Extended Longevity

Scientific White Paper

Sentophagy Formula, increases autophagy and mitophagy. Includes phytotherapeutic extracts of: *Taraxacum officinale*, *Camellia sinensis*, *Berberis vulgaris*, *Curcuma longa*, *and Cinnamomum verum*. Biological Actions, Molecular Mechanisms, and Their Effects.

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Sentophagy Formula is a senolytic formulation of five (5) plant species known to induce autophagy and mitophagy, including ***Taraxacum officinale, Camellia senensis, Curcuma longa, Berberis vulgaris and Cinnamomum verum***. This formula is a synergistic herbal analog providing senolytic cellular support, increasing the efficiency of autophagy and mitophagy.

Cellular Senescence

Cellular senescence is a permanent state of cell cycle arrest induced by cellular stresses. When the cell is in senescence, its protein expression profile usually changes. When DNA damage occurs, the cell cycle promoter gene is down-regulated, and the cell cycle inhibitory gene is up-regulated, and cells are induced to block and lead in through different signaling pathways.

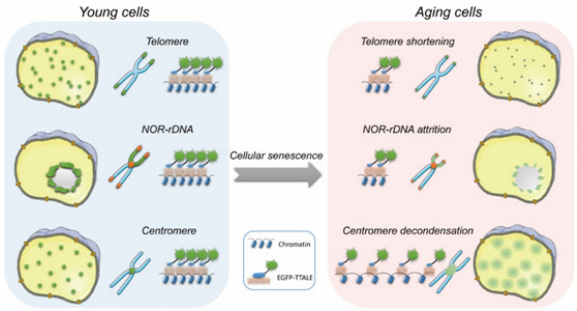

Figure 1. Structure changes of cell senescence

Cellular senescence provides sufficient time for DNA damage repair to maintain the stability of the cell's genome. Cellular senescence requires signal transduction. When the phenotype associated with cell senescence occurs, such as growth arrest, and formation of senescence-associated heterochromatin sites, the maintenance of the pathway is no longer required.

Senescence During the Aging Process

During the aging process, senescent cells (SCs) increasingly accumulate in tissues, causing a loss of tissue-repair capacity because of cell cycle arrest in progenitor cells and produce proinflammatory molecules which are known as the senescence-associated secretory phenotype (SASP) which contribute to the development of various age-related diseases.

Pathway regulation

The process of cellular senescence can be regulated by various factors and pathways in the cellular senescence signaling pathway. Cell senescence is similar to the state of cell cycle arrest, and the difference is that senescent tumor cells do not have activation of the apoptotic pathway.

The Function of Cell Autophagy

Autophagy is a fundamental biological process by removing damaged organelles, but disordered autophagy is involved in a variety of diseases including

neurodegeneration and microbial infection. Autophagy is activated in response to adverse environmental conditions such as the deprivation of nutrients, hypoxia, pathogen infection, radiation and oxidative stress as a survival mechanism. This process plays a role in cellular homeostasis, development, and longevity and has many effects on cellular renovation. Autophagy can contribute to whole-body rejuvenation and is considered a physiologic cytoprotective or pro-survival mechanism. Completely uncontrolled or excessive autophagy has been associated with cell death. Defective autophagy has been linked to aging and neurodegenerative disorders.

Autophagy is an evolutionary conserved catabolic process used by eukaryotic cells for the degradation of damaged or superfluous proteins and organelles. There are roughly three main forms of autophagy: Macro-autophagy is the major type of autophagy. It involves the sequestering of cellular constituents in double-membrane vesicles (autophagosomes) and subsequent delivery to lysosomes for degradation. It can be nonselective or selective. Nonselective autophagy is used for the turnover of bulk cytoplasm under starvation conditions. Selective autophagy targets damaged or superfluous mitochondria (mitophagy), peroxisomes (pexophagy), lipid droplets (lipophagy) and microbes (xenophagy). Micro-autophagy is the second type of autophagy. The lysosome itself engulfs small components of the cytoplasm by inward invagination of the lysosomal membrane. Chaperone-mediated autophagy does not involve membrane reorganization, instead, substrate proteins directly translocate across the lysosomal membrane during chaperone-mediated autophagy.

Mitophagy

Mitophagy is an essential catabolic pathway by which cytoplasmic materials are delivered to and degraded in the mitochondrial lysosome. This highly regulated pathway is physiologically essential, ensuring nutrient recycling, and cellular and organismal homeostasis during stress. It is activated by various endogenous and exogenous stimuli. Mitophagy is required to allow sessile organisms to cope with biotic or abiotic stress conditions. Defective mitophagy has been associated with aging and neuronal degeneration disorders.

Senolytic drugs

Senolytic drugs are agents that selectively induce apoptosis of senescent cells. These cells accumulate in many tissues with aging and at sites of pathology in multiple chronic diseases. Targeting senescent cells using genetic, pharmacological, or herbal plant-based approaches delays, prevents, or alleviates multiple age-related phenotypes, chronic diseases, geriatric syndromes, and loss of physiological resilience. Among the chronic conditions successfully treated by depleting senescent cells are frailty, cardiac dysfunction, diabetes, liver steatosis, osteoporosis, vertebral disk degeneration, and pulmonary fibrosis. Genetic evidence has demonstrated that clearance of SCs can delay aging and extend healthspan. Senolytics have been developed to treat various age-related diseases. Natural compounds have been discovered to be effective senolytic agents, such as quercetin, fisetin, spermidine, and curcumin.

Active Herbal Ingredients

Taraxacum officinale, A source of the autophagy inducing flavonoids quercetin, luteolin, apigenin, and luteolin-7-glucoside Extracts have anti-influenza, anti-retrovirus activity, antioxidant and hepatoprotective effects. Ethanol extracts reduce inflammation and inhibit angiogenesis. Dandelion contains sesquiterpene lactones (believed to have anti-inflammatory and anti-cancer effects).

Camellia sinensis, A source of the autophagy inducing polyamine Spermidine. *Camellia sinensis* has diverse pharmacological activities, including anti-hyperglycemia, antioxidative, anti-obesity and antitumor activities. the major theaflavins in black tea are theaflavin (TF1), theaflavin-3-gallate (TF2A), theaflavin-3'-gallate (TF2B) and theaflavin-3,3'-digallate (TF3) Cs also has a beneficial effect on immunomodulatory activity that is attributed to dietary fibers and specific polyphenols. The polyphenolic compounds in tea demonstrate potential antitumor and anti-oxidant effects in various cancer cell lines, including gastric, colon, and lung.

Berberis vulgaris, Berberine, a major isoquinoline alkaloid present in ***Berberis vulgaris***, is a potent inhibitor of inflammation and has shown anti-diabetic activity. Type 2 diabetes and obesity are rapidly becoming a worldwide epidemic and they are associated with the development of insulin resistance. Insulin resistance is believed to be an underlying feature of type 2 diabetes and metabolic syndrome. Berberine has a wide range of pharmacologic actions, such as antidiarrheic, anticancer, and antiinflammation. It has been used for the treatment of infective and inflammatory disorders. It improves insulin resistance, lowers blood sugar, and treats lipid metabolism disorders by activating the AMP activated protein kinase (AMPK) pathways. Berberine inhibits gene expression of proinflammatory cytokines in adipose tissue of obese mice and suppresses inflammatory response through AMPK activation in macrophages, while demonstrating its anti-inflammatory potency.

Curcuma longa, *or* Turmeric root, and it's curcuminoid constituents have demonstrated properties consistent with decreases in inflammatory stress signaling and increases in protective signaling. Curcumin is known to have anti-aging, anti-oxidant, anti-inflammatory, anti-arthritic, and anti-cancer effects and increases BDNF while having a positive effect on Alzheimer's disease and depression. It is also anti-rheumatic, and anti-microbial.

Cinnamomum verum, Cinnamon health benefits are attributed to its content of a few specific types of antioxidants, including polyphenols, phenolic acid, and flavonoids. These compounds work to fight oxidative stress in the body and aid in the prevention of chronic disease. the effects of cinnamon on life span implicated major longevity pathways. These include the DAF-16 transcription factor in the insulin signaling pathway, which promotes the expression of stress resistance and the longevity genes. Cinnamon activates the insulin signaling pathway, anti-oxidative pathway and serotonin signaling for its lifespan prolonging effect.

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Emerging senolytic agents derived from natural products

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ABSTRACT

Cellular senescence is a hallmark of aging, it is a permanent state of cell cycle arrest induced by cellular stresses. During the aging process, senescent cells (SCs) increasingly accumulate in tissues, causing a loss of tissue-repair capacity because of cell cycle arrest in progenitor cells and produce proinflammatory and matrix-degrading molecules which are known as the senescence-associated secretory phenotype (SASP), and thereby contribute to the development of various age-related diseases. Genetic evidence has demonstrated that clearance of SCs can delay aging and extend healthspan. Senolytics, small molecules that can selectively kill SCs, have been developed to treat various age-related diseases. In recent years, emerging natural compounds have been discovered to be effective senolytic agents, such as quercetin, fisetin, piperlongumine and the curcumin analog. Some of the compounds have been validated in animal models and have great potential to be pushed to clinical applications. In this review, we will discuss cellular senescence and its potential as a target for treating age-related diseases, and summarize the known natural compounds as senolytic agents and their applications.

1. Introduction

Aging is an irreversible process characterized by a progressive loss of physiological integrity, causing impaired function and increased vulnerability to death (López-Otín et al., 2013). It has been shown to be the primary risk factor for major age-related diseases, such as cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases. The hallmarks of aging, such as cellular senescence, genomic instability, telomere attrition, epigenetic alterations, and mitochondrial dysfunction, have been described previously (López-Otín et al., 2013). Accumulating evidence suggests that targeting some of the aging hallmarks, for example, cellular senescence, can significantly improve human health and extend healthspan (Childs et al., 2017; He and Sharpless, 2017; Kirkland and Tchkonia, 2017; Naylor et al., 2013; Niedernhofer and Robbins, 2018).

Cellular senescence is a phenomenon where normal cells stop dividing. Senescent cells (SCs) accumulate in various tissues during the aging process. On one hand, cellular senescence blocks the propagation of damaged cells in order to maintain tissue homeostasis (Demaria et al., 2014). On the other hand, it plays a causative role in irreparable, deleterious cellular damage and loss of tissue homeostasis, which relates to aging and aging-associated diseases (Campisi and d'Adda di Fagagna, 2007). Accumulating evidence demonstrates that elimination of SCs can reduce age-dependent deterioration in tissues and organs, which is useful in improving the treatment of age-associated diseases and alleviating the side effects of therapy-induced senescence (Baker et al., 2011; Campisi and d'Adda di Fagagna, 2007; Childs et al., 2015; He and Sharpless, 2017; Kirkland and Tchkonia, 2017; Naylor et al., 2013; Niedernhofer and Robbins, 2018).

Small molecules that can selectively kill SCs, called senolytics, have the potential to both prevent and treat age-related diseases, thereby extending healthspan. Until now, several classes of senolytic agents, including natural compounds such as quercetin (Geng et al., 2018; Hwang et al., 2018; Zhu et al., 2015), fisetin (Yousefzadeh et al., 2018), piperlongumine (Wang et al., 2016b; Zhang et al., 2018), and curcumin analog EF24 (Li et al., 2019), and targeted therapeutics, which are mainly senolytic target inhibitors, have been identified. Compared to the targeted senolytics, natural senolytic compounds are less potent, but

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Senolytics Improve Physical Function and Increase Lifespan in Old Age

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Author contributions: M.X., T.T., and J.L.K. conceived and designed the study. M.X. performed and analyzed most of the transplanted animal experiments and human adipose tissue explant experiments. N.G. and T.T. contributed to the human adipose tissue explant experiments. J.N.F., D.G.F., J.L.O., and S.K. contributed to the study of aged mice treated with senolytics. A.K.P. contributed to the bioluminescence studies. M.B.O. and D.J. contributed to ear fibroblast isolation. T.P., M.X., T.T., and C.L.I. performed the survival experiments using senolytics in aged mice. B.M.W., M.M.W., C.M.H., N.K.L., H.C., V.D.G., X.H., S.J.W., K.O.J., M.W., L.G.P.L., G.C.V., N.K.L., P.D.R., L.J.N., and J.D.M. contributed to the animal studies. R.J.S. contributed to androgen measurement. D.B.A. and K.E. contributed to lifespan analysis. G.B.H. and Y.I. contributed to mouse pathology analysis. M.X. and J.L.K. wrote the manuscript with input from all co-authors. J.L.K., M.X., and T.T. oversaw all experimental design, data analysis, and manuscript preparation.

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Epigenetic clock analyses of cellular senescence and ageing

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ABSTRACT

A confounding aspect of biological ageing is the nature and role of senescent cells. It is unclear whether the three major types of cellular senescence, namely replicative senescence, oncogene-induced senescence and DNA damage-induced senescence are descriptions of the same phenomenon instigated by different sources, or if each of these is distinct, and how they are associated with ageing. Recently, we devised an epigenetic clock with unprecedented accuracy and precision based on very specific DNA methylation changes that occur in function of age. Using primary cells, telomerase-expressing cells and oncogene-expressing cells of the same genetic background, we show that induction of replicative senescence (RS) and oncogene-induced senescence (OIS) are accompanied by ageing of the cell. However, senescence induced by DNA damage is not, even though RS and OIS activate the cellular DNA damage response pathway, highlighting the independence of senescence from cellular ageing. Consistent with this, we observed that telomerase-immortalised cells aged in culture without having been treated with any senescence inducers or DNA-damaging agents, re-affirming the independence of the process of ageing from telomeres and senescence. Collectively, our results reveal that cellular ageing is distinct from cellular senescence and independent of DNA damage response and telomere length.

INTRODUCTION

While ageing at the level of the organism is obvious and easily understood, the biological aspect of ageing is far from clear. Even the definition of ageing is not self-evident. It is reasonable to consider ageing as a natural biological process that in time, leads to the eventual failure of organs, as it is this that gives rise to the phenotypic features of ageing; from the benign, such as thinning of the skin and greying of the hair, to the pathological, such as cataracts and cardiovascular disease. Understanding why tissues and cells function sub-optimally and eventually fail in time, will greatly aid our understanding of ageing.

One model of ageing posits that the failure of tissues to function properly is due to the depletion of stem cells [1]. Stem cells, which are the reservoir cells of tissues, may have finite capacities of proliferation such as being limited by the lengths of their telomeres. Their eventual depletion leads to the deficit of properly functioning cells, causing phenotypic changes that constitute ageing. While this model is plausible and supported by strong circumstantial evidence, it is presently difficult to prove or refute directly, not least because the identification of specific tissue stem cells is difficult. Similarly, the association between telomere length and ageing, although widely reported, is not without inconsistencies [2-4].

There is however, another model of ageing which is based on the observation that the number of senescent cells in the body increases in function of organism age [5-7]. While this could be interpreted to mean that senescent cells cause ageing, it could also equally mean that senescent cells are a consequence of ageing. In this regard, it is noteworthy that there is increasing evidence to demonstrate that senescent cells are not benign. Instead they secrete bio-chemicals that are detrimental to normal functioning of neighbouring cells. The senescence-associated secretory phenotype (SASP) proteins include cytokine, chemokines and proteases [8, 9] and their paracrine activities are very diverse and include oncogenic characteristics that stimulate cellular proliferation and

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Mitophagy in neurodegeneration and aging

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Macroautophagy is a cellular catabolic process that involves the sequestration of cytoplasmic constituents into double-membrane vesicles known as autophagosomes, which subsequently fuse with lysosomes, where they deliver their cargo for degradation. The main physiological role of autophagy is to recycle intracellular components, under conditions of nutrient deprivation, so as to supply cells with vital materials and energy. Selective autophagy also takes place in nutrient-rich conditions to rid the cell of damaged organelles or protein aggregates that would otherwise compromise cell viability. Mitophagy is a selective type of autophagy, whereby damaged or superfluous mitochondria are eliminated to maintain proper mitochondrial numbers and quality control. While mitophagy shares key regulatory factors with the general macroautophagy pathway, it also involves distinct steps, specific for mitochondrial elimination. Recent findings indicate that parkin and the phosphatase and tensin homolog-induced putative kinase protein 1 (PINK1), which have been implicated in the pathogenesis of neurodegenerative diseases such as Parkinson's disease, also regulate mitophagy and function to maintain mitochondrial homeostasis. Here, we survey the molecular mechanisms that govern the process of mitophagy and discuss its involvement in the onset and progression of neurodegenerative diseases during aging.

Keywords: aging, autophagy, neuron, mitochondria, mitophagy, neurodegeneration, parkin, PINK1

INTRODUCTION

Macroautophagy (henceforth referred to as autophagy) is a high-regulated catabolic process responsible for the lysosomal degradation of cytoplasmic constituents. The main characteristic of the autophagic pathway is the formation of a double-membrane structure known as autophagosome, which engulfs cytoplasmic cargo and delivers it to lysosomes for degradation (Klionsky, 2007). In direct correlation with the large variety of autophagy substrates, including cytoplasmic proteins, ribosomes, organelles, bacteria and viruses, autophagy defects have been associated with a wide range of human disorders, such as cancer, autoimmune and neurodegenerative diseases (Mizushima et al., 2008). The main physiological role of autophagy is to supply the cell with essential materials and energy by recycling intracellular components, under conditions of nutrient deprivation when nutrients cannot be obtained from the extracellular environment. Selective types of autophagy, including pexophagy (Sakai et al., 2006), ribophagy (Kraft et al., 2008), ER-phagy (Bernales et al., 2007), protein selective chaperone-mediated autophagy (Cuervo et al., 2004), nucleophagy (Mijaljica et al., 2010), mitochondrial autophagy (mitophagy; Lemasters, 2005) take place under nutrient-rich conditions to rid the cell of damaged organelles or protein aggregates that would otherwise compromise cell viability.

Mitochondria are double-membrane-bound organelles, essential for energy production and cellular homeostasis in eukaryotic cells. In addition, mitochondria have vital roles in calcium signaling and storage, metabolite synthesis, and apoptosis (Kroemer et al., 2007). Thus, mitochondrial biogenesis, as well as, elimination of damaged and superfluous mitochondria are highly regulated processes. Mitophagy is a selective type of autophagy that mediates the removal of mitochondria. Through mitophagy cells regulate mitochondrial number in response to their metabolic state and also implement a quality control system for proper elimination of damaged mitochondria. The process of mitophagy is highly regulated and conserved from yeast to mammals (**Table 1**). While mitophagy shares key regulatory factors with the general autophagy pathway, it also involves distinct steps, specific for mitochondrial elimination. Studies in yeast identified specific genes that are required for mitophagy, but not for other types of autophagy (Kanki et al., 2009a; Kanki and Klionsky, 2010), demonstrating the selective regulation of this process. Despite the fact that the actual selection of mitochondria for degradation is a still obscure part of the process, recent studies shed light on the mechanisms that govern mitophagy and regulate removal of mitochondria during developmental processes or upon mitochondrial damage. In this review, we survey the molecular mechanisms that mediate mitophagy and also highlight how defects in this process may contribute to the onset and progression of neurodegenerative diseases during aging.

MOLECULAR MECHANISMS OF MITOPHAGY

The molecular mechanisms of mitophagy were studied in the yeast *Saccharomyces cerevisiae*. The yeast *uth1* gene encodes a Sad1p/UNC-84 (SUN)-domain protein that is located in the outer mitochondrial membrane and is essential for the specific autophagic elimination of mitochondria upon nitrogen starvation or rapamycin treatment, without influencing general autophagy (Kissova et al., 2004). The protein Aup1, a member of protein phosphatase 2C (PP2C) superfamily that is located in the

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Review

Autophagy mediates pharmacological lifespan extension by spermidine and resveratrol

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Abbreviations: AMPK, AMP-activated protein kinase; ATG, autophagy-related; BH3, Bcl-2 homology; dsRNA, double-stranded RNA; eIF2α, eukaryotic translation initiation factor 2α; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; HIF-1, hypoxia-inducible factor 1; IKK, IκB kinase; IGF, insulin-like growth factor; IP_3, inositol 1,4,5-trisphosphate; IP_3R, IP_3 receptor; JNK, c-JUN N-terminal kinase; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; PI3K, phoshatidylinositol-3-kinase; RNAi, RNA interference; TOR, target of rapamycin.
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Abstract: Although autophagy has widely been conceived as a self-destructive mechanism that causes cell death, accumulating evidence suggests that autophagy usually mediates cytoprotection, thereby avoiding the apoptotic or necrotic demise of stressed cells. Recent evidence produced by our groups demonstrates that autophagy is also involved in pharmacological manipulations that increase longevity. Exogenous supply of the polyamine spermidine can prolong the lifespan of (while inducing autophagy in) yeast, nematodes and flies. Similarly, resveratrol can trigger autophagy in cells from different organisms, extend lifespan in nematodes, and ameliorate the fitness of human cells undergoing metabolic stress. These beneficial effects are lost when essential autophagy modulators are genetically or pharmacologically inactivated, indicating that autophagy is required for the cytoprotective and/or anti-aging effects of spermidine and resveratrol. Genetic and functional studies indicate that spermidine inhibits histone acetylases, while resveratrol activates the histone deacetylase Sirtuin 1 to confer cytoprotection/longevity. Although it remains elusive whether the same histones (or perhaps other nuclear or cytoplasmic proteins) act as the downstream targets of spermidine and resveratrol, these results point to an essential role of protein hypoacetylation in autophagy control and in the regulation of longevity.

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Molecular Basis of the 'Anti-Aging' Effect of Spermidine and Other Natural Polyamines – A Mini-Review

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Key Words
Spermidine · Polyamines · Longevity · Aging · Autophagy · Inflammation · Metabolism · Cell survival

Abstract
Background: Spermidine, a naturally occurring polyamine, has recently emerged as exhibiting anti-aging properties. Its supplementation increases lifespan and resistance to stress, and decreases the occurrence of age-related pathology and loss of locomotor ability. Its mechanisms of action are just beginning to be understood. ***Objectives:*** An up-to-date overview of the so far identified mechanisms of action of spermidine and other polyamines on aging is presented. ***Methods:*** Studies of aging and of the molecular effects of polyamines in general and spermidine in particular are used to synthesize our knowledge on what molecular mechanisms spermidine and other polyamines trigger to positively affect aging. ***Results:*** Autophagy is the main mechanism of action of spermidine at the molecular level. However, recent research shows that spermidine can act via other mechanisms, namely inflammation reduction, lipid metabolism and regulation of cell growth, proliferation and death. It is suggested that the main pathway used by spermidine to trigger its effects is the MAPK pathway. ***Conclusions:*** Given that polyamines can interact with many molecules, it is not surprising that they affect aging via several mechanisms. Many of these mechanisms discovered so far have already been linked with aging and by acting on all of these mechanisms, polyamines may be strong regulators of aging.

Introduction

Aging is a multifaceted process, probably caused by a myriad of interacting factors and with consequences at all levels of the organism. Research into the subject has revealed that factors leading to aging are as varied as sustained exposure to cellular stress, chronic inflammation, dysregulation of lipid metabolism, autophagy and cell survival and death. These factors will impinge upon each other in complex interactions. Effective interventions against aging will need to be able to impact as many as possible of the factors causing aging and their interactions. Dietary restriction may be one such intervention with its wide-ranging effects. Polyamines, especially spermidine, have also emerged as strong potential candidates

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Review

Natural Compounds from Herbs that can Potentially Execute as Autophagy Inducers for Cancer Therapy

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Abstract: Accumulated evidence indicates that autophagy is a response of cancer cells to various anti-cancer therapies. Autophagy is designated as programmed cell death type II, and is characterized by the formation of autophagic vacuoles in the cytoplasm. Numerous herbs, including Chinese herbs, have been applied to cancer treatments as complementary and alternative medicines, supplements, or nutraceuticals to dampen the side or adverse effects of chemotherapy drugs. Moreover, the tumor suppressive actions of herbs and natural products induced autophagy that may lead to cell senescence, increase apoptosis-independent cell death or complement apoptotic processes. Hereby, the underlying mechanisms of natural autophagy inducers are cautiously reviewed in this article. Additionally, three natural compounds—curcumin, 16-hydroxycleroda-3,13-dien-15,16-olide, and prodigiosin—are presented as candidates for autophagy inducers that can trigger cell death in a supplement or alternative medicine for cancer therapy. Despite recent advancements in therapeutic drugs or agents of natural products in several cancers, it warrants further investigation in preclinical and clinical studies.

Keywords: autophagy inducer; autophagy inhibitor; natural compound; cancer therapy

1. Introduction

Cancer is a group of diseases involving out-of-control of cell growth due to the accumulation of defects, or mutations, in their DNA and with an impendence to invade or spread to other parts of the body [1]. In 2015, about 90.5 million people were diagnosed with cancer [2]. About 14.1 million new cases occur each year (not including skin cancer other than melanoma) [3]. Consequently, it causes about 8.8 million (15.7%) human deaths [4]. Anti-cancer drugs including 5-fluorouracil (5-FU), cisplatin, etoposide, paclitaxel, and doxorubicin are commonly used to treat various cancers, such as cisplatin and doxorubicin in ovarian cancer, 5-FU in colon and gastric cancer, paclitaxel and doxorubicin in breast cancer, and etoposide in small-cell lung cancer. However, these chemotherapeutic agents have evident side effects such as nausea, vomiting, loss of appetite, decreased immunity, oral ulcers, and other adverse effects [5]. In general, the anti-cancer drugs, such as cisplatin and doxorubicin favor abnormal triggering via programmed cell death (PCD) such as apoptosis, necrosis, necroptosis, and autophagy in normal cells as well as abolishing inflammation of damaged cells. Remarkably, apoptosis and

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Induction of autophagy by spermidine promotes longevity.

Eisenberg T[1], Knauer H, Schauer A, Büttner S, Ruckenstuhl C, Carmona-Gutierrez D, Ring J, Schroeder S, Magnes C, Antonacci L, Fussi H, Deszcz L, Hartl R, Schraml E, Criollo A, Megalou E, Weiskopf D, Laun P, Heeren G, Breitenbach M, Grubeck-Loebenstein B, Herker E, Fahrenkrog B, Fröhlich KU, Sinner F, Tavernarakis N, Minois N, Kroemer G, Madeo F.

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Abstract

Ageing results from complex genetically and epigenetically programmed processes that are elicited in part by noxious or stressful events that cause programmed cell death. Here, we report that administration of spermidine, a natural polyamine whose intracellular concentration declines during human ageing, markedly extended the lifespan of yeast, flies and worms, and human immune cells. In addition, spermidine administration potently inhibited oxidative stress in ageing mice. In ageing yeast, spermidine treatment triggered epigenetic deacetylation of histone H3 through inhibition of histone acetyltransferases (HAT), suppressing oxidative stress and necrosis. Conversely, depletion of endogenous polyamines led to hyperacetylation, generation of reactive oxygen species, early necrotic death and decreased lifespan. The altered acetylation status of the chromatin led to significant upregulation of various autophagy-related transcripts, triggering autophagy in yeast, flies, worms and human cells. Finally, we found that enhanced autophagy is crucial for polyamine-induced suppression of necrosis and enhanced longevity.

Comment in

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Active extracts of black tea *(Camellia Sinensis)* induce apoptosis of PC-3 prostate cancer cells via mitochondrial dysfunction

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Abstract. Cancer of the prostate gland is the most common invasive malignancy and the second leading cause of cancer-related death in human males. Many studies have shown that black tea reduces the risk of several types of cancer. We studied the effects of active extracts of black tea and the black tea polyphenols theaflavins (TFs), on the cellular proliferation and mitochondria of the human prostate cancer cell line PC-3. Our studies revealed that Yinghong black tea extracts (YBT), Assam black tea extracts (ABT) and TFs inhibited cell proliferation in a dose-dependent manner. We also showed that TFs, YBT and ABT affected the morphology of PC-3 cells and induced apoptosis or even necrosis in PC-3 cells. In addition, it was observed that the samples significantly caused loss of the mitochondrial membrane potential, release of cytochrome *c* from the intermembrane space into the cytosol, decrease of the ATP content and activation of caspase-3 compared with the control. Taken together, these findings suggest that black tea could act as an effective anti-proliferative agent in PC-3 cells, and TFs, YBT and ABT induced apoptosis of PC-3 cells through mitochondrial dysfunction.

Introduction

Tea, one of the most widely consumed beverages in the world, has diverse pharmacological activities, including anti-hyperglycemia, antioxidative, anti-obesity and antitumor activities (1-4). Green tea, consumed at high levels in Asian countries, and black tea, consumed primarily in Western countries, are derived from leaves of *Camellia sinensis* (5).

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Key words: theaflavins, black tea, PC-3 cells, mitochondria, cytochrome *c*

During the past decades, numerous *in vitro* and *in vivo* studies have showed the possible protective effects of tea and tea polyphenols on cancer and other diseases (6-8). Catechins are the most abundant polyphenol in green tea, whereas the typical pigments in black tea are formed from catechin oxidation during fermentation which includes theaflavins, thearubigins and theabrownins (9). Among them, the major theaflavins in black tea are theaflavin (TF1), theaflavin-3-gallate (TF2A), theaflavin-3'-gallate (TF2B) and theaflavin-3,3'-digallate (TF3) (Fig. 1). Therefore, the monomeric polyphenol content of black tea is low. However, it is not diminished for black tea during partial polymerization or other alterations in the fermentation of tea leaves (10), as it was proved that TF3 showed higher antioxidative activity than EGCG (11).

Apoptosis can occur via the mitochondria. The mitochondria is essential for energy production, and is involved in reactive oxygen species (ROS) generation and induction of apoptosis (12). In most tissues, the mitochondria accounts for the generation of ~90% of the ATP needed by the cells (13). Mitochondrial dysfunction causes permeabilization of the outer mitochondrial membrane (14) and then leads to the release of the intermembrane space proteins such as cytochrome *c*, which ultimately triggers apoptotic cell death (15). Therefore, the mitochondrial permeability transition event could serve as an early indicator of the initiation of apoptosis. This mitochondrial permeability transition process results in the collapse of the electrochemical gradient across mitochondrial membrane and thus could be measured by noting the changes of the mitochondrial membrane potential (14).

Prostate cancer is one of the leading causes of human male deaths throughout the world (16). It is a group of cancerous cells (a malignant tumor) that grow mostly from the outer part of the prostate (17). In our previous study, we evaluated time- and dose-dependent cytotoxicity of EGCG and Zn^{2+} on PC-3 cells by the MTT assay (18). Whereas, in another study, we found that EGCG, Zn^{2+} and EGCG+Zn^{2+} induced apoptosis or even necrosis of PC-3 cells through the mitochondria-mediated pathway, and free Zn^{2+} enhanced the effects of EGCG on PC-3 cells due to its interactions with mitochondria (19). In the present study, we investigated effects of black tea extracts on the viability and morphology of PC-3 cells, the functions of mitochondria of PC-3 cells,

References

1.Adams, B.K., Cai, J., Armstrong, J., Herold, M., Lu, Y.J., Sun, A., Snyder, J.P., Liotta, D.C., Jones, D.P., Shoji, M., 2005. EF24, a novel synthetic curcumin analog, induces apoptosis in cancer cells via a redox-dependent mechanism. Anticancer Drugs 16, 263–275.

2. Aggarwal, B.B., Harikumar, K.B., 2009. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int. J. Biochem. Cell Biol. 41, 40–59.

3. Anand, P., Thomas, S.G., Kunnumakkara, A.B., Sundaram, C., Harikumar, K.B., Sung, B., Tharakan, S.T., Misra, K., Priyadarsini, I.K., Rajasekharan, K.N., Aggarwal, B.B., 2008. Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. Biochem. Pharmacol. 76, 1590–1611.

4. Anand David, A.V., Arulmoli, R., Parasuraman, S., 2016. Overviews of biological importance of quercetin: a bioactive flavonoid. Pharmacogn. Rev. 10, 84–89.

5. Arai, Y., Watanabe, S., Kimira, M., Shimoi, K., Mochizuki, R., Kinae, N., 2000. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. J. Nutr. 130, 2243–2250.

6. Assuncao, M., Andrade, J.P., 2015. Protective action of green tea catechins in neuronal mitochondria during aging. Front. Biosci. (Landmark Ed.) 20, 247–262.

7. Baar, M.P., Brandt, R.M.C., Putavet, D.A., Klein, J.D.D., Derks, K.W.J., Bourgeois, B.R.M., Stryeck, S., Rijksen, Y., van Willigenburg, H., Feijtel, D.A., van der Pluijm, I., Essers, J., van Cappellen, W.A., van IJcken, W.F., Houtsmuller, A.B., Pothof, J., de Bruin, R.W.F., Madl, T., Hoeijmakers, J.H.J., Campisi, J., de Keizer, P.L.J., 2017. Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging. Cell 169 132-147.e16.

8. Baker, D.J., Wijshake, T., Tchkonia, T., LeBrasseur, N.K., Childs, B.G., van de Sluis, B., Kirkland, J.L., van Deursen, J.M., 2011. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature 479, 232–236.

9. Baker, D.J., Childs, B.G., Durik, M., Wijers, M.E., Sieben, C.J., Zhong, J., Saltness, R.A., Jeganathan, K.B., Verzosa, G.C., Pezeshki, A., Khazaie, K., Miller, J.D., van Deursen, J.M., 2016. Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. Nature 530, 184–189.

10. Belinha, I., Amorim, M.A., Rodrigues, P., de Freitas, V., Moradas-Ferreira, P., Mateus, N., Costa, V., 2007. Quercetin increases oxidative stress resistance and longevity in Saccharomyces cerevisiae. J. Agric. Food Chem. 55, 2446–2451.

11. Bent, E.H., Gilbert, L.A., Hemann, M.T., 2016. A senescence secretory switch mediated by PI3K/AKT/mTOR activation controls chemoprotective endothelial secretory responses. Genes Dev. 30, 1811–1821.

12. Bernatoniene, J., Kopustinskiene, D.M., 2018. The role of catechins in cellular responses to oxidative stress. Molecules 23.

13. Bezerra, D.P., Militão, G.C.G., de Castro, F.O., Pessoa, C., de Moraes, M.O., Silveira, E.R., Lima, M.A.S., Elmiro, F.J.M., Costa-Lotufo, L.V., 2007. Piplartine induces inhibition of leukemia cell proliferation triggering both apoptosis and necrosis pathways. Toxicol. In Vitro 21, 1–8.

14. Bezerra, D.P., Pessoa, C., de Moraes, M.O., Saker-Neto, N., Silveira, E.R., Costa-Lotufo, L.V., 2013. Overview of the therapeutic potential of piplartine (piperlongumine). Eur. J. Pharm. Sci. 48, 453–463.

15. Boots, A.W., Haenen, G.R.M.M., Bast, A., 2008. Health effects of quercetin: from antioxidant to nutraceutical. Eur. J. Pharmacol. 585, 325–337. Campisi, J., d'Adda di Fagagna, F., 2007. Cellular senescence: when bad things happen to good cells. Nat. Rev. Mol. Cell Biol. 8, 729–740.

16. Chandrashekara, K.T., Popli, S., Shakarad, M.N., 2014. Curcumin enhances parental reproductive lifespan and progeny viability in Drosophila melanogaster. Age (Dordr) 36, 9702.

17. Chang, J., Wang, Y., Shao, L., Laberge, R.-M., Demaria, M., Campisi, J., Janakiraman, K., Sharpless, N.E., Ding, S., Feng, W., Luo, Y., Wang, X., Aykin-Burns, N., Krager, K.,Ponnappan, U., Hauer-Jensen, M., Meng, A., Zhou, D., 2016. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. Nat. Med. 22, 78–83.

18. Childs, B.G., Durik, M., Baker, D.J., van Deursen, J.M., 2015. Cellular senescence in aging

and age-related disease: from mechanisms to therapy. Nat. Med. 21, 1424–1435.
19. Childs, B.G., Baker, D.J., Wijshake, T., Conover, C.A., Campisi, J., van Deursen, J.M., 2016. Senescent intimal foam cells are deleterious at all stages of atherosclerosis. Science 354, 472–477.
20. Childs, B.G., Gluscevic, M., Baker, D.J., Laberge, R.-M., Marquess, D., Dananberg, J., van Deursen, J.M., 2017. Senescent cells: an emerging target for diseases of ageing. Nat. Rev. Drug Discov. 16, 718–735.
21. Cormenier, J., Martin, N., Deslé, J., Salazar-Cardozo, C., Pourtier, A., Abbadie, C., Pluquet, O., 2018. The ATF6α arm of the Unfolded Protein Response mediates replicative senescence in human fibroblasts through a COX2/prostaglandin E2 intracrine pathway. Mech. Ageing Dev. 170, 82–91.
22. Demaria, M., Ohtani, N., Youssef, S.A., Rodier, F., Toussaint, W., Mitchell, J.R., Laberge, R.-M., Vijg, J., Van Steeg, H., Dollé, M.E.T., Hoeijmakers, J.H.J., de Bruin, A., Hara, E., Campisi, J., 2014. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. Dev. Cell 31, 722–733.
23. Dinkova-Kostova, A.T., Cory, A.H., Bozak, R.E., Hicks, R.J., Cory, J.G., 2007. Bis (2 hydroxybenzylidene) acetone, a potent inducer of the phase 2 response, causes apoptosis in mouse leukemia cells through a p53-independent, caspase-mediated pathway. Cancer Lett. 245, 341–349.
24. Druelle, C., Drullion, C., Deslé, J., Martin, N., Saas, L., Cormenier, J., Malaquin, N., Huot, L., Slomianny, C., Bouali, F., Vercamer, C., Hot, D., Pourtier, A., Chevet, E., Abbadie, C., Pluquet, O., 2016. ATF6α regulates morphological changes associated with senescence in human fibroblasts. Oncotarget 7, 67699–67715. Formica, J.V., Regelson, W., 1995. Review of the biology of Quercetin and related bioflavonoids. Food Chem. Toxicol. 33, 1061–1080.
25. Fuhrmann-Stroissnigg, H., Ling, Y.Y., Zhao, J., McGowan, S.J., Zhu, Y., Brooks, R.W., Grassi, D., Gregg, S.Q., Stripay, J.L., Dorronsoro, A., Corbo, L., Tang, P., Bukata, C., Ring, N., Giacca, M., Li, X., Tchkonia, T., Kirkland, J.L., Niedernhofer, L.J., Robbins, P.D., 2017. Identification of HSP90 inhibitors as a novel class of senolytics. Nat. Commun. 8, 422.
26. Geng, L., Liu, Z., Zhang, W., Li, W., Wu, Z., Wang, W., Ren, R., Su, Y., Wang, P., Sun, L., Senolytics derived from nature plants may help delay aging, reduce age-related diseases and extend healthspan through eliminating SCs. W. Li, et al. Mechanisms of Ageing and Development 181 (2019) 1–65
27. Ju, Z., Chan, P., Song, M., Qu, J., Liu, G.-H., 2018. Chemical screen identifies a geroprotective role of quercetin in premature aging. Protein Cell.
28. Grill, A.E., Shahani, K., Koniar, B., Panyam, J., 2018. Chemopreventive efficacy of curcumin-loaded PLGA microparticles in a transgenic mouse model of HER-2-positive breast cancer. Drug Deliv. Transl. Res. 8, 329–341.
29. Gupta, S.C., Patchva, S., Aggarwal, B.B., 2013. Therapeutic roles of curcumin: lessons learned from clinical trials. AAPS J. 15, 195–218.
30. Harvey, A.L., 2008. Natural products in drug discovery. Drug Discov. Today 13, 894–901. Hatcher, H., Planalp, R., Cho, J., Torti, F.M., Torti, S.V., 2008. Curcumin: from ancient medicine to current clinical trials. Cell. Mol. Life Sci. 65, 1631–1652.
31. Hayflick, L., Moorhead, P.S., 1961. The serial cultivation of human diploid cell strains. Exp. Cell Res. 25, 585–621.
32. He, S., Sharpless, N.E., 2017. Senescence in health and disease. Cell 169, 1000–1011.
33. He, Y., Li, Y., Zhao, T., Wang, Y., Sun, C., 2013. Ursolic acid inhibits adipogenesis in 3T3-L1 adipocytes through LKB1/AMPK pathway. PLoS One 8, e70135.
34. He, Y., Li, W., Li, Y., Zhang, S., Wang, Y., Sun, C., 2014. Ursolic acid increases glucose uptake through the PI3K signaling pathway in adipocytes. PLoS One 9, e110711.
35. He, Y., Li, W., Hu, G., Sun, H., Kong, Q., 2018. Bioactivities of EF24, a novel curcumin analog: a review. Front. Oncol. 8, 614.
36. Hernandez-Segura, A., Nehme, J., Demaria, M., 2018. Hallmarks of cellular senescence. Trends Cell Biol. 28, 436–453.
37. Howitz, K.T., Bitterman, K.J., Cohen, H.Y., Lamming, D.W., Lavu, S., Wood, J.G., Zipkin, R.E., Chung, P., Kisielewski, A., Zhang, L.-L., Scherer, B., Sinclair, D.A., 2003. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature 425, 191–196.
38. Hwang, H.V., Tran, D.T., Rebuffatti, M.N., Li, C.-S., Knowlton, A.A., 2018. Investigation of quercetin and hyperoside as senolytics in adult human endothelial cells. PLoS One 13, e0190374.
39. Jeon, O.H., Kim, C., Laberge, R.-M., Demaria, M., Rathod, S., Vasserot, A.P., Chung, J.W., Kim, D.H., Poon, Y., David, N., Baker, D.J., van Deursen, J.M., Campisi, J., Elisseeff,

J.H., 2017. Local clearance of senescent cells attenuates the development of posttraumatic osteoarthritis and creates a pro-regenerative environment. Nat. Med. 23, 775–781.
40. Justice, J.N., Nambiar, A.M., Tchkonia, T., LeBrasseur, N.K., Pascual, R., Hashmi, S.K., Prata, L., Masternak, M.M., Kritchevsky, S.B., Musi, N., Kirkland, J.L., 2019. Senolytics in idiopathic pulmonary fibrosis: results from a first-in-human, open-label, pilot study. EBioMedicine.
41. Kashyap, D., Sharma, A., Sak, K., Tuli, H.S., Buttar, H.S., Bishayee, A., 2018. Fisetin: a bioactive phytochemical with potential for cancer prevention and pharmacotherapy. Life Sci. 194, 75–87.
42. Khan, N., Syed, D.N., Ahmad, N., Mukhtar, H., 2013. Fisetin: a dietary antioxidant for health promotion. Antioxid. Redox Signal. 19, 151–162.
43. Kimira, M., Arai, Y., Shimoi, K., Watanabe, S., 1998. Japanese intake of flavonoids and isoflavonoids from foods. J. Epidemiol. 8, 168–175.
44. Kirkland, J.L., Tchkonia, T., 2017. Cellular senescence: a translational perspective. EBioMedicine 21, 21–28.
45. Knutson, M.D., Leeuwenburgh, C., 2008. Resveratrol and novel potent activators of SIRT1: effects on aging and age-related diseases. Nutr. Rev. 66, 591–596.
46. Lall, R.K., Adhami, V.M., Mukhtar, H., 2016. Dietary flavonoid fisetin for cancer prevention and treatment. Mol. Nutr. Food Res. 60, 1396–1405.
47. Lämmermann, I., Terlecki-Zaniewicz, L., Weinmüllner, R., Schosserer, M., Dellago, H., de Matos Branco, A.D., Autheried, D., Sevcnikar, B., Kleissl, L., Berlin, I., Morizot, F., Lejeune, F., Fuzzati, N., Forestier, S., Toribio, A., Tromeur, A., Weinberg, L., Higareda Almaraz, J.C., Scheideler, M., Rietveld, M., El Ghalbzouri, A., Tschachler, E., Gruber, F., Grillari, J., 2018. Blocking negative effects of senescence in human skin fibroblasts with a plant extract. NPJ Aging Mech. Dis. 4, 4.
48. Li, T., Chen, S., Feng, T., Dong, J., Li, Y., Li, H., 2016. Rutin protects against aging-related metabolic dysfunction. Food Funct. 7, 1147–1154.
49. Li, W., He, Y., Zhang, R., Zheng, G., Zhou, D., 2019. The curcumin analog EF24 is a novel senolytic agent. Aging (Albany NY) 11, 771–782.
50. Liao, V.H.-C., Yu, C.-W., Chu, Y.-J., Li, W.-H., Hsieh, Y.-C., Wang, T.-T., 2011. Curcuminmediated lifespan extension in Caenorhabditis elegans. Mech. Ageing Dev. 132, 480–487.
51. Liu, X., Lin, X., Mi, Y., Li, J., Zhang, C., 2018. Grape seed proanthocyanidin extract prevents ovarian aging by inhibiting oxidative stress in the hens. Oxid. Med. Cell. Longev. 2018, 9390810.
52. López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks of aging. Cell 153, 1194–1217.
53. Maheshwari, R.K., Singh, A.K., Gaddipati, J., Srimal, R.C., 2006. Multiple biological activities of curcumin: a short review. Life Sci. 78, 2081–2087.
54. Marković, Z.S., Mentus, S.V., Dimitrić Marković, J.M., 2009. Electrochemical and density functional theory study on the reactivity of fisetin and its radicals: implications on in vitro antioxidant activity. J. Phys. Chem. A 113, 14170–14179.
55. Molinski, T.F., Dalisay, D.S., Lievens, S.L., Saludes, J.P., 2009. Drug development from marine natural products. Nat. Rev. Drug Discov. 8, 69–85.
56. Mukherjee, P.K., Maity, N., Nema, N.K., Sarkar, B.K., 2011. Bioactive compounds from natural resources against skin aging. Phytomedicine 19, 64–73.
57. Muñoz-Espín, D., Serrano, M., 2014. Cellular senescence: from physiology to pathology. Nat. Rev. Mol. Cell Biol. 15, 482–496.
58. Muñoz-Espín, D., Rovira, M., Galiana, I., Giménez, C., Lozano-Torres, B., Paez-Ribes, M., Llanos, S., Chaib, S., Muñoz-Martín, M., Ucero, A.C., Garaulet, G., Mulero, F., Dann, S.G., VanArsdale, T., Shields, D.J., Bernardos, A., Murguía, J.R., Martínez-Máñez, R., Serrano, M., 2018. A versatile drug delivery system targeting senescent cells. EMBO Mol. Med. 10.
59. Naylor, R.M., Baker, D.J., van Deursen, J.M., 2013. Senescent cells: a novel therapeutic target for aging and age-related diseases. Clin. Pharmacol. Ther. 93, 105–116.
60. Newman, D.J., Cragg, G.M., 2016. Natural products as sources of new drugs from 1981 to 2014. J. Nat. Prod. 79, 629–661.
61. Niedernhofer, L.J., Robbins, P.D., 2018. Senotherapeutics for healthy ageing. Nat. Rev. Drug Discov. 17, 377.
62. Pal, H.C., Pearlman, R.L., Afaq, F., 2016. Fisetin and its role in chronic diseases. Adv. Exp. Med. Biol. 928, 213–244.
63. Piska, K., Gunia-Krzyżak, A., Koczurkiewicz, P., Wójcik-Pszczoła, K., Pękala, E., 2018. Piperlongumine (piplartine) as a lead compound for anticancer agents - Synthesis and properties of analogues: a mini-review. Eur. J. Med. Chem. 156, 13–20.

64. Sastre, J., Millán, A., García de la Asunción, J., Plá, R., Juan, G., Pallardó, Null, O'Connor, E., Martin, J.A., Droy-Lefaix, M.T., Viña, J., 1998. A Ginkgo biloba extract (EGb 761) prevents mitochondrial aging by protecting against oxidative stress. Free Radic. Biol. Med. 24, 298–304.
65. Selvendiran, K., Kuppusamy, M.L., Bratasz, A., Tong, L., Rivera, B.K., Rink, C., Sen, C.K., Kálai, T., Hideg, K., Kuppusamy, P., 2009. Inhibition of vascular smooth-muscle cell proliferation and arterial restenosis by HO-3867, a novel synthetic curcuminoid, through up-regulation of PTEN expression. J. Pharmacol. Exp. Ther. 329, 959–966.
66. Sharpless, N.E., Sherr, C.J., 2015. Forging a signature of in vivo senescence. Nat. Rev. Cancer 15, 397–408.
67. Shoba, G., Joy, D., Joseph, T., Majeed, M., Rajendran, R., Srinivas, P.S., 1998. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta Med. 64, 353–356.
68. Singh, A., Naidu, P.S., Kulkarni, S.K., 2003. Reversal of aging and chronic ethanol-induced cognitive dysfunction by quercetin a bioflavonoid. Free Radic. Res. 37, 1245–1252.
69. Sundarraj, K., Raghunath, A., Perumal, E., 2018. A review on the chemotherapeutic potential of fisetin: in vitro evidences. Biomed. Pharmacother. 97, 928–940.
70. Takano, K., Tatebe, J., Washizawa, N., Morita, T., 2018. Curcumin inhibits age-related vascular changes in aged mice fed a high-fat diet. Nutrients 10.
71. Tamvakopoulos, C., Dimas, K., Sofianos, Z.D., Hatziantoniou, S., Han, Z., Liu, Z.-L., Wyche, J.H., Pantazis, P., 2007. Metabolism and anticancer activity of the curcumin analogue, dimethoxycurcumin. Clin. Cancer Res. 13, 1269–1277.
72. Wang, W., Sun, C., Mao, L., Ma, P., Liu, F., Yang, J., Gao, Y., 2016a. The biological activities, chemical stability, metabolism and delivery systems of quercetin: a review. Trends Food Sci. Technol. 56, 21–38.
73. Wang, Y., Chang, J., Liu, X., Zhang, Xuan, Zhang, S., Zhang, Xin, Zhou, D., Zheng, G., 2016b. Discovery of piperlongumine as a potential novel lead for the development of senolytic agents. Aging (Albany NY) 8, 2915.
74. Wood, J.G., Rogina, B., Lavu, S., Howitz, K., Helfand, S.L., Tatar, M., Sinclair, D., 2004. Sirtuin activators mimic caloric restriction and delay ageing in metazoans. Nature 430, 686–689.
75. Xu, Z., Feng, W., Shen, Q., Yu, N., Yu, K., Wang, S., Chen, Z., Shioda, S., Guo, Y., 2017. Rhizoma coptidis and Berberine as a natural drug to combat aging and aging-related diseases via anti-oxidation and AMPK activation. Aging Dis. 8, 760–777.
76. Xu, M., Pirtskhalava, T., Farr, J.N., Weigand, B.M., Palmer, A.K., Weivoda, M.M., Inman, C.L., Ogrodnik, M.B., Hachfeld, C.M., Fraser, D.G., Onken, J.L., Johnson, K.O., Verzosa, G.C., Langhi, L.G.P., Weigl, M., Giorgadze, N., LeBrasseur, N.K., Miller, J.D., Jurk, D., Singh, R.J., Allison, D.B., Ejima, K., Hubbard, G.B., Ikeno, Y., Cubro, H., Garovic, V.D., Hou, X., Weroha, S.J., Robbins, P.D., Niedernhofer, L.J., Khosla, S., Tchkonia, T., Kirkland, J.L., 2018. Senolytics improve physical function and increase lifespan in old age. Nat. Med. 24, 1246–1256.
77. Yang, Y.-C., Lin, H.-Y., Su, K.-Y., Chen, C.-H., Yu, Y.-L., Lin, C.-C., Yu, S.-L., Yan, H.-Y., Su, K.-J., Chen, Y.-L.S., 2012. Rutin, a flavonoid that is a main component of Saussurea involucrata, attenuates the senescence effect in D-galactose aging mouse model. Evid. Complement. Alternat. Med. 2012 980276.
78. Yang, L., Zheng, Z., Qian, C., Wu, J., Liu, Y., Guo, S., Li, G., Liu, M., Wang, X., Kaplan, D.L., 2017. Curcumin-functionalized silk biomaterials for anti-aging utility. J. Colloid Interface Sci. 496, 66–77.
79. Yosef, R., Pilpel, N., Tokarsky-Amiel, R., Biran, A., Ovadya, Y., Cohen, S., Vadai, E., Dassa, L., Shahar, E., Condiotti, R., Ben-Porath, I., Krizhanovsky, V., 2016. Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL. Nat. Commun. 7, 11190.
80. Yousefzadeh, M.J., Zhu, Y., McGowan, S.J., Angelini, L., Fuhrmann-Stroissnigg, H., Xu, M., Ling, Y.Y., Melos, K.I., Pirtskhalava, T., Inman, C.L., McGuckian, C., Wade, E.A., Kato, J.I., Grassi, D., Wentworth, M., Burd, C.E., Arriaga, E.A., Ladiges, W.L., Tchkonia, T., Kirkland, J.L., Robbins, P.D., Niedernhofer, L.J., 2018. Fisetin is a senotherapeutic that extends health and lifespan. EBioMedicine 36, 18–28.
81. Zhang, Xin, Zhang, S., Liu, X., Wang, Y., Chang, J., Zhang, Xuan, Mackintosh, S.G., Tackett, A.J., He, Y., Lv, D., 2018. Oxidation resistance 1 is a novel senolytic target. Aging Cell, e12780.
82. Zhu, Y., Tchkonia, T., Pirtskhalava, T., Gower, A.C., Ding, H., Giorgadze, N., Palmer, A.K., Ikeno, Y., Hubbard, G.B., Lenburg, M., O'Hara, S.P., LaRusso, N.F., Miller, J.D., Roos, C.M., Verzosa,

G.C., LeBrasseur, N.K., Wren, J.D., Farr, J.N., Khosla, S., Stout, M.B., McGowan, S.J., Fuhrmann-Stroissnigg, H., Gurkar, A.U., Zhao, J., Colangelo, D., Dorronsoro, A., Ling, Y.Y., Barghouthy, A.S., Navarro, D.C., Sano, T., Robbins, P.D., Niedernhofer, L.J., Kirkland, J.L., 2015. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell 14, 644–658.

83. Zhu, Y., Tchkonia, T., Fuhrmann-Stroissnigg, H., Dai, H.M., Ling, Y.Y., Stout, M.B., Pirtskhalava, T., Giorgadze, N., Johnson, K.O., Giles, C.B., Wren, J.D., Niedernhofer, L.J., Robbins, P.D., Kirkland, J.L., 2016. Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. Aging Cell 15, 428–435.

84. Zhu, Y., Doornebal, E.J., Pirtskhalava, T., Giorgadze, N., Wentworth, M., Fuhrmann-Stroissnigg, H., Niedernhofer, L.J., Robbins, P.D., Tchkonia, T., Kirkland, J.L., 2017. New agents that target senescent cells: the flavone, fisetin, and the BCL-XL inhibitors, A1331852 and A1155463. Aging (Albany NY) 9, 955–963.