Elimination of Senescent Cells by Polyphenols and Flavonoids

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Abstract

Oxidative stress, oncogenic signaling, DNA damage, or shortened telomeres, among other stimuli, can lead to cellular senescence. Senescent cells develop a secretory phenotype (SASP) that secretes factors such as inflammatory cytokines, growth factors, or metalloproteases into the surrounding tissue. These factors can create an environment that favors tumor growth. Senescent cells are more resistant to apoptosis than young cells, and this state may be a refuge for cancer cells to survive chemotherapies by switching between states. Polyphenols can mitigate SASP, but also act directly against senescent cells. Substances that selectively kill senescent

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cells are called senolytics. Quercetin and fisetin are substances that are thought to have these properties, and these substances are already being studied in clinical trials. Polyphenols may interfere with cellular senescence and its consequences through different mechanisms and alleviate age-associated diseases such as cancer.

Keywords

Cellular senescence · Polyphenols · Senescence-associated secretory phenotype · Herbal preparations · Cancer introduction

Introduction

In 2018, the widespread disease cancer affected about 18 million people of the world's population. Due to the great progress in the treatment of cancer, the mortality rate, at least in developed countries, has decreased sharply in recent years. For example, it has been achieved that 70% of breast cancer patients reach a survival time of 10 years from the time of diagnosis. In addition, many blood cancers have high cure rates (Short et al. 2019).

Because there are more and more cancer survivors, it is very important for the medical community to enable these people to live with a high quality after the disease. Radiation and chemotherapy not only have short-term effects on constantly renewing tissues such as the bone marrow but also have much more far-reaching consequences on the organ system, such as general debilitation, mortality, and accelerated aging (Short et al. 2019).

There are still cancers with very low survival rates – malignant gliomas, for example. Glioblastoma patients survive only a little more than a year despite a combination of surgery, high-dose radiation therapy, and chemotherapy. But here, too, great efforts are being made to improve the situation (Short et al. 2019).

Cancer therapy aims to inactivate cancer cells. This is achieved through various mechanisms, including surgery, ionizing radiation, or chemotherapeutic agents. In most cases, chemotherapeutic agents or radiation therapy induces apoptosis in cancer cells, but there are other cellular mechanisms to stop cancer cell growth (Campisi 2013).

Substances such as reactive oxygen species (ROS) can cause DNA damage that causes cells to stop the cell cycle and repair the damage. If the damage is irreparable, a cellular program called cellular senescence is activated. Senescent cells often arise as a byproduct of chemotherapeutic treatments, and in some cases, cellular senescence is also the target of these treatments (Campisi 2013).

Cellular Senescence

Originally, cellular senescence was identified as an irreversible growth arrest. It seems that this growth arrest serves to prevent cancer. Cells enter cellular senescence when exposed to oncogenic stimuli or stress (Campisi and Di d'Adda Fagagna

2007a). Growth arrest is mainly induced and maintained by two tumor suppressor pathways: p53/p21 and $p16^{INK4a}$/pRB. Furthermore, there are far-reaching changes in chromatin organization and gene expression. Part of these changes is the senescence-associated secretory phenotype (SASP), which is characterized by the secretion of numerous pro-inflammatory cytokines, chemokines, growth factors, and proteases. This SASP is not only involved in the development of cancer but also in prevention of cancer (Campisi 2013).

A more modern differential view of cellular senescence defines cellular senescence as a stress response characterized by at least three interacting signaling pathways.

- Ongoing DNA damage response (DDR) which can be induced by shortened telomeres, for example.
- Senescence-associated mitochondrial dysfunction (SAMD) activated by the DDR and characterized by decreased respiratory activity and membrane potential and mitochondrial ROS production.
- Senescent cells are further characterized by the senescence-associated secretory phenotype (SASP) (Short et al. 2019).

As mentioned, one reason for cellular senescence is telomere shortening. This represents a kind of clock for the number of divisions a healthy cell can undergo. This limit of divisions is called the Hayflick limit after a researcher who discovered this phenomenon (Hayflick 1965). Functional telomeres prevent repair mechanisms that recognize chromosome ends as double-strand breaks. If the protecting telomere structure can't be maintained anymore because telomeres get to short, the DNA damage response (DDR) is induced by the activation of p53. Without functional telomerase, these telomeres are irreparable and cause permanent growth arrest. Many cells are also forced into senescence when DNA damage occurs at other sites of the genome than the telomeres. Ionizing radiation, chemicals, or reactive oxygen species (ROS) that damage DNA are able to cause senescence in normal tissue as well as in tumor tissue (Campisi 2013).

Lesions caused by oxidative stress, such as damage to bases or single-strand breaks, which also cause double-strand breaks when they are repaired, can also drive cells into senescence. Even a single unrepaired double-strand break can trigger senescence (Di Leonardo et al. 1994). Oxidative stress can also cause telomere shortening. The G-rich sequences in the telomeres are very susceptible to oxidative damage. However, the exact damage that causes senescence is unclear but a permanent DDR signal is essential (Campisi 2013).

In order to establish a permanent growth arrest, two main signaling pathways p53/p21 and $p16^{INK4a}$/pRB must be activated. There may be other signaling pathways that can also induce cellular senescence but these two are the most dominant. Genomic damage and dysfunctional telomeres activate the DDR, which induces the p53/p21 pathway that provides a rapid response (within minutes to 1 hour) which is also transient (24–48h) (Levine and Oren 2009). When the damage is severe and irreparable and causes senescence, a low p53 activated p21 signal is maintained. This rapid

response is also accompanied by slow (days-long) signaling pathways such as p38 MAPK (p38 mitogen-activated protein kinases) and protein kinase C signaling and ROS, which are also involved in signaling. Thus, $p16^{INK4a}$ is also activated which acts via pRB and maintains an irreversible growth arrest (Beausejour 2003).

With regard to senescent cells and disease, widely recognized publications by Baker et al. have created strong evidence that cellular senescence is involved in the aging process itself and related diseases (also cancer), using a mouse model (INK-ATTAC) where a $p16^{INK4a}$ promoter element regulates the expression of a caspase-8 coupled to an FK506 binding protein. This fusion protein dimerizes in response to the drug AP20187 which activates caspase-8 and induces apoptosis in senescent (p16 expressing) cells (Baker et al. 2011, 2016). This allows the elimination of senescent cells in various aging and degenerative disease models, for example, in osteoarthritis (Jeon et al. 2017).

Markers of Cellular Senescence

How can senescent cells be recognized? There is no specific marker for senescent cells so far; nevertheless, senescent cells show certain characteristics. Senescent cells show a flattened, enlarged cell shape that is observable with an ordinary light microscope. A defining characteristic of senescent cells is irreversible growth arrest, generally in response to oncogenic stimuli. This distinguishes them from quiescent and differentiated cells (Campisi and Di d'Adda Fagagna 2007b). This growth arrest is, for example, detected by BrdU/EdU incorporation assay.

Another factor that can lead to senescent cells is (epi) genomic stress resulting from DNA damage (e.g., stress-induced cellular senescence (SIPS) (Toussaint et al. 2000), dysfunctional telomeres (replicative cellular senescence), or even strong mitogenic signals (oncogenes)). These stimuli lead to a cellular DDR mediated by the damage sensor "ataxia telangiectasia mutated" (ATM) that stabilizes the tumor suppressor p53, which in turn upregulates its transcriptional target gene p21 (Galbiati et al. 2017). This signaling pathway is also called ATM-p53-p21-Axis. The p53-p21 signaling pathway can also occur in a DDR-independent manner, e.g., through loss of the tumor suppressor PTEN (Chen et al. 2009). Additionally, a tumor suppressor is expressed, namely, $p16^{INK4a}$, a cyclin-dependent kinase inhibitor. The protein $p16^{INK4a}$ gradually accumulates during the physiological aging process (Zindy et al. 1997) and inhibits cell growth through activation of the p16INK4a/retinoblastoma (p16/pRb) signaling pathway. Binding of $p16^{INK4a}$ to the cyclin-dependent kinase 4-6/cyclin D complex inhibits phosphorylation from pRB family proteins, resulting in G1 cell cycle arrest (Overhoff et al. 2014). This mechanism can occur either alone or together with the previously mentioned p53-p21 signaling pathway depending on the nature of the stress and cell type. It appears that p21 is often upregulated temporally before p16INK4a and represents an earlier phase of cellular senescence (van Deursen 2014). Proteins that arrest the cell cycle can be detected by immunoblotting-based methods.

Senescent cells do have an increased lysosomal mass. Therefore, senescence-associated ß-galactosidase (SA-ß-Gal) (Dimri et al. 1995) activity is increased and detectable even at a suboptimal pH value 6. This effect is detected by chromogenic substrate X-Gal used on adherent cells and in tissues, but there are also fluorescent dyes that can be detected with a flow cytometer.

Senescent cells secrete numerous cytokines, growth factors, and proteases that induce autocrine and paracrine signaling activity. This phenomenon is called senescence-associated secretory phenotype, as mentioned earlier. The SASP is mainly activated by NF-kB and p38 PAPK signaling and maintained by interleukin 1α, in an autocrine pathway (Nelson et al. 2012). SASP factors are mainly detected by ELISA, SILAC (stable isotope labeling by/with amino acids in cell culture)-based quantitative proteomics, antibody arrays, multiplex assays, and mRNA profiling.

However, to date, no specific marker for cellular senescence has been found. To uniquely identify senescent cells, an elaborate process is required that considers all of the above factors and more (Childs et al. 2015; Lawless et al. 2010). There are additional factors that might point at senescence of cells normally more than one factor is used to define senescence of cells.

Senescence-Associated Mitochondrial Dysfunction (SAMD)

Mitochondrial dysfunction is a "hallmark of aging." Mitochondrial dysfunction and cellular senescence are closely intertwined. Mitochondrial dysfunction promotes and ensures that cellular senescence is maintained, whereas cellular senescence in turn influences and maintains senescence-associated mitochondrial dysfunction (SAMD) by maintaining the DNA damage response. In summary, mitophagy, SAMD, and SASP are closely linked to cell senescence through feedback signaling pathways. Metabolic dysfunction in aging can be attributed to SAMD. In the senescent cell, mitochondrial mass increases in addition to cell size. The increase in mitochondrial mass is a rapid process, but not an immediate one. The increase in mitochondrial mass occurs approximately 2–3 days after the peak of DNA damage, but before the establishment of a robust SASP. Dysfunctional mitochondria in senescent cells produce increased amounts of ROS; this stimulates both DNA damage and DNA damage response signaling pathway; thus, growth arrest is maintained (Korolchuk et al. 2017).

In oncogene-induced senescence, redox stress is increased by increasing the consumption of pyruvate in the tricarboxylic acid cycle, which in turn increases cellular respiration. Activation of the master regulator of peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1β) by p53 upon ionizing radiation (IR)-induced senescence induces the increase in mitochondrial mass (Passos et al. 2010). Furthermore, SAMD not only leads to increased ROS production but also increases the release of other molecules such as the "intracellular danger-associated molecular patterns" (DAMPs) and mtDNA fragments. NLRP3 (NLR family pyrin domain containing 3), a NOD-like receptor, recognizes these molecules, and triggers inflammasome assembly and activation of the pro-inflammatory

cytokines IL-1β and IL-18 (Salminen et al. 2011). Enhanced autophagic clearance of mitochondria suppresses NLRP3 activation. This signaling pathway positively interacts with NF-κB signaling leading to an inflammatory response. Thus, decreased mitophagy leading to SAMD stimulates for the pro-inflammatory arm of SASP. All of the above findings were reviewed by Korolchuk et al. (Korolchuk et al. 2017).

Senescence-Associated Secretory Phenotype (SASP)

A fundamental hallmark of most senescent cells is their SASP. The SASP can have a variety of biological activities. With respect to cancer-inducing activities, the SASP can stimulate cell proliferation by proteins such as GROs (growth-regulated oncogenes) (Coppé et al. 2010). Blood vessel growth can also be stimulated, for example, by VEGF (vascular endothelial growth factor) (Coppé et al. 2006). IL-6 and IL-8 can stimulate or inhibit the WNT signaling pathway depending on the biological context. Differentiated cells as well as stem cells can be driven into senescence by chronical WNT signaling. Furthermore, many SASP components lead to inflammation. These inflammations can also become chronic and may have local or systemic effects. Age-associated diseases, degenerative as well as hyperplastic, can be promoted by this (Rodier et al. 2009).

DDR is regulating many but not all SASP components positively. ATM, NBS1 (Nijmegen breakage syndrome 1), and CHK2 (checkpoint kinase 2) (Rodier et al. 2009) are such DDR proteins that act upstream of p53. Nuclear structures that contain the activated DDR proteins (DNA-SCARS (segments with chromatin alternations reinforcing senescence)) establish persisted DDR signaling. The immediate DDR after DNA damage, which is rapid and robust, does not induce SASP. SASP is also positively regulated by nuclear factor κB (NF-κB) and C/EBP-ß. NF-κB, for example, induces transcription of IL-6 and IL-8 (Coppe et al. 2008). In contrast, p53 is regulating the SASP negatively or restrains the SASP. Hence, if p53 in senescent cells is decreased by RNAi, a hyperincrease of several SASP factors (Coppe et al. 2008) is the consequence.

In more detail, in an early phase of SASP, NOTCH1 (notch receptor 1) signaling causes a repression of C/EBP-ß and induction of an immunosuppressive and pro-fibrotic SASP with high TGF-ß (transforming growth factor beta) levels. Later, NOTCH1 is downregulated in favor of a C/EBP-ß and NfκB-driven SASP with high inflammatory cytokine levels and matrix metalloprotease expression. This pro-inflammatory part of the SASP is strongly linked to the SAMD by positive feedback loops (Short et al. 2019). Deletion of mitochondria from senescent cells (Correia-Melo et al. 2016) as well as ROS scavenging (Passos et al. 2010) suppresses the complete senescent phenotype including inflammatory cytokine production, whereas sustained activation of NF-κB-induced SASP leads to increased ROS levels and DNA damage in senescent cells. SAMD and SASP are furthermore interconnected by rewriting the epigenome (Cruickshanks et al. 2013).

Like cellular senescence, also the SASP is a double-edged mechanism, on one hand, secreting inflammatory factors but also preventing cancer and activating the

immune system (Velarde et al. 2013). M1-type macrophages are attracted by the SASP secreted factors; these immune cells can, for example, prevent the transformation of epithelial cells (Krizhanovsky et al. 2008). M1-type macrophages have a strong inflammatory phenotype from which it plays an important role in eliminating cancer cells. Additionally, in the process of normal repair response to heal tissues, inflammation is an important part.

Bystander Effect

The effect that senescent cells can also convert surrounding cells into senescent cells is called the bystander effect. Via ROS- and NF-κB-dependent signaling pathways, senescent cells are able to induce a senescent phenotype in surrounding bystander cells (da Silva et al. 2019; Nelson et al. 2012). Senescent cells accumulate with age in various tissues and can cause age-associated diseases and functional decline. It has been shown that autologous transplantation of senescent fibroblasts into healthy knee joints resulted in the development of an osteoarthritis-like condition in mice (Xu et al. 2017). The significance for the accumulation of senescent cells in vivo has been investigated in several studies (da Silva et al. 2019; Nelson et al. 2012).

In a study by da Silva et al., senescent cells were xenotransplanted into the skeletal muscle and skin of immunosuppressed NSG mice (immunodeficient laboratory mice). This study provided direct evidence that transplanted or preexisting senescent cells induce senescence in surrounding tissues. After 3 weeks, the examined cells (dermal fibroblasts and myofibers) of the mice showed numerous senescence markers in the area where senescent cells were transplanted (da et al. 2019). Overall, there is much evidence that senescent cells can induce a deleterious bystander effect in vivo.

Therapy-Induced Senescence (TIS)

Cellular senescence plays an important role in the prevention of cancer. On the one hand, it prevents uncontrolled growth of genetically unstable cells, and on the other hand, it inhibits the growth of cells that overexpress oncogenes. In addition, harmful stimuli such as oxidative stress lead to growth arrest by cellular senescence. Cells that have already become cancer cells can also be forced into cellular senescence. Chemotherapeutic agents or radiation therapy is a tremendous stressor for these cells, and they may respond by developing cellular senescence. This variant of cellular senescence is often referred to as therapy-induced senescence (TIS) or chemotherapy-induced senescence (CIS).

Since cancer cells stop growing by inducing cellular senescence, this effect is definitely seen as positive in the fight against cancer (Lee and Lee 2019). Several compounds are able to increase senescence in cancer, and that selective induction of senescence in cancer cells is a potent antitumor response. Therefore, many of these compounds are currently being tested in preclinical studies to thus develop

promising pro-senescence therapies. For such pro-senescence therapies to be effective, they must induce senescence in tumor cells without affecting normal cells (Calcinotto and Alimonti 2017).

TIS Induced by Polyphenols

Polyphenols are able to induce senescence in multiple ways. In cell culture experiments with glioma cells, an induction of cellular senescence could be achieved, by a combination of resveratrol and quercetin (two polyphenols). In the same cell type, a combination of resveratrol with temozolomide (which is an alkylating cytostatic drug administered orally and intravenously; temozolomide is used for the palliative therapy of glioblastomas in combination with radiotherapy) also enhanced senescence and reduced temozolomide drug resistance. Cells that were resistant to gefitinib (gefitinib is an antineoplastic drug from the tyrosine kinase inhibitor drug class used to treat bronchial carcinoma) or paclitaxel (paclitaxel is a cytostatic agent from the taxane group used to treat various types of cancer; the effects are based on the disruption of the microtubule network and the inhibition of mitosis, which leads to the inhibition of cell division) could also be resensitized by resveratrol. A herbal extract of Chinese medicine (ASMq) was able to induce senescence in fibroblast and mouse lymphoblast cells by inducing ROS (Kühnel et al. 2015). Resveratrol also induced double-strand breaks and ROS in lung cancer cells and enhanced ionizing radiation-induced cellular senescence, thereby reducing radioresistance (Bian et al. 2020). Besides the induction of DNA damage and ROS, other mechanisms of polyphenols are suspected to play a role in cancer control. For example, gene products responsible for proliferation and anti-apoptotic activity are downregulated. Quercetin, for example, binds directly to PI3K (phosphoinositide 3-kinase) and inhibits PI3K/AKT (protein kinase B) signaling pathway (Song et al. 2014). Resveratrol has also positive effects on T-cell function which exerts anticancer properties (Verdura et al. 2020).

Because of multiple modes of action, nearly all types of senescence induction are addressed by polyphenols. Oncogene-induced senescence (OIS) was induced, for example, by targeting of oncogene AKT-inducing cell cycle arrest mediated by beta-naphthoflavone (synthetic flavonoid). The inactivation of PI3K/AKT signaling resulted in inhibition of cyclin D1/D3, and cyclin-dependent kinase 4 (CDK4) could arrest breast cancer cells (Wang et al. 2013). Oxidative stress-induced senescence, ROS produced by endoplasmic reticulum stress (ER stress), mitochondrial damage, or increased intracellular ROS level by loss of histone lysine methyl transferase 2D in prostate cancer cells (Bian et al. 2020) are also induced by polyphenols. ER stress-induced senescence can also be activated by compounds like cristacarpin (cristacarpin is a phenolic compound found in the herbs of Erythrina burana) targeting chaperones like binding immunoglobulin protein (BiP) also known as (GRP-78) or heat shock protein 90kDa beta member 1 (HSP90B1) (Bian et al. 2020).

Induction of cellular senescence appears as a promising strategy in the fight against cancer because it stops proliferation of tumor cells. Combining this treatment

with senolytic agents that specifically kill senescent cells seems to be an approach with great prospects for success. Furthermore, non-phenolic-natural substances can also induce senescence, such as sulforaphane, which is considered a potent drug candidate against esophageal squamous cell carcinoma (ESCC) and exploits these mechanisms to fight cancer (Zheng et al. 2020).

Escape From TIS

As Senescence program has to be inhibited for progression of cancer. Incomplete TIS might occur because cancer cells in contrast to primary cells undergo a non-definitive arrest. Essentially, effects are certainly more complex in a cancer cell. So recently, there has been increasing evidence that permanent growth arrest is not always maintained in cancer cells, as demonstrated by in vivo and in vitro experiments (Saleh et al. 2019). In malignant cells, most TIS parameters are still detectable but this does not guarantee definitive growth arrest (Saleh et al. 2019).

Malignant cells have to overcome apoptotic pathways or modify them to cause therapeutic failure. As described above, p16 protein is necessary to maintain senescence. More importantly, upregulation of this protein leads to apoptosis inhibition. By inhibition of caspase-3, the blockade of cytochrome c release, or the inactivation of stress kinases, p16 acts as a pro-survival factor. In addition, the p21 protein shows anti-apoptotic properties. This leads to the conclusion that senescent cells are resistant to apoptotic pathways (Saleh et al. 2019).

By downregulation of p16, an escape of cancer cell lines of senescence proliferative arrest is possible. Furthermore, the maintenance of senescence also relies on the stability of epigenetic modifications and histone turnover, which can be modified by successive rounds of chemotherapy leading to clones that could bypass TIS. Furthermore, histone demethylation by LSD1 (lysine-specific histone demethylase 1A) and JMJD2C (lysine-specific demethylase 4C) can reactivate proliferative promoters for E2F targets which is inactivated by a constant trimethylation of histone H3K9 in senescent cells. Co-expression of Ras with these proteins allows senescence bypass even if cells already entered suppressive arrest (Guillon et al. 2019). In addition, abnormal JAK-STAT pathway activity can favor senescence escape.

Senescent Cells: A Survival Niche Generating a Complex Tumor Environment

Senescent cells are able to provide a survival niche; thus, cells can be generated that are more aggressive. TIS can have different effects. On the one hand, cells go into complete senescence, while others just express proliferative arrest markers; unfortunately, these cells are sometimes able to resume proliferation (Guillon et al. 2019).

Signal transducer and activator of transcription proteins (STATs) could be involved in TIS heterogeneity. Inactivating JAK (Janus kinases) signaling induces immune surveillance which prevents tumor progression. By excretion of IL-6 by the

SASP, STAT3 could be activated which is an activator of bcl-2 (B-cell lymphoma 2) pro-survival family members which could make cancer cells more resistant to treatment. Besides, STAT3 is well-known to restrain antitumor activity of immune cells. However, STAT1 pathway can activate immune cell populations like NK of CD8-T-cells, which eliminate senescent cells.

There is evidence that resistance to chemotherapy is highly correlated with the presence of senescent cells. Cells that escape TIS become more transformed and invasive and show increased metastatic spreading (Guillon et al. 2019).

A SASP-mediated survival niche can favor escape from senescence of premalignant clones and allow the survival of clones with lower fitness. It is known that tumor progression is linked to cooperation with the microenvironment. SASP induces epithelial-mesenchymal transition (EMT) and dedifferentiation, which are factors that induce chemotherapy resistance. Vascular endothelial growth factor (VEGF) metalloproteases or IL-6 is playing an important role in carcinogenesis. For instance, IL-6 is inducing anti-apoptotic proteins like BCL-xL making cells fit for survival of treatments they wouldn't survive without SASP factors in tumor environment (Guillon et al. 2019).

Overall, tumor progression relies on cooperation between different malignant and senescent cells indicating a significant genetic diversity among tumor subclones. There is evidence that the accumulation of senescent cells can promote an unfavorable outcome of cancer therapy. Senescent cells can also lead to a more malignant phenotype (Guillon et al. 2019).

Importance of TIS by the Example of Radiotherapy-Induced Senescence

Approximately 50 percent of all cancer patients receive radiation. Radiation is given either alone or in combination with surgery or chemotherapy (Khor et al. 2015). Survival rates for patients treated with radiation therapy are roughly comparable to those after surgical resection, but lower costs occur with radiation (Tabasso et al. 2019).

With SBRT (stereotactic body radiation therapy), for example, recurrence rates are higher than in patients whose tumor was surgically resected at the same stage. One reason for this could be that irradiation results in more senescent cells (Tabasso et al. 2019).

As mentioned, cellular senescence can prevent further proliferation of cancer cells. In some cancers, such as lung cancer and glioblastoma, senescence is preferentially induced over apoptosis; thereby, cellular senescence is becoming increasingly important in the treatment of cancer. Due to antagonistic pleiotropy, the consequences of SASP and senescent cells may be initially beneficial by triggering an immune response that renders the treated cells more immunogenic in various ways, and this process is called immunosurveillance (Wennerberg et al. 2017). However, these initially beneficial effects can also promote cancer development and growth. The initial supportive recruitment of immune cells can create a

pro-inflammatory microenvironment. Furthermore, this process can lead to tissue remodeling and vascularization and regrowth of transformed cells. Thus, overall tumor growth may be promoted (Tabasso et al. 2019).

Endothelial tissue surrounding the tumor may be forced into senescence by ionizing radiation, leading to fibrosis and cardiovascular disease (Borghini et al. 2013). The overall survival of cancer patients is related to the accumulation of senescent cells in the surrounding normal tissue.

Role of TIS in Other Cancer Treatments

DNA damage is often induced by chemotherapy; if severe, this leads to cell death. When the DDR does not lead to cell death, cellular senescence can be induced depending on the extent and duration of the stimulus. Functional tumor suppressors such as p53 or p16 are required for entry into senescence. Furthermore, microvesicles, such as exosomes, may play a role in promoting tumor growth; these are also secreted by senescent cells. Exosomes are vesicles that contain a variety of proteins, mRNAs, and miRNAs that can influence the behavior of surrounding cells. For example, these extracellular vesicles (EVs) secreted by DNA-damaged cells can induce telomeric dysregulation. Furthermore, secretion of cytoplasmic DNA can cause inflammation. Also, senescent cells stimulate the mitogenic pathway in cancer cells; this occurs through EV-associated EPHA2 (EPH receptor A2 (ephrin type A receptor 2)) (Takasugi 2018). Chemotherapeutic agents that induce senescence include doxorubicin, vinca alkaloids, taxanes, and cyclophosphamide and also CAF chemotherapy (cyclophosphamide, adriamycin, and fluorouracil) or adriamycin, and carboplatin, among many others (Wyld et al. 2020).

With regard to hormonal therapies – antiestrogens and anti-androgens – senescence may also play a role (Wyld et al. 2020).

Senescence even plays a role in surgical procedures to remove solid tumors. Wound healing, for example, is a complex multistep process in which senescence plays a critical role. After an initial hemostatic phase, an inflammatory reaction begins. This inflammatory reaction causes a large number of cells to migrate into the injured tissue. These differentiate locally to support the healing process. Collagen synthesis and remodeling processes also take place. Especially in old organisms but also in people with diabetes, wound healing is not optimal; the accumulation of senescent cells in old tissues may play a role here. Liver regeneration after hepatectomy correlates negatively with p16 expression in the cells involved. Regarding surgical interventions and cellular senescence, data are insufficient and further studies are needed (Wyld et al. 2020).

Polyphenols Inhibiting SASP (Senostatics)

Senostatics inhibit paracrine signaling and block the spread of senesce due to the bystander effect. As abovementioned antioxidants can be efficient senostatics.

Furthermore, inhibitors of NF-κB can act also as senostatics. SAMD has an essential role in regulating the SASP. Interventions that improve mitochondrial function have fundamental senostatic potential, for example, mTOR pathway inhibitors (Campisi 2013).

Polyphenols can actually be used to influence SASP. Fibroblasts are the main cell type in the tissue stroma, and when they become senescent, they can create an environment that promotes tumor growth. In order to create an antitumor environment, a reduction in the secretion of SASP factors has been achieved, for example, through chronic administration of resveratrol (Menicacci et al. 2018), apigenin significantly inhibited SASP in the kidneys of aged rats (Lim et al. 2015), and combination of dasatinib and quercetin reduced plasma SASP factors (Hickson et al. 2019).

Apigenin

In cell culture experiments, decreased expression of IL-6, IL-8, and IL-1β at the mRNA level in a concentration-dependent manner was observed in human foreskin fibroblasts during bleomycin-induced senescence (a premature senescence model) (Lim et al. 2015). In stress-induced premature cellular senescence by UVA irradiation, decreased level of MMP-1, a metalloprotease, and decreased activity of Sa-B-gal, an important senescence marker, were observed. These experiments were also performed in fibroblasts (Maria and Ingrid 2017).

Epigallocatechin-3-Gallate (EGCG)

Likewise in fibroblast cells in several replicative senescence models a reduced activity of Sa-B-galactosidase in a concentration-dependent manner is observed. Furthermore a decreased amount of cells in G0/G1 phase, and an increased amount of cells in S-phase in a concentration-dependent manner as well as a decreased level of p53 and acetylated p53, and a decreased level of p21 are shown. A decreased level of MDA and a reduced mRNA level of NF-κβ and mRNA and protein level of COX-2 and iNOS were observed in a SIPS model with H2O2; all these studies are reviewed by Janubová Mária and Žitňanová Ingrid (Maria and Ingrid 2017).

Resveratrol

Resveratrol, a polyphenol produced by various plants, can inhibit the mTOR pathway at concentrations close to cytotoxicity. A cytostatic effect occurs at concentrations greater than 10 μM. The mTOR pathway is affected via sirtuin activation, PI3K inhibition, AMPK activation, and AKT and MAPK inhibition. Transient inhibition of the mTOR pathway is sufficient to slightly suppress senescence.

Furthermore, resveratrol is able to protect adipose-derived mesenchymal stem cells from H2O2 and D-glucose-induced senescence. Here, the senescence-associated genes p53, p21 cyclin D1, IL-6, and MMP1 are attenuated (Prašnikar et al. 2020).

SIRT1 (sirtuin 1, also known as NAD-dependent deacetylase sirtuin-1) expression is increased, and additionally the expression of Pim-1 (proto-oncogene serine/threonine-protein kinase) is upregulated by the PI3K/AKT signaling pathway. SIRT1

plays an important role in senescence. Therefore, it represents an attractive therapeutic target. Resveratrol is a substance that can activate SIRT1 (Prašnikar et al. 2020). Resveratrol can counteract changes in the cell associated with senescence, but does not prevent the onset of replicative senescence (Prašnikar et al. 2020). Genistein, kaempferol, and quercetin show similar effects.

Quercetin

In the abovementioned study from Lim et al., decreased expression of IL-6, IL-8, and IL-1β at mRNA level in a concentration-dependent manner and a reduced activity of SA-B-galactosidase in a concentration-dependent manner were observed. Interestingly, quercetin also reduced viability of senescent cells by 70% to 50% and decreased expression of p21, BCLxl at protein level. Furthermore PAI-2 at mRNA and protein level was also decreased in a ionizing radiation model wiht preadipocytes (Maria and Ingrid 2017).

In addition, senostatics can also have senolytic effects. As an example apart from chemical and biological substances, dietary restriction has proven senostatic effects decreased cells positive for a variety of markers of senescence below the initial values before treatment (da et al. 2019).

Polyphenols as Senolytics (Polyphenols Killing Senescent Cells)

"Senolytics" are a new class of drugs. Prof. James Kirkland and his team at the Mayo Clinic initially developed them. The name senolytica, a neologism for "senescence" and "lytic" destruction, derives from their ability to eliminate senescent cells. Senescent cells are dependent on pro-survival signaling pathways. This dependence is exploited to kill senescent cells. Optimally, proliferating or resting differentiated cells remain unaffected. Senolytics tested to date include dasatinib (an FDA-approved tyrosine kinase inhibitor), navitoclax, A1331852, and A1155463 (inhibitors of the Bcl-2 pro-survival family) and two natural compounds of great importance, namely, fisetin (a flavonoid) and quercetin (a flavonoid found in many fruits and vegetables). However, not only pro-survival pathways are under investigation, any difference in senescent cell metabolism to normal cells can be used to kill senescent cells and favor the survival of normal cells. The use of senolytic agents and the concomitant elimination of senescent cells have been shown to improve physical function and prolong health and lifespan in mice using fisetin as an example (Yousefzadeh et al. 2018). These preclinical studies in mice have also shown that it leads to delay, prevention, or alleviation of several age- and senescence-related diseases, and with dasatinib and quercetin, the first promising clinical studies in humans are already underway (Hickson et al. 2019).

Fisetin

Fisetin is a naturally occurring polyphenolic compound and member of the flavonoid family. Strawberries contain a high amount of fisetin but it is also present in other fruits and vegetables in low amount. The average dietary intake is approximately 0.4 mg/day, apparently without any adverse effects. In a study, fisetin was even

administered as a nutritional supplement as 100 mg capsules daily. This dose was also used in a colorectal cancer chemotherapy study where it reduced inflammatory markers (Farsad-Naeimi et al. 2018). Not even with these high dosages, there were safety issues. There are also studies that test concentrations up to 20 mg/kg orally for 2 days (Wyld et al. 2020). Fisetin has anticancer activity through its topoisomerase inhibitor activity and by blocking the PI3K/AKT/mTOR pathway. In vitro experiments show that fisetin increases the catalytic activity of hSIRT1. In addition, it also inhibits pro-inflammatory cytokines such as TNFα, IL-6 and the transcription factor NF-κB. Fisetin is a radical scavenger and has antioxidant effects by stimulating the synthesis of glutathione. It also has other antihyperlipidemic, anti-inflammatory, and neurotrophic effects (Yousefzadeh et al. 2018).

In various cancer types, fisetin is extensively tested. In vitro effects like growth arrest and apoptosis of hepatic and colorectal and gastric cancer, breast cancer and pancreatic cancer as well as cervical and lung adenocarcinoma cells are observed. In mouse models the in vivo investigations with chemotherapeutics like 5FU, sorafenib, cisplatin, oxiplatin, and capecitabine, or cyclophosphamide showed effects like reduced proliferation, increased apoptosis, reduced metastases, reduced incidence of colorectal cancer, and reduced inflammatory mediators (IL-8, CRP und MMP7) (observed in a study by Wyld et al. (2020)). It is suspected that fisetin might cause aneuploidy in cultured non-senescent cells (Gollapudi et al. 2014); nevertheless, fisetin delays cancers in cancer-prone Ercc1-/D mice that have a DNA repair enzyme mutation (Yousefzadeh et al. 2018).

More generally, the senolytics dasatinib and quercetin in combination and fisetin delay cancer death in mice, which would otherwise die in 50% of the cases by cancer (Kirkland and Tchkonia 2020). When these animals are given dasatinib and quercetin and fisetin, the death of old mice is delayed by up to 35 and 17%, respectively, which might be true because of the abovementioned reasons.

One of the clinical studies fisetin is tested in is named “Alleviation by Fisetin of Frailty, Inflammation, and Related Measures in Older Adults” (AFFIRM-LITE). The study is currently in the recruitment phase and hopes to recruit 40 participants aged 70–90 who will receive an oral 2-day dose of a placebo or fisetin at a dosage of 20 mg/kg/day. Parameters observed are frailty, inflammation, insulin resistance, and bone metabolism (Kaur et al. 2020).

Quercetin

The physiological effects of quercetin and fisetin are relatively similar. With the exception of a hydroxyl group in position 5, the chemical structure of fisetin is almost the same as that of quercetin. Nevertheless, fisetin has a higher senotherapeutic activity in cultured cells than quercetin (Yousefzadeh et al. 2018).

Dasatinib and quercetin belong to the first generation of senolytics that are designed to induce apoptosis preferentially in senescent cells. This combination induces apoptosis in certain types of senescent cells. In progeria and naturally aged mice, a combination of dasatinib and quercetin alleviates several senescence-associated phenotypes. This demonstrates the feasibility of senolytic applications for the improvement of life expectancy (Zhu et al. 2015).

A combination of quercetin and dasatinib is used in most applications. This combination has made it into clinical trials. Currently, there are several clinical trials of senolytics, planned or ongoing. These trials include phase I or II trials of quercetin and dasatinib where among others one study deals with childhood cancer survivors in their late 30s and 40s with accelerated age-like syndrome (Kirkland and Tchkonia 2020).

There is also evidence against the effect of quercetin as senolytic. Hwang et al. (2018) argue that quercetin at a concentration that reduced senescent endothelial cells also caused significant early passage endothelial cell death. Furthermore, there was no evidence of quercetin-mediated senescent cell-specific cell death in this study. So in this study, there is no senolytic activity detectable.

Epigallocatechin-3-Gallate (EGCG)

In a study by Dong-Wook Han et al., the effect of EGCG on replicative senescent primary cells was investigated. RVSMCs (vascular smooth muscle cells), human dermal fibroblasts (HDFs), and human articular chondrocytes (HACs) were studied here. Replicative senescence in RVSMCs and HACs could be significantly prevented with 50μM EGCG, while HDFs 100μM EGCG could significantly prevent senescence and restore cell cycle progression to near normal levels. Moreover, it was discovered that replicative senescent and H2O2-induced senescent cells' (HDFs') p53 acetylation was prevented, but Sirt1 activity was not affected. The uptake of FITC-conjugated EGCG into the cytoplasm of young cells is comparable to that of senescent cells. Differential nuclear translocation of EGCG could be the reason for differences in the responses of proliferating and senescent cells. The authors suggest the use of this substance as senolytic or senomorphic (Han et al. 2012).

Curcumin

In human senescent disc cells, treatment with curcumin induced a significant decrease in senescent cells. This was determined by p16INK4a staining. In addition, an induction of proliferation of the remaining cells was observed. Inflammatory markers such asvIL-6, IL-8, MMP3, and MMP13 associated with SASP were also reduced (Cherif et al. 2019). The efficacy of curcumin is increased by chemical manipulation. For example, a curcumin analog has been developed called EF24 which shows better bioavailability. In aged animal models, curcumin improved cognitive function by reducing oxidative stress and increasing the expression of p-CaMKII (CaMKII is an important member of the calcium-/calmodulin-activated protein kinase family, functioning in neural synaptic stimulation and T-cell receptor signaling) and p-NMDAR1, which is a protein that is a critical subunit of N-methyl-D-aspartate receptors, in the hippocampus (Li et al. 2019).

Herbal Extracts with Senolytic and Senomorphic Properties

Extract of the Plant Solidago virgaurea Subsp. alpestris

Lammermann et al. showed that an alcoholic extract of the plant Solidago virgaurea subsp. alpestris has a weak senolytic activity. Solidago virgaurea is also known as

goldenrod. In traditional Western medicine, it was used as an anti-inflammatory herbal medicine. By pathway analysis of RNA-Seq data, evidence was found that the plant extract is able to influence several signaling pathways that enhance SASP. Caffeoylquinic acids are expected to act on the SASP with their anti-inflammatory property. Three derivatives of quercetin appear to cause a slow but significant selective elimination of about one-third of senescent cells (Lammermann et al. 2018).

Alcoholic Extract of Ajuga taiwanensis

The alcoholic extract of Ajuga taiwanensis (ATE) reduced senescence-associated biomarkers such as SA-β-gal and p53 in old human dermal fibroblasts (HDFs), and no significant cytotoxicity was observed. G1 arrest was resolved and a reactivation of the cell cycle was observed. Furthermore, growth rate of the cells was increased. Fractions with n-butanol (BuOH), ethyl acetate (EA), and water were prepared. These fractions showed the following behavior: BuOH and water subfractions showed less effect on cell growth arrest than EA subfraction. The suppression of SA-β-gal and p53 from old HDFs was achieved by all fractions. A major active component was identified, isolated, and identified as 8-O-acetylharpagide by structural analysis. This substance was also able to suppress Sa-β-gal and p53 of old HDFs with concentrations below 10 μM. In addition, ATE suppressed intracellular reactive oxygen species (ROS) in old HDFs. However, the EA subfraction showed little ability to suppress ROS. In addition, an in vivo study was conducted with aging mice treated with ATE and the subfractions followed by immunohistochemical (IHC) staining. The expression of p53 and SA-β-gal was significantly reduced in multiple tissue sections, including the skin, liver, kidney, and spleen. In conclusion, the current data demonstrate that A. taiwanensis can suppress cellular senescence in HDFs (Hsu et al. 2020).

Pinellia ternata Tuber

Pinellia ternata tuber is a traditional medicinal plant in China and is used as antiemetic, sedative-hypnotic, anticancer, anti-asthma, antitussive, and anti-inflammatory medicine. In this study, an ethanol extract of P. ternata tubers (PTE) was tested. H2O2 and 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH) were used to establish cellular senescence models. In this study, the researchers found out that PTE showed significant effect on cell senescence, as evidenced by the inhibition of Sa-β-gal expression, lipofuscin accumulation, cell cycle arrest in G2/M phase, oxidative damage and apoptosis, and increase in telomerase activity. The mechanisms were associated with the increase in the expression of SIRT1, forkhead box 3a (Foxo3a), Bcl-2, the active regulator of SIRT1, RPS19BP1 (AROS), and Hu antigen R (HuR), but with the decrease in the levels of Bax and p53. In addition, adenosine and succinic acid, as the critical compounds in PTE, were also able to inhibit SA-β-gal expression and cell cycle arrest, downregulate Bax expression, and upregulate Bcl-2, SirT1, and Foxo3a (Tang et al. 2020).

Olive Plant

Phenolic compounds from virgin olive oil have anti-inflammatory and antioxidant activity. In particular, hydroxytyrosol and oleuropein are the most abundant and

intensively studied phenolic compounds. Pre-senescent human lung (MRC5) and neonatal human skin (NHDF) fibroblasts were used as in vitro cellular models of chronic treatment with 1 μM hydroxytyrosol (HT) or 10 μM oleuropein aglycone (OLE) on senescence and inflammation markers. The markers of senescence p16 and β-galactosidase-positive cell number could be reduced by both compounds. Additionally, SASP markers like IL-6 and metalloproteases were decreased. Levels of cyclooxygenase type 2 (COX-2) and α-smooth-actin were reduced furthermore. In NHDF, COX-2 expression levels of the nuclear factor κ-light-chain-enhancer of activated B cells (NFκB) and nuclear localization were also decreased by treatment with OLE and HT. In cells (NHDF) pretreated with OLE and HT, the inflammatory effect of tumor necrosis factor α (TNFα) was almost completely abolished (Menicacci et al. 2017). Also, the SA-β-galactosidase activity induced by UVA irradiation could be reduced in a concentration-dependent manner by a polyphenol named hydroxytyrosol. Also, the increased expression of the metalloproteases of SASP (MMP-1 and MMP-3) by UVA was also decreased depending on the dose. Furthermore, interleukins IL-1β, IL-6, and IL-8 were analyzed. Quantitative RT-PCR showed that hydroxytyrosol decreased the expression of IL-1β, IL-6, and IL-8 genes. It can be concluded that hydroxytyrosol has effects on anti-inflammatory and antiaging in HDFs damaged by UVA (Jeon and Choi 2018). Further studies reported reduction of oxidative stress and inhibition of mTOR by OLE as well as proteasome activity improvement as reviewed by Kaur et al. (Kaur et al. 2020).

Conclusion

Polyphenols possess various effects on mammalian cells. It is not always easy to obtain unambiguous results, as there are several modes of action involved in the concentration- and cell type-specific effects of these natural products, for example, their antioxidant potential, which is a hallmark of polyphenols. At low concentrations, these compounds protect against oxidative damage, whereas at higher concentrations, polyphenols can also react as oxidants, leading to cellular senescence and even cell death (Kühnel et al. 2015). Polyphenols like quercetin and curcumin are often applied in cell culture experiments in near or above toxic concentration, which might be a point to consider when evaluating effects.

Another interesting question is how mixtures of different substances can increase healing success. These mixtures can be mixtures of polyphenols, other natural substances, or chemically synthesized substances, but also herbal extracts, which should not be ignored. The natural composition of the plants is biologically validated and may contain additives that are nontoxic and show, for example, stabilizing effects on the active ingredients of the mixture. In addition, blending different plant extracts could yield interesting results. Determining which substances in plant extracts are necessary for action and efficacy is an important goal for further research. Intensive investigations should lead to new insights into the optimal composition of natural substances. Why not creating drugs that contain ten or

more substances? Substances have to be evaluated in animal models anyway, and the experimental design for the testing of effects of such a drug will be challenging but very interesting. Maybe fine-tuning of single compounds with regard to personalized medicine could be adapted individually.

As already mentioned, the effects of polyphenols are manifold and cell type dependent (Hwang et al. 2018). The broad spectrum of effects and the different effects from cell type to cell type make the data appear contradictory and confusing. Nevertheless, there are undeniable effects. Focusing on cell senescence does not simplify the issue; on the contrary, recent studies show that senescent cells exhibit greater variability in mRNA levels than quiescent cells and that gene expression correlations change during cellular senescence (Wiley et al. 2017). For this reason, experiments with natural products should be conducted with special caution to avoid damaging the reputation of natural products research.

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