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# 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.



*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-1$\beta$, interleukin-1 beta; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; MTs, metallothioneins; NF-$\kappa$B, 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 underlining 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 $p21^{CIP1}$[13]. Stress-induced senescence works mainly through the activation of $p16^{INK4a}$ [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 $\beta$-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-