let7b) (Olivieri et al. 2015; Lawrence and Ceccoli 2017).

### 23.5.4 Nutritional Supplementation

Saturated fat consumption is associated with increased CRP, sTNFRII, TNF-α, and IL-1β. On the other hand, EPA+DHA supplementation is associated with diminished CRP and TNF-α; additionally EPA+DHA plus supplements are associated with diminished IL-1β. Higher consumption of saturated fat is positively correlated with CRP, IL-6, and ICAM-1. Omega-6 is generally pro-inflammatory, whereas omega-3 shows inverse association and less inflammatory effects (Marquez et al. 2010). Alcohol consumption is negatively associated with sTNFRII. It may act as a buffer by taking away TNF-α from circulation but also itself is complicated in apoptotic and pro-inflammatory signaling in response to TNF-α binding (Schumacher et al. 2008).

Dietary fiber may decrease inflammation by the various mechanisms such as sluggish absorption of glucose and management of insulin sensitivity, inducing favorable shifts in gut microbiota (Geng et al. 2011).

It has been shown that curcumin has a positive effect on longevity and reduces ROS in *C. elegans* through antioxidant and anti-inflammatory effects. It has been widely known that curcumin has potent anti-inflammatory and protective effects against atherosclerosis and anticancer effects (Rahman and Bagchi 2013).

### 23.5.5 Vitamins

In humans, 800 mg of vitamin E has been shown to improve cell-mediated immunity when used in healthy elderly people (Meydani et al. 1990). In addition, other antiaging properties of vitamin E can be summarized as (1) upregulation of apoptotic genes and downregulation of apoptotic genes due to aging and (2) protection of telomerase shortening caused by hydrogen peroxide by restoring telomerase activity in fibroblasts (Park et al. 2008).

Vitamin C also acts as a cofactor in many enzymatic reactions and neutralizes free radicals. Vitamin D has a vital function in the immune system, and lack of vitamin D is known to cause many inflammatory diseases. Interaction of vitamin D3 with its receptor blocks NF-κB activation and signaling (Panickar and Jewell 2015).

### 23.5.6 Calorie Restriction (CR)

CR has the feature of sustaining the mitochondrial biogenesis and increasing resistance of the muscles to inflammation. CR also declines inflammation and insulin resistance and exhibits anti-inflammatory activity (López-Lluch and Navas 2016). In a recent study, two beneficial effects of resveratrol in terms of inflammation have been shown: (1) pro-inflammatory cytokine production can be reduced and (2) liver inflammation can be reduced (Tung et al. 2015).

### 23.5.7 Epigenetic Diets

Recent studies have shown that epigenetic diets can be positively associated with aging. "Epigenetic diets" are termed as nutritional interventions caused by epigenetic changes. These interventions can be classified as (1) calorie restriction, (2) diet supplementation with nutrients involved in one-carbon metabolism, and (3) diet supplementation with bioactive food components. All these interventions are thought to contribute to decreasing DNA methylation or hypermethylation. According to current knowledge, global and locus-specific DNA methylation levels indicate that human aging is promising. This effect can be caused by reduction of intracellular methyl reserve, AdoMet, and/or inhibition of DNA methyl transferase enzymes (DNMTs). Experimental studies and their current results seem to be promising, but it is still not clear that a tissue (or even cellular) specificity of age-related epigenetic changes and their functional contributions to aging are should also be furtherly clarified (Bacalini et al. 2014).

### 23.5.8 Exercise

Exercise is the phenomenon of aging process. Some of the literature shows that exercise has beneficial effect in aging process, but other literatures show adverse results but not with unfortunate health effect. In recent years, researchers have suggested that athletes have relatively fewer immune system cells, such as neutrophils, lymphocytes, and leukocytes (Moro-García et al. 2014). In other respects, according to some studies, long-term exercise helps obtain a high baseline level of antioxidants. It has not been proved that exercise is an antiaging method, which is not denied that it is beneficial for health. In an experimental study, it has been shown that PGC1-a-dependent mechanism with exercise inhibits the age-related increase in systemic IL-6 and TNF-a levels. Exercise enhances antioxidant defense systems. Physical activity is negatively associated with CRP and sTNFRII (Yoon et al. 2015).

## 23.6 Conclusions

Chronically increased circulating inflammatory markers are commonly seen in elderly individuals, but its molecular mechanism is still unclear. Age-related impaired inflammatory process is usually related to increased predisposition to

illness and mortality. It has been known that the persistent low level of inflammation, seen in elderly individuals, may lead to increased levels of circulating pro-inflammatory and anti-inflammatory cytokines two- to fourfold. However, the type of lifestyle of elderly people (cultural factors in nutrition and type of diet, e.g., Mediterranean diet, northern European diet, personal habits such as exercise and smoking) may be considered the primary cause for systemic variations in the level of systemic pro-inflammatory cytokines.

For this reason, preventive interventions, anti-inflammatory therapeutic strategies, and successful aging achievement are urgently needed to slow down age-related inflammatory processes.

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# The Place of Geroprotective Agents in Life Quality and Longevity of Companion Animals

24

Alev Akdoğan Kaymaz

**Abstract**

Aging is a series of progressive degenerative body changes associated with decreased physiological functionality and a decline in the ability to adapt to metabolic stress. Geroscience particularly focuses on understanding the mechanisms of aging and multiple genetic and pharmacological therapies which have been developed in order to modulate the pace of natural aging and to prevent the age-related disorders such as cancer, kidney diseases, cardiovascular disorders, and many others; these geroprotective and senolytic interventions have shown to be beneficial for extending the life span and delaying age-related functional declines in rodents. Recent developments in geroscience also contributed to the improvement of quality of life and the extension of expected life span of cats and dogs. Like in humans, the life expectancy of cats and dogs varies significantly based on racial disparities; breed is an important determinant of life expectancy in pets. Other factors such as gender, genetics, environment, and stressors also play an important role throughout the aging process. Use of geroprotective agents by veterinarians is becoming more common day by day, extending the life span of pets and contributing to the establishment of a more peaceful home environment by decreasing the disease burden. This chapter will focus on the use of these geroprotective and senolytic agents and their overall impact on the prevention of age-related degenerative changes and disorders in companion animals: cats and dogs.

**Keywords**

Aging · Geroscience · Longevity · Life span · Quality of life · Cat · Dog

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## 24.1 Aging in Cats and Dogs

Although aging pattern of cats and dogs is a little different than of humans, the basic principles are similar. Molecular and cellular alterations that occur in aging pets cause a decline in physiological functions and tissue damage; this leads to diseases and eventually death. The aging process of pets must be well evaluated as the risk of developing cardiovascular diseases, cancer, hormonal alterations, and neurodegenerative diseases increases with aging.

Pets are referred as "senior" usually after the age of 7 (Fortney 2004). In dogs, life stage classification is convertible due to breed and size. Small-sized dogs after 11 years of age, medium-sized dogs after 9–10 years of age, and giant breed dogs after 7 years of age can also be considered as senior (Chastain 2004). The life expectancy in cats and dogs shows significant variations between breeds; multiple factors such as genetics, gender, weight, spaying, environment, stressors, and access to quality health care are also important determinants of aging.

## 24.2 Factors That Influence the Rate of Aging in Companion Animals

### 24.2.1 Sex

According to the most comprehensive study on sex-dependent longevity in cats which was conducted with nearly 4000 cats, median longevity of females was 2 years or about 15% greater than the longevity of all males (15.0 versus 13.0 years) (Egenvall et al. 2009). However, the impact of neutering on life span extension was greater than the impact of sex (O'Neill et al. 2015). The current information on sex-dependent longevity in dogs is slightly more conflicting: one small study ($n = 287$) on laboratory beagles has detected no significant sex differences in longevity (Albert et al. 1994), while in a much larger study conducted with thousands of Swedish dogs younger than 10 years of age, females were found to have a slightly longer life span (Egenvall et al. 2005).

### 24.2.2 Neutering (Spaying)

Neutering was found to be associated with 0.6 years greater longevity in females and 1.7 years greater longevity in males (O'Neill et al. 2015). Early-age neutering (<5.5 months of age) is considered to be beneficial as it also decreases the occurrence of some diseases. Spain et al. (2004) have detected that in male cats that underwent early-age gonadectomy, the occurrence of abscesses was decreased compared to the cats that were neuterized at an older age. The rate of developing asthma and gingivitis was also decreased in cats that underwent early-age gonadectomy. Neutering also had some behavioral impacts on cats; aggression toward veterinarians, urine spraying, and hyperactivity were less observed in early-age neuterized cats, while hiding and shyness increased.

### 24.2.3 Breed and Body Size

Certain diseases seem to develop more commonly in specific dog and cat breeds; this difference in prevalence also gave rise to breed-specific causes of deaths. For example, death from neoplasia was overrepresented in Golden Retrievers and Boxers, whereas cardiac diseases were the leading cause of death in Chihuahuas and Maltese, which was expectable as the prevalence of mitral valvular disease was high in toy breeds (Fleming et al. 2011). Crossbred cats are found to have a higher median life span than purebred cats. Being crossbred, having a lower body weight and being neutered were also found to be associated with a longer life (O'Neill et al. 2015).

A correlation between body size and longevity also exists: dogs from smaller breeds are found to live longer than dogs from larger breeds in general (Table 24.1). Greer et al. (2007) found a negative correlation between height and life span and also between weight and life span on 700 dogs. However, according to the same study, weight is the main significant predictor of longevity: the correlation between life span and adult body mass has been shown to be stronger than the correlation between life span and adult height of the breed (Sutter et al. 2007; Chase et al. 2009; Greer et al. 2011).

**Table 24.1** Average and maximum life span of cats and dogs

| Species | Life span (years) | | References |
|---|---|---|---|
| | Average | Maximum | |
| Cat | | >27 | Comfort (1956) |
| | 13–17 | 21 | Spector (1956) |
| | | 22 | Hideki et al. (1988) |
| | 15 | 38 | Grimm (2015) |
| | | 30 | http://genomics.senescence.info/species* |
| | | 24 | Cozzi et al. (2017) |
| Dog | 13–17 | 34 | Spector (1956) |
| | | >24 | Comfort (1956) |
| | 10 | | Eichelberg and Seine (1996) |
| | 6.7–8.5 | | Patronek et al. (1997) |
| | 12 | | Michell (1999) |
| | 10 | | Proschowsky et al. (2003) |
| | 12 | | O'Neill et al. (2013) |
| | 12 | 29 | Grimm (2015) |
| | | 24 | http://genomics.senescence.info/species* |
| | | 28 | Cozzi et al. (2017) |
| Small- to medium-sized dogs | | 24 | Comfort (1960) |
| | 10 | >15 | Li et al. (1996) |
| Large-sized dogs | | 14 | Comfort (1960) |
| | 7 | >10 | Li et al. (1996) |

*http://genomics.senescence.info/species is a website dedicated to genetic research in aging

### 24.2.4 Body Weight, Obesity, and Associated Comorbidities

Obesity is becoming more prevalent day by day, not only among humans but also among our companion animals: almost 50% of dogs and cats between 5 and 10 years of age are overweight or obese (Laflamme 2012). As obesity is also associated with a wide variety of morbidities such as diabetes mellitus, cardiovascular disease, hypertension, dyslipidemia, gallstone, and even certain forms of cancer, it poses a great threat to longevity (Jaso-Friedmann et al. 2008; Laflamme 2012; Zoran 2010; German 2006; O'Neill 2016). An increase in adult body weight is also known to be associated with a shorter life span (Zoran 2010; O'Neill et al. 2015). Adipose tissue inflammation is closely related with aging; this makes obesity a significant factor in longevity. In humans, oxidative stress increases as the BMI (body mass index) increases; increased oxidative damage and chronic inflammatory status are believed to be the underlying mechanism which gave rise to a dysfunctional metabolism seen in obese humans, but still there are only very few studies examining the pathophysiologic mechanism in pets (Wonisch et al. 2012; Tanner et al. 2007; Grant et al. 2011; LaFlamme 2012).

Obesity is mainly caused by an imbalance between energy intake and expenditure; environmental, genetic, and hormonal factors contribute to the development of the disease (Radin et al. 2009). Some breeds are more prone to becoming obese, whereas some are more resistant to developing obesity: Labrador Retriever, Boxer, Cairn Terrier, Scottish Terrier, Shetland Sheepdog, Basset Hound, Cavalier King Charles Spaniel, Cocker Spaniel, Dachshund (Miniature Long-Haired), Beagle, and some giant breed dogs are under a greater risk, while Greyhounds are more resistant (Gossellin et al. 2007; Diez and Nguyen 2006; Zoran 2010). Having a coexisting endocrine morbidity (such as hypothyroidism and hyperadrenocorticism), use of anticonvulsants or glucocorticosteroids is also a risk factor for developing obesity (Murphy et al. 2011; Courcier et al. 2009; Montoya-Alonso et al. 2017). This disease is known for being more prevalent among neutered pets; it is believed to be caused by reduced metabolic rate decrease with the loss of sex hormones (Zoran 2010; Hoenig 2014).

Obesity was found to be more common in younger female dogs, but as both sexes reach an old age (>12 years), obesity also tends to increase with age because of reduced metabolic rate (Zoran 2010; German 2006). In cat, males seem to be more prone to become obese than females (O'Neill 2016). However, the contribution of body weight was much less significant than sex for the development of obesity (Slingerland et al. 2009).

Interestingly, caregiver-related factors such as the age, income, and lifestyle habits of the caregiver were also found to be important risk factors for the development of obesity in their companion animals; the incidence of obesity was higher among pets with an elderly caregiver (Edney and Smith 1986). Dog owners who consume nutrient-rich, calorie-poor diets had dogs with normal weight, while obese owners were found to be more likely to have obese dogs (Heuberger and Wakshlag 2011). The caregiver's misinterpretation of normal pet behavior may also pose a risk for the development of obesity in pet, particularly in cats. In fact, increasing the

frequency of regular meals as a response to the perceived hunger behavior, in addition to sharing their own meals and rewarding them with treats, owners may contribute to the development of obesity (German 2015).

Unlike in humans, obesity may not always result in diabetes mellitus in pets. Diabetes in dogs present in a type 1 diabetes-like manner, obese dogs may develop insulin resistance, but they do not develop diabetes due to obesity. On the other hand, obese cats are very prone to developing DM just like human type 2 diabetes (Osto and Lutz 2015; Hoenig 2014; Zoran 2010). The risk for development of diabetes increases about twofold in overweight cats and about fourfold in obese cats (Laflamme 2012; Hoenig 2014). Obese cats are also known to be prone to have comorbidities such as hepatic lipidosis, urinary tract disease, lameness, and dermatopathies (Raffan 2013).

Some anti-obesity agents have also been recently proposed for the management of obesity. Dirlotapide and mitratapide have been recently licensed for the treatment of obesity in dogs. As microsomal triglyceride transfer protein inhibitors, these drugs function by disabling lipid intake at enterocyte level. These drugs also decrease the sensation of appetite. As both the absorption of lipids and the desire to eat lessen, a reduction in caloric intake occurs. So, it causes crease decreasing in the body up to 20–40% without ill effects (Wren et al. 2007).

## 24.3 Age-Associated Changes and Diseases in Cats and Dogs

Aging is a multifactorial process which leads to a variable decline in the functions of organs and tissues; it is associated with several physiological changes. Behavioral changes, body weight changes, decreased physical activity, and reduced vision and hearing are commonly encountered in aging cats and dogs (Davies 2012; Landsberg and Araujo 2005) (Fig. 24.1).

Chronic inflammation is believed to be the main pathologic event that causes aging and age-related diseases. Aging predisposes our companion animals to most of the chronic diseases such as atherosclerosis (a common cause of heart attacks, strokes, and peripheral vascular disease), endocrine disorders (such as obesity and diabetes), dementias, cancers, arthritis, cataract, age-related muscle dysfunction (sarcopenia), and loss of resilience. All these conditions are associated with a sterile, low-grade, chronic inflammation which is also seen in aging tissues (Chung et al. 2009; Freeman 2012).

Age-related changes such as increased need for sleep, loss of hearing or sight, and slowing down are easily recognized by the caregivers; however signs of potentially life-threatening diseases are often failed to be noticed. Respiratory distress and palpable masses can be easily missed in aging pets (Davies 2012). As the signs of organ dysfunction easily go unnoticed by an untrained eye, regular veterinary visits are beneficial for the early detection and management of age-related diseases. Unfortunately, older animals usually suffer from reduced functionality in multiple organs with different degrees of dysfunction (Fortney 2004).

The most recognized diseases of geriatric cats were found to be urinary system disorders, whereas cardiovascular system disorders were mostly observed in dogs

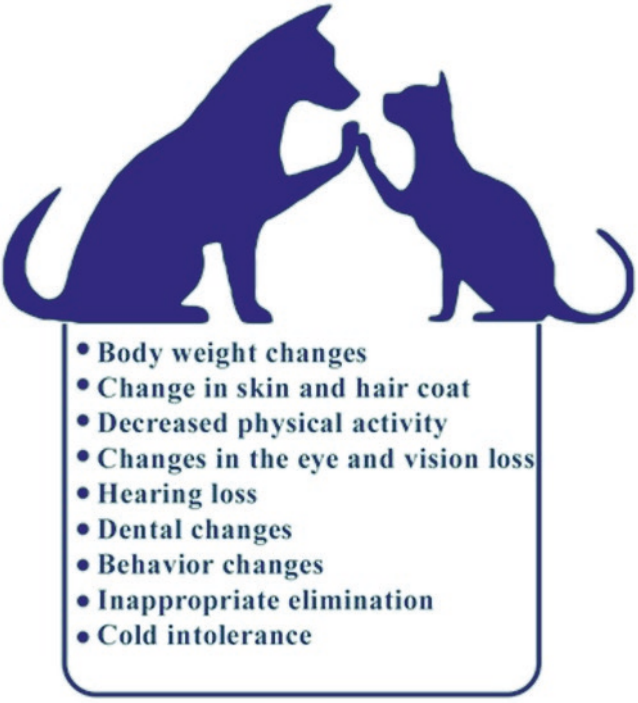


**Fig. 24.1** Expected physiological changes in elderly cats and dogs

(Ulgen et al. 2014). The prevalence of cardiopulmonary disease increases with advancing age in dogs: degenerative valvular disease, chronic obstructive pulmonary disease, and arrhythmias are particularly common in the geriatric dog (Miller et al. 1989). Chronic kidney disease (CKD) is the most frequent urinary system disorder encountered in elderly cats (Quimby et al. 2013). Particularly diseases which affect the kidneys pose an important risk for the development of CKD; systemic hypertension, hyperthyroidism, dental disease, and inflammatory bowel disease are some of the known threats (DiBartola et al. 1996; van Hoek and Daminet 2009; Williams et al. 2010, 2014).

Nutritional needs of cats and dogs change as they age; planning of an adequate feeding regime is needed, particularly if an age-related morbidity is seen (Dzanis 2004). For that reason geriatric cats (>12 years of age) may need a highly digestible nutrient-dense diet, or overweight or obese dogs may benefit from diets with lower fat and calories (Laflamme 2005). Henegar et al. (2001) showed that feeding dog by a high-fat diet causes increased arterial pressure, hyperinsulinemia, activation of the renin-angiotensin system, and structural changes in the kidney which may be the precursors of more severe glomerular injury associated with prolonged obesity.

As stated earlier, aging cats are under a greater risk of developing diabetes. Diabetes in dogs are more like type 1 diabetes, but DM in cat generally present with a type 2 diabetes-like syndrome; it is characterized with a relative insulin deficiency

combined with an insulin resistance (Hoenig et al. 2003; Hoenig 2014; Slingerland et al. 2009). Obese cats have an altered expression of several insulin signaling genes and glucose transporters; they are also resistant to leptin: this gives rise to a defective glucose transportation. Pathologic changes such as beta cell failure and deposition of amyloid within pancreas islets are also seen; along with the amyloid deposition, a reduction in the concentration of adipokine is also observed. Every one of these pathologic events contributes to the development of insulin resistance and eventually diabetes. (Hoenig et al. 2003; Zoran 2010). Particularly indoor cats are under a greater risk of developing obesity as they have a more sedentary lifestyle (Nelson and Reusch 2014; O'Neill 2016).

Obesity is also a risk factor for orthopedic diseases, combined with the degeneration of joints in aging pet; orthopedic diseases are more prevalent among elderly, heavy pets (Muir et al. 2004). A recent study by Van Hagen et al. (2005) found a link between neutering and hip dysplasia in boxers.

Advancing age creates a predisposition for neoplasias; the incidence of benign and malign neoplasia is higher among elderly pets. Cancer-associated deaths are also commonly seen in elderly pets. Obesity is a known risk factor for developing neoplasias; obese elderly pets are under a greater risk of developing mammary tumors and transitional cell carcinoma (German 2006). Most commonly encountered cancers in cats are lymphoma with feline leukemia, mammary tumors, skin cancer, mast cell cancer, and osteosarcoma. Lymphoma, osteosarcoma, mammary tumors, and hemangiosarcoma are commonly encountered in dogs (Dobson et al. 2002; Egenvall et al. 2005; Merlo et al. 2008). Incidence of cancer is three times higher in female than in male dogs (Merlo et al. 2008).

Motility disorders are very common in elderly cats and dogs. Gastrointestinal diseases which are associated with neoplasia (dog and cat), stomatitis (cat), idiopathic megaesophagus (dog), gastric motility disorders (dilatation-volvulus and gastroparesis) (dog), lymphocytic-plasmacytic enteritis (dog and cat), and idiopathic megacolon (cat) are of major concern in small animals (Neiger 2004).

A decline in the physical or mental health of older dogs can be a challenge for the owners due to cognitive and behavioral changes which occur with aging (Chapagain et al. 2017). Any pathology can affect the body and cause a behavioral change such as posture changes, circling, and vocalization because of chronic pain (Houpt and Beaver 1981). In addition to this, in geriatric patients, a dog's sight (such as lens sclerosis, cataracts) and joints can cause both physical and behavioral changes (Landsberg and Araujo 2005; Godfrey 2005; Lascelles and Robertson 2010). However age-related hearing loss is the most common cause of deafness in pets (Houpt and Beaver 1981; Strain 2017).

Cognitive abilities may decline with aging, causing deficits in learning and memory. Most research on cognitive aging in dogs has focused on the translational approaches to human aging and Alzheimer's disease (AD) (Kaeberlein et al. 2016; Head 2013; Adams et al. 2000; Davis and Head 2014). Like in humans, genetics, diet, and lifestyle choices influence the prevalence, the pattern of pathologic neuronal changes (particularly senile plaque formation), and the nature of cognitive dysfunction in companion animals. Neuropathological changes present as some

behavioral changes such as referring to eating, drinking, grooming, disorientation, elimination habits, lack of self-hygiene, sleep-wake cycles, aggression, anxiety, aimless activity, excess vocalization, and alteration in social interactions (Golini et al. 2009; Gunn-Moore et al. 2007; Landsberg et al. 2012; Landsberg and Malamed 2017). Changes in social interaction with people and other pets are commonly seen in 11–14-year-old cats. Excessive vocalization and aimless activity are common among cats older than 15 years (Gunn-Moore et al. 2007). It should also be kept in mind that behavioral changes do not always arise from neuropathological events; loss in sight due to age-related eye diseases such as cataracts and age-related hearing loss (which is the most common cause of deafness in pets) can also cause behavioral changes (Landsberg et al. 2012; Godfrey 2005; Lascelles and Robertson 2010; Houpt and Beaver 1981; Strain 2017).

## 24.4 Geroprotective Agents That Are Used in Animals

Improving the quality of care and nutrition of pet animals and advanced diagnosis and treatment by developing technologies lead to an increase in survival rate (Akdoğan Kaymaz et al. 2014).

Our companions, dogs and cats, become a part of our families right on the first day that they enter our life; every little suffering of them hurts us deeply. This arises two main questions: "What can we do to prevent diseases?" and "How can we make them live longer?"

Even though the occurrence of diseases is associated with multiple factors such as breed, genetics, size, sex, and aging, it is known that proper nutrition, exercise, and quality health care reduce the prevalence of morbidities and increase life expectancy of companion animals.

Geroprotective, in other words, antiaging, interventions also seem promising for the prevention of early aging. Mitochondria-permeable or mitochondria-targeted antioxidants are one of these agents; they slow down the aging process by reducing mitochondrial oxidative stress and by eliminating senescent cells from tissues. Edaravone, idebenone, α-lipoic acid, carotenoids, vitamin E and coenzyme Q10, MitoQ, SkQ, and astaxanthin (a ketocarotenoid from the xanthophyll family) are examples for these agents (Cakatay and Kayalı 2005; Ademowo et al. 2017; Williams et al. 2015). Dietary antioxidants such as vitamin C, vitamin E, selenium, and β-carotene and other carotenoids (α-carotene, β-cryptoxanthin, lutein, lycopene, and zeaxanthin) are also very helpful for counteracting oxidative damage and senescent cells. For this reason, pet food manufacturers start to improve their senior pet food options with the addition of selenium, copper, iron, manganese, vitamins (particularly E and C), beta carotene, and lutein. And also those commercial senior diets consist of reduced protein, macrominerals (phosphorus and calcium), and salt (Hesta et al. 2006; Kim et al. 2000). Chondroprotective agents such as glucosamine and chondroitin sulfate are routinely added to diet of senior pets (Dzanis 2004). On the other hand, dietary management is also used for the treatment of age-related diseases, combined with medicine or not (Chew and Park 2004; Lenox and Bauer

2013; Nolan et al. 2014). When antioxidants and vitamin supplementations are combined with cognitive training and regular physical exercise, they are shown to be beneficial for reducing brain-derived neurotrophic factor (BDNF), for this reason (Fahnestock et al. 2012). These agents are also preferred in the treatment of cognitive dysfunction syndrome (CDS) which presents with aging. Recent studies also have shown that long-term supplementation with medium-chain triglycerides can improve cognitive function in aged dogs. Carotenoids lutein (L), zeaxanthin (Z), and meso-zeaxanthin are known as macular pigment (MP). MP's constituent carotenoids are also important for cognitive function (Chew and Park 2004; Head et al. 2009; Manteca 2011; Lenox and Bauer 2013; Nolan et al. 2014 Snigdha et al. 2015).

Owner-requested euthanasia is performed often due to chronic illnesses which occur with aging. Each caregiver has a different perception about the quality of life; they decide on euthanasia based on their perception about the severity of their companion's diseases, their own cultural values, and their life experiences. Age and financial status of the owner and numbers of medicine pills used in the treatment also affect their decision (Boyd et al. 2008; Reynolds et al. 2010). Total cost of planned treatment is also an important factor for the caregivers; aging and age-related morbidities are known to raise the financial burden (Kirkland 2013). Because of the high rate of euthanasia of aging pets, it is also harder to investigate whether senolytic medicines and nutrients are really beneficial or not; an important number of aging cats and dogs are euthanized before having chance to try out these interventions.

A number of probiotic products are commercially available in shape of powder, tablet, capsule, paste, and liquid forms for use in dogs and cats. Some commercial dog and cat foods also claim to contain prebiotics and probiotics (Weese and Arroyo 2003). In dogs, prebiotics such as fructooligosaccharides (FOS) and mannanoligosaccharides (MOS) cause an increase in neutrophil activity; hence they may affect immune systems or undesirable microbial communities in the gut. Previously, acute and chronic enteropathic dysbiosis (disbacteriosis) of dogs and cats was treated with probiotics (Kelley et al. 2009; Herstad et al. 2010). A related study conducted on shelter cats has shown that probiotic administration leads to a decrease in the duration of diarrhea (Bybee et al. 2011).

The owners are sometimes doubtful whether the commercial diets are safe for their pet or not; for that reason pet owners have shown increased interest in holistic and natural diets containing oats and barley (Di Cerbo et al. 2017). But actually quality commercial diets seem to be more suitable with the needs of the aging pets.

Recent advances in geroscience have identified several hallmarks of aging that have a role in the molecular mechanisms which may give rise to diseases (Lopez-Otin et al. 2013; Pitt and Kaeberlein 2015; Urfer et al. 2017). This discovery initiated the development of therapeutic strategies for delaying age-related disability and diseases. Rapamycin (an immunosuppressant and nutrient-responsive mTOR inhibitor agent) and metformin (an antihyperglycemic) are two examples of these agents (Castillo-Quan and Blackwell 2016; Kaeberlein et al. 2016; Pitt and Kaeberlein 2015). Metformin is regarded as an effective agent to prolong the life span in humans, but its effectivity in animals is still not well established. The other agent,

rapamycin, at low doses is found to be significantly effective on diminishing the mortality and delaying age-related diseases in several studies (Larson et al. 2016; Wilkinson et al. 2012; Kaeberlein et al. 2016). "The Dog Aging Project" is currently conducting an intervention trial with middle-aged dogs using rapamycin in order to enhance longevity and healthy life span (Kaeberlein et al. 2016; Urfer et al. 2017). The main aim of this study was to use dogs as an animal model for human aging, presenting an effective geroprotective agent both for humans and their companion animals. Dogs are counted as highly valuable for these studies because of both their genetic and phenotypic characteristics and social environment that they share with humans. Dogs also have much more short life span than humans (approximately 7–10 times); both the benefits and side effects of these agents can be seen much more earlier in dogs.

## 24.5 Conclusion

Numerous studies have focused on not only prolongation of life but also providing a disease-free survival. Companion dogs have been considered as an optimal model for understanding the variables of morbidity and mortality which are specific for aging. On the other hand, these life partners in which we share our homes provide great contribution to our physical and mental health and also social communications. The studies performed in dogs may also play important roles not only in the exploration of the structural background of the diseases due to aging but also in the improvements in the longevity and quality of life outcomes of these animals and their owners. Moreover, these potential advances may prevent unethical euthanasia procedures performed upon decision of "owners," mainly due to the mental and the financial burden of age-associated diseases. With studies on aging that continues in ethical approach, it will be possible to provide a healthy and long life for both companion animals and their caregivers.

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