Modulators of cellular senescence: mechanisms, promises, and challenges from in vitro studies with dietary bioactive compounds

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Abstract

Cellular senescence is considered an important mechanism to prevent malignant transformation of potentially mutated cells but, persistence of senescent cells within tissues alters microenvironment in ways that can promote cancer and aging phenotype thus underlining pathophysiologic processes of different age-related diseases. Coincident with this increased knowledge, understanding and finding modulators of the dynamics that control senescent-cell formation, fate and subsequent effect on tissue function has gained critical interest in experimental gerontology and cancer research. The purpose of this review is to discuss the evidence that various dietary bioactive compounds can modulate cellular senescence in vitro and to summarize findings and mechanisms that might be useful for the development of health-promoting nutraceuticals. An overview of cellular senescence and its impact in aging and cancer is described along with the strategies and pathways that are currently being investigated to target cellular senescence. Particular emphasis is given to the mechanisms by which bioactive dietary factors (i.e. most polyphenols) can delay or induce cellular senescence in vitro and how this knowledge could be used to explain the opposite effects shown in cancer lines and primary cells by some of these compounds. In addition, the problems to translate findings from modulation of cellular senescence in vitro into experimental treatments or clinical trials able to prevent or counteract age-related diseases are briefly described. The information herein provided might be useful to design further research in the field as well as to develop new nutraceuticals to be tested in experimental models and clinical trials.

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Keywords: Cellular senescence, Bioactive dietary compounds, Senescence modulators, Aging, Cancer

Abbreviations: AMPK, adenosine monophosphate–activated protein kinase; CS, cellular senescence; Cu, copper; DDR, DNA damage response; EGCG, epigallocatechin gallate; hF, human fibroblasts; hTERT, human telomerase reverse transcriptase; IL-1β, interleukin-1 beta; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; MTs, metallothioneins; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; OIS, oncogene-induced senescence; ROS, reactive oxygen species; RS, replicative senescence; SASP, senescence-associated secretory phenotype; SIPS, stress-induced premature senescence; SIRT-1, NAD-dependent deacetylase sirtuin-1; Zn, zinc.

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1. Introduction

The discovery of cellular senescence (CS) in cultured cells and the evidence that senescence occurs in vivo under pathophysiological conditions have raised exponential interest around the relevance of this process for tumorigenesis and the aging phenotype [1]. Cellular senescence is considered an important mechanism to prevent malignant transformation of potentially mutated cells; but persistence of senescent cells within tissues alters microenvironment in ways that can promote cancer and the aging phenotype, thus underlying pathophysiologic processes of different age-related diseases [2]. Modulators of the dynamics that control senescent-cell formation, fate, and subsequent effect on tissue function are clearly an attractive target for the pharmaceutical and food industry. Convincing evidence around the impact of caloric restriction on processes involved in CS suggests that food-derived compounds able to interfere with the same pathways of caloric restriction could be evaluated as potential senescence modulators to increase health span [3]. Indeed, various micronutrients including polyphenols, flavonoids, and vitamins have been claimed to modulate CS in vitro; but critical collection and overview of the results have never been afforded. Interestingly, some of these compounds have been shown to induce the appearance of CS characteristics in cancer cells as well as to delay CS in normal primary cells. In this review, we summarize the most relevant studies focused on modulation of CS in vitro by dietary bioactive compounds and discuss critical aspects related to their putative mechanisms of action. We used the electronic bibliographical database PubMed until January 2014 (without any methodological restrictions) to identify studies using the following keywords: cellular senescence, senescence, bioactive dietary compounds, aging, cancer, telomere, telomerase, mTOR, mTORC1, resveratrol, curcumin, vitamin C, morin, polyphenols, EGCG, ginsenoside, quercetin, bisdemethoxycurcumin, berberine, carnosine, tocotrienols. In addition, we reviewed the references of identified studies and of selected narrative review articles.

2. Overview of CS

2.1. Pathways leading to CS

Cellular senescence is defined as a status of irreversible growth arrest usually mediated by a persistent DNA damage response, insensibility to mitogen stimuli, and upregulation of tumor suppressor pathways. The observation that human diploid fibroblasts have a finite replicative life span in vitro paved the way toward the term replicative senescence (RS) [4]. Telomere attrition was firstly identified as possibly responsible for this phenomenon, as overexpression of the catalytic subunit of the enzyme telomerase (human telomerase reverse transcriptase [hTERT]), a reverse transcriptase that corrects normal telomere erosion, was shown to overcome RS in human cells [5]. However, the observation that single or repeated short exposure to various subcytotoxic stressors (UV, hyperoxia, hydrogen peroxide, etc) can accelerate CS [6,7] led to the introduction of the term stress-induced premature senescence (SIPS), which can also occur independent of telomere length and hTERT expression [8]. Overexpression of hTERT cannot bypass also another type of stress-induced CS [9], named oncogene-induced senescence (OIS) [10] and prompted by aberrant activation of oncoproteins (ie, RAS, BRAF). Similar to aberrant oncogene activation, loss of tumor suppressors (ie, phosphatase and tensin homolog, neurofibromatosis type 1, and von Hippel-Lindau tumor suppressor gene) can also trigger senescence in mouse and human cells [1]. Although the division between RS, SIPS, and OIS is useful, these processes have multiple areas of overlap. Compelling evidence obtained in recent years, with noted exception [11], demonstrates that DNA damage is a common mediator for both RS and SIPS and that a persistent DNA damage response (DDR) appears in most experimental models of CS [12]. Replicative senescence leads to the recognition of telomere ends as DNA breaks that induce DDR, prime the stabilization of p53, and activate the cyclin-dependent kinase inhibitor p21CIP1[13]. Stress-induced senescence works mainly through the activation of p16INK4a [1], but an interplay between this pathway and DDR itself has been reported [14]. However, both pathways converge on the inhibition of Rb phosphorylation, which results in the inactivation of the E2F transcription factor and target genes involved in cell cycle progression [15]. Another important aspect, which seems to be pivotal to induce senescence in the presence of cell cycle arrest, is the activation of the growth pathways via the mammalian target of rapamycin (mTOR), in particular via mTOR complex 1 (mTORC1) [16]. Indeed, mTOR activation converts quiescent cells into senescent cells, whereas rapamycin (the most known mTOR inhibitor) reverses this process [17]. This does not seem to be a universal feature, as inhibition of mTORC1 was reported to induce senescence in particular cancer lines [18,19]. There are also controversial findings related to the role of autophagy in CS. Inhibition of autophagy, as it may occur downstream mTOR signaling, results in the accumulation of protein aggregates, ER stress, and mitochondrial dysfunction, each of which could promote senescence. However, other studies suggest that autophagy may be required for an efficient senescence response [20]. The controversial aspects on senescence mechanisms suggest that cell-type and context-specific responses are involved in the establishment of CS [11]. These controversial aspects are reflected in the absence of a universal marker of CS. Therefore, the best way to characterize CS appears to be to use a pool of senescence-associated biomarkers. In addition to the features exposed above, other hallmarks that can be used to identify senescent cells include an altered morphology, activation of senescence-associated β-galactosidase, chromatin aggregates involving the formation of heterochromatin foci, markers of DNA damage and production of the senescence-associated secretory phenotype (SASP), which in turn includes several proteins involved in the inflammatory processes [21].

2.2. Role of CS in aging

Evidence is rising that senescent cells accumulate in different organs during patho- and physiological processes of aging [22]. However, the biological role of senescent cells is still not completely clarified. Studies of human tissues and cancer-
prone mice argue strongly that CS is one of the most important processes to suppress cancer in vivo [23]. Promoting tissue remodeling during embryonic development [24] and limiting the extent of fibrosis following liver damage [25] are additional possible beneficial roles of CS. Nevertheless, the altered functional profile of senescent cells might alter tissue microenvironment in ways that can promote both cancer and aging phenotypes. Indeed, the proinflammatory products of the SASP can induce deleterious effects in the neighboring cells, thus facilitating tumor development and aggressiveness [26], mediating paracrine transmission of CS [27], and promoting age-related dysfunctions [28]. Taking into account that senescent cells can display long-term survival and resistance to apoptosis [29], it is likely that this represents one of the most intriguing examples of antagonistic pleiotropy in the context of aging. A direct demonstration that senescent cells can drive age-related pathologies has been recently provided with the development of a transgenic mouse model in which p16INK4a-expressing cells can be specifically eliminated upon drug treatment [30]. In the BubR1 progeroid mouse background, this strategy was shown to delay age-related dysfunction in organs such as adipose tissue (loss subcutaneous fat), skeletal muscle (sarcopenia), and eye (cataracts) as well as to attenuate progression of already established age-related disorders [30]. Hence, modulation of CS appears to be one of the most promising targets for the development of therapies against cancer and age-related diseases.

3. Modulators of CS with potential therapeutic interest

Several target proteins and pathways required to induce or prevent CS in various experimental settings have been identified up to now. Together with this increasing knowledge, the development of modulators of CS with a potential therapeutic interest is rapidly growing. New concepts and new terminology around the central term of gerogenic conversion or geroconversion have been recently introduced. This term was introduced to emphasize the concept that a simple cell cycle arrest is not yet senescence without an active mTOR [31]. Hence, geropromoters were defined as small molecules (including bioactive dietary factors or drugs) that accelerate geroconversion by activating mTOR. Conversely, gerosuppressants are those molecules that suppress geroconversion by inhibiting mTOR. However, because the role of mTOR in CS is still controversial [18,19], we preferred to categorize modulators of CS according to their potential therapeutic target. In this sense, most prominent targets and specific aims of these modulators are as follows: (1) senescent cells, with the aims (a) to rejuvenate these cells, (b) to remove these cells by apoptosis or to elicit senescence immunosurveillance for their clearance, and (c) to reduce the deleterious effects of the SASP in the microenvironment; (2) cancer cells, with the aim to promote a senescence phenotype (as in the case of geropromoters); and (3) normal cells, with the aim to “delay” the appearance of the senescent phenotype (as in the case of gerosuppressants). These concepts are shown in Fig. 1.

4. Modulators of CS that target senescent cells

4.1. Senescence rejuvenators

Although the senescence program is considered a barrier for efficient cell reprogramming, induced pluripotent stem cells, functionally indistinguishable from embryonic stem cells, have been obtained from in vitro senescing cells [32]. Other strategies have been reported to partially rejuvenate senescent cells in vitro. This could be the consequence of removal of the cell cycle arrest, such as by inactivation of p53 pathway in senescent fibroblasts that display low expression of p16INK4A [33] or by inactivation of some interleukins (such as interleukin-6) in particular models of OIS [1]. In addition, pathways not strictly related to those used by cells to evade the cell cycle arrest during tumorigenesis can be targeted. Inhibitors of mTOR, such as rapamycin (a natural drug isolated from

![Fig. 1 – Modulators of CS with potential therapeutic impact.](image-url)
bacteria), have been reported to partially reverse the senescent phenotype in mouse embryonic fibroblasts [34] and primary human fibroblasts (hF) [35]. Therefore, independent of their possible consequences in vivo, it sounds possible that bioactive compounds derived from food sources might be used to rejuvenate senescent cells in vitro. As a consequence of the novelty of this concept, very limited reports exist around bioactive dietary compounds that might achieve this target. However, more than 10 years ago, a partial rejuvenation of senescent hF has been reported using L-carnosine (20-50 mmol/L), a naturally occurring dipeptide [36,37]. The transfer of cells approaching senescence from normal medium to the medium supplemented with L-carnosine was reported to partially rejuvenate these cells with a variable extension of the life span. This was not an isolated case, as terminally senescent fibroblasts (0 population doublings per 2 weeks) treated with 6 to 7 μmol/L of quercetin for 5 consecutive days were shown to restart proliferation compared to the control cultures [38]. More recently, incubation of human senescent fibroblasts at various passages with a tocotrienol-rich extract (0.5 mg/mL for 24 hours) was shown to reverse the senescent morphology, decrease the activity of SA–β-gal, and reelongate telomeres [39]. Further research to clarify the mechanisms involved in these processes might be useful to design pharmacological or nutritional interventions to ameliorate the problems related to dysfunction/exhaustion of aging stem cell populations. However, because rejuvenation of a cancer-prone damaged cell would represent a possible undesired adverse effect, there is a growing field of research that is searching for bioactive dietary compounds able to revert the general resistance to apoptosis shown by senescent cells.

### 4.2. Senescence ablators and senescence immune surveillance modulators

Finding ways to eliminate senescent cells by causing apoptotic cell death or by improving senescence immune surveillance [40] might deserve a great impact on future therapeutic strategies. The development of “senescent ablators” is still in its infancy, but the potential benefits that might be obtained after removing senescent cells from the organism have been already verified in animal models [30]. Preliminary experiments using ganciclovir combined with the herpes simplex virus thymidine kinase suggest that it is possible to kill senescent cells in vivo with strategies similar to the suicide gene therapies used for cancer [41]. The possibility that similar effects could be elicited by dietary bioactive compounds is perhaps one of the greatest challenges in this field. Given that many bioactive molecules have already been characterized for their specific anticancer activities [42], the possibility to modulate the altered metabolic pathways of senescent cells could be not such a prohibitive task. By the way, various models of senescence display metabolic features—such as enhanced glucose metabolism—that overlap with cancer cells and that are currently exploited in cancer therapy. In line with this concept, chemotherapy-induced senescent cells were found to be selectively susceptible to inhibition of glucose transporters and of the energy sensor adenosine monophosphate–activated protein kinase (AMPK) as well as to suppression of fatty acid oxidation and adenosine triphosphate depletion [43]. Remarkably, a specific vulnerability of senescent cells consists of the high energetic needs required for production of SASP factors. It is not a simple coincidence that the natural phenol phloretin, an inhibitor of glucose transporters, was found to reduce specifically the viability of senescent cells [43]. Thus, dietary-derived compounds claimed for their anticancer properties mediated by an inhibitory effect on glucose uptake, such as the recently characterized graviola extracts [44] or the widely known phophenols fisetin, myricetin, quercetin, apigenin, genistein, cyaniding, daidzein, hesperetin, naringenin, and catechin [42], would be useful candidate as senescent ablators. In consideration of these aspects, it is foreseeable that the future production of nutraceuticals would be able to induce apoptosis in senescent cells by making use of compounds that have been accepted as safe for human consumption and already approved by the Food and Drug Administration.

Regarding the possibility to enhance senescence immune surveillance, no specific compounds or strategies have been implemented. However, it cannot be excluded that particular drugs or compounds able to burst immune response in the macrophage, T cell, and NK cell compartments might display these features. Possible candidates might include the drug lenalidomide, which was shown to reverse T-cell abnormalities of immunosenescence [45] and to induce hair repigmentation in a clinical case with multiple myeloma [46], and zinc (Zn), which was shown to burst multiple parameters of innate immunity in elderly patients [47].

### 4.3. Modulators of SASP

Another interesting approach to target CS regards the possible development of SASP modulators. At this moment, these compounds are not available; but it is possible to interfere with the general proinflammatory pathway mediated by nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Metformin, a widely used antidiabetic drug that has been linked to a reduced cancer incidence, seems a viable candidate as SASP modulator because it has been shown to prevent events required for activation of the NF-κB pathway [48]. However, other experimental settings have shown that metformin can induce SASP in cancer and normal human cells [49]. Hence, it should be still clarified if metformin can really achieve an anti- or pro-SASP activity, especially in vivo, where induction of SASP could contribute to immune-mediated clearance of premalignant senescent cells [50]. Corticosterone and the related glucocorticoid cortisol have also been shown to decrease the production and secretion of selected SASP components in human senescent fibroblasts via inhibition of NF-κB [51]. These data suggest that nutraceuticals currently used to inhibit this pathway during the treatment of osteoarthritis might be promising candidates for anti-SASP activity [52]. Among these candidates can be included phycocyanobilin (a tetrapyrrole chromophore commonly found in the blue-green algae spirulina), berberine (an alkaloid usually found in the roots of berberis), and glucosamine (an amino sugar precursor for glycosaminoglycans, which are the major component of joint cartilage). This last compound seems particularly interesting because it was shown to inhibit interleukin-1 beta (IL-1β)-induced activation of NF-κB by specific epigenetic changes [53]. In agreement
with this putative role of SASP inhibitor, a decreased risk for cancer and total mortality was observed in regular users of this nutraceutical by prospective epidemiological studies [54,55]. Regarding berberine, the inhibition of NF-κB signaling may be mediated by several downstream targets of AMPK [56].

Similar mechanisms could be also argued for other known or putative AMPK activators (see next chapter). Although these strategies lack specificity for senescent cells, these nutraceuticals might be particularly useful to alleviate part of the undesirable systemic effects induced by SASP in cancer patients treated with radio- or chemotherapy [57]. A possible drawback of “anti-SASP” compounds could be related to the inhibition of the inflammatory reaction necessary to activate the clearance of senescent cells by the immune system [58].

5. Modulators of CS for targeting cancer cells

Escape of senescence response in premalignant lesions is a well-established mechanism for tumor progression. Although, for many years, the role of senescence in opposing tumor growth has been underestimated, an increasing body of evidence suggests that engagement of senescence response may represent a key component for therapeutic intervention in the eradication of cancer [59]. Indeed, reactivation of the senescence program triggers an innate immune response in vivo that contributes to tumor clearance [58]. Therefore, it is not surprising that compounds potentially able to induce the senescent phenotype in tumor cells have been widely studied in the last decade. Targeting the most common pathways that tumor uses to escape the cell cycle block is behind the strategy for prosenescence therapy [59]. The possibility to target similar pathways with bioactive dietary compounds has been extensively investigated so that polyphenols and other natural products are among the most widely compounds tested in vitro to induce senescence in cancer models.

5.1 Dietary bioactive factors that can induce CS by modulating telomerase and epigenetic changes

Induction of CS by inhibition of telomerase activity has been claimed as one of the major mechanisms underlying the anticancer effects of tea catechins. Nontoxic concentrations (15 μmol/L) of epigallocatechin gallate (EGCG), compatible with the levels observed in serum after drinking few cup of tea, can shorten telomeres; increase senescence-associated β-galactosidase staining; induce chromosomal abnormalities; and, most importantly, limit the life span of U937 monoblastoid leukemia and HT29 colon adenocarcinoma cell lines [60]. Further experiments in MCF-7 and HL60 cell lines confirmed an inhibitory activity on telomerase activity by EGCG [61] and curcumin [62]. Alterations in histone modifications, decreased methylation of hTERT promoter, and increased binding of the hTERT repressor E2F-1 at the promoter were proposed as mediators of the observed bioactivity [61]. Tocotrienols (concentration range, 10-20 μmol/L) were also shown to inhibit hTERT in human colorectal adenocarcinoma cell lines, possibly by involving inhibition of protein kinase C and downregulation of c-Myc [63] By the way, Myc is known to induce epigenetic changes leading to transcriptional activation of genes that suppress key drivers of CS, such as p21 [64]. In agreement with these findings, tocotrienols (range, 50-100 μmol/L) were shown to induce expression of senescent markers, such as p53, p21, and p16, in breast cancer cells [65]. These results were confirmed in the HER2/neu breast cancer mouse model, where administration of annatto tocotrienols delayed tumor onset and reduced tumor number and size through enhancing in situ both apoptosis and senescence markers [66]. Epigenetic downregulation of telomerase by the naturally occurring isothiocyanate sulforaphane (20 μmol/L) in human hepatocellular carcinoma Hep3B cells [67] or by the flavone apigenin in human leukemia cells [68] provides further example that this mechanism can be targeted by dietary compounds. Correspondingly, green tea catechins have been demonstrated to induce tumor suppressor activity by targeting p53 [69], p21 [69], p16 [70], and Rb [71]. Such a multtarget activity is compatible, at least in part, with epigenetic changes that involve inhibition of DNA methyltransferases, modulation of histone acetyltransferases and deacetylases, [72] as well as alteration of selected microRNAs [73]. Similar mechanisms appear to be involved also in the bioactivity of siliibinin [74,75], quercetin [76], geneistein [77], selenium compounds [78], and resveratrol [79,80]. Epigenetic changes have been recognized as some of the primary events detected during carcinogenesis and senescence [81], thus representing important novel targets for cancer chemoprevention or treatment. In contrast to tumor suppressor genes permanently inactivated by genetic mutations, those genes silenced by epigenetic changes (used by tumor cells to overcome the senescence barrier) can be potentially reactivated by small molecules or bioactive dietary factors. In addition to unsilencing tumor suppressor genes, dietary bioactive compounds able to target epigenome can also inactivate nonhistone proteins that are required for DNA stability and induce reactive oxygen species (ROS) as well as DNA double-strand breaks. Normal cells are able to counteract these effects by antioxidant mechanisms and effective DNA repair, whereas cancer cells, which are known to be defective in some of these mechanisms, might fail to repair DNA damage [82] and undergo cell death or senescence. A schematic representation of these mechanisms is reported in Fig. 2A.

5.2 Dietary bioactive factors that can induce CS by inhibiting mTOR and its related pathways

Inhibition of mTOR pathway can suppress geroconversion, maintaining quiescence and eventually enhancing susceptibility to apoptosis. Conversely, cell cycle arrest in the presence of an active mTOR may lead to CS. Hence, it is not surprising that some prosenescence anticancer strategies have been developed by hyperactivating mTOR pathway [59]. However, the normal function of mTOR in cancer cells is to promote extensive growth, proliferation, and survival in response to nutrients. This offers a possible target for bioactive dietary compounds. Early studies have shown that inhibition of mTOR with rapamycin can synergize with chemotherapeutic reagents to induce apoptosis in breast and ovarian cancer cells [83]. Further investigations confirmed the potential of this strategy so that rapamycin derivatives are currently studied in
clinical trials [84]. Now, it is well known that the nutrient-sensitive signaling pathways are frequently dysregulated in various types of cancers [85]. These pathways include the AMPK/tuberous sclerosis 1/tuberous sclerosis 2 that links energy depletion with growth control by inhibiting mTORC1 signaling, as well as the phosphoinositide 3-kinase/protein kinase B and protooncogene serine/threonine-protein kinase/mitogen-activated protein kinase/extracellular signal–regulated kinases signaling cascades that are critically involved in activating mTORC1 downstream [86]. Targeted activation of AMPK and inhibition of individual players involved in the mTOR activating pathways is a potential strategy for cancer therapy and clinical trials are already underway [87,88].

Because reactivation of CS has been only recently proposed as an anti-cancer strategy, large part of the studies on nutritional compounds that affect these pathway have focused their attention on apoptosis. Various flavonols including fisetin, quercetin, myricetin and kaempferol have been claimed to inhibit mTOR directly and/or by the phosphoinositide 3-kinase/protein kinase B pathway, leading to toxic or apoptotic effects in cancer models [89,90]. Another common mechanism by which plant-derived bioactive compounds (ie, berberine, ECGC, resveratrol, curcumin, galegine, capsaicin, hispidulin, and garlic oil) are able to interfere with mTOR signaling involves the inhibition of mitochondrial functions, likely as a consequence of their natural role as antiparasites. The consequence is a starvation-like condition that activates AMPK and its downstream effects, including mTOR inhibition and autophagy. This is particularly important because, under certain circumstances, induction of autophagy can prompt a specific mode of cell death (autophagic cell death) [91].

Most recently, in circumstances that still need to be clarified, it has been found that inhibition of mTOR, instead of driving apoptosis, can prompt a sustained autophagic response that can lead even radioresistant cancer cells into a paradoxical senescence [19]. Targeted inhibition of mTORC1 by everolimus (a drug similar to rapamycin) has been also

Fig. 2 – Modulation of CS by some dietary bioactive compounds in normal primary cells and cancer cells via epigenetic mechanisms and modulation of telomerase. A, In cancer cells, epigenetic changes leading to repression of tumor suppressors and upregulation of telomerase might eventually be targeted by bioactive dietary compounds able to revert these changes. Additionally, dietary phytochemicals that affect the epigenome can trigger DNA damage and repair mechanisms. A sustained DDR coupled with insufficient repair may be a pivotal mechanism to induce senescence or apoptosis in cancer cells exposed to dietary phytochemicals. B, In normal cells, particular dietary compounds have been reported to induce epigenetic mechanisms leading to repression of retrotransposable elements (ie, biotin) or to activate telomerase (ie, TA-65). Abbreviations: HDAC = histone deacetylases; HAT = histone acyltransferases; DNMT = DNA methyltransferases.
reported to induce CS in a model of lymphoma driven by Myc deregulation [18]. There is also indirect evidence that this process might be targeted by bioactive dietary compounds that are able to activate AMPK. For example, curcumin was previously reported to exert cytotoxic effects in various cancer cells by activating AMPK pathway [92] and inhibiting mTORC1 signaling [93]. More recently, curcumin was shown to exert antitumor activity by inducing CS in human colon cancer cells with associated induction of p53 (in p53 responsive lines), p21, and autophagy [94]. Although, in this study, mTORC1 signaling and AMPK were not considered, a possible involvement of these mechanisms appears foreseeable and would deserve further investigation. The mechanisms affecting the decision between apoptosis, senescence, and quiescence following AMPK activation are not completely elucidated but can be greatly influenced by p53 status. Experiments set up to test the chemopreventive activity of EGCG in hepatoma cells are explicative in this sense. Epigallocatechin gallate blocked the progression of cell cycle by inducing p53 and p21 expression in p53-positive Hep G2 cells, whereas induction of apoptosis was the main outcome in p53-negative Hep 3B cells [95]. The critical aspect that needs to be addressed is in what circumstances a claimed geroprotective compound could lead to a paradoxical prosenescent activity or apoptosis. For example, the alkaloid berberine, in agreement with its AMPK/mTOR inhibitory activity, can suppress senescence in lymphoblastoid and cell lung cancers models of SIPS (at 5-60 μmol/L) [96]. Nonetheless, berberine (50 μmol/L) was also shown to both induce apoptotic death and increase the expression of some senescence hallmarks including p53, p21, and p16 in HER2/neu tumor cell lines [97]. The question that needs to be answered is if inhibition of pathways upstream of mTOR in tumor cells could result into cell linespecific effects that can even terminate in CS or the observed phenotype is a simple reestablishment of quiescence. By the way, various feedback mechanisms involving p53, mTOR, AMPK, and autophagy are known, or are likely, to be dysfunctional in cancer cell lines. This could offer a “weak point” of cancer cells to induce chronic activation of autophagy or p53 and the resulting senescent or apoptotic response. Another implication of this observation is that the use of bioactive dietary compounds as chemopreventive or adjuvants for cancer therapy might be strictly “personalized.” A schematic representation of how bioactive dietary compounds can modulate this pathway in cancer cells is reported in Fig. 3A.
5.3. Dietary bioactive factors that induce CS by promoting ROS

Defects in genome stability of cancer cells increase their sensitivity to DNA-damaging agents and provide an effective target for therapy. Although dietary polyphenols and other bioactive compounds modulate drug metabolizing enzymes and scavenge free radicals, under some conditions, they have been shown to generate ROS and cause oxidative DNA damage [98]. Increased ROS and epigenetic changes induced by these compounds can trigger a sustained DDR, which, in the presence of defects in DNA repair (such as in cancer cells), can lead to cell death or CS [82]. Given that a primary regulator of cellular DDR is the ATM kinase, which in turn phosphorylates and activates p53, it is not surprising to find that that many bioactive dietary compounds (ie, polyphenols) have been described as genotoxic activators of the p53 pathway. The genotoxic response is usually observed at micromole-per-liter concentrations for these compounds, sometimes equals and sometimes slightly above the concentrations shown to delay senescence in normal cells (Table). In lung cancer cells, increasing doses of resveratrol (10-50 μmol/L) were found to induce a proportionally increased ROS production and DNA damage with subsequent induction of the senescent phenotype [99]. Similarly, a naturally occurring gallotannin from Oriental herbs, penta-1,2,3,4,6-O-galloyl-β-D-glucose, was shown to induce cell senescence (at 25 μmol/L) in some hepatoma and breast cancer cell lines by increasing ROS levels [100]. In this context, an increase of intracellular ROS could partly explain why treatment with different natural compounds can induce a similar inhibition of telomerase. An additional underscored feature of cancer cells, which might help to explain why antioxidant bioactive compounds are able to induce intracellular ROS preferably in these cells, consists of the specific alterations of the machinery that regulates intracellular metal homeostasis. A high-throughput method able to screen a library of 4160 known bioactive compounds and natural products (at 10 μmol/L) for their potential to induce senescence in prostate cancer cells identified 4 lead compounds not previously associated with senescence [101]. Interestingly, one of these compounds is the Zn ionophore pyrithione. Zinc was shown to induce in vitro a strong prosenescence response in vascular smooth muscle cells [102] and in dermal fibroblasts [103]. In this last model, it was shown that intracellular concentration of 125 nmol/L Zn (adjusted by pyrithione treatment) induced premature senescence by activating the mTOR pathway, the transcriptional activity of p53, and its target gene p21, as well as by inducing the expression of p16. Similar results were also shown for other trace elements including copper (Cu) [104] and selenium [105]. The way Zn induces senescence in various cell models suggests, at glance, the possibility to use Zn supplementation during chemotherapy. However, our body regulates the uptake of Zn and its intracellular homeostasis using at least 30 different proteins, most of which are promptly regulated to maintain Zn homeostasis following exposure, increased dietary intake of Zn, or supplementation [106]. A class of these proteins, metallothioneins (MTs), is increased following supplementation with Zn and has been involved in resistance to chemotherapy [107]. Nevertheless, various types of cancer cells display strong abnormalities in the Zn homeostatic machinery [108] that could be involved in conferring resistance to Zn-induced senescence in certain cell lines [109] while increasing susceptibility to Zn-induced senescence [101] or apoptosis in others [110]. Some dietary bioactive compounds (ie, organosulfur compounds such as sulforaphane) have been shown to induce RNA and protein expression of MTs [111], a process which is usually the consequence of increased intracellular free Zn. By the way, there is some evidence that several bioactive dietary factors, especially phenolic compounds, are able to interfere with Zn, Cu, and iron through their oxydryl and chetoester groups. The polyphenols luteolin, apigenin, EGCG, resveratrol, olive oil polyphenols, and caffeic acid were shown to inhibit cell proliferation and induce apoptosis in different cancer cell lines through mobilization of intracellular Cu and generation of ROS [112,113] or by downregulating MTs [114]. Hence, conversely to normal primary cells, the elevated Cu levels found in certain cancer cells could potentiate the redox cycling between Cu ions and polyphenols to generate ROS responsible for DNA breakage. Similar mechanisms could be hypothesized for Zn and might partly explain why the same bioactive compound behave as prooxidant or antioxidant in cancer and normal primary cells, respectively [112]. Further consideration should be directed to understand the influence of Zn, Cu, and iron ions in mediating the pleiotropic effects of plant polyphenols in CS. Fig. 4A represents schematically this possibility.

6. Modulators of CS for targeting normal cells

Delaying the onset of CS is considered nowadays among the most promising future strategies to counteract age-related syndromes (ie, cataract, sarcopenia, arthritis) and age-related diseases including cancer. Hence, the field of research around the effects of putative nutraceuticals claimed as gerosuppressive or antiaging is exponentially growing. The pathways targeted by dietary bioactive compounds with a potential to delay CS are mostly the same observed to induce senescence and apoptosis in cancer cells. Most intriguingly, these compounds seem to prevent also neoplastic transformation of normal cells in culture. Taking into account that genomic instability is a fundamental process that drives both cancer and CS, a likely explanation is that these effects are mediated by modulation of pathways that converge to maintain a stable genome in normal cells.

6.1. Dietary bioactive factors that delay CS by modulating telomerase and epigenetic changes

Whereas cancer cells rely on telomerase to sustain their proliferation, studies in laboratory animals and human cells confirm a causal mechanism for cell or tissue dysfunction triggered by critically short telomeres, suggesting that telomerase activation may be an approach to delay CS and increase health span [115]. Among dietary bioactive modulators of telomerase, TA-65, a small molecule purified from the root of an Asian medicinal herb, has received particular attention. TA-65 is capable of increasing average telomere...
## Table – Examples of divergent effects of some bioactive dietary compounds on CS in vitro

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<th>Reference</th>
<th>Concentration (timing)</th>
<th>Model a (type of senescence)</th>
<th>Effect c</th>
<th>Dietary bioactive compound</th>
<th>Effect c</th>
<th>Model a (type of senescence)</th>
<th>Concentration (timing)</th>
<th>Reference</th>
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<tr>
<td>[186]</td>
<td>50-100 mmol/L (24 h)</td>
<td>hCCC</td>
<td>I</td>
<td>1-L-Carnosine</td>
<td>D/R</td>
<td>hF (RS)</td>
<td>20-50 mmol/L (chronic)</td>
<td>[153]</td>
</tr>
<tr>
<td>[37]</td>
<td>20-30 mmol/L (chronic)</td>
<td>mTC</td>
<td>I</td>
<td>1-L-Carnosine</td>
<td>I</td>
<td>hMSC</td>
<td>&gt; 10 μmol/L (4 d)</td>
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</tr>
<tr>
<td>[187]</td>
<td>10-20 μmol/L (chronic)</td>
<td>hGC</td>
<td>I</td>
<td>Resveratrol</td>
<td>D</td>
<td>hMSC (RS)</td>
<td>0.1 μmol/L (30 d)</td>
<td>[128]</td>
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<td>30 μmol/L (chronic)</td>
<td>hGCC</td>
<td>I</td>
<td>Resveratrol</td>
<td>I</td>
<td>hEC</td>
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<td>[100]</td>
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<td>[189]</td>
<td>75-250 μmol/L (72 h)</td>
<td>hGCC</td>
<td>I</td>
<td>Resveratrol</td>
<td>I</td>
<td>hMSC</td>
<td>20 μmol/L (chronic)</td>
<td>[125]</td>
</tr>
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<td>[90]</td>
<td>10-50 μmol/L (chronic)</td>
<td>NSCCL</td>
<td>I</td>
<td>Resveratrol</td>
<td>D</td>
<td>hF, hRPE (SIPS)</td>
<td>50 μmol/L (30 min during H2O2 stress or 3 d during other stress)</td>
<td>[125]</td>
</tr>
<tr>
<td>[80]</td>
<td>50 μmol/L (96 h, then left recovery for 48 h)</td>
<td>hOC, HACC</td>
<td>I</td>
<td>Resveratrol</td>
<td>D</td>
<td>pTM (SIPS)</td>
<td>25 μmol/L (chronic during 40% O2 stress)</td>
<td>[193]</td>
</tr>
<tr>
<td>[191]</td>
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<td>mSCC</td>
<td>I</td>
<td>Resveratrol</td>
<td>D</td>
<td>hF (RS)</td>
<td>60 μmol/L (24 h)</td>
<td>[137]</td>
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<td>[192]</td>
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<td>hHC</td>
<td>I</td>
<td>Tocotrienols</td>
<td>D/R</td>
<td>hF (RS)</td>
<td>Gold Tri E 50 0.5 mg/mL (24 h at various PD including senescent hF)</td>
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<tr>
<td>[65]</td>
<td>50-100 μmol/L (chronic)</td>
<td>mBCC</td>
<td>I</td>
<td>Berberine</td>
<td>D</td>
<td>hF (RS)</td>
<td>60 μmol/L (24 h)</td>
<td>[137]</td>
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<td>[88]</td>
<td>100 μmol/L (chronic)</td>
<td>mBCC</td>
<td>I</td>
<td>Berberine</td>
<td>D</td>
<td>hF (RS)</td>
<td>60 μmol/L (24 h)</td>
<td>[137]</td>
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<td>[95]</td>
<td>10-60 μmol/L (cotreatment with mitoxantrone stress)</td>
<td>hNSCLC (SIPS)</td>
<td>D</td>
<td>Berberine</td>
<td>D</td>
<td>hF (RS)</td>
<td>6-7 μmol/L (chronic)</td>
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<tr>
<td>[194]</td>
<td>20-40 μmol/L (24-72 h)</td>
<td>hBCC</td>
<td>I</td>
<td>Bisdemethoxycurcumin</td>
<td>D</td>
<td>hF (RS, SIPS)</td>
<td>20 μmol/L (pretreatment 48 h)</td>
<td>[129]</td>
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<tr>
<td>[195]</td>
<td>25 μmol/L (24-72 h)</td>
<td>hGC</td>
<td>I</td>
<td>Quercetin</td>
<td>D/R</td>
<td>hF (RS, SIPS)</td>
<td>6-7 μmol/L (chronic)</td>
<td>[38]</td>
</tr>
<tr>
<td>[196]</td>
<td>300 μmol/L (2 h cotreatment with H2O2)</td>
<td>mFCL (SIPS)</td>
<td>D</td>
<td>Quercetin</td>
<td>D</td>
<td>hF (RS, SIPS)</td>
<td>6-7 μmol/L (chronic)</td>
<td>[38]</td>
</tr>
<tr>
<td>[197]</td>
<td>300 μmol/L (2 h cotreatment with H2O2)</td>
<td>mFSC (SIPS)</td>
<td>D</td>
<td>Quercetin</td>
<td>D</td>
<td>hF (RS, SIPS)</td>
<td>6-7 μmol/L (chronic)</td>
<td>[38]</td>
</tr>
<tr>
<td>[144]</td>
<td>0.15-0.3 mmol/L (5 d cotreatment with peroxide)</td>
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<td>D</td>
<td>Vitamin C</td>
<td>D</td>
<td>Vitamin C</td>
<td>D (RS)</td>
<td>hEmC, hF</td>
</tr>
<tr>
<td>[159]</td>
<td>1 mmol/L (cotreatment with 4 μmol/L phenylaminonaphthoquinones)</td>
<td>hBCC (SIPS)</td>
<td>D</td>
<td>Vitamin C</td>
<td>D (RS)</td>
<td>hEmC, hF</td>
<td>0.2 mmol/L (chronic)</td>
<td>[142]</td>
</tr>
<tr>
<td>[146]</td>
<td>0.1 mmol/L (2 d cotreatment with CKII inhibitor stress)</td>
<td>hCC (SIPS)</td>
<td>D</td>
<td>Vitamin C</td>
<td>D (RS)</td>
<td>hEmC, hF</td>
<td>0.13 mmol/L (chronic)</td>
<td>[145]</td>
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<tr>
<td>[120]</td>
<td>20 μmol/L (48-72 h)</td>
<td>hLC (SIPS)</td>
<td>I</td>
<td>Ginsenoside Rg1</td>
<td>D (SIPS)</td>
<td>hF</td>
<td>5-20 μmol/L (chronic pretreatment from 24-30 PD)</td>
<td>[122]</td>
</tr>
<tr>
<td>[121]</td>
<td>20 μmol/L (chronic)</td>
<td>hGC (SIPS)</td>
<td>I</td>
<td>Ginsenoside Rg1</td>
<td>D (SIPS)</td>
<td>hF</td>
<td>5-20 μmol/L (24 h pretreatment)</td>
<td>[119]</td>
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<tr>
<td>[60]</td>
<td>15 μmol/L (chronic)</td>
<td>hCCC, hLC</td>
<td>I</td>
<td>EGCG</td>
<td>D (RS, SIPS)</td>
<td>rVSMC, hF, hAC</td>
<td>50-100 μmol/L (chronic)</td>
<td>[147]</td>
</tr>
</tbody>
</table>

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### Notes:

a Model: hF = human fibroblasts; hMSC = human mesenchymal stem cells; hEC = human endothelial cells; hGC = human glioma cells; hT = human tenocytes; hRPE = retinal pigment epithelial cells; pTM = porcine trabecular meshwork; mER2 = mouse breast cancer cells; hKSC = human keratinocyte stem cells; hNSCLC = human pulmonary non-small cell lung carcinoma; mSCC = murine squamous cell carcinoma; mTC = mouse teratocarcinoma cells; mESC = mouse embryonic stem cells; hOC = human osteosarcoma cells; hACC = human adenocarcinoma cells; hamF = hamster fibroblasts; mFCL = mouse fibroblasts cell line; hBCC = human bladder cancer cells; hLC = human lymphoblastoid cells; hEmC = human embryonic cells; hEPC = human endothelial progenitor cells; hOAC = human osteoarthritic chondrocytes; mFC = mouse fibroblastoma cells; rVSMC = rat vascular smooth muscle cells; hAC = human articular chondrocytes.

b Type of senescence: SIPS = stress-induced premature senescence; RS = replicative senescence.

c Effect: I = inducer of a senescent-like phenotype; R = rejuvenator of the senescent state; D = delay of senescence markers onset.
length and decreasing the percentage of critically short telomeres and DNA damage in mouse embryonic fibroblasts that harbor critically short telomeres [116]. The mechanisms of action of TA-65 are still unclear, but it seems to regulate telomerase at the transcription level. Most importantly, TA-65 can improve health span in mice without significantly increasing global cancer incidence [116]. Preliminary results from a commercial health maintenance program, which included TA-65 supplementation (10-50 mg daily), reported that the protocol lengthened critically short telomeres and remodeled the relative proportions of circulating leukocytes subjects toward a more “youthful” profile [117]. These results are explicative to show how bioactive compounds that delay senescence in vitro might be used to develop dietary strategies to prevent age-related dysfunctions. Other potential modulators of telomerase derived from oriental herbs include ginsenosides [118,119], which have been reported to display opposite effects on survival and proliferation of cancer vs normal cells (Table). Indeed, while acting as prosenescent compounds in cancer cell models [120,121], they were reported to protect against IL-1β, H₂O₂, and tert-butyl hydroperoxide-induced senescence in human chondrocytes [118], endothelial progenitor cells [119], and fibroblasts [122], respectively. Pleiotropic effects on telomere length in normal [39] and cancer cells [65] have been also reported for ϒ- and δ-tocotrienols. Although the mechanisms involved in these effects are still mostly unknown, the involvement of epigenetic changes is foreseeable. Some bioactive dietary compounds are able to activate the epigenetic regulator NAD-dependent deacetylase sirtuin-1 (SIRT1), which includes hTERT among its targets [123]. The SIRT1-related epigenetic changes in vitro have been shown to mediate the suppression of p16 and inhibition of CS. These mechanisms were proposed to explain the cellular life span extension of glucose-restricted HF compared to cells receiving normal glucose in the culture medium [124]. Conversely, high-glucose conditions were
shown to induce CS in a process that can be antagonized by a resveratrol derivative [125]. By the way, resveratrol (5 μmol/L) was shown to activate the SIRT1/telomerase pathway in human umbilical cord fibroblasts, thus resulting in possible prevention of CS [123]. Although the legitimacy of resveratrol as direct SIRT1 activator has been widely debated [126], a new study demonstrates that resveratrol can allosterically activate SIRT-1 and that a single amino acid in SIRT-1 located in the terminal N-domain is critical for this activation [127]. This could explain why resveratrol, at low concentration (0.1 μmol/L), has been recently provided to delay CS of human mesenchymal stem cells [128]. Another natural compound that might act through the same pathway is bisdemethoxycurcumin, which was shown to increase SIRT1 and to antagonize SIPs in fibroblasts [129].

Human diploid fibroblasts approaching RS have been shown to undergo epigenetic changes leading to gene silencing and activation of transposable elements [130]. Retrotransposons are expressed at very low levels in normal cells, and their activation is counteracted by epigenetic mechanisms that include DNA methylation and microRNA targeting of transcripts. Taking into account that epigenetic changes are potentially reversible, it is not excluded that this process could be targeted by nutritional bioactive compounds. Indeed, biotinylation of lysine-12 in histone H4 has been proposed as an epigenetic mechanism to repress long terminal repeat retrotransposons in human and mouse cell lines [131]. In line with these studies, biotin-deficient primary human lung fibroblasts were shown to senescence before biotin-sufficient cells [132]. Whether similar mechanism of action could be involved in the effects of other bioactive dietary factors is currently unknown. A schematic representation of these mechanisms is reported in Fig. 2B.

6.2 Dietary bioactive factors that delay CS by inhibiting the mTORC1 pathways

Inhibition of mTOR pathway in cancer cells has been reported to increase susceptibility to apoptosis and to induce quiescence or even a paradoxical senescence-like response. Conversely, inhibition of mTOR in normal cells has been reported to be a potent tool to delay the appearance of the senescent phenotype. This could be in part the consequence of a nonchronic autophagy-sustained metabolism and reduced byproducts of metabolism that are associated with genomic stability in normal cells [133]. By the way, not only CS can be delayed; but some aspects of senescence can be reversed either using the mTOR inhibitor rapamycin or by depletion of mTORC1 [35]. Inhibition of mTOR pathway can be the consequence of various types of metabolic stress that lead to adenosine triphosphate depletion, such as caloric restriction. Bioactive compounds that inhibit mitochondrial function and activate AMPK might display similar effects. Interestingly, whereas persistent AMPK activation can lead to accelerated p53-dependent CS, glucose restriction was shown to induce a p53-dependent and reversible cell cycle arrest that promoted cell survival [134].

Resveratrol that is claimed to mimic the effects of caloric restriction was proposed as a valuable candidate for delaying senescence in cellular models. At a concentration claimed to inhibit mTOR (50 μmol/L), resveratrol was reported to delay senescence of hF [135]. Unfortunately, this effect seems to be the consequence of the “superpharmacological” dose [136]; but other mechanisms could be involved in cellular life span extension by resveratrol at lower doses (ie, modulation of SIRT1; see previous chapter). The extravirgin olive oil secoiridoids, which provide an effective defense against plant attack by herbivores and pathogens, are able to activate the AMPK and trigger numerous resveratrol-like antiaging transcriptional signatures. Crude extravirgin olive oil phenolic extracts rich in secoiridoids prevented senescence-associated morphological changes and SA-β-GAL staining of normal diploid hF at the end of their proliferative life spans [114]. An additional bioactive compound derived from food sources, berberine, has been reported to display gerosuppressive properties [96,137] and to attenuate SIPs in human pulmonary non–small cell lung carcinoma cells by activation of AMPK. It has been also suggested that curcumin can inhibit mTOR pathway, thus exerting proapoptotic anticancer activity [138]. However, although curcumin has attracted the attention of researchers and clinicians as an anti-inflammatory and antioxidant agent [139–141], its effects on RS of human primary cells still need to be specifically addressed.

Anyway, the field of research around dietary bioactive compounds able to inhibit mTOR signaling and to activate downstream players (ie, autophagy) or other processes (ie, physiological function of p53) involved in genome maintenance (see Fig. 3B for a schematic representation) holds promise for the development of nutraceuticals that can delay CS without harmful consequences.

6.3 Dietary bioactive factors that delay CS by inhibiting ROS

In addition to other specific mechanisms, a number of bioactive dietary compounds are likely to delay CS through inhibiting ROS and oxidative DNA damage. One of the most intensely studied antioxidants able to delay CS is vitamin C. In primary cell culture, physiological concentrations of vitamin C (10⁻⁵ mol/L) promote growth, cell division, and cell differentiation while delaying the onset of CS [142]. Importantly, vitamin C enhances also primary fibroblast reprogramming to pluripotency; but the underlying mechanisms are unclear [143]. These effects could be related to a possible inhibition of intracellular ROS, prevention of DNA damage, and subsequent repression of cell cycle blocks. This mechanism was proposed in a model of p53-induced senescence of human bladder cancer cells [144], in a model of RS of human vascular endothelial cells [145], and in a model of CKII inhibition-mediated senescence in human colon cancer cells (hCCC) [146]. Prevention of ROS generation could also explain why various natural antioxidants independent of their nature or chemical structure have been shown to prevent SIPs. However, it is difficult to establish whether inhibition of ROS is the cause or consequence of signaling mechanisms that regulate CS. For example, EGCG was shown (at concentration of 50–100 μmol/L) to prevent replicative and stress-induced senescence in various primary cells by repressing p53 activity [147]. Interference with p53 transcriptional activity was also proposed to explain the protective mechanism of morin.
against ultraviolet B–induced CS in human keratinocyte stem cells [148]. Yet, this could be the consequence of suppression of ROS that are strictly related to p53 signaling [149]. In this context, it is important to consider that direct inhibition of p53 could not be a preferential target for delaying the onset of cells senescence because this is the same mechanism used by many cancer lines to overcome cell cycle blocks [150]. But, in the absence of genomic instability, it is also reasonable to hypothesize that p53 can be transiently activated and repressed by feedback mechanisms, as shown in Fig. 1. In this case, in contrast to the prosenescence effects of stable expression of p53, modest levels of p53 expression transiently induced by nutrient-limiting conditions or by bioactive dietary factors can be useful to maintain genome stability and prevent senescence or neoplastic transformations [151]. Another interesting connection between ROS and p53 activity is likely offered by the mechanisms that control Zn and Cu homeostasis. A multitude of nutritional compounds display Zn and Cu binding sites in their structure. Because of their weak binding sites, these compounds act more likely as mobilizers of intracellular trace elements rather than effective chelators [112]. Conversely, a compound that might provide higher Cu and Zn binding activity (via imidazole and amino groups) is carnosine [152], which was claimed to delay senescence and to partially rejuvenate replications senescent human foreskin fibroblasts without a clear elucidation of the mechanisms involved [36,153]. Interestingly, in the same cellular model, inhibition of p53 was shown to reverse the senescent phenotype [33] with effects similar to those reported by carnosine. Given the relevance of Zn for p53 transcriptional activity [154], this paves the way to a possible speculation on the involvement of Zn homeostasis in the senescence-modulatory effect of this nutritional compound. By the way, if intracellular mobilization of Cu and Zn by dietary flavonoids (see previous chapter on cancer cell targets) can accelerate CS, improving intracellular control of Zn and Cu homeostasis might potentially display opposite effects. In this context, there is evidence that upregulation of proteins involved in intracellular buffering of Zn, that is, MTs, can accelerate CS, improving intracellular control of Zn homeostasis might potentially display opposite effects. In this context, there is evidence that upregulation of proteins involved in intracellular buffering of Zn, that is, MTs, can delay senescence-related processes in both in vitro and in vivo models [155]. This might be also potentially achieved by “training” the cells with transient exposure(s) to physiological doses of Zn, which are known to induce MTs [156]. The possibility to take advantage of this mechanism to improve intracellular control of Zn homeostasis by transient exposure to polyphenolic compounds [157] would deserve further investigations. The proposed mechanism is included in Fig. 4B.

7. Senescence “dichotomic” effects: putative mechanisms and challenges

The chapters above have pointed out the possible dichotomic effect of various food-derived compounds on modulation of CS: the same compound might act as senescence inducer on cancer cell models while acting as senescence delay in normal primary cells. This effect, shown with different compounds and in different experimental settings, is likely to be not the consequence of chance or artifacts. Rather, there are several explanations that might be provided. First of all, hermetic-like effects could explain opposite responses. This is likely the case of vitamin C, which delays CS in the 0.1- to 0.2-mmol/L range while displaying proapoptotic effects at doses greater than 0.6 mmol/L [158] and even prosenescence effects at a dose of 1 mmol/L in combination with redox cyclers able to form ROS (phenylaminonaphthoquinones) [159]. Also, for polyphenolic compounds, biological effects at nanomole-per-liter concentrations could be different from those observed with doses in the order of tens of micromoles per liter. A useful example has been shown using resveratrol in a wide concentration range in human mesenchymal stem cells [128]. Moreover, it is important to consider eventual differences between the uptake of the compound by normal vs cancer cells. This may result in a different intracellular accumulation in response to similar extracellular concentrations. For example, tocotrienols in the micromoles per liter range have been shown to exert anticancer effects [160], whereas nanomole-per-liter concentrations of tocotrienols have been associated with cellular protective functions [161]. But tocotrienols have been shown to specifically accumulate in cancer tissues of Her2/neu mice at a very high rate than observed in normal tissues. Hence, the specific intracellular accumulation of these compounds in cancer cells might be responsible for the prosenescence effects shown by tocotrienols in particular experimental models [66], whereas protective function in normal cells [39] might be related to a lower uptake.

Another potential explanation is offered by the interference with mTOR pathway that is relevant to sustain growth and survival of cancer cells while being at the same time a “proaging” pathway in normal cells. The altered profile of expression of cancer cells combined with mTOR inhibition can lead to the persistence of an autophagic flux that can conduct apoptosis or senescence. Conversely, induction of autophagy by inhibition of mTOR in normal cells can represent a mechanism to prevent genomic instability and to delay the onset of CS. These effects could be related to the different metabolism of normal and cancer cell lines as well as to specific defects of cancer cells in the regulatory feedbacks that involve mTOR, p53, and AMPK [16]. Also, the different regulation of Zn and Cu homeostasis shown in cancer cells might contribute to explain the differential effects of some polyphenols. Although these mechanisms have been poorly investigated, there is evidence that mobilization of intracellular trace elements by plant polyphenols could contribute to explain the anticancer and chemopreventive properties of these compounds [112]. Importantly, these results suggest that the same bioactive compound may be differentially effective against tumors with specific mutational pattern. Identification of the mutation patters that make susceptible specific cancer cell lines to bioactive compounds at concentrations that are harmless or beneficial for normal cells would be useful for the future improvement of therapies.

Finally, it should be taken into account that there is not a universal marker of senescence and that most senescence biomarkers have been found also in nonsenescent cells during particular conditions. This makes rather difficult a uniform characterization of the senescent state and might introduce bias in the interpretation of experimental results.
8. Modulators of CS and potential impact for active health in aging

Although modulation of CS holds great promise for the development of strategies aimed at improving health in aging individuals, it should be kept in mind that the exact role of senescent cells, the way they are formed, and the effectiveness of targeting this process in vivo to achieve potential health benefits are still unclear. A major obstacle that still remains is the problem of characterizing the senescent phenotype; but knowledge around methods to detect hallmarks of CS in vitro and in vivo is exponentially increasing, and it is likely that this gap could be covered soon. But even given that this problem would be addressed in the immediate future, the possibility to target CS with food-derived compounds could result into much less health improvements than expected. Indeed, extending life span of cells in culture with micronutrients does not necessarily mean that the same treatment could improve health span. Moreover, the “multitarget” ability of nutritional compounds that have been reported to modulate CS in vitro could result in a mix of beneficial and deleterious effects that might produce minimal or negligible effects in vivo. Nonetheless, it should be noted that several compounds discussed in this work have been reported to extend life span or health span of laboratory animal models [3,163]; and part of them has already proven to display health benefits in humans or is currently being used in clinical and nutritional trials [164,165]. In the case of resveratrol, its efficacy in clinical populations, including obese individuals and individuals with cardiometabolic dysfunction, is well supported [166,167]. However, when resveratrol clinical trials have been applied to healthy and elderly populations, the results on metabolic functions are often disappointing [169,170]. Regarding curcumin, some clinical trials support its potential as preventive agent for certain types of cancer [165,171], whereas others reported an improvement of vascular health in type 2 diabetes patients [172] and postmenopausal women [173]. However, despite in vitro, preclinical, and clinical evidence, a recent systematic meta-analysis of randomized controlled trials with curcumin has shown a lack of evident effects on blood lipid profile [174]. Although there have been numerous conflicting results [175], data available from various clinical trials with green tea polyphenol EGCG also provide a rationale to support this compound in primary chemoprevention for certain types of cancer [176]. There is also a consistent body of evidence that EGCG could have a role in improving cardiovascular health [177]; but epidemiological inferences are sometimes conflicting, and some clinical studies may appear discrepant [178].

In this context, issues regarding the effective bioavailability of various bioactive compounds and the dose response required for a beneficial effect on the organism may hold the key to answer the frequent discrepancy between in vitro and in vivo studies [162,179]. The molecular structure of a bioactive compound is known to affect its absorption considerably. For example, the sugar moiety of flavonoids seems to be pivotal in this process because most flavonoids are attached in nature to β-glucosides. In this form, they can be absorbed to a very small extent and are poorly metabolized by enzymes in the small intestine [162]. However, in the case of quercetin, it has been reported that its glycosylated form from onions is more bioavailable than the parent flavonol [180]. To overcome poor bioavailability of some bioactive compounds (ie, curcumin, anthocyanins) that display limited intestinal uptake and/or rapid metabolism, encapsulation systems and micronized powder and liquid micellar formulations have been recently developed [181,182]. These formulations are able to improve significantly the bioavailability of the compounds without altering safety parameters and may thus be ideally suited for use in human intervention trials. There is also interesting perspective of application in cancer prevention and treatment using nanoparticles containing nutraceuticals, such as green tea polyphenols and curcumin [183].

Further challenges in primary prevention or antiaging clinical trials with bioactive compounds arise from the problem of interpreting intervention-induced changes in the absence of pathological deviations from healthy reference range. For this reason, it has been recently proposed to compare the effects of hypothetical antiaging interventions with the treatments that provide theoretical maximal response, such as caloric restriction and physical exercise [184]. Additional tools that might be useful in future interventions could arise from projects aimed to identify human biomarkers of aging (ie, Mark-Age Project: http://www.mark-age.eu/).

Another concern regards the pathways modulated by dietary bioactive compounds, which sometimes are the same ones used by cancer cells to escape the senescence block. One representative example is telomerase. Telomerase activation is likely a system to delay senescence both in vitro and in vivo. However, telomerase activation in the presence of genomic instability can also enable evolving cancers to progress and acquire new biological properties, including central features of advanced cancer [185]. Thus, although telomerase does not cause cancer, its activation in the presence of precancerous transformations requires further investigations. Targeting CS and its putative deleterious consequences is a very attractive field of research that will require combined multidisciplinary efforts in the field of biochemistry; pharmacy; biology; medicine; and, in particular, gerontology. Indeed, studies around the mechanisms involved in CS are currently underpinning unexplored processes involved in the mechanism of aging and age-related diseases that have not been addressed in the past. This knowledge could be used to develop new therapies and to unravel the pathogenesis of relevant age-related syndromes and diseases including sarcopenia, arthritis, neurodegenerative diseases, cardiovascular diseases, and cancer. The information herein provided might be useful to design further research in the field as well as to develop new nutraceuticals to be tested in experimental models and clinical trials.

9. Unknown aspects, missing knowledge, and future research

9.1. Unknown aspects on modulation of CS by dietary bioactive compounds and missing knowledge

Literature analysis revealed that many dietary bioactive compounds can affect CS in vitro by acting on telomerase,
epigenetic changes, mTOR pathways, and ROS. Several studies have reported an opposite modulatory effect between cancer cell lines and primary cells by some of these compounds. These studies suggest a potential benefit of selected dietary bioactive compounds in both cancer and primary prevention of age-related diseases. The main weaknesses of most studies in vitro with dietary bioactive compounds regards the lack of standardized procedures to assess CS, the short concentration range usually explored, and the use of a limited number of cell lines and primary cells. Moreover, the translation of the results in vitro into experimental or clinical intervention is complicated by our poor knowledge of the role of CS in aging, by the “multitarget” ability of nutritional compounds that might display different effects in different cells and tissues, problems related to the bioavailability of these compounds, and by the problem of interpreting intervention-induced changes in the absence of pathological deviations from healthy reference range.

9.2. Future research needs
A priority of this research field is to improve our knowledge on the role of CS, on senescence biomarkers in vitro and in vivo, and on standardized procedures to assess CS. The development of animal models and cell lines that allow to monitor the accumulation of senescent cells in vivo and to monitor progress towards the senescent state in vitro would greatly improve the progress of this field of research. Moreover, the knowledge around the mechanisms by which bioactive dietary factors can delay or induce CS in vitro is still largely underestimated. Last but not least, the impact of dietary bioactive compounds on the epigenome and genome stability (both pivotal to modulate CS) should be considered among the priority research areas in this field.

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REFERENCES


Lopez HL. Nutritional interventions to prevent and treat osteoarthritis. Part II: focus on micronutrients and supportive nutraceuticals. PM R 2012;4:S155–68.


