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Review

Anti-aging pharmacology: Promises and pitfalls

Alexander M. Vaiserman[a,*], Oleh V. Lushchak[b], Alexander K. Koliada[a]

[a] D.F. Chebotarev Institute of Gerontology, NAMS, Kiev, Ukraine
[b] Vasyl Stefanyk Precarpathian National University, Ivano-Frankivsk, Ukraine

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ABSTRACT

Life expectancy has grown dramatically in modern times. This increase, however, is not accompanied by the same increase in healthspan. Efforts to extend healthspan through pharmacological agents targeting aging-related pathological changes are now in the spotlight of geroscience, the main idea of which is that delaying of aging is far more effective than preventing the particular chronic disorders. Currently, anti-aging pharmacology is a rapidly developing discipline. It is a preventive field of health care, as opposed to conventional medicine which focuses on treating symptoms rather than root causes of illness. A number of pharmacological agents targeting basic aging pathways (i.e., calorie restriction mimetics, autophagy inducers, senolytics etc.) are now under investigation. This review summarizes the literature related to advances, perspectives and challenges in the field of anti-aging pharmacology.

Contents

* Corresponding author at: Institute of Gerontology, Vyshgorodskaya st. 67, Kiev 04114, Ukraine.
E-mail address: vaiserman@geront.kiev.ua (A.M. Vaiserman).

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1. Introduction

Human longevity had dramatically increased during the past century when implementation of vaccination, disinfectants and antibiotics caused significant reduction in the importance of infectious diseases as a cause of death worldwide. The continuing decline in the mortality rates among the elderly had most probably occurred due to the preventative factors, including improved nutrition, as well as exercise and reduction of smoking (de Magalhães, 2014; Vijg and de Grey, 2014). As a consequence, the majority of modern societies are characterized by rapid population aging, and the rise of age-related diseases prevalence such as cancer, stroke, heart failure, as well as Alzheimer's and Parkinson's diseases becomes a considerable socio-economic challenge (Beard and Bloom, 2015; Harper, 2014).

Over the decades, the compression of morbidity was the basic strategy in geriatric research. According to this strategy, it is assumed that morbidity should be restricted to a short period late in life by delaying the onset of age-associated chronic pathologies, thereby reducing the disability burden (Seals et al., 2016). Recently, a new field focused on healthspan extension has started to develop within the aging research. This field is referred to as 'geroscience' (Kennedy et al., 2014). Extending healthspan is a major component of 'optimal longevity', defined as living long, but with satisfactory health, well-being and life quality (Seals et al., 2016). The attempts to extend healthspan are now focused on slowing the fundamental biological processes underlying aging such as mitochondrial dysfunction, impaired proteostasis, cellular senescence, age-associated decline of stress resistance, dysregulation of pathways involved in growth and cellular energy sensing, deteriorating the function and/or bioavailability of stem cells, as well as oxidative stress and inflammation (Fontana et al., 2014; Kirkland, 2013a). The investigation dedicated to extending the human lifespan is a part of novel research field, 'anti-aging medicine' which is an increasingly debated topic throughout the last years (Anton et al., 2005; Barazzetti and Reichlin, 2011; de Cabo et al., 2014). Traditionally, the research aimed at human life extension raises the concern that it can result in the rise of the elderly population and, thereby, in the high prevalence of aging-related chronic diseases. Experimental studies, however, have repeatedly demonstrated that life extension is generally accompanied by delayed and/or reduced morbidity (Fontana et al., 2010). Consistent with these animal findings, centenarian studies have demonstrated that the majority of centenarians not only exhibit the extraordinary longevity, but usually remain free from chronic disorders and disability until the very advanced age (Willcox et al., 2008).

The development of pharmacological treatments targeting age-related functional decline and pathological manifestation (so-called 'anti-aging drugs') is currently in the focus of biogerontological research (Kennedy and Pennypacker, 2014; Verdaguer et al., 2012). A number of genes playing pivotal roles in regulating aging and longevity have been identified over this time period, and most of these genes represent promising drug targets (Lindborg et al., 2015; Moskalev et al., 2014; Paul et al., 2010; Shadyab and LaCroix, 2015). Another area in anti-aging pharmacology is evaluation of pharmacological potential of agents which were approved by the U.S. Food and Drug Administration (FDA) and other regulatory agencies for the treatment of particular pathological conditions related to aging. Beta-blockers, metformin, thiazolidinediones, renin-angiotensin-aldosterone system inhibitors and several anti-inflammatory drugs (Seals et al., 2016) are among these medications. These preparations are commonly used for the treatment of persons with particular chronic illnesses and their safety and efficiency was confirmed in numerous clinical trials. In several trials, they have also demonstrated the improvement of physiological functioning, well-being and health status in patients with chronic diseases (Seals et al., 2014). Such medications are not used now in treatment of age-related decline in physiological and cognitive functions in the absence of clinical manifestations of particular diseases. However, these drugs would likely be redirected to preventing or treating specific conditions and/or syndromes that are commonly associated with aging.

By slowing and/or delaying the process of aging *per se*, it apparently would be possible to prevent the majority of age-related pathological changes rather than to overcome them one by one, which is the current approach of a disease-centered paradigm of drug development (Seals and Melov, 2014). Furthermore, preventing the progression of a particular age-associated chronic pathology, e.g. cardio-vascular disease, would apparently have only a modest impact on the population life expectancy since comorbidity such as cancer or neurodegenerative disorders would to a great extent substitute the reduction of the mortality risk due to the prevention of targeted pathology. As a consequence, the longevity dividend, i.e. the idea that extending healthspan by slowing aging can prove to be the most effective way to combat the disabling and fatal disorders that plague us today (Olshansky, 2013), may provide a great opportunity to revitalize the pipeline for drug development.

This review is focused on current advances and perspectives in the field of anti-aging pharmacology.

2. The free radical theory of aging: conceptual issues and clinical applicability

Historically, the free radical theory of aging has probably been the most influential theory of aging. This theory was first postulated by Denham Harman in the mid of the last century (Harman, 1956). In modern literature, this concept is more commonly termed the oxidative damage theory of aging. It has been one of the earliest concepts to try to explain the basic cause of age-related functional decline and accompanying pathology. This hypothesis supposes that free radicals and other reactive oxygen species (ROS), formed in the course of mitochondrial metabolism as inevitable side products of the oxidative energy metabolism, may result in damaged molecules such as carbonylated proteins, lipid peroxides, and oxidized DNA (Liu et al., 2014a; Payne and Chinnery, 2015; Pinto and Moraes, 2015). The accumulation of this damage is believed to be the leading cause of cellular senescence, age-dependent telomere attrition and aging-related diseases (Koliada et al., 2015). In addition, it is assumed that longevity is determined by the rate at which such damage occurs.

Physiologically, ROS excess in the body can be eliminated by endogenous antioxidative defense systems, including superoxide dismutase (SOD), the enzyme detoxifying superoxide radical ($O_2^{\bullet -}$), glutathione reductase (GR) involved in the regeneration of glutathione, as well as catalase (CAT) and glutathione peroxidase (GPX) both implicated in the detoxification of peroxides. Together these antioxidant systems allow to keep the balance between

oxidative and anti-oxidative processes. In the conditions of chronic oxidative stress, the endogenous antioxidant systems are, however, believed to be insufficiently effective. According to Harman's theory, it is assumed that in such conditions supplementation with exogenous antioxidants such as vitamins E and C, beta-carotene, lipoic acid, coenzyme Q10, glutathione, polyphenols, phytoestrogens, as well as several minerals including zinc, manganese and selenium can play a role in maintaining homeostasis (Pandey and Rizvi, 2010; Bouayed and Bohn, 2010; Sadowska-Bartosz and Bartosz, 2014). Thereby, the proponents of this theory believe that there is a demand for exogenous antioxidant sources to prevent oxidative stress, representing an imbalance in the redox state in the favor of oxidation. However, excessive doses of these substances may be toxic due to their pro-oxidative effects at high concentrations or their potential to eliminate beneficial concentrations of ROS normally occurring at physiological conditions and required for the effective cellular functioning (Bouayed and Bohn, 2010).

According to the oxidative damage theory of aging, it is supposed that longevity could be extended by activation of endogenous antioxidant defense systems or by exogenous antioxidant supplementation. On the basis of this assumption, a large number of studies have been attempted to delay aging and promote lifespan by dietary supplementation with synthetic or natural antioxidants (Sadowska-Bartosz and Bartosz, 2014; Si and Liu, 2014). The ones that have been the most studied in this regard are vitamins C and E. Vitamin C (ascorbic acid) is the major hydrophilic (water-soluble) antioxidant. It effectively reduces α-tocopheroxyl radicals and level of low-density lipoprotein (LDL) in cell membranes, thereby restoring α-tocopherol and inhibiting the generation of free radicals (Niki et al., 1995). Vitamin E (α-tocopherol) is the main hydrophobic (fat-soluble) antioxidant protecting cell membranes from oxidative damage by reaction with lipid radicals produced in the course of the lipid peroxidation chain reaction. The dietary vitamin E supplementation has been shown to be associated with reduced risk of atherosclerosis through reducing oxidative stress and inhibiting LDL oxidation (Meydani, 2001). Currently, the development of novel means of antioxidants delivery into cells or construction of novel antioxidant compounds is believed to open new prospects for the modulating the aging rate and healthspan extension. Because mitochondria are the main source of ROS in the cell, mitochondria-targeted antioxidants are suggested to be more effective than traditional ones. Such compounds are suggested by several authors (see, e.g., Oyewole and Birch-Machin, 2015; Skulachev, 2013) to have a considerable potential since they can cross the mitochondrial phospholipid bilayer and eliminate ROS in the heart of the source. Some preliminary evidence favoring that conclusion originates from recent *in vivo* findings of effects of mitochondria-targeted antioxidants including plastoquinone (SkQ) and mitoquinone mesylate (MitoQ) (Apostolova and Victor, 2015; Skulachev, 2013).

2.1. ARE/Nrf2 pathway

Generally, cellular protection against oxidative stress is provided by both direct and indirect antioxidant activities. Direct antioxidants are compounds that directly neutralize ROS. These compounds are themselves redox active and are consumed upon reaction with ROS. Indirect antioxidants activate the body's own natural detoxification systems, causing transcriptional activation of a battery of cytoprotective proteins acting catalytically (Dinkova-Kostova and Talalay, 2008).

The nuclear factor-erythroid 2-related factor 2 (Nrf2) is a master transcriptional regulator of indirect antioxidant activity (Jung and Kwak, 2010). In response to oxidative stress, it regulates a battery of protective genes by binding to regulatory antioxidant response elements (AREs) in the promoter regions of antioxidant genes, as well as genes implicated in other cytoprotective functions (Xue et al., 2015). SOD, catalase and glutathione peroxidase are among the antioxidant genes activated by Nrf2 (Pall and Levine, 2015). The Nrf2-signaling pathway promotes cytoprotection by inducing the transcription of more than 200 genes involved in the metabolism of toxins and drugs, protection against inflammation and oxidative stress, stability of proteins and removal of damaged proteins via proteasomal degradation or autophagy (Lewis et al., 2010). In addition, Nrf2 interacts with other crucial cell regulators including nuclear factor-kappa beta (NF-kappaB) and tumor suppressor protein 53 (p53). Nrf2 is constitutively expressed in all tissues, although key detoxification organs such as liver and kidney exhibit the highest levels (Lewis et al., 2010).

Studies in transgenic mice showed that elevated level of Nrf2 activity can promote healthspan and longevity, whereas lowered level is associated with lifespan shortening (Pall and Levine, 2015). This apparently is because of the fact that activation of Nrf2 can positively influence pathways involved in cellular senescence (Kapeta et al., 2010). Because of these properties, it is proposed to be the master regulator of the aging process, protecting against many age-related diseases including cardiovascular disorders (Silva-Palacios et al., 2015) and neurodegeneration (Johnson and Johnson, 2015). In addition, Nrf2 plays a dual role in carcinogenesis. On the one hand, it is known to be required for systemic protection against redox-mediated injury and tumorigenesis. On the other hand, however, its constitutive activation is strongly associated with cancer progression and aggressiveness. Therefore, a better understanding of Nrf2 regulation will be necessary to maintain the pro-oxidant/antioxidant balance to inhibit tumor progression (Moon and Giaccia, 2015).

Several factors were identified demonstrating health-promoting properties attributed to the Nrf2 activation. Among these factors, there are phenolic antioxidants, carotenoids, γ, δ-tocopherols and tocotrienols, isothiocyanates from cabbage, broccoli and other cruciferous food, terpenes, fish oil and sulfur compounds (Kumar et al., 2014; Pall and Levine, 2015). Taking that into account, the ARE/Nrf2 pathway seems to be a promising target for the pharmacological control of chronic diseases, such as cardiovascular diseases as well as immunological and neurodegenerative disorders (Al-Sawaf et al., 2015; Gao et al., 2014).

2.2. Life extension by antioxidants

In experimental studies, supplementation with antioxidant substances caused controversial effects on longevity. Major findings on the effects of exogenous antioxidants on lifespan in various experimental models are summarized in Table 1.

As we can see from this table, antioxidant supplementation led in most cases to the life extension, and also to reduced levels of aging-related oxidative stress, enhanced antioxidant enzyme activity, reproductive activity and stress resistance, as well as to changed expression of aging-associated genes. In some studies, however, the lifespan and fertility were found to be unchanged or even diminished. In some cases, life-promoting capacity of antioxidants was observed in superoxide dismutase (SOD)-deficient mutants with compromised antioxidant defense or in normal animals under stressful conditions, but it has not been demonstrated if wild-type animals reared in normal conditions have been studied. In addition, antioxidants have been shown to be able to influence the mean lifespan but they have failed to show consistent effects in extending maximal lifespan of wild-type animals, assuming that these effects would be caused by reduction of mortality rate via delaying or preventing aging-related pathologies rather than via interfering with the aging process *per se*. Moreover, it is necessary to consider that life extension in many animal models can be caused by mechanisms other than those attributable to aging *per se*. For example, antibi-

Table 1
Summary of the effects of substances having antioxidant properties on the lifespan and associated functional traits of model organisms.

Model/Agent	Longevity effect	Associated functional traits	Reference
Rotifer			
Vitamin E	N.D.	extended pre-reproductive stage	Sawada and Enesco (1984)
Indolepropionamide	increased lifespan	N.D.	Poeggeler et al. (2010)
Caenorhabditis elegans			
Vitamin E; CoQ	increased lifespan	reduced superoxide anion levels	Ishii et al. (2004)
Vitamin E	increased lifespan	delayed reproduction	Harrington and Harley (1988)
Polydatin, a natural resveratrol glycoside	increase of mean lifespan by 31% and 62% under normal and acute stress conditions, respectively	N.D.	Wen et al. (2014)
Curcumin	increased lifespan	N.D.	Liao et al. (2011)
Quercetin, a major flavonoid in the human diet	increased lifespan	increased reproductive capacity; enlarged body size	Surco-Laos et al. (2011)
Quercetin-3-*O*-glucoside	increase of mean lifespan by 23% by low concentrations, decrease of lifespan by high concentrations	N.D.	Dueñas et al. (2013)
Flavonoids myricetin, quercetin, kaempferol, naringenin	increased lifespan in wild-type worms	N.D.	Grünz et al. (2012)
Caffeic acid; rosmarinic acid	increased lifespan	reduced body size; altered lipid metabolism; delayed reproduction	Pietsch et al. (2011)
Flavanol catechin	increased lifespan	enhanced stress resistance; reduced body length	Saul et al. (2009)
Epigallocatechin gallate	increased lifespan	enhanced stress resistance	Abbas and Wink (2009)
Epigallocatechin gallate	increased lifespan under stress conditions; no effect under normal conditions	N.D.	Zhang et al. (2009a,b)
Ferulsinaic acid	increased lifespan	improved resistance for heat and oxidative stresses; attenuated lipid peroxidation	Sayed (2011)
EUK-8 and EUK-134, SOD/CAT mimetics	increased mean lifespan of wild-type worms by 44% and prematurely aging worms by 67%	N.D.	Melov et al. (2000)
Tyrosol, an olive oil phenol	increased lifespan	N.D.	Cañuelo et al. (2012)
KPG-7, herb mixture complex	increased lifespan	protection against oxidative stress	Moriwaki et al. (2013)
EUK-8	dose-dependent reduction of lifespan	dose-dependent reduction of fertility	Keaney and Gems (2003)
Platinum nanoparticles (nano-Pt), a SOD/CAT mimetic	increased lifespan	reduced accumulation of lipofuscin and ROS induced by paraquat	Kim et al. (2008)
N-acetyl-L-cysteine	increased lifespan	increased resistance to oxidative, UV and heat stresses	Oh et al. (2015)
Drosophila melanogaster			
Lipoic acid and resveratrol	increased lifespan	N.D.	Bauer et al. (2004)
Melatonin	increased lifespan	N.D.	Izmaylov and Obukhova (1999)
EUK-8 and EUK-134; MitoQ	increased lifespan in SOD-deficient flies; no effect in wild type flies	N.D.	Magwere et al. (2006)
SkQ1	increased lifespan	N.D.	Krementsova et al. (2012)
Epitalon	increase of mean lifespan by 11–16%	N.D.	Khavinson et al. (2000)
Carnosine, S,S-Trolox-carnosine, STC	20% increase of male mean lifespan, no effect on females; increase of male mean lifespan by 16%, female by 36%	N.D.	Stvolinsky et al. (2010)
Curcumin	increased lifespan	enhanced SOD activity	Suckow and Suckow (2006)
Curcumin	increased lifespan	reduced oxidative stress; improved locomotion; modulated expression of aging-related genes	Lee et al. (2010)
Curcumin	increased lifespan	increased SOD activity; reduced accumulation of malondialdehyde and lipid peroxidation	Shen et al. (2013)
Sesamin, a major lignan in sesame oil	increase of mean lifespan	up −regulation of SOD1, SOD2, CAT and Rpn11 genes; attenuation of neurodegeneration	Zuo et al. (2013)
Musca domestica			
EUK-8 and EUK-134	no effect on lifespan under normoxic conditions; shortened lifespan under hyperoxic conditions	N.D.	Bayne and Sohal (2002)
Mus musculus			
Vitamin E	increased median lifespan in C57BL/6 mice by 15%	anti-cancer effect via inducing the P21 signaling pathway	Banks et al. (2010)
Vitamin E	no effect on lifespan	N.D.	Morley and Trainor (2001)
Vitamin E	increase in mean but not maximum lifespan	lower fatal tumor incidence	Blackett and Hall (1981)
Tetrahydrocurcumin; polyphenol from green tea	increased survival in male C57BL/6 mice	N.D.	Kitani et al. (2007)
Commercial nutraceutical combinations	no or detrimental effect on lifespan	N.D.	Spindler et al. (2014)
Carboxyfullerene, SOD mimetic	increased lifespan;	reduced age-associated oxidative stress; rescued age-related cognitive impairment	Quick et al. (2008)
Alpha-lipoic acid	decreased lifespan in senescent prone SAMP8 mice	reduced oxidative stress and improved memory in old mice	Farr et al. (2012)
SkQ1	increased lifespan	prevented age-dependent disappearance of estrous cycles; retardation of age-linked immune decline	Anisimov et al. (2011a)

In this and subsequent tables, N.D. – not determined.

otics may promote longevity in *C. elegans* by killing pathogenic bacteria, and agents reducing egg-laying rate may increase lifespan in *Drosophila*. Life-extending effects of antioxidants may be also caused by non-specific (hormetic) response, a dose-response relationship phenomenon characterized by low-dose stimulation and high-dose inhibition (Calabrese et al., 2015; Rattan, 2014). Noteworthy, lifespan-extending effects were so far mainly observed in species that are evolutionarily distant from mammals. All life-extending strategies used till now, however, did not result in similar lifespan extension in mammals, where more complex control systems are apparently working over the aging process, probably requiring more elaborated interventions.

2.3. Melatonin

Among the agents with antioxidant activity, melatonin stands out due to its highly pleiotropic properties. It is a natural hormone produced predominantly by pineal gland in the brain and released into circulation in a pulsatile fashion with the sharpest peaks in the early morning hours (Claustrat and Leston, 2015). In addition to the direct free radical scavenging and indirect antioxidant effects, it demonstrates a variety of metabolic and physiological activities. It was shown to modulate activity of antioxidant and pro-oxidant enzymes thereby reducing the oxidative damage. These actions of melatonin are believed to be mediated by the Keap1-Nrf2-ARE pathway. This highly pleiotropic agent is also well known as circadian rhythm regulator, as well as a modulator and protectant of mitochondrial electron flux, a coregulator of metabolic sensing and antagonist of insulin resistance, immunoregulating and anti-inflammatory molecule, and oncostatic agent (Hardeland, 2015; Manchester et al., 2015). In addition, its effects are mediated by autophagy and regulation of some signaling pathways that influence energy metabolism, including insulin/IGF1, mammalian target of rapamycin (mTOR), and Akt and phosphoinositide 3 kinase (PI3K) signaling (Jenwitheesuk et al., 2014).

The role of melatonin in aging processes is evident from the observation that both aging and aging-related pathologies are linked to the loss of melatonin secretion and decline of circadian amplitude of the melatonin rhythm. Recently, it was also assumed that lifespan-promoting effects of melatonin could be attributed to activation of sirtuin 1 (SIRT1) (Ramis et al., 2015). Its potential aging-modulating properties were studied in a range of experimental models including *Drosophila melanogaster* and mice (Anisimov et al., 2006; Hardeland, 2013).

Findings from these studies are summarized in Table 2.

2.4. Antioxidant intake: contradictions, controversies and paradoxes

Findings from the research of antioxidant effects of dietary supplements collectively suggest that these effects could be explained by mechanisms other than antioxidant activity *per se*. To maintain the redox homeostasis, exogenous antioxidants act synergistically with endogenous antioxidant systems (Bouayed and Bohn, 2010). In several cases, the effects of antioxidant compounds may be linked not with their own antioxidant activity, but with inducing the endogenous antioxidant mechanisms; the intake of excess levels of exogenous antioxidants may, however, suppress the synthesis of endogenous antioxidant enzymes (Sadowska-Bartosz and Bartosz, 2014). This is an important point since a fine-tuned equilibrium among the oxidative and antioxidative processes is critical in maintaining homeostatic stability (Bouayed and Bohn, 2010). The excessive antioxidant supplementation can result in destroying the delicate control mechanisms of homeostasis thereby leading to deteriorating health and life shortening (Vaiserman, 2014). Moreover, since it is difficult to evaluate the quality and quantity of food consumed by experimental organisms, intake of high amounts of antioxidants may likely modulate their eating behavior and thereby result in malnutrition or even starvation (Le Bourg, 2001). If so, life-extending effects of exogenous antioxidants could be explained, at least partly, by dietary restriction (DR) known to dramatically slow the aging rate in various experimental models and delay the onset of age-associated diseases, such as neurodegenerative disease, cancer, cardiovascular disease and type 2 diabetes. Furthermore, life-promoting effects of antioxidants could be a by-product of other, rather adverse, effects such as reduced level of metabolic rate, lower weight and decreased rate of development (Le Bourg, 2001). This is all the more true that most substances with antioxidant properties are multi-functional. The mechanisms which are dominant in a certain situation are dependent on the particular biological conditions and those conditions may influence the kinetics and thereby the antioxidant activity. The ascorbic acid, e.g., may act, depending on conditions, as antioxidant, but also as oxygen scavenger, metal chelating agent or reducing agent (Frankel, 1996). The active substance in turmeric, curcumin, is another example. Although it is known to demonstrate great antioxidant activity, its lifespan-extending properties can also be caused by induction of exogenous antioxidant enzymes, or by interference with various cellular signaling pathways including apoptosis-associated and cell-cycle proteins, growth factors and their receptors, inflammatory cytokines, adhesion molecules, transcription factors such as a major transcription factor for inflammatory responses, NF-kappaB, AP-1 and STAT, as well as with several longevity-associated

Table 2
Summary of the effects of melatonin on the lifespan and associated functional traits of model organisms.

Model	Longevity effect	Associated functional traits	Reference
Drosophila melanogaster	increase of lifespan up to 19% in males and up to 12% in females	N.D.	Izmaylov and Obukhova (1999)
	13.5% increase in median lifespan; 33.2% increase in maximum lifespan;	increased resistance to paraquat and to heightened temperature (36° C)	Bonilla et al. (2002)
	27.8% decrease in mean lifespan, 25.4% increase in maximum lifespan	increased eclosion rate and the locomotor activity; decreased concentration of malondialdehyde	Terán et al. (2012)
Mus musculus	5.4% increase in mean lifespan of female CBA mice	decreased locomotor activity and body temperature; delay of the age-related switching-off of estrous function; 20% increase in malignant tumor incidence	Anisimov et al. (2001)
	10% increase in maximum lifespan of female SHR mice	delay of the age-related switching-off of estrous function	Anisimov et al. (2003a)
	17.4% increase in maximum lifespan of SAMP8 mice	normalized redox and bioenergetic status of the mitochondria and increased ATP levels	Rodríguez et al. (2008)

enzymes like mTOR, mitogen-activated protein kinase (MAPK) and protein kinase B (Akt) (Sadowska-Bartosz and Bartosz, 2014). Furthermore, antioxidants demonstrate inconsistent behavior in varying environments. Depending on their concentrations and on the nature of neighboring molecules, they can act as pro-oxidants (Carocho and Ferreira, 2013). In addition, while high ROS concentrations are hazardous and damage basic cellular components, evidence have been obtained that at proper concentrations they play a key mediator role in signaling processes such as signal transduction from membrane receptors and control of gene expression, as well as in cellular proliferation and differentiation, cell cycle regulation and autophagy stimulation (Juránek et al., 2013; Mao and Franke, 2013). Therefore, excessive antioxidant supplementation can lead to disruption of the pro/antioxidant balance.

Similarly to all hormesis-inducing agents (hormetins), antioxidants act in certain concentration range, and their higher concentrations are commonly toxic to most organisms. On the contrary, longevity hormesis may paradoxically be mediated by increased ROS formation (Mao and Franke, 2013). According to the hypothesis of 'mitohormesis', while high ROS level generated in the mitochondria can cause cellular damage and promote aging, their moderate level might induce an adaptive response thus improving the systemic defense mechanisms. This concept postulates that ROS act as important signaling molecules to promote health and lifespan, and that consumption of exogenous antioxidants may be worthless or even harmful (Ristow and Schmeisser, 2014). Based on these facts, Bouayed and Bohn (2010) stated in their review that intake of antioxidants causes double-edged effects depending on their concentrations: physiologic doses lead to beneficial effects while high doses cause harmful effects.

Given these contradictions, it is not surprising that conflicting data were obtained on the health consequences of long-term antioxidant intake in human populations. Oxidative macromolecular damage is known to be significantly involved in the etiology of age-related disorders including metabolic, cardiovascular and neurodegenerative diseases, as well as cancer (Richardson and Schadt, 2014). Several authors therefore suppose that enhancement of the antioxidant defense systems may be valuable in reducing oxidative stress and risk for aging-associated pathological conditions. Indeed, it has been found in animal studies that intake of exogenous antioxidants can reduce the progression of aging-related pathologies such as Parkinson disease, amyotrophic lateral sclerosis and heart failure (Jung et al., 2001; Kawakami et al., 2009; Makino et al., 2011; Peng et al., 2005; Redout et al., 2010; van Empel et al., 2006). Applying that idea to humans, however, appeared rather problematic. Most clinical trials failed to show any clinical benefits of antioxidant therapy for aging-related disorders such as cardiovascular disease (Kris-Etherton et al., 2004; Sahebkar et al., 2015), stroke (Schürks et al., 2010), and type 2 diabetes (Sheikh-Ali et al., 2011; Suksomboon et al., 2015). This controversy could likely be related to the excessive antioxidant intake or to insufficient antioxidant bioavailability in relevant organs to reduce oxidative damage (Doss, 2012).

The excessive levels of exogenous antioxidants can disturb endogenous signaling mechanisms and thereby might be deleterious (Halliwell, 2011). So, the use of multivitamins more than seven times per week has been demonstrated to be related to a significantly enhanced risk for prostate cancer in comparison to never users (Lawson et al., 2007). Dietary intake of vitamins C and E combined precluded the health-promoting effects of exercise in the Ristow et al. (2009) study. A substantial increase in lung cancer diagnosis has been found in those Finnish heavy smokers who were at high risk for lung cancer and who took the beta-carotene supplement relative to those taking a placebo (Albanes et al., 1996). An increased incidence of lung cancer and elevated risk of death from lung cancer, cardiovascular disease, and any cause were found in both heavy smokers and workers exposed to asbestos taking the combination of beta carotene and vitamin A (Omenn et al., 1996). In meta-analyses of randomized controlled trials and observational studies in healthy and well-nourished populations, antioxidant supplementation was repeatedly shown to be associated with disadvantageous health consequences (Bjelakovic et al., 2014; Curtis et al., 2014; Dolara et al., 2012). Collectively, these findings demonstrate that supplementation with antioxidants can not always be beneficial to the human health status (Bast and Haenen 2013; Liu, 2014).

The contradiction lies in the fact that ROS are involved in chronic human disorders, but intake of large doses of dietary antioxidants leads to little or no preventive effect in most cases. Currently, this contradiction is commonly referred to as 'antioxidant paradox'. Based on that, some authors suggest that manipulation of endogenous antioxidants level (e.g., by intake of mild pro-oxidants) is likely a more appropriate approach for prevention and treatment of ROS-mediated diseases than the consumption of large doses of dietary antioxidants (Halliwell, 2013). While benefits of dietary antioxidant intake seem to be clear in case of enhanced oxidative stress and endogenous antioxidant deficiency, further research is required to determine the potential risks and benefits linked to the supplement of antioxidants by healthy persons.

In summary, it should be noted that a body of recent evidence raised serious doubts whether the oxidative damage theory remains still valuable for understanding the processes underlying aging and longevity (Gladyshev, 2014; Liochev, 2015; Speakman and Selman, 2011). One example is the research on mice with knockout of antioxidant genes. These mice exhibited expected rise of oxidative damage but any impact on longevity (Zhang et al., 2009a,b). Some authors claim that the oxidative damage theory of aging is fundamentally wrong, though this has been a topic of intense debate within the field over the past decade. This theory, however, still has many adherents and needs to be decisively verified in further studies.

3. Calorie restriction mimetics

Current doubts about the efficiency of antioxidant consumption led to a growing interest in alternative healthspan-promoting interventions. Among them, DR- or, more specifically, calorie restriction (CR)-based strategies are likely the most hopeful now. CR refers to a reduced calorie intake without malnutrition in animals normally fed *ad libitum* (Masoro, 2005). Generally, CR is a diet providing all essential nutrients, vitamins and minerals but having 30–70% reduced amount of calories.

Presently, DR is the most well-established experimental procedure for life extension, although some authors suggest that the restriction of specific dietary components such as protein and particular amino acids like methionine can play a crucial role (Fontana and Partridge, 2015). The ability of CR to promote lifespan and slow aging have attracted significant scientific attention since the pioneering work conducted by McCay and colleagues eighty years ago (McCay et al., 1935). In this research, it has been shown that rats subjected to 40% CR had up to 50% longer median and maximum lifespans than rats fed a standard diet. Over the years, a number of studies have shown that DR can retard aging and extend lifespan in various experimental models including yeast, worms, insects and rodents (Ingram and Roth, 2015; Selman, 2014; Testa et al., 2014). More recently, evidence has been obtained that DR can lead to a delayed onset of age-associated pathologies in non-human primates such as rhesus monkeys (Colman et al., 2009; Kemnitz, 2011; Mattison et al., 2012). Specifically, DR leads to reduced levels of body fat, slower rate of aging-related muscle loss, reduced incidence of cardio-vascular disease, type 2 diabetes and cancer,

as well as improved glucose tolerance and insulin sensitivity. In addition, DR resulted in reducing the age-related mortality of monkeys (Colman et al., 2014). Accumulating data demonstrate that moderate DR may reduce the risk of cancer, and also cause powerful protective effect against inflammation, hypertension, obesity, type 2 diabetes and cardio-vascular disease in humans (Cava and Fontana, 2013; Holloszy and Fontana, 2007).

However, although DR was shown to clearly reduce risk factors associated with human aging, it is still a subject of debate whether it may increase the human life expectancy. One challenge of the DR model is that the DR-mediated lifespan extension is not universal and can not even be shared between various strains of the same species. In addition, some authors suggest that control animals which fed *ad libitum* are overweight and tend to have associated health problems, and thus they could not be appropriate control animals for longevity studies. Increasing evidence suggest that DR can promote lifespan of genotypes that develop energy imbalance through the *ad libitum* feeding (Sohal and Forster, 2014). In such groups, DR results in decrease of body temperature, metabolic rate, and oxidant production and retardation of the aging-associated pro-oxidative shift of the redox state. Nevertheless, despite these challenges and limitations, the DR concept continues to be one of the leading paradigms in modern gerontology (Ingram et al., 2006).

The beneficial effects of DR were found to be mediated by the same pathways which are also involved in regulating immunity, tissue repair, metabolism, thermoregulation and appetite (Le Couteur et al., 2012). These effects of DR likely result not from passive metabolic changes, but rather from highly regulated processes associated with activation of specific effectors (Mouchiroud et al., 2010). Adenosine monophosphate (AMP)-activated protein kinase (AMPK), the insulin/insulin-like growth factor signaling (IIS) and mTOR pathways, as well as sirtuins, especially SIRT1 are the basic nutrient-sensing pathways related to the control of the lifespan by DR. These pathways are known to be crucial regulators of mitochondrial function, cell growth, proliferation and autophagy, and are also known to be regulated by crosstalk with one another. Importantly, the long-term DR was shown to be necessary to produce beneficial effects on healthspan and lifespan. It is apparently problematic for most modern people. Thus, such interventions may not be readily applicable.

The doubt on the applicability of DR-based treatments has generated the growing interest in developing alternative treatment strategy which can provide pro-healthspan and pro-longevity DR benefits without the substantial reduction in the long-term food intake. Compounds used in this treatment strategy are referred to as CR mimetics. Basic characteristic properties of CR mimetics are as follows: (1) inducing the hormonal, metabolic and physiological effects similar to those of CR; (2) activation of the stress response pathways like the CR; (3) lifespan extension and reducing the incidence or delaying the onset of age-associated disorders (Ingram et al., 2006).

The main signaling pathways mediating the CR effects and the most promising pharmacological substances modulating these pathways and thus mimicking the CR effects are reviewed in the subsections below.

3.1. Inhibitors of glycolytic metabolism

Hyperinsulinemia and hyperglycemia are widespread in old age. Age-associated metabolic disorders such as type 2 diabetes, which are accompanied by disturbance in glucose homeostasis and insulin signaling, are known to produce various aging-like phenotypes. Untreated diabetes patients commonly have enhanced glucose levels and they are more susceptible to chronic disorders such as vascular and microvascular damage, heart disease, kidney problems, impaired wound healing and cataracts, and neurological degeneration, which typically occur in non-diabetics much later in life (Anisimov, 2013; Bonomini et al., 2015).

Oxidative stress induced by hyperglycemia is known to promote the generation of advanced glycosylation end products (AGEs), thus substantially accelerating the aging rate (Anisimov, 2013). The accumulation of AGEs has been found to be enhanced in persons with type 2 diabetes and this parameter was proposed as a reliable biomarker for both aging rate and 'metabolic memory' (the persistence of adverse effects of prior hyperglycaemia on the onset and progression of diabetic vasculopathies even after the normalization of glucose levels) (Rajaobelina et al., 2015; Sato et al., 2001). Furthermore, hyperinsulinemia, a marker of insulin resistance that also shown to increase with age is a substantial causal factor in the cancer etiology (Gristina et al., 2015).

In a number of studies, CR has been demonstrated to reduce the levels of both glucose and insulin, and to improve the insulin sensitivity (Anisimov, 2003). Therefore, the inhibition of enzymes of the glycolytic pathway is believed to be a promising strategy to produce CR-like effects (Longo et al., 2015). In this pharmacological category, an analog of glucose 2-deoxy-D-glucose is the most studied candidate agent (Minor et al., 2009). In the course of glycolysis, 2-deoxy-D-glucose is converted by hexokinase to 2-deoxyglucose 6-phosphate that may in turn inhibit phosphoglucose isomerase and imped processing of glucose to fructose 6-phosphate. Owing to these properties, the capability of 2-deoxy-D-glucose to affect glycolysis has been investigated for several decades in a variety of model organisms from bacteria to mammals. In these studies, the therapeutic benefits of this preparation for treating viral infection, epilepsy and cancer have been revealed (Xi et al., 2014). It is considered to be especially promising in anti-cancer therapy. Indeed, tumor cells exhibit elevated glycolysis and use this metabolic pathway for generating ATP as a primary source of the energy supply. The enhanced dependence of malignant cells on glycolytic pathway for the generation of ATP could provide a mechanistic basis for the development of novel therapeutic strategies to preferentially kill cancer cells by the pharmacological inhibition of glycolysis (Ganapathy-Kanniappan and Geschwind, 2013; Ingram and Roth, 2010).

2-Deoxy-D-glucose has also been found to reproduce some aspects of the CR phenotype including reduced levels of circulating insulin and body temperature in rodents, whereas its apparent toxic effect has been demonstrated until now only in one chronic feeding research (Minor et al., 2009). In this study, CR-like phenotype has been revealed in rats treated with this medication, such as reduced dietary intake and decreased weight gain. These changes have been accompanied, however, by cardiac vacuolization and increased mortality in treated rats. The potential toxicity of 2-deoxy-D-glucose leads to serious doubts in the CR-based therapeutic application of this preparation.

3.2. Inhibitors of GH/IGF-1 pathway

Among the signaling pathways linked to DR, insulin/insulin-like growth factor-1 (IGF-1) pathway is known to play a crucial role in the lifespan regulation. The bulk of the research findings suggests that CR, as well as intermittent fasting and protein or amino acid restriction, all can extend the mammalian lifespan via modulating the IGF-1 signaling (Taormina and Mirisola, 2015). Several polymorphic variants of the IGF-1 receptor gene have been found to be linked to the human longevity (Pawlikowska et al., 2009), in particular, to the exceptional lifespan of centenarians (Suh et al., 2008). In addition, low circulating IGF-I bioactivity was shown to be associated with longevity of the exceptionally long-lived individuals (Milman et al., 2014), and their offspring (Vitale et al., 2012). The long-lived growth hormone (GH) receptor knockout Laron dwarf mice (GHR−/−) have been found to be insulin sensitive despite

obesity, and were also shown to have substantially reduced IGF-1 levels and decreased risks of cancer and diabetes (Zhou et al., 1997; Ikeno et al., 2009). The Laron syndrome patients, who have reduced IGF1 levels despite normal or elevated levels of GH, have been similarly found to be protected from cancer and diabetes (Guevara-Aguirre et al., 2011; Steuerman et al., 2011). As a consequence, it was suggested that treatments targeted at reducing IGF-1 levels may be a promising pharmacological strategy for the extension of healthspan.

Recently, inhibitors of the GH/IGF-1 axis have been proposed for the treatment of patients with acromegaly, a disease caused by GH/IGF-I hypersecretion and associated with a high mortality rate (Giustina et al., 2014). Such medications include, e.g., somatostatin analogues which may reduce the GH serum levels via suppressing GH secretion by pituitary somatotrophs, thus resulting in reduced serum IGF-1 levels. Such therapy, however, can potentially cause severe unfavorable effects since such agents may suppress secretion of some hormones including insulin.

Indeed, the adverse health consequences such as diarrhoea, anorexia and gallstones, were revealed in 20–50% of treated acromegalic patients. Another medicine from that pharmacological class is the GH receptor antagonist pegvisomant known to inhibit GH secretion and GH action by binding to and blocking the GH receptor (Kopchick et al., 2002; Kopchick, 2016). In patients with acromegaly treated with pegvisomant, a significant (up to 90%) dose-dependent decrease of IGF-1 levels was revealed (van der Lely and Kopchick, 2006). Pegvisomant is believed to be a promising agent for healthspan and lifespan extension because it led to beneficial effects on the glucose metabolism by lowering serum IGF-1 levels and increasing the insulin sensitivity. However, even though no harmful effects have been reported in the long-term research of pegvisomant (van der Lely et al., 2012), further research is needed to prove its safety. Moreover, this medication requires daily injections and is very expensive (Higham et al., 2009).

Modulating the IGF-1 availability could be another way to inhibit IGF-1 action. For that, pharmaceuticals modulating the pregnancy-associated plasma protein-A (PAPP-A), a zinc metalloproteinase known to enhance the local bioavailability of the IGFs, have been developed. Such medications are believed to be promising as PAPP-A knockout mice have been previously found to have substantially extended healthspan, suggesting an important role of PAPP-A in aging and associated disease risk (Conover, 2010, 2013). Healthspan-promoting effects of these medications, however, have not been examined yet and are the subject of further research.

Recently, a high life-extending potential of modulating the insulin/IGF-1 pathway was reported by Slack et al. (2015). In this study, a role for Ras-Erk-ETS pathway, implicated in the effects of reduced insulin/IGF-1 signaling, was identified. Specifically, the adult-onset supplementation with an FDA-approved medication for the treatment of melanoma, trametinib, which is a highly specific inhibitor of Ras-Erk-ETS signaling, caused significant life extension in *Drosophila*.

3.3. Pharmacological inhibition of mTOR pathway

The mammalian target of rapamycin (mTOR) pathway is another nutrient-sensing pathway which can play a role in mediating advantageous effects of CR on the aging rate and longevity (Ehninger et al., 2014). mTOR is a serine/threonine protein kinase evolutionarily conserved from yeast to human in the catalytic domain and known to be implicated in cell growth, metabolism and regulation of energy homeostasis (Johnson et al., 2015). Within the cell, it exists as a part of two multiprotein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (Laplante and Sabatini, 2012). The mTORC1 regulates important cellular processes including transcription, translation and autophagy (Inoki et al., 2012). The mTORC1 was also found to be the main mTOR complex involved in lifespan regulation (Kaeberlein, 2014). In the presence of sufficient amounts of nutrients, growth factors and energy availability, mTOR switches off stress resistance and autophagy, and switches on cell growth pathways, including translation and ribosomal biogenesis (Kapahi et al., 2010). Since it has been demonstrated in lower animal models that decreasing protein synthesis can increase lifespan (Hipkiss, 2007), inhibition of protein synthesis by reducing the activity of mTOR pathway can likely be involved in life-extending effects across species. In several studies, it was shown that mTOR can be activated by single amino acids. It may provide a causal mechanism for recent findings that decrease in the proportion of protein in the diet, rather than CR *per se*, can cause lifespan-promoting effects in various experimental models (Lee, 2015; Solon-Biet et al., 2014, 2015). In addition, mTOR signaling was shown to play a central role in mediating aging and associated metabolic disorders, including obesity, type 2 diabetes and cancer (Albert and Hall, 2015). Therefore, mTOR is supposed to be a leading target for pharmacological intervention to modulate the nutrient response pathways and to decelerate the aging process. Inhibition of this pathway has been repeatedly demonstrated to confer protection against aging-associated pathological conditions and to extend lifespan in different model organisms (Johnson et al., 2013). Genetic inhibition of mTOR signaling caused life extension in worms (Jia et al., 2004; Vellai et al., 2003), fruit flies (Kapahi et al., 2004), and mice (Lamming et al., 2012). Recent gene expression analysis showed that mTOR pathway is strongly associated with human health and longevity (Passtoors et al., 2013).

3.4. Rapamycin

Rapamycin (also known as sirolimus), a natural compound produced by bacteria *Streptomyces hygroscopicus* that was first found in an Easter Island soil sample around 1970, is the most widely used inhibitor of mTOR. It has been initially developed as an antifungal agent but has subsequently been found to have substantial regulatory effects on crucial biological processes such as cellular growth, proliferation, and inflammation via its inhibitory action on mTOR (Lamming et al., 2013). Since rapamycin was shown to inhibit the immune response, it was subsequently used in immunosuppressive therapy to prevent graft rejection and to treat autoimmune disorders (Ingle et al., 2000). Currently, rapamycin and its analogs (rapalogs), e.g., temsirolimus and everolimus, are suggested to be among the most promising anti-aging drugs (Blagosklonny, 2007). Rapamycin therapy has also been revealed to delay aging-related changes in mice, including tendon stiffening, accumulation of subcellular alterations in the myocardium, endometrial hyperplasia, liver degeneration, and decline in physical activity (Wilkinson et al., 2012). Furthermore, it proved to be efficient against aging-linked pathologies including cognitive decline, retinopathy, neurodegenerative disorders, cardiac hypertrophy, and loss of stem cell function (Chen et al., 2009; Halloran et al., 2012; Kolosova et al., 2012; Shioi et al., 2003; Spilman et al., 2010).

Life-extending properties of rapamycin have been examined in many studies from yeast to mammals. A summary of these studies is presented in Table 3.

The potential mechanisms of rapamycin-induced life extension are assumed to include antitumor effect (Blagosklonny, 2012; Saran et al., 2015), autophagy induction (Perluigi et al., 2015), stem cell guidance (Maiese, 2015), as well as immune-modulating and anti-inflammatory activity (Araki et al., 2011). In addition, these effects can be mediated by the reduction of the rate of protein synthesis, because the decrease of the overall translation rate may prevent the accumulation of damaged or misfolded proteins which might in turn affect lifespan (Hipkiss, 2007). The mTOR was also shown to be negatively regulated by other nutrient-sensing pathways such

Table 3
Summary of the effects of rapamycin on the lifespan and associated functional traits of model organisms.

Model	Longevity effect	Associated functional traits	Reference
Saccharomyces cerevisiae	extension of chronological lifespan up to 54%	N.D.	Powers et al. (2006)
	15% increase in replicative lifespan	N.D.	Medvedik et al. (2007)
	19% increase in replicative lifespan	N.D.	Ha and Huh (2011)
Cenorhabditis elegans	19% increase in mean lifespan	N.D.	Robida-Stubbs et al. (2012)
Drosophila melanogaster	extension of female lifespan up to 17% and 23% (median and maximum, respectively) on rich media and up to 54% and 36% during starvation	increased resistance to starvation and paraquat stress	Bjedov et al. (2010)
	standard diet: increase in the male mean lifespan on 6% and increase in the female mean lifespan on 26%; low-calorie diet: decrease in the male mean lifespan on 12% and increase in the male mean lifespan on 57%	N.D.	Sun et al. (2012)
Mus musculus	14% increase in 90% mortality for female and 9% for male genetically heterogeneous mice fed rapamycin beginning at 600 days of age heterogeneous mice	N.D.	Harrison et al. (2009)
	increased mean lifespan (male: 10%; female: 18%) in genetically heterogeneous mice fed rapamycin from the age of 9 months	attenuating age-associated decline in spontaneous activity in males but not in females	Miller et al. (2011)
	increase of mean lifespan of genetically heterogeneous mice by 23% (males) to 26% (females)	N.D.	Miller et al. (2014)
	11% increased maximum lifespan in females, prevention of age-related weight gain	decreased rate of aging and suppressed carcinogenesis in transgenic HER-2/neucancer-prone mice	Anisimov et al. (2010a)
	8% increased maximum lifespan,	inhibited age-related weight gain, decreased aging rate, and delayed spontaneous cancer in inbred females	Anisimov et al. (2011b)
	improved survival of C57BL/6 mice after the late-life rapamycin feeding	reduced total and resting metabolic rate during the light (inactive) phase	Zhang et al. (2014)
	increased median lifespan in male C57BL/6J mice	N.D.	Neff et al. (2013)

as the AMPK and SIRT1 pathways (Cetrullo et al., 2015). Rapamycin treatment was demonstrated to inhibit both mTORC1 and mTORC2. Its life-extending effects, however, are mediated by the inhibition of TORC1 activity only.

Remarkably, the rapamycin treatment starting in later life may be sufficient to extend lifespan; similar benefits were also achieved by transient late-life treatments (Kaeberlein, 2014). In mice studies, rapamycin supplementation initiated late in life (20 months of age) was nearly as efficient as if beginning it at 9 months of age (Harrison et al., 2009; Miller et al., 2011). Late-life transient rapamycin treatment was also demonstrated to reverse the age-linked heart dysfunction, and also caused the beneficial motor, skeletal and behavioral changes in 24-month-old female C57BL/6J mice (Flynn et al., 2013). The benefits of a mid- or late-life rapamycin therapy seem particularly promising since such interventions are preferable when considering potential translation of anti-aging therapies to humans (Kaeberlein, 2014). Currently, some inhibitors of mTOR pathway are already clinically approved, and others are under development.

There are some concerns, however, on the applicability of rapamycin in anti-aging therapy. In the study by Wilkinson et al. (2012), treating with rapamycin starting at 9 months of age resulted in significantly higher incidence of testicular degeneration and cataracts in mice. It has been also found that rapamycin-induced mTORC2 suppression can cause insulin resistance and impaired glucose homeostasis through adverse effect on hepatic gluconeogenesis (Lamming et al., 2012). Side effects of chronic rapamycin administration such as hyperglycemia, glucose intolerance and insulin resistance, have been revealed in some rodent studies (Deblon et al., 2012; Houde et al., 2010). Some side effects were also observed in clinical trials with cancer patients in which rapamycin was used as a monotherapy (Richardson, 2013). These effects included metabolic abnormalities such as decreased insulin sensitivity, glucose intolerance, hyperlipidemia, hypertension, and enhanced incidence of new-onset diabetes (Lamming et al., 2013), as well as anaemia, diarrhoea, skin rash, thrombocytopenia, stomatitis, and malignancies (lymphoma and skin cancers) (Lamming et al., 2012; Zafar et al., 2009). The rapamycin-induced metabolic impairments were demonstrated, however, to be fully reversible. In both obese and lean mice, those effects have been found to be almost completely leveled within a few weeks of rapamycin cessation (Liu et al., 2014b).

Currently, the most serious concerns on the clinical applicability of rapamycin are related to its immunosuppressive properties. Even though rapamycin and other rapalogs are widely useful in cancer prevention and therapy, the fear of cancer is the main concern about their clinical application (Blagosklonny, 2013). Therefore, these drugs should be used cautiously to avoid potentially dangerous levels.

3.5. AMPK pathway

mTORC1 complex is regulated by cellular energy levels through AMPK pathway, which is another key target in CR-based therapies. This pathway maintains the cell energy balance by modulating the ATP production. AMPK is a cellular energy sensor that monitors the AMP/ATP ratio. The reduced ATP concentrations cause the increase of AMP levels and AMPK activation (Hardie, 2015). Recent studies show that adenosine diphosphate (ADP) is also significantly contributed to AMPK activation (Gowans and Hardie, 2014). Activated

AMPK increases the cellular ATP production and decreases the ATP consumption by shutting down energy-consuming processes such as the mTOR-dependent protein translation (Towler and Hardie, 2007). Upon activating, AMPK switches on catabolic pathways that generate ATP, both in a short time frame by promoting glycolysis and fatty acid oxidation and in a long time frame by enhancing mitochondrial content and using mitochondrial substrates as an energy source, and switches off ATP-consuming processes including cell growth, biosynthesis and proliferation (Cantó and Auwerx, 2010). All these processes occur to respond to the metabolic stress at the cellular level. Cytokines and hormones such as leptin, insulin and adiponectin interact with this system, and these processes likely play an important role in maintaining energy balance at the whole-body level. AMPK responds to energy stress by suppressing cellular growth and biosynthetic processes, partially through inhibiting the mTORC1 pathway (Gwinn et al., 2008; Hindupur et al., 2015). Moreover, AMPK activation results in enhanced glucose uptake in skeletal muscles, and in decreased hepatic glucose production and increased fatty acid oxidation in various tissues (Ruderman et al., 2013). Consistent data indicate that AMPK inhibits mTOR through the phosphorylation and activation of the tuberous sclerosis complex (TSC), and also through direct phosphorylation of the RAPTOR subunit of mTORC1 (Potter et al., 2010).

Recently, the tumor suppressor LKB1 was identified as the major upstream kinase in the AMPK cascade in mammalian cells (Hardie et al., 2012). So, AMPK is a key player in control of energy homeostasis at both cellular and whole-body levels through the regulation of mitochondrial biogenesis and insulin sensitivity, as well as in the processes of autophagy and stress resistance (Ha et al., 2015). Therefore, it seems promising in development of novel treatments for obesity, type 2 diabetes and metabolic syndrome. It was also demonstrated to be the key mediator and integrator of processes linking metabolism to longevity (Burkewitz et al., 2014; Salminen and Kaarniranta, 2012). Because changes in AMPK signaling result in modulating key anti-aging pathways such as the peroxisome proliferator-activated receptor (PPAR) gamma coactivator a (PGC-1a), SIRT1 and TOR (Verdaguer et al., 2012), this pathway is supposed to be a promising drug target for health- and lifespan extension.

3.6. Metformin

Several drugs demonstrating the AMPK-activating properties have been recently developed. These substances include some FDA-approved drugs including biguanides, resveratrol, thiazolidinediones, salicylates, glucagon-like peptide-1 receptor agonists etc (Coughlan et al., 2014). Among them, antidiabetic biguanide metformin (1,1-dimethylbiguanide hydrochloride), a medication derived from French lilac, is currently in the focus of research activity. Metformin is known to specifically inhibit the hepatic gluconeogenesis without enhancing insulin secretion, inducing weight gain and posing a risk of hypoglycaemia (Madiraju et al., 2014; Moreira, 2014). Therefore, it is considered as one of the most effective preparation for treating type 2 diabetes. It may also act as a CR mimetic since it can decrease hepatic glucose production, mostly through mild inhibition of the mitochondrial respiratory-chain complex 1 (Viollet et al., 2012). In experimental studies, treatment with metformin and other biguanides, including phenformin and buformin, caused life extension in many animal models such as nematode worms, fruit flies and rodents, although findings have been contradictory (Table 4).

Evidence for beneficial effects of metformin has been provided in human studies as well. For example, significant improvement in total cholesterol, body mass index, low-density lipoprotein cholesterol, and non-high density lipoprotein cholesterol was observed in children with metabolic syndrome treated with metformin (Luong et al., 2015). In both diabetic and non-diabetic patients, anti-cancer effect of metformin has been also revealed (Coperchini et al., 2015). Metformin is assumed to directly affect tumor cells, primarily influencing the mitochondrial respiratory chain and, thereby, activating AMPK.

It is far from clear, however, to what extent the beneficial effects of metformin are mediated by activation of AMPK. AMPK-independent pathways can also be involved in both beneficial and unfavorable effects of this drug (Zheng et al., 2015). These pathways are believed to include suppression of glucagon signaling, inhibition of mitochondrial shuttles, induction of autophagy and mitochondrial stress, attenuation of inflammasome activation, reduction of terminal endoplasmic reticulum stress and changes in intestinal microbiota (Hur and Lee, 2015). Remarkably, microarray analysis showed marked similarity between gene expression patterns induced by both metformin and CR (Dhahbi et al., 2005).

By arguing that CR and its mimetics may promote their beneficial effects through various signaling pathways, Ingram and Roth (2015) have proposed to apply the “cocktails” of CR mimetics to contemporaneously affect multiple pathways. One example of such treatment strategy would be the usage of the metformin to block the glucose dysmetabolism caused by rapamycin treatment via inhibiting the gluconeogenesis by downregulation of expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (Mendelsohn and Larrick, 2012). This combined approach would likely be more secure and provide a more balanced therapeutic strategy, especially in healthy subjects.

4. Pharmacological induction of autophagy

Autophagy is a process by which defective or damaged cellular components are transferred into the lysosomes for degradation and recycling; then they may be used for protein synthesis and cellular repair (Gelino and Hansen, 2012). Autophagy is commonly classified into three major categories depending on the mode of delivery of cellular material to lysosomes: macroautophagy, microautophagy and chaperone-mediated autophagy (Jia and Sowers, 2015). Among them, macroautophagy (usually referred to as ‘autophagy’) is the most studied form of autophagy. It is a degradative pathway by which aggregated/misfolded proteins and organelles are engulfed within double-membrane vesicles (autophagosomes) and then delivered to lysosomes for degradation.

For decades, this process has been regarded as a largely unspecific and destructive, contributing to autophagic cell death. Recent research, however, has revealed important cytoprotective functions of this pathway including cellular differentiation, fuel utilization and protection against cell death (Martinez-Lopez et al., 2015). The cytoprotective effects of autophagy are commonly attributed to the digestion of potentially harmful intracellular structures, including aggregates of misfolded dysfunctional proteins and damaged organelles (Martins et al., 2011; Morselli et al., 2009). Moreover, cytoprotection by autophagy may be attributed to buffering of cellular stress in conditions of fluctuating nutrient availability (Madeo et al., 2015).

On the organismal level, these effects can be mediated by immune regulation, suppression of carcinogenesis and hormetic responses (Madeo et al., 2015). Energy resources mobilized through autophagic cytoprotective response allow cell to cope with stresses like genotoxic stress, amino acid and/or glucose deprivation, hypoxia and viral infection (Filomeni et al., 2015; He and Klionsky, 2009). As a consequence, autophagy enhances cellular survival, thereby improving cellular fitness, while inhibiting this pathway may cause bioenergetic failure and cell death (Tasdemir et al., 2008). The induction of autophagy is assumed to be contributed

Table 4
Summary of the effects of biguanides on the lifespan and associated functional traits of model organisms.

Model	Biguanide	Longevity effect	Associated functional traits	Reference
Caenorhabditis elegans	Metformin	extended median lifespan	slowed lipofuscin accumulation, prolonged youthful locomotory ability	Onken and Driscoll (2010)
	MetforminPhenformin	increase of mean lifespan by 18%, 36%, and 3% in worms treated with doses of 25, 50, and 100 mM, respectively; increase of mean lifespan by 5%, 21%, and 26% in worms treated with doses of 1.5, 3, and 4.5 mM, respectively	altered microbial folate and methionine metabolism	Cabreiro et al. (2013)
	Metformin	significant life extension	45% increase in respiration and 38% increase in metabolic heat production	De Haes et al. (2014)
Drosophila melanogaster	Metformin	males: no effect on lifespan of flies supplemented with 1–50 mM, decreased lifespan at 100 mM; females: no effect on survival of flies supplemented with 1–10 mM, dose-dependent decrease in lifespan at doses above 10 mM	activation of AMPK and reduced lipid store	Slack et al. (2012)
Mus musculus	Metformin	C57BL/6 strain: 5.83%, increase of mean lifespan in males treated with 0.1%; 14.4% decrease of mean lifespan in mice treated with 1%; B6C3F1 strain: 4.15% increase of mean lifespan in males treated with 0.1%	N.D.	Martin-Montalvo et al. (2013)
	Metformin	8% increase of mean lifespan in female transgenic HER-2/neu mice supplemented with 100 mg/kg	decreased food consumption; slowdown of the age-related rise in blood glucose and triglycerides level, as well as the age-related switch-off of estrous function	Anisimov et al. (2005)
	Metformin	Increase of mean lifespan up to 37.8% and maximum lifespan up to 10.3% in female outbred SHR mice supplemented with 100 mg/kg; slowdown the age-related switch-off of estrous function	N.D.	Anisimov et al. (2008)
	Metformin	8% increase of mean lifespan in transgenic HER2/neu mice supplemented with 0.5 mg/ml	increased latency of mammary adenocarcinoma by 13.2%	Anisimov et al. (2010b)
	Metformin	13.4% decreased mean lifespan of inbred 129/Sv male mice treated with 100 mg/kg; no significant effect on female lifespan	3.5 times decreased incidence of malignant neoplasms in female mice	Anisimov et al. (2010c)
	Metformin	14% increase of mean lifespan in mice treated started at the age of 3 months; no effects on longevity in mice treated starting from the ages of 9 or 15 months	decreased body temperature and postponed age-related switch-off of estrous function in female outbred SHR mice; delayed detection of the first tumor by 22% and 25% in mice treated since the ages of 3 and 9 months, respectively	Anisimov et al. (2011c)
	Phenformin	Increase of mean lifespan of female C3H/Sn mice by 21.1% and the maximum lifespan by 26%.	N.D.	Anisimov et al. (2003b)
Rattus norvegicus	Buformin	9% increase of mean lifespan in LIO rats	1.6-fold reduction in cumulative incidence of spontaneous tumors	Anisimov (2001)
	Metformin	no effect on lifespan in Fischer-344 rats	reduced body weight	Smith et al. (2010)

to the beneficial effects of fasting and hormetic effects induced by low doses of radiation, toxins and other stressors (Martins et al., 2011; Moore et al., 2015; Szumiel, 2012). It may also counteract the age-related accumulation of damaged proteins and organelles. Since the capacity of the cell to autophagic degradation declines with age, it is not surprising that autophagy has a strong impact on pathological processes common to the elderly such as glucose intolerance, excessive lipid accumulation, neurodegeneration, sarcopenia, and cardiac malfunction (Martinez-Lopez et al., 2015). Autophagy-related genes (ATGs) have been demonstrated to be substantially implicated in the life-extending effects reported in fruit flies, nematodes and mice (Yamaguchi and Otsu, 2012). In experimental models, autophagy has been shown to be triggered by genetic manipulations similar to that required to extend longevity, such as mutations in signaling pathways like TOR, SIRT1 and insulin/IGF-1 (Gelino and Hansen, 2012; Morselli et al., 2010a). Moreover, life extending effects were often accompanied by autophagy in experimental studies (Madeo et al., 2015). Although biological mechanisms underlying the role of autophagy in health and disease are not clear yet, there are some plausible explanations for that. Enhanced autophagy may improve the stress resistance of the cell by elevating its metabolic buffering ability or, alternatively, it may enhance the organelle turnover, thereby preventing the accumulation of damaged or old organelles (Levine and Kroemer, 2009). Moreover, the reduced autophagy may contribute to cancer progression. The increased autophagy levels are characteristic to cancerous cells where the availability of oxygen and nutrients is restricted, and this represents a mechanism of cell survival aimed to overcome the cellular stress and drug-induced cytotoxicity (Tucci, 2012).

Pharmacological induction or suppression of autophagy may cause therapeutic effects, depending on the pathophysiological context, through promoting either survival or death of particular cell groups that can be critically important to prevent and treat chronic diseases including cancer (Cheng et al., 2013). Modulating the autophagy activity by targeting specific components of its molecular machinery may affect the onset and progression of chronic pathological processes, thereby representing a promising pharmacological target in treatment of age-related disorders. Recent findings indicate that induction of autophagy may mediate both DR- and drug-induced life extension in various experimental models (He et al., 2013). In yeast, rapamycin was not able to extend the chronological lifespan in mutants lacking the essential autophagic genes, *ATG1* and *ATG7* (Alvers et al., 2009). Role of autophagy in life extension by rapamycin was also demonstrated in *Drosophila melanogaster* (Bjedov et al., 2010).

Autophagy is also believed to mediate health-promoting effects of resveratrol. It is suggested that this substance increase the level of SIRT1 that is a key regulator of both autophagy and longevity. For example, resveratrol caused life extension in the autophagy-proficient nematodes in conditions of CR. This effect, however, was abolished by knockdown of the autophagic modulator Beclin-1 (Morselli et al., 2010b). Similarly to the resveratrol, which is the histone deacetylase activator, the histone acetylase inhibitor, spermidine, was shown to extend lifespan and induce autophagy by up-regulation of ATG genes in a range of model organisms from yeast to mice (He et al., 2013; Mariño et al., 2011). Spermidine is a naturally occurring polyamine interacting with negatively charged molecules, including DNA, RNA and lipids, thereby influencing basic cellular functions such as DNA stability, as well as cell growth, proliferation and death. The intracellular concentrations of this substance have been shown to decline throughout the aging process (Eisenberg et al., 2009). Recently, spermidine has emerged as promising anti-aging agent (Madeo et al., 2010; Minois, 2014). Dietary supplementation with this compound was found to increase the stress resistance and lifespan as well as to decrease the age-related loss of locomotor activity and the incidence of aging-linked impairments in yeast, worms and fruit flies (Minois et al., 2014). Supplementation with spermidine and other polyamines, such as spermine and putrescine, also increased both healthspan and lifespan in mice (Soda et al., 2009, 2013), although role of autophagy has not been examined in these studies. Since spermidine may influence acetylation profiles of many proteins (primarily belonging to the autophagy protein network), autophagy is currently postulated to be the main mechanism underlying its anti-aging effects.

Involvement of autophagy in the life-extending effects of spermidine is evident from the fact that these effects were abolished in yeast, worms and fruit flies if autophagy has been blocked by knockout or knockdown of autophagy genes *ATG7* or *BECN1* (He et al., 2013). The deletion of SIRT1 did not abolish the life-extending capability of spermidine, indicating that it may promote longevity by pathways other than SIRT1. Other candidate pathways, AMPK and mTOR, are also unlikely to play a crucial role because spermidine does not alter the phosphorylation of these enzymes and their substrates (Morselli et al., 2011). The signaling pathway mediating the effects of spermidine could be the MAPK pathway, known to interact with polyamines. This pathway is involved in the control of cellular functions such as inflammation, cell proliferation, programmed cell death, and also in autophagy modulation (Vellai et al., 2009; Yang et al., 2013).

Remarkably, health benefits of spermidine are mediated by inhibition of histone acetylase activity, whereas similar beneficial effects of resveratrol are due to the activation of the histone deacetylase, SIRT1. This can indicate on the role of histone acetylation and other epigenetic pathways in both the autophagy and longevity regulation (Eisenberg et al., 2009). Recently, other autophagy-inducing compounds have emerged as exhibiting potent life-extending capacities. For example, β-guanidinopropionic acid (a creatine analog that causes ATP depletion) was shown to extend lifespan of *Drosophila* through the AMP-activated protein kinase-dependent increase in autophagy (Yang et al., 2015). Supplementation with ethanolamine (a precursor of phosphatidylethanolamine that is an essential component of biological membranes) was also found to positively regulate the autophagy and longevity in yeast (Rockenfeller et al., 2015).

Experimental findings on the lifespan-modulating effects of autophagy-inducing agents are summarized in Table 5.

5. Senolytic drugs

Intracellular damage occurring during the aging process, such as genomic instability, epigenetic alterations, telomere attrition and loss of proteostasis, can lead to loss of functionality of the cells. Cellular senescence, a permanent proliferative cell-cycle arrest, occurs in damaged cells and prevents their propagation in the organism (Ovadya and Krizhanovsky, 2014). Under normal conditions, senescent cells recruit the immune system that provides their removal from tissues. During aging, however, senescent cells tend to accumulate, and can adversely affect their microenvironment (Tchkonia et al., 2013). Even though proportion of senescent cell constitutes a maximum of 15 percent in aged tissues (Herbig et al., 2006), these cells secrete pro-inflammatory chemokines, cytokines, and extracellular matrix proteases, which collectively constitute the senescence-associated secretory phenotype (SASP), likely contributing to systemic dysfunction and chronic disease (Byun et al., 2015). It is thought that chronic inflammation triggered by senescent cells is among the main drivers of aging-associated pathologies. Increasing evidence suggests that immune cells recruited through proinflammatory chemotactic factors secreted as a part of the SASP, can effectively remove damaged

Table 5
Summary of the effects of autophagy-inducing agents on the lifespan and associated functional traits of model organisms.

Model	Substance/dose	Longevity effect	Associated functional traits	Reference
Saccharomyces cerevisiae	Rapamycin (0.1–40 nM)	extension of chronological lifespan; no effects on lifespan in autophagy-deficient mutant strains, *ATG1* and *ATG7*	induction of autophagy in wild-type strain	Alvers et al. (2009)
	Spermidine (1 mM)	extension of chronological lifespan	induction of autophagy	Eisenberg et al. (2009)
	Ethanolamine (10 mM)	extension of chronological lifespan	induction of autophagy	Rockenfeller et al. (2015)
Caenorhabditis elegans	Spermidine (0.2 mM)	extension of lifespan	induction of autophagy	Eisenberg et al. (2009)
Drosophila melanogaster	Spermidine (1 mM)	extension of lifespan	induction of autophagy	Eisenberg et al. (2009)
	Spermidine (0.1 and 1 mM)	increased median lifespan	enhances starvation resistance, improved survival on paraquat, reduced female fecundity; abolished life extension by downregulation of autophagy through the reduction in expression of *ATG5* gene by RNA interference	Bjedov et al. (2010)
	β-guanidinopropionic acid (>900 mM)	life extension; abolished life extension by downregulation of autophagy through the reduction in expression of *ATG5* gene by RNA interference	AMP-activated protein kinase-dependent increase in autophagy	Yang et al. (2015)
Mus musculus	Mixture of spermine, spermidine and putrescine (374–1540 nmol/g)	increased survival	lower incidence of glomerulosclerosis in kidney	Soda et al. (2009)
	Mixture of spermine, spermidine and putrescine (374–1540 nmol/g)	reduced mortality in advanced age	decreased age-associated pathological changes, suppressed age-associated increase in pro-inflammatory status, inhibited age-associated global alteration in DNA methylation status and 1,2-dimethylhydrazine-induced tumorigenesis	Soda et al. (2013)

and senescent cells. Moreover, although the senescence response apparently originally appeared as a tumor suppressor mechanism, it may become a cancer promoter as a consequence of aging (Loaiza and Demaria, 2016). Indeed, senescent cells, similarly to tumor cells, exhibit enhanced expression of "pro-survival networks" which help them to resist apoptosis. Thereby, SASP, along with age-related immune decline and genomic instability, may contribute to deterioration of the effectiveness of the anti-cancer response. In addition, cell-autonomous and paracrine mediators produced by senescent cells can influence the processes of self-renewal and differentiation of stem cells, thus, persistent growth arrest may contribute to the decline of tissue regenerative potential (Childs et al., 2015). For example, in BubR1 progeroid mice, fat and muscle progenitor cells are shown to be highly susceptible to cellular senescence (Baker et al., 2011). Optimal stem cell function is known to strongly depend on their microenvironment (niche), and, therefore, SASP can impair this function by modulating the niche (Jang et al., 2011). As a result, senescent cells are regarded as attractive targets for therapeutic interventions (Childs et al., 2015; Kirkland, 2013b).

Identification of small molecules with potential to selectively induce death of senescent cells (senolytic drugs) seems to be a promising therapeutic approach in anti-aging medicine (Kirkland and Tchkonia, 2015). Recently, by using a bioinformatics-based approach, Zhu et al. (2015) identified potent senolytic agents triggering apoptosis preferentially in human senescent cells without damaging other cells throughout the body. An increased expression of "pro-survival networks" was the main criteria for this search. Using siRNA to inhibit expression of critical junctions of these networks, including PI3Kd, p21, BCL-xL, ephrins (EFNB1 or 3), or plasminogen-activated inhibitor-2, the authors eliminated the senescent but not normal cells in *in vivo* mice model. On this basis, two compounds of 46 agents tested were identified as senolytic drugs, namely, quercetin, a natural polyphenol flavonoid known as antihistamine, and also anti-inflammatory and anti-cancer agent, and dasatinib, an inhibitor of multiple tyrosine kinases commonly used for treating cancers. In this research, quercetin was found to be efficient against senescent human endothelial cells and mouse bone marrow stem cells, whereas dasatinib effectively eliminated senescent human fat cell progenitors. In addition, the combination of these agents was effective for eliminating senescent mouse embryonic fibroblasts, as well as for reducing the burden of senescent cells in radiation-exposed, chronologically aged, and progeroid $Ercc1^{-/\Delta}$ mice. Periodic treatment with this drug combination resulted in delayed aging-related symptoms including osteoporosis and loss of intervertebral disk proteoglycans in this mice model. Moreover, treatment with a single dose of this drug combination significantly improved cardiovascular function in old mice as well as exercise capacity in animals weakened by radiation therapy used for cancer. The later effect lasted for at least 7 months following the treatment. Furthermore, the senolytic potential of the inhibitors of B-cell lymphoma-extra large (Bcl-xl) pathway known to regulate mitochondrial-dependent apoptosis has been tested (Zhu et al., 2016). This potential was revealed in an anti-cancer drug navitoclax (also known as ABT263) which is an inhibitor of the anti-apoptotic proteins BCL-2, BCL-w, and BCL-xL. This drug acted in a potentially predictable cell type-restricted manner. Specifically, it reduced viability of senescent human umbilical vein epithelial cells, IMR90 human lung fibroblasts, and murine embryonic fibroblasts, but not human primary preadipocytes. Navitoclax administration to either sublethally irradiated or normally aged mice reduced the amount of senescent cells, including senescent muscle stem cells and bone

marrow hematopoietic stem cells. That, in turn, diminished the radiation-induced premature aging of the hematopoietic system and rejuvenated the aged muscle stem cells and hematopoietic stem cells in normally aged mice.

Data demonstrating that selective elimination of senescent cells may prevent or delay age-related functional impairments and extend healthspan were obtained in transgenic progeroid mouse that expresses low amounts of the mitotic checkpoint protein, BubR1 (Baker et al., 2011; Naylor et al., 2013). In this research, a biomarker of senescence, p16(Ink4a), was used to design a novel transgene, INK-ATTAC, for inducible elimination of p16(Ink4a)-positive senescent cells. The authors based their approach on the FAT-ATTAC (fat apoptosis through targeted activation of caspase) mouse model, where adipocytes are selectively killed by apoptosis upon the administration of the synthetic membrane-permeable drug AP20187. In tissues such as skeletal muscle, adipose tissue and eye in which p16(Ink4a) contributes to age-associated disorders, long-term removal of p16(Ink4a)-expressing cells resulted in delayed onset of aging-related phenotypes, such as cataracts, lipodystrophy and lordokyphosis (curvature of the spine). Moreover, later-life clearance retarded the progression of already established chronic pathologies. In continuing these studies, the authors conducted experiments in normal (i.e. non-BubR1 mutant) mice. Specifically, they used a previously established transgene, INK-ATTAC, to induce apoptosis in p16Ink4a-expressing cells of wild-type mice by injections of cell-permeant small molecule ligand, AP20187, since one year of age (Baker et al., 2016). In both male and female mice belonged to two distinct genetic backgrounds (one of mixed genetic origin and one a pure C57BL/6 line), AP20187 extended median lifespan by 17–35% compared with the vehicle alone. Furthermore, the clearance of p16Ink4a-positive cells led to a delay of age-related decline in kidney function and improvement in some markers of cardiac function. Deleting senescent cells also resulted in decreased expression of inflammatory markers and protection from cataract formation, as well as in delayed tumor appearance. Remarkably, extension of median lifespan in AP20187-treated mice dying without tumors ranged from 24% to 42%, indicating that this life extension was not merely owing to the tumor-protective effect. Collectively, these findings demonstrate the feasibility of selectively removing of senescent cells and effectiveness of senolytics for mitigating the symptoms of frailty and healthspan extension. Another related approach is pharmacological SASP suppression, which might in part explain the anti-aging properties of rapamycin (Serrano, 2015; Tomimatsu and Narita, 2015).

Recently, a pro- aging role of mitochondria in cellular senescence was reported (Correia-Melo and Passos, 2015). Induction of mitochondria-dependent pro-oxidant and pro-inflammatory pathways is known to occur at both cellular senescence-related processes such as tumor suppression and aging. To study to what extent mitochondria are implicated in senescence, Correia-Melo et al. (2016) have completely eliminated the mitochondrial compartment by inducing mitophagy [a kind of autophagy ensuring the preservation of healthy mitochondria due to the removal of damaged or excessive mitochondria (Patergnani and Pinton, 2015)] in mice model. For this, they used the depolarization of mitochondria by uncouplers, such as carbonyl cyanide m-chlorophenyl hydrazone (CCCP), a chemical inhibitor of oxidative phosphorylation, targeting the ubiquitin ligase Parkin to mitochondria and promoting their degradation through proteasome and autophagy pathways. Elimination of mitochondria caused reduction of a range of senescence effectors and phenotypes, including reduced oxidative stress, inflammation markers and expression of the cyclin-dependent kinase inhibitors p21 and p16 (Correia-Melo et al., 2016). These data suggest that mitochondria are a promising candidate target for interventions aimed to reduce the adverse impacts of senescence in aging tissues. The concern is, however, whether there is a possibility that targeting mitochondria would adversely affect non-senescent cells too. This issue should be addressed in further studies.

6. Telomerase activators

Pharmacological targeting of telomerase activity is another promising anti-aging approach. Proper maintenance of telomeres (nucleoprotein structures at the end of linear eukaryotic chromosomes) is crucial for genome stability (Chiodi and Mondello, 2016). Age-related telomere shortening is known to play a major role in senescence and aging-associated conditions (Zhang et al., 2016). Telomerase is the specialized reverse transcriptase capable of maintaining telomere length through the template-mediated addition of telomeric repeats onto the ends of newly synthesized chromosomes (Sishc et al., 2015). In multicellular organisms, telomerase activity is limited to specific cell types. Activation of telomerase in somatic cells is a key step toward cell immortalization and transformation. Targeting telomerase is believed to be a promising antitumor therapeutic strategy, and also may provide clinical benefits in non-cancer pathological conditions, including aging-related ones (Sprouse et al., 2012). The ectopic expression of the telomerase catalytic subunit (telomerase reverse transcriptase, TERT) in mice epithelial cells was shown to extend lifespan by up to 40% in the cancer-resistant animals (Tomás-Loba et al., 2008); it, however, resulted in increased cancer risk in wild-type mice (Gonzalez-Suarez et al., 2001; Artandi et al., 2002). Reactivation of telomerase in the telomerase-negative normal human cells, exhibiting telomere shortening and senescence, by transfection with vectors encoding the human TERT (hTERT), resulted in elongation of telomeres and reduced level of beta-galactosidase (a biomarker for senescence) in these cells (Bodnar et al., 1998). Retroviral introduction of hTERT into senescent normal human diploid fibroblasts lacking telomerase activity also resulted in reconstitution of telomerase activity, telomere maintenance, and significant cell life extension *in vitro* (Forsythe et al., 2002; Steinert et al., 2000; Vaziri and Benchimol, 1998). Treatment with an adeno-associated virus (AAV) carrying the mouse TERT (mTERT) cDNA resulted in substantial beneficial effects on health and fitness in mice (Bernardes de Jesus et al., 2012). These effects included decreased osteoporosis risk and enhancement of insulin sensitivity, neuromuscular coordination and several molecular biomarkers of aging. Importantly, AAV9-mTERT-treated 1- and 2-year old mice did not develop more cancers than control animals, and their median lifespans were increased by 24 and 13%, respectively. These beneficial effects were not revealed with a reporter virus or a catalytically inactive TERT, indicating the strong dependence of an active telomerase complex. Interestingly, CR caused significantly decreased cancer incidence in TERT transgenic (TgTERT) mice and extended their lifespan compared with the wild-type controls, demonstrating a synergy between TgTERT and CR in increasing the longevity (Vera et al., 2013).

A pharmacological telomerase-based approach to enhance immune function was first applied by Fauce et al. (2008). In this study, exposure with a small molecule telomerase activator TAT2 retarded telomere shortening and enhanced proliferative potential, cytokine/chemokine production and antiviral activity of human CD8(+) T lymphocytes. A small-molecule telomerase activator, TA-65, extracted from the roots of the plant *Astragalus membranaceus* was shown to be able to activate telomerase in human fibroblasts, keratinocytes, and immune cells (Harley et al., 2011). In human T cells treated with TA-65, MAPK-specific telomerase activation and significant increase in proliferative activity were observed (Molgora et al., 2013). Dietary supplementation with TA-

65 resulted in telomerase-dependent elongation of short telomeres and rescue of associated DNA damage in mice (Bernardes de Jesus et al., 2011). Moreover, supplementation of female mice with TA-65 led to an improvement of several healthspan indicators such as osteoporosis, glucose tolerance and skin fitness, without increase in cancer incidence. More recently, the stimulating effect of TA-65 on the telomerase activity and telomere lengths, as well as flight feather renewal capacity and faster feather regeneration was revealed in captive zebra finches, *Taenopygia guttata* (Reichert et al., 2014). 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).

Collectively, these findings indicate that activation of telomerase can be an effective treatment option, especially for disorders related to impaired telomere maintenance such as telomeropathies. Both direct and indirect strategies of telomerase activation, however, have raised safety concerns because constitutive reactivation of endogenous telomerase is apparently associated with risk of cancer development. In particular, it has been reported in several studies that activation of TERT by TA-65 is mediated by inducing mitogenic pathways causing activation of the oncogene c-myc (Bernardes de Jesus et al., 2011; Molgora et al., 2013), thereby driving tumorigenesis. Therefore, the development of safe clinical applications for telomerase activation represents an important goal for further research. AAV-based gene therapy is suggested to be of particular value for TERT activation, because of the non-integrative and replication incompetent properties of AAVs allow for cell division-associated telomere elongation and subsequent loss of TERT expression as cells divide, thereby restricting TERT expression to a few cell divisions (Ramunas et al., 2015). Indeed, the unfavorable side effects of telomerase activation were shown to be avoided via direct delivery of TERT using AAV vectors (Bernardes de Jesus et al., 2012). Thus, AAV-based treatment can apparently provide a transient and relatively safe strategy to TERT activation. Another prospective way to extend telomeres and increase cell proliferative capacity without risk of insertional mutagenesis by transient delivery of modified mRNA encoding TERT was recently reported in the *in vitro* human fibroblast study (Ramunas et al., 2015).

7. Epigenetic drugs

In the last years, pharmacological compounds targeted to epigenetic regulators of gene expression are actively studied in the context of geroscience. Epigenetic mechanisms including histone modifications, DNA methylation, and changes in microRNA (miRNA) expression play central role in regulating gene expression and genomic instability throughout the lifespan. Epigenetic modifications are known to be finely balanced in normal tissues. They can be, however, unbalanced in malignant and other transformed cells. Epigenetic dysregulation was shown to contribute to the pathogenesis of age-associated pathologies such as cancer, atherosclerosis, type 2 diabetes, psychiatric and neurodegenerative diseases, as well as decline in immune response (Brunet and Berger, 2014; Cacabelos and Torrellas, 2015; Vaiserman, 2015). Therefore, the modulators of the activity of enzymes involved in epigenetic regulation might be clinically applicable. Epigenetic modifications are known to be potentially reversible; this feature makes them attractive targets for pharmacological intervention (Cacabelos and Torrellas, 2015).

Over the past years, a series of medications have been developed targeted to epigenetic regulators, including modulators of DNA methyltransferases (DNMTs), histone deacetylases (HDACs), histone acetyltransferases (HATs) and noncoding miRNAs, with potential effects against various types of tumors, myelodysplastic syndromes, and neurodegenerative disorders (Arguelles et al., 2016; Cacabelos, 2014).

7.1. DNMT inhibitors

Over the past years, DNMT inhibitors have become the promising drugs in the treatment of several chronic diseases, primarily cancers; their preventive role in carcinogenesis, however, remains unclear. It was shown that inhibition of DNMT1 is able to reverse the malignant phenotype of transformed cells by restoring expression of aberrantly silenced genes involved in differentiation, senescence, and apoptosis (Zhong et al., 2016). Among the drugs targeted to inhibit DNMTs, FDA-approved drugs such as 5-azacytidine (azacitidine) and 5-aza-2′-deoxycytidine (decitabine) are the most intensively studied. Several clinical trials have demonstrated that these medications have therapeutic potential against leukemias including myelodysplastic syndrome, chronic myelogenous leukemia, acute myeloid leukemia and chronic myelomonocytic leukemia, while their efficacy in solid tumors seems rather limited (Ghoshal and Bai, 2007). A major problem in the usage of these substances as anti-cancer agents is their instability *in vivo* and toxicity, causing cell cycle arrest. DNMT inhibitors were also demonstrated to be able to modulate vascular biology and atherosclerosis development (Dunn et al., 2015). It should be noted, however, that DNA-demethylating agents can beneficially reactivate tumor suppressor genes, but also activate silenced prometastatic genes thereby inducing metastasis (Cheishvili et al., 2015). Therefore, caution is required in using DNMT inhibitors in cancer patients.

7.2. HDAC inhibitors

Histone acetylation is the main histone modification affecting gene transcription. The main enzymes controlling process of histone acetylation are HDACs and histone acetyltransferases (HATs). HATs are acetylate lysines of histone proteins, causing relaxation of chromatin structure and promoting gene activation. HDACs, conversely, remove acetyl groups from hyperacetylated histones and thereby suppress gene transcription. Currently, HDACs are divided into four classes (Eom and Kook, 2014). Class I HDACs undergo posttranslational modifications including sumoylation, phosphorylation and S-nitrosylation. Class II HDACs work mainly in association with other transcription factors to target binding elements in a phosphorylation-dependent manner. In addition, HDACs belonging to class I demonstrate much higher enzymatic activity than class II HDACs and target many non-histone proteins as well as the histone-core complex. In recent years, increasing evidence has been accumulated that HDACs play key role in diverse biological processes such as cell proliferation, apoptosis, inflammation and carcinogenesis (Chen et al., 2015). Several modulators of HDAC activity (primarily, HDAC inhibitors), among other drugs targeting epigenetic machinery, have been recently examined in human clinical trials, and some have been proposed as promising anti-aging drug candidates (Vaiserman and Pasyukova, 2012). HDAC inhibitors include four chemical classes: cyclic peptides, hydroxamic acids, short chain fatty acids and synthetic benzamides, and they substantially vary in biological activity, structure and specificity (Lakshmaiah et al., 2014). The anti-tumor effects of these medications may be attributed to the transcriptional reactivation of silent tumor suppressor genes and the transcriptional repression of proto-oncogenes (Boumber and Issa, 2011).

Among the chemicals affecting HDAC activity, HDAC inhibitors seem to be the most promising in the field of geroscience. Since the level of transcription of many genes, primarily metabolic

and biosynthetic ones, is known to decrease with aging (Seroude et al., 2002), the restoration of the transcriptional activity via HDAC inhibitors could likely delay the age-related functional decline. Moreover, HDAC inhibition can cause up-regulation of genes involved in response to stress and inflammation – pathways generally involved in the regulation of longevity (Kourtis and Tavernarakis, 2011).

Several convincing lines of experimental evidence have also been obtained indicating substantial life-extending potential of HDAC inhibitors. A significant increase in both mean and maximum lifespans by up to 30–50% without diminution of stress-resistance, locomotor activity and reproductive ability has been revealed by feeding *Drosophila* with HDAC inhibitor, 4-phenylbutyrate (PBA), throughout adulthood (Kang et al., 2002). Flies fed PBA exhibited a global increase in histone acetylation and dramatically changed patterns of gene expression of various genes, including those supposedly implicated in the life extending effects: elongation factor 1, superoxide dismutase, cytochrome P450, glutathione S-transferase, and three chaperones. All these genes were up-regulated by PBA. Life extension accompanied by elevated expression of *hsp22* gene was observed in flies fed with HDAC inhibitor such as trichostatin A (TSA) (Tao et al., 2004). TSA and other HDAC inhibitor, sodium butyrate, have also been demonstrated to significantly extend longevity and promote expression of *hsp22* and *hsp70* in fruit flies (Zhao et al., 2005). In a more recent study, significant increase of the mean lifespan was revealed in both males and females fed sodium butyrate during both pre-adult and adult stages; treatment with sodium butyrate during the adult stage only caused an increase in male (but not female) lifespan (Vaiserman et al., 2012). The effects of sodium butyrate on the flies' longevity were also studied simultaneously in normal- and long-lived strains of *Drosophila melanogaster* (Arking, 2015; McDonald et al., 2013). This agent increased longevity in the normal-lived Ra strain when administered in the 'transition' or 'senescent' spans, but decreased longevity when administered over the 'health' span only or over the entire adult lifespan. Mostly deleterious effects were revealed when administered to the long-lived La strain. Late-life extending effects were observed in the normal-lived strain administered with another HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA). On the basis of these findings, the authors concluded that use of HDAC inhibitors may significantly alter the mortality rate of the 'senescent' span by decreasing its vulnerability, or short-term risk of death, in a manner similar to that of CR (McDonald et al., 2013).

Since the onset and progression of various cancers involve substantial dysregulation of HDAC activity, a wide range of HDAC inhibitors are emerging as promising anticancer pharmaceuticals. HDAC inhibitors such as belinostat, panobinostat, SAHA and FK228 (Ma et al., 2016), as well as trichostatin A (TSA), sodium butyrate, vorinostat, valproic acid and romidepsinor (Chun, 2015) showed substantial activity in both haematological and solid tumors in different tissues. Their effects are apparently mediated by the regulation of gene expression and DNA repair, inducing cell cycle arrest and apoptosis and inhibiting angiogenesis. They can also be caused by the long-term stimulation of immune response (Leggatt and Gabrielli, 2012). In the last years, HDAC inhibitors have undergone a rapid phase of clinical development in different cancer types, either as monotherapy or combined with other anticancer modalities. To date, three HDAC inhibitors have been approved by FDA for the treatment of cutaneous/peripheral T-cell lymphoma (Mottamal et al., 2015), and four HDAC inhibitors, namely vorinostat, belinostat, romidepsin and panobinostat have been approved by FDA for the treatment of hematologic cancers (Yoon and Eom, 2016). Many other HDAC inhibitors are at different stages of clinical development for the treatment of haematological malignancies and solid tumors (Mottamal et al., 2015).

The FDA's approval of HDAC inhibitors as anticancer agents has provided the motivation to use these medicines as treatment options for non-malignant diseases. The beneficial outcomes of HDAC inhibition were obtained in treatment of various types of inflammatory, neurodegenerative and cardiovascular disorders (Yoon and Eom, 2016). In particular, experimental evidence has indicated that inhibitors of Class I HDACs can attenuate the development of cardiac hypertrophy and preserve cardiac function in several small animal models (Abend and Kehat, 2015). In addition, HDAC inhibitors have been found to be beneficial in preventing myocardial infarction, hypertension, atherosclerosis, vascular calcification, supraventricular arrhythmia, cardiac remodeling, fibrosis, and neointima formation (Eom and Kook, 2014). The putative mechanisms mediating beneficial effects of HDAC inhibitors on the heart function include suppression of oxidative stress and inflammation, enhancement of cardiac protein aggregate clearance and autophagic flux, as well as inhibition of MAP kinase signaling (Ferguson and McKinsey, 2015). In addition, since HDAC inhibitors were reported to promote β-cell proliferation, differentiation and function and positively affect late diabetic microvascular complications, HDAC inhibition was proposed as a novel treatment strategy for type 2 diabetes (Christensen et al., 2011).

HDAC inhibitors show great promise to combat the aging-associated cognitive decline and to ameliorate the symptoms of posttraumatic stress disorder and depression (Penney and Tsai, 2014). Neurodegenerative diseases such as amyotrophic lateral sclerosis, polyglutamine-related diseases, as well as Parkinson's and Alzheimer's diseases are known to be accompanied by transcriptional dysfunctions, leading to neuronal death (Selvi et al., 2010). Several recent studies have highlighted the importance of HDACs in neuronal memory, learning, synaptic plasticity and neural regeneration (Ganai et al., 2016). Accumulating evidence indicate that histone acetylation plays a crucial role in the etiology of neurodegenerative disorders. Some studies demonstrate that increase of histone acetylation can be involved in Alzheimer's disease, and that HDAC inhibitors may be neuroprotective by regulating memory and synaptic dysfunctions in both *in vitro* and *in vivo* models of this pathology (Lu et al., 2015). HDAC inhibitors were also reported to cause beneficial effects in both *in vitro* and *in vivo* models of Parkinson's disease. The potential mechanisms underlying these effects include maintenance of histone acetylation homeostasis and transcriptional activation of neuronal survival genes (Sharma and Taliyan, 2015). In the last years, clinical trials have been initiated to examine the effectiveness of HDAC inhibitors in patients with Parkinson's disease. The loss of functional activity of HATs (enzymes with activities antagonistic to those of HDACs) is likely a common mechanism related to the impairment of the chromatin acetylation status throughout the lifetime of neurons. The therapeutic potential of HAT activators in the treatment of neurodegenerative disorders has been established in preclinical studies. Substantial neuroprotective properties were revealed for one of the HATs termed cAMP response element binding protein (CREB)-binding protein (CBP), an also for several other HATs which were shown to be essential for processes of neuronal plasticity and memory formation (Selvi et al., 2010).

Non-specific HDAC inhibitors also demonstrated anti-inflammatory effects in both *in vitro* and *in vivo* models. Recently, evidence was obtained for the role of the NF-κB signal transduction pathway in mediating the effects of HDAC inhibitors on inflammatory responses (Dekker et al., 2014). The important point is that such effects were reported at concentrations which were 10–100-fold lower than anti-cancer effects of these compounds. Clinical application of these substances for treating inflammatory diseases is, however, hampered due to their low specificity and a wide variety of HDACs that they affect throughout the body (Cantley and Haynes, 2013).

Overall, the therapeutic strategy based on HDAC inhibition seems very promising. However, as most HDAC inhibitors lack specificity (Pirooznia and Elefant, 2013), their wide applicability is still questionable.

7.3. Sirtuin-activating compounds

The term 'sirtuins' is derived from the 'sir-two-ins' in reference to homology to the yeast Sir2 protein (silent information regulator) (North and Sinclair, 2007). Silent information regulators (SIRTs) are nicotinamide adenine dinucleotide (NAD +)–dependent protein deacetylases playing a crucial role in epigenetic regulation of gene expression by mediating the assembly of the silenced chromatin. The yeast deacetylase Sir2 was initially identified as a molecule with potent anti-aging properties (Imai et al., 2000). Subsequently, it has been demonstrated to be implicated in the control of lifespan in yeast, nematode, and *Drosophila*. In mammals, a homologue of Sir2, SIRT1, has been found to act as anti-aging molecule in the context of CR (Boily et al., 2008). Apart from gene silencing, SIRT1 was shown to regulate other important biological processes including stress resistance, cell survival, inflammation, mitochondrial biogenesis, glucose and lipid metabolism, apoptosis and autophagy (Giblin et al., 2014; Liu and McCall, 2013). Moreover, the control of SIRT1 activity is closely related to those of the AMPK, that regulates glucose, lipid and cholesterol metabolism in specialized metabolic tissues, such as muscle, liver and adipose tissue and which is one of the key regulators of eukaryotic metabolism (Shackelford and Shaw, 2009). AMPK is activated in conditions where nutrition is limited and promotes oxidative metabolism. It has also been demonstrated that AMPK is activated by increasing NAD+ production, and activation of SIRT1 in turn leads to AMPK activation through the deacetylation of liver kinase B1 (LKB1), which is the major upstream AMPK kinase (Cantó et al., 2009; Hardie, 2015).

The importance of SIRT1 for the CR effects has been shown in SIRT1 transgenic mice that manifest a CR-like phenotype, showing lowered levels of adipokines, insulin, fasting glucose blood cholesterol, and greater glucose tolerance compared to the control animals (Bordone et al., 2007). In a controlled intervention trial conducted in healthy individuals, with measurements before and after the CR treatment, it has been shown that 25% reduction in calorie intake during six months leads to the overexpression of SIRT1 and peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α (PGC-1α) in the skeletal muscles, accompanied by the enhanced mitochondrial function and reduced levels of the body temperature, metabolic rate, oxidative stress, visceral fat mass and insulin resistance in non-obese young adults (Civitarese et al., 2007). However, in several studies in yeast, nematodes and *Drosophila*, CR was found to be able to extend lifespan in the absence of Sir2 (Burnett et al., 2011; Kaeberlein et al., 2004; Smith et al., 2007). Therefore, the role of sirtuins, particularly SIRT1, in CR-mediated life extension still remains unclear.

7.3.1. Resveratrol

Large-scale screening of drugs capable of activating SIRTs led to the discovery of 18 molecules demonstrating such properties (Kitada and Koya, 2013). Among them, resveratrol (3,5,40-trihydroxystilbene), a naturally occurring polyphenolic phytoalexin present mostly in red wine and grape skins, but also in berries and peanuts, has been investigated most thoroughly (de Ligt et al., 2015). The research of this compound was initially started through the "French paradox" which describes improved cardiovascular outcomes despite a high-fat diet in the French population (Catalgol et al., 2012).

Dietary resveratrol administration in doses of 100–1000 μM caused life extension in short-lived model organisms, such as yeast, worms, honey bee and fruit flies (Table 6).

Resveratrol has also been shown to prolong lifespan and retard the onset of aging-associated markers in short-lived vertebrates such as fish (Valenzano et al., 2006; Yu and Li, 2012). In mice model, the beneficial effects of resveratrol on metabolism and lifespan were revealed in high-fat diet-induced obese animals (Baur et al., 2006). In mice fed a standard diet, resveratrol administration did not prolong life, but resulted in delayed aging-related impairments and transcriptional changes similar to those observed in CR animals (Pearson et al., 2008). Resveratrol has also been shown to be able to protect against a variety of aging-related disorders including type 2 diabetes, Alzheimer's disease and cancer (Bhullar and Hubbard, 2015). In obese male individuals, supplementation with resveratrol during 30 days prevented the obesity-associated metabolic dysregulation by activating the AMPK/SIRT1 pathway in skeletal muscle (Timmers et al., 2011). Furthermore, resveratrol intake was associated with elevated intramyocellular lipid levels, and decreased intrahepatic lipid content, triglycerides, circulating glucose, alanine-aminotransferase and inflammatory markers. More recently, 30-day supplementation with the same dose of resveratrol caused a significantly decreased adipocyte size, with a shift toward a decrease in the proportion of large adipocytes and an increase in the proportion of small adipocytes (Konings et al., 2014). A widespread view is that longevity effects of resveratrol are mediated by activation of sirtuins. It, however, had no detectable effects on Sir2 activity in *in vivo* yeast studies (Kaeberlein et al., 2005). Thereby, the direct activation of sirtuins by resveratrol is still under debate (Fernández and Fraga, 2011).

Currently, resveratrol is one of the most popular nutritional supplements on the market. In the U.S., two-thirds of persons consuming multiple dietary supplements, consume this supplement (Hubbard and Sinclair, 2014). However, despite the high expectations placed on resveratrol, some challenges are related to its application. Recently, in discussing these issues Vang (2015) stressed that: '(1) many different cellular effects are observed for resveratrol, but it is not known whether they arise from one point of action (or a few) or whether resveratrol is non-specific in its action; (2) the health-promotion effect of dietary resveratrol is likely a combinatory effect of various bioactive components in the mixture (diet); (3) the known cell biological response to resveratrol is presently based on exposure to short-term high levels, and better in vitro analyses have to be developed; (4) the actual level of resveratrol and resveratrol metabolites present *in vitro* and *in vivo* during experiments may be over- and underestimated, respectively, because resveratrol is not very soluble in water; and (5) only a few small clinical studies have been published to date, focusing on the therapeutic effects of resveratrol.'

7.3.2. Synthetic sirtuin activators

Several synthetic SIRT1 activators such as SRT1460, SRT1720, SRT2104 and SRT2183 have been synthesized over the last decade. Among them, SRT1720 is the most studied. This agent has been shown to cause life extension and to ameliorate the metabolic impairments in mice fed both a normal diet (Mitchell et al., 2014) and a high-fat diet (Minor et al., 2011). Moreover, it has also been found to be able to reverse adverse aging-associated changes including vascular endothelial dysfunction, excessive superoxide production and inflammation (Gano et al., 2014). Recently, helpful effects of another SIRT1 activator, SRT2104, on muscle and bone mass as well as on the survival rate in male mice have been detected (Mercken et al., 2014). Based on this research background, small SIRT1 activators such as SRT501, SRT2104 and SRT2379 are currently undergoing clinical trials for the treatment of obesity, type 2 diabetes and metabolic syndrome, among other chronic disorders (Camins et al., 2010).

As the activation of SIRT1 has been shown in several studies to be able to delay the progression of aging-associated conditions

Table 6
Summary of the effects of resveratrol on the lifespan and associated functional traits of model organisms.

Model	Longevity effects	Associated functional traits	Reference
Saccharomyces cerevisiae	extension of replicative lifespan by 70%	N.D.	Howitz et al. (2003)
Caenorhabditis elegans	9–18% increase in mean lifespan	transcriptional induction of a family of genes encoding prion-like glutamine/asparagine-rich proteins involved in endoplasmic reticulum stress response to unfolded proteins	Viswanathan et al. (2005)
	10–14% increase in mean lifespan	non-reduced fertility	Wood et al. (2004)
Drosophila melanogaster	29% increase in mean lifespan, 20–22% increase in maximum lifespan	non-reduced fertility	Wood et al. (2004)
	10–17% increase in mean lifespan	N.D.	Bauer et al. (2004)
	10–15% increase in mean lifespan	downregulation of genes in aging-related pathways, including antioxidant peroxiredoxins, insulin-like peptides involved in insulin-like signaling and several downstream genes in Jun-kinase signaling involved in oxidative stress response	Wang et al. (2013)
Apis mellifera	33–38% increase in mean lifespan	ingestion of fewer quantities of food under ad libitum feeding conditions	Rascón et al. (2012)
Nothobranchius furzeri	33–56% increase in median lifespan, 27–59% increase in maximum lifespan	delaying the age-dependent decay of locomotor activity and cognitive performances and reducing the expression of neurofibrillary degeneration in the brain	Valenzano et al. (2006)
Nothobranchius guentheri	19% increase in mean lifespan, 28% increase in maximum lifespan	enhanced cognitive ability and locomotor activity; protection from neurodegeneration; reduced rate of lipofuscin formation	Yu and Li (2012)
Mus musculus	31% reduced risk of death from the high calorie diet until the 114 weeks of age	increased insulin sensitivity, reduced insulin-like growth factor-1 levels, increased AMPK and peroxisome proliferator-activated receptor-gamma coactivator 1alpha activity, increased mitochondrial number, improved motor function	Baur et al. (2006)
	no effect on lifespan	marked reduction in signs of aging, including reduced albuminuria, decreased inflammation, and apoptosis in the vascular endothelium, increased aortic elasticity, greater motor coordination, reduced cataract formation, and preserved bone mineral density	Pearson et al. (2008)

thus extending healthspan, SIRT1 regarded as a potential target for CR mimetics (Baur et al., 2012). Several recent controversial findings regarding the role of SIRTs in the effects of CR, however, cast doubt on their relevance to aging, particularly as mediators of the longevity effects induced by CR (Park et al., 2013). Therefore, the potential of SIRTs as therapeutic targets is being questioned by many researchers and is a subject of intense debate now (Baur et al., 2012; Dang, 2014; Park et al., 2013).

7.4. Micro RNA

MicroRNAs (miRNAs) are small non-coding RNAs which play a crucial role in epigenetic regulation of gene expression. They are known to be crucially involved in various biological processes and are dysregulated in a variety of aging-related pathologies (Caravia and López-Otín, 2015; Kato and Slack, 2013). In experimental studies, miRNAs have been observed to be able to extend lifespan in model organisms such as *C. elegans* (Boehm and Slack, 2005; de Lencastre et al., 2010; Wang et al., 2015) and mice (Du et al., 2014). Thereby, miRNAs are believed to be promising targets for therapeutic applications (van Rooij and Kauppinen, 2014). Since single miRNAs may regulate the expression of many genes, and multiple miRNAs may target the same mRNA, they act as "network-level" regulators affecting complex phenotypes by integrating multiple inputs and outputs (Inukai and Slack, 2013). As aging is a multifactorial process influenced, on the one hand, by many factors and, on the other hand, subjected to central regulation, such features of miRNAs seem to be very important in the context of geroscience. Furthermore, miRNAs are quite easy to target therapeutically and they generally have a variety of targets within cellular networks, thereby allowing modulation of integral disease pathways through therapeutic targeting of miRNAs associated with particular disease states.

Currently, two main methodical approaches are used for modulating miRNA activity. The first one is modulating the miRNA function by either viral vector-based overexpression or synthetic double-stranded miRNAs and the second one is inhibiting miRNA function by chemically modified anti-miRNA antisense oligonucleotides (van Rooij and Kauppinen, 2014). In addition, the RNA editing pathway might likely be used to affect the double-stranded RNA-mediated silencing machinery to suppress disadvantageous RNA interference activity in the process of aging (Kato and Slack, 2013). In the last years, the investigation of miRNA-modulating agents has been started both in preclinical studies and in clinical trials (Grillari and Grillari-Voglauer, 2010).

Summarizing, it should be noted that the problem of fine-tuning of gene expression when treated with epigenetic drugs remains still unsolved. Indeed, the unbalanced drug action on epigenetic pathways, similarly to unbalanced supply of vitamins, antioxidants, or hormones, may disrupt delicate regulatory mechanisms providing homeostatic balance. However, as epigenetic control of gene expression is a highly coordinated process mediated by central regulatory mechanisms, this can provide a proper orchestration of responses to epigenetic-modifying interventions (Vaiserman, 2011). It makes epigenome-targeted agents the promising drug candidates for modulating a highly integrated biological process such as aging (Vaiserman and Pasyukova, 2012). In addition, it may be expected that stage- and tissue-specific epigenetic drugs will be developed in future, suggesting that eventually the epigenetic treatments will become more safe and effective.

Table 7
Summary and categorization of the most promising anti-aging agents.

Preparation	Effects observed									
	AO	DR	AI	AC	AP	NP	AA	GHS	TA	LE
Agents with no observed demographic effects until now										
2-deoxy-D-glucose		+		+	+					
Pegvisomant	+			+				+		
TA-65							+		+	
Agents suggesting to promote longevity mainly by inhibiting particular disease(s)										
Statins			+	+	+	+	+			+
Rapamycin	+	+	+	+	+	+		+		+
Metformin	+	+	+	+			+	+		+
Agents with both anti-aging and pro-longevity effects										
Aspirin			+				+			+
Coenzyme Q10	+		+			+	+			+
Spermidine	+		+		+			+		+
Vitamin E				+						+
Sodium butyrate	+		+	+	+	+				+
Suberoylanilide hydroxamic acid	+		+	+	+	+				+
Melatonin	+		+	+	+	+		+		+
Epigallocatechin-3-gallate	+	+	+	+			+	+		+
Curcumin	+		+	+	+		+	+		+
Quercetin		+	+	+	+	+	+	+		+
Resveratrol	+	+	+	+	+	+	+	+	+	+

AO: antioxidant; DR: dietary restriction; AI: anti-inflammatory; AC: anti-cancerous; AP: autophagy; NP: neuroprotective; AA: anti-atherogenic; GHS: growth hormone suppression; TA: telomerase activation; LE: life extension.

8. Conclusions

Few decades ago, it was generally believed that damage induced by free radicals is a primary cause of aging and, thereby, that antioxidants may protect against this damage, consequently slowing the aging process and improving health. Currently, it is becoming apparent that a single cause could not properly explain the health- and lifespan-promoting effects of different pharmacological classes. It is increasingly clear that pro-longevity effects of many prospective anti-aging agents are complex and could be attributable to numerous interacting signaling pathways (Vaiserman, 2014). Taking into account the exceptional complexity of mechanistic pathways underlying aging, the identification of these pathways and development of relevant anti-aging interventions seems to be a challenging task. Considerable progress, however, has been made in this research field throughout the past years. A large number of pharmacological substances having the potential to target molecular pathways underlying aging and to induce protective responses against aging-associated diseases including, e.g., mTOR inhibitors such as rapamycin, senolytics, etc, are presently under investigation.

One important issue in the study of potential geroprotective agents is that their pro-longevity effects might be mediated by the prevention and/or delay of onset of age-associated, life-shortening diseases, rather than through inhibiting the aging processes itself. For example, Neff et al. (2013) and Ehninger et al. (2014) based on a large-scale assessment of more than 150 cellular, molecular, histopathological, and functional traits, which typically change during the course of aging, in rapamycin-treated mice, concluded that rapamycin can extend longevity, but it has only limited effect on the aging process *per se*. Another promising anti-aging drug candidate, resveratrol, was found to be able to extend life span in mice fed with high-fat diet by reversing the initial steps of particular age-related diseases induced by that diet, but had no effect on animals fed with healthy diet (Baur et al., 2006).

Thus, all known pro-longevity drugs can be divided in three categories in accordance with the suggested mode of action:

- those demonstrating anti-aging effects, but without any evidence yet of their ability to prolong life;
- those that are suggested to extend longevity primarily because they can prevent or postpone the progression of particular age-related disease, such as cancer, but which are not proven to delay the aging process(es) *per se*;
- those that extend longevity because they are actually suggested to reverse the aging process itself, at least in certain environments.

Based on these considerations, the most promising agents with suspected anti-aging properties are categorized into three main groups in Table 7. Certainly, the classification presented in this table is quite conditional since only relatively few research findings have been reported until now in this area of investigation, thus any new data can move a particular substance from one category to another.

In summarizing it may be suggested that targeting aging *per se* may be more efficient approach to prevent or postpone age-related diseases than treatments targeted to particular pathological conditions. Because of the aging population, this therapeutic strategy undoubtedly can be an area of increasing relevance for the pharmaceutical industry and public health organizations. It is currently assumed that great socio-economic benefits can be obtained from the approach based on the longevity dividend paradigm in comparison with modern public health strategy aimed on the prevention of particular diseases. Presently, the consensus is among medical and health professionals that the optimization of physiological and mental functioning through the life course should be a main focus of public health policy addressing the problem of global aging. A healthy lifestyle including a balanced diet, regular exercise and smoking cessation is the first-line strategy. Using the pharmacological substances, both potential and existing, may serve as a prospective additional approach (Seals et al., 2016).

The great expectations related to further development of anti-aging interventions, however, must certainly be critically discussed and explored in the light of their economic, social and ethical implications. The implementation of these approaches in clinical practice will be possible only after in-depth examination and further comprehensive discussion. To meet the needs caused by the rapid population aging across the globe, a novel emphasis in physiological geroscience is needed which will require the joint efforts of interdisciplinary investigators working throughout the continuum

of translational medicine from basic research to clinical applications.

Conflict of interests

The authors have no conflict of interests.

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