



Senotherapeutics in Malignant Brain Cancer Therapy

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Abstract

Purpose of Review Malignant brain cancer, the most severe form is glioblastoma (GBM), has a dismal prognosis, despite maximal resection followed by radio-chemotherapy. First line therapeutics are alkylating drugs, notably the DNA-methylating temozolomide (TMZ), administered concomitantly with radiation. Radio-chemotherapy induces not only apoptosis, but also cellular senescence in GBM cells. Senescent cells change the tumor microenvironment, cause an inflammatory response in the affected area and can be reactivated, contributing to recurrences. To eliminate therapy-induced senescent cells, senotherapeutics have gained attention. Here, we describe the pathways triggered in GBM cells leading to cellular senescence and update drugs and natural compounds acting as senolytics, senomorphics and senopreventives.

Recent Findings There is an increasing amount of data showing that temozolomide induces cellular senescence, which is even the main response of GBM cells following treatment. We outline the mechanism of senescence in glioblastoma cells and show that it rests on some unique cellular responses that may explain the low curability and aggressiveness of glioblastoma. Thus, senescent GBM cells are incompletely blocked in G2 following temozolomide treatment and undergo endoreduplications. This is presumably fostered by inactivation of CDKN2A, which is frequently mutated in gliomas.

Summary Since cellular senescence is a key event induced by temozolomide and radiation in GBM cells, it is reasonable to conclude that glioma cells cannot be completely eliminated, neither by radiation or chemotherapy alone nor in combination. Based on the data, new treatment options with senopreventives, senolytics and senostatics/senomorphics as important supportive medication during or after radiochemotherapie are discussed.

Keywords Senotherapeutics · Senopreventives · Senolytics · Senomorphics · Glioblastoma · Temozolomide · Senescence · Apoptosis

Introduction

DNA-alkylating agents are well-established drugs in the treatment of malignant brain tumors [1]. The group of methylating anticancer drugs comprises temozolomide (TMZ), procarbazine, dacarbazine and streptozotocin. TMZ is the front-line drug for the treatment of WHO grade 3 and grade 4 gliomas, including anaplastic astrocytoma and glioblastoma [2]. Procarbazine, which is administered in the PCV scheme together with the chloroethylnitrosourea

chloroethyl-cyclohexyl-nitrosourea (CCNU, Lomustine) and the mitosis inhibitor vincristine, is also frequently used in glioma therapy [3]. While procarbazine (Natulan) needs metabolic activation, TMZ spontaneously decomposes into the reactive metabolites. In both cases, carbonium ions are generated, which methylate the DNA at various sites producing, among others, the critical lesion O⁶-methylguanine [4]. The conversion of the primary DNA damage into a cytotoxic lesion needs DNA replication and mismatch repair (MMR), leading downstream to DNA double-strand breaks (DSB) and finally the activation of the DNA damage response (DDR), which triggers apoptosis, senescence and autophagy pathways (Fig. 1) [5]. TMZ is given after resection concomitantly with radiation and in subsequent adjuvant treatment cycles. Chemotherapy can extend over long periods of time since TMZ is relatively well tolerated [6], and the effects are accumulating if the primary damage is not repaired [7].

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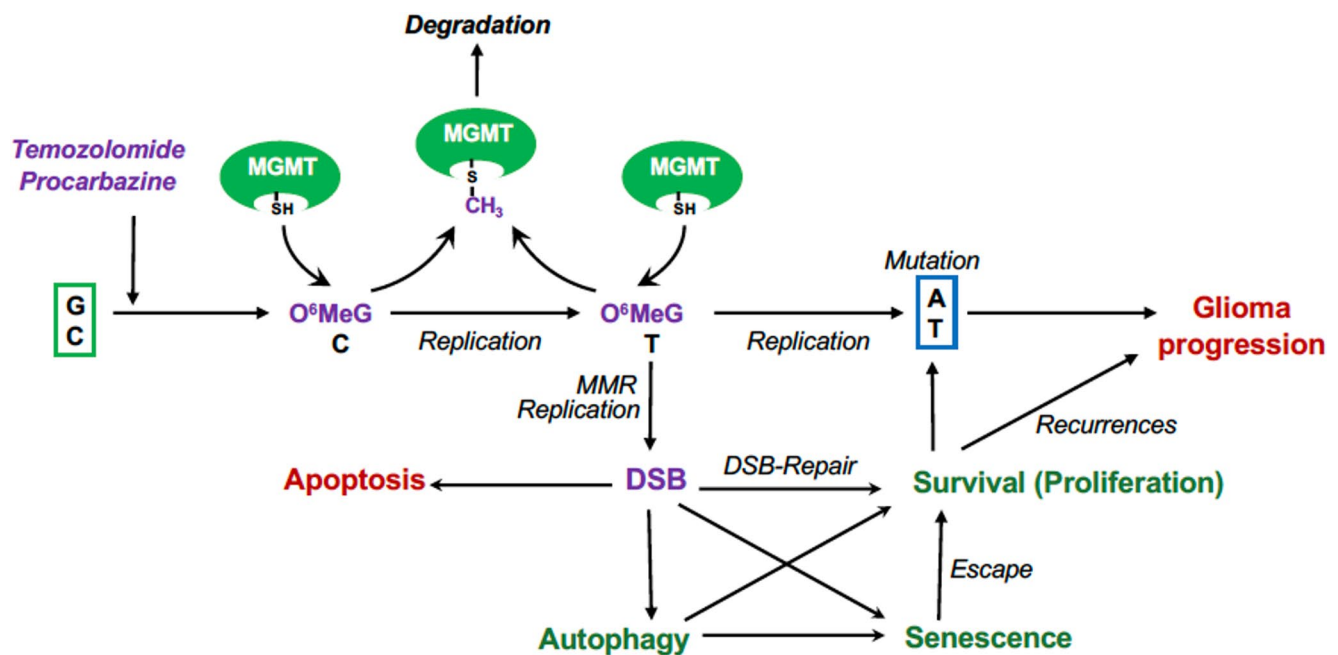


Fig. 1 Responses triggered by temozolomide, procarbazine and other methylating drugs and defense by MGMT

Mechanism of Cytotoxicity

The critical primary damage O⁶MeG is, because of its mispairing properties, mutagenic (Fig. 1), but not cytotoxic per se. Cytotoxicity needs conversion of the damage into DSBs. This occurs by the mediation of MMR. A model based on reliable data states that during the first DNA replication cycle after treatment with TMZ or other DNA methylating drugs O⁶MeG/thymine mismatches are formed, which are the substrate of repetitive, futile MMR cycles. This gives rise to gaps and other distortions in the DNA that block replication in the subsequent cell cycle leading to DSBs at arrested replication forks. If not processed by MMR, mutations can arise. On the other hand, blocked replication forks and free DSBs activate the DNA damage response (DDR) pathway and induce downstream apoptosis, senescence and autophagy [8]. During cancer therapy, successful repair and escape from senescence leading to reuptake of proliferation can lead to recurrences, tumor progression and more aggressive tumor growth (Fig. 1).

A key target of DDR kinases is p53, which regulates genes that control apoptosis pathways. It also regulates some genes involved in DNA repair causing drug resistance [9]. p53 becomes phosphorylated at various sites, which influences its activity as a transcription factor. Phosphorylation of serine 15 of the protein causes preferential activation of repair genes such as *DDB2* and *XPC* [10], while phosphorylation on serine 46 stimulates pro-apoptosis genes such as *FAS-R*, *BAX* and *NOXA* [11]. p53^{Ser46} is the result of stress activation of the kinase HIPK2. Under normal conditions,

the kinase is inactive due to binding to SIAH1. Activation of ATM and ATR leads to phosphorylation of SIAH1, which releases HIPK2 that phosphorylates p53 [12]. We showed that glioblastoma cells treated with therapeutically relevant doses of TMZ (< 50 μM) activate the ATR/ATM-SIAH1/HIPK2-p53^{Ser46} axis and thus trigger apoptosis [8]. In addition to this signaling pathway, the Jun kinase pathway also becomes activated, which regulates both receptor and mitochondrial mediated apoptosis via the Fas ligand (FAS-L) and BIM [13]. The apoptotic signaling pathways activated by O⁶MeG upon TMZ are outlined in Fig. 2.

TMZ may also cause necrosis, which may occur under hypoxic conditions together with RT, or ferroptosis which was reported following autophagy inhibition [17] or high NRF2 expression [18]. However, these are not major traits, and there is no evidence that the pathways involved are triggered by O⁶MeG. Necroptosis rests on ATP depletion, which is caused by excessive activation of PARP1. This is a specific response following high level of DNA damage that are repaired by base excision repair (BER), causing PARP-activating BER intermediates. At clinically relevant doses this process plays no significant role.

Mechanisms of Drug Resistance: MGMT, MMR and DSB Repair

Both O⁶-methylguanine (O⁶MeG) and O⁶-chloroethylguanine are repaired by the suicide repair O⁶-methylguanine-DNA methyltransferase (MGMT) [19]. Repair occurs in a fast and single-step reaction. In repair competent cells,

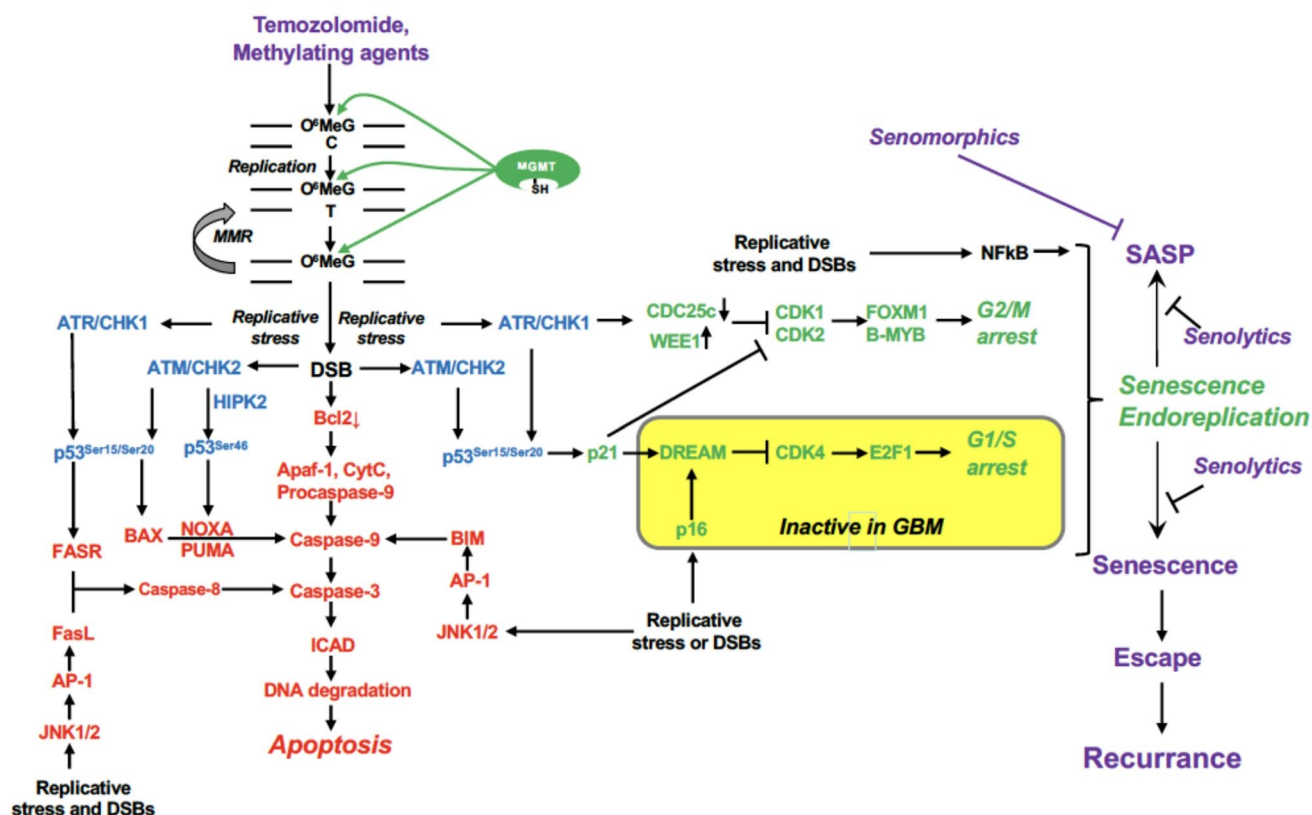


Fig. 2 Pathways triggered by O⁶MeG in glioblastoma cells leading either to survival or death through apoptosis during therapy. It should be noted that in conserved models CDK inhibition leads to lack of Rb phosphorylation and missing activation of E2F1, which represses S-phase genes, causing arresting cells in G1. This was not the case in our study with TMZ, in which Rb was still phosphorylated and G1/S genes were normally expressed in senescent glioma cells. FOXM1 and

B-MYB control G2/M genes and, therefore, inhibition of this pathway results in arrest in G2 and early mitosis [14]. The observed endoreplication occur likely in the G2 arrested cell population that restart replication of the genome. NF-κB is a master regulator of TIS [15], which is also activated in TMZ-induced senescent glioma cells [16]. Further explanations see text

O⁶-alkylating agents are nearly non-toxic [20], with TMZ at least in the therapeutic low dose range (<50 μM) being completely ineffective [21, 22]. On the other hand, in MGMT repair deficient cells, O⁶-alkylguanine in the tumor DNA accumulates during treatment, resulting in stepwise increase in cytotoxic effects [7]. This applies to about 40% of glioblastoma, which are MGMT promoter methylated and therefore deficient in MGMT [23], having significant impact on therapeutic outcome [24]. MGMT is otherwise expressed in all tissues, but to varying degrees: strongly in the liver, intestine, and peripheral lymphocytes, and less in the brain and very low in CD34 hematopoietic stem cells [25], which explains therapy-limiting hematotoxic side effects. The different expression levels of MGMT in the tissues and tumors as well as the inter-individual variability [26] indicates that the repair protein is subject to strong regulation on epigenetic and genetic level. Actually, hardly any other repair gene is as strongly epigenetically regulated as MGMT [27].

As outlined before, TMZ needs MMR for conversion of primary lesions into a cytotoxic damage. Therefore,

enzymes involved in MMR necessarily determine the level of drug resistance. Proteins involved are MSH2, MSH6, MLH1 and PMS2. They are also regulated in cancer cells, and quantitative changes have already an impact on TMZ resistance [28–30].

During conversion of MMR-mediated secondary lesions DSBs are formed if replication meets repair intermediates (Fig. 2). These DSBs are subject to repair by homologous recombination (HR) involving Rad51, BRCA1, BRCA2 and other players, and lack or downregulation of either one of these leads to strong sensitization to TMZ [31–33]. In summary, MGMT, MMR and DSB repair by HR are the most important upstream determinants of glioma drug resistance.

Induction of Cellular Senescence (CSEN)

While initial studies on glioblastoma cell responses were focused on cell death through apoptosis [34, 35], we and others became aware that TMZ also induces cellular

senescence (CSEN) [5, 16, 36, 37] with O⁶MeG to be the primary inducing lesion [5, 16]. Apoptosis and CSEN are late events that occur in proliferating glioblastoma cells at the earliest 3 days after TMZ treatment. Senescence runs parallel to apoptosis, but with a significantly higher proportion (10–20% apoptosis versus 80–90% senescence (SA- β GAL) measured 6 days after the onset of treatment). Under optimal conditions, 10 days after treatment >90% of the cells in the population are in the senescence stage, which is impressively visible morphologically (giant cells, large nuclei), while apoptotic cells have been successively eliminated. The remaining senescent cells are arrested in the G2 phase. Obviously, senescence is the main trait triggered by O⁶MeG in glioblastoma cells. We determined that a dose of 20 μ M TMZ caused about 14,000 O⁶MeG adducts per cell, which gave rise to 7% apoptosis and 35% senescent cells in the population [21].

TMZ-induced senescent glioma cells show high levels of ROS production, oxidative DNA damage, and DSBs (visible as γ H2AX foci) that are not preferentially located in telomeres [38]. Although the DSB level is high, senescent cells can still repair DSBs [38], indicating that senescence-associated DSBs are stabilized breaks not accessible to repair that were formed during senescence induction.

In line with this notion is the finding that expression of MGMT (using an inducible tet-on system) after damage induction by TMZ prevents from CSEN while expression of MGMT in senescent cells had no impact on the CSEN level [38]. This indicates that O⁶MeG is essential as a primary trigger for CSEN, but not for maintaining the senescent state. TMZ-induced senescent glioblastoma cells are also characterized by proinflammatory cytokine production, including the cytokines IL-1, IL-6, IL-8 and TNF- α [16, 39] and thus display the senescence-associated secretory phenotype (SASP).

Data on apoptosis and CSEN in vivo, following therapy of gliomas, are scarce. We pursued to determine apoptosis and senescence in primary tumor specimens and corresponding recurrences. The proportion of senescent cells was significantly higher, while apoptosis was lower in recurrences compared to the primary tumor [38]. The result indicates that after radio-chemotherapy a significant proportion of tumor cells remain in the senescent stage and become visible even in the relapse, while apoptotic cells were cleared in the post-treatment period.

It should be noted that O⁶MeG is also an autophagy-inducing lesion, with the upstream pathways being identical: involvement of MMR and DSBs, and protection by MGMT. Inhibition of autophagy increased the apoptosis rate; it therefore exerts a protective function in glioblastoma cells [5]. Furthermore, we should note that ionising radiation is also a potent inducer of senescence in glioma

cells [40–42]. Since radiation and TMZ are concomitantly applied for treatment, the question arises as to additive or synergistic effects in this therapeutic setting, which is worth to be studied.

Nitrosoureas such as CCNU (lomustine) are frequently applied in glioma therapy, either alone or in combination with procarbazine (PCV scheme). Whether nitrosoureas induce CSEN in GBM cells and how toxic lesions induced by the drugs are interacting is to our very best knowledge not yet explored. The same is true for tumor-treating fields (TTF), which are being used in GBM maintenance therapy, alone or together with TMZ and CCNU [43]. TTF is thought to be cytotoxic by inhibiting mitosis and DNA repair through homologous recombination [44], which is a key drug resistance mechanism. Thus, it is reasonable to hypothesize that TTF together with TMZ enhances the therapeutic response through amelioration of cell death, but as a side effect also through the induction of CSEN.

Molecular Mechanism of CSEN Induction in Glioblastoma Cells

It has been shown that the upstream pathway triggering apoptosis and CSEN following TMZ is identical [5]. Both endpoints need MMR causing sustained replication blockage and DSBs that are the ultimate upstream trigger. They activate the ATR-CHK1- and the ATM-CHK2- p53 axis, causing transcription of p21, which acts as inhibitor of cyclin-dependent kinases driving cells through the cell cycle. To be more precise: ATR-CHK1, which become primarily activated in the O⁶MeG pathway [45], provoke upregulation of WEE1 and downregulation of CDC25c, causing inhibition of CDK1 and CDK2 that usually activates FOXM1 and B-MYB causing a G2 arrest. At the same time, ATM-CHK2 and ATR-CHK1 activate p53 through phosphorylation of ser15 and ser20, giving rise to transcriptional activation of the CDKN1A gene and thus p21 overproduction. p21 is a CDK inhibitor blocking CDK1, CDK2, CDK4 and cyclin E through physical interaction, thus inhibiting cells in the G2 phase (Fig. 2).

An important target of p21 is the multi-repressor complex DREAM [46]. The role of DREAM in induction and maintenance of CSEN in glioblastoma cells following TMZ has been studied recently [14]. It was shown that TMZ-induced senescence does not require activation of the DREAM complex, but is bound on a G2-specific response. Thus, we showed that p21 upregulated via the ATR/ATM-p53 axis does not directly interact with CDK4, but as outlined above, with CDK1- and CDK2-cyclin E, causing abrogation of the B-Myb and FOXM1-signaling pathway, which leads to arrest of cells in the G2-phase. Interestingly,

the induced G2-arrest was incomplete, presumably because of incomplete gene repression due to lack of DREAM. As a consequence, DNA synthesis in senescent cells can be resumed leading to endoreduplications. This process is preceded by reactivation of the G1/S-specific E2F1-signaling pathway, which is facilitated due to lack of functional DREAM. Incomplete DREAM activation may also explain the finding that senescent glioblastoma cells are able to repair DSBs [38] and do not show general downregulation of DNA repair, as reported for other cell systems [47–49]. The finding that CDK4 is not blocked and E2F1 still active may be taken as explanation for the incomplete G2 arrest, explaining endoreduplications, the giant cell morphology and the increase of DNA content, which is a hallmark of TMZ-induced senescent glioma cells [14].

Interestingly, the process of endoreduplication in senescent cells was subject to inhibition by the CDK4-blocking drug palbociclib, thereby stabilizing cells in the senescent stage [14]. This might be harnessed in therapy as endoreduplications and presumably also reactivation of senescent cells is prevented.

The incomplete activation of DREAM in glioblastoma cells following TMZ is very likely due to lack of CDKN2A/p16^{INK4A}, which acts as specific inhibitor of CDK4/6-cyclin D [50]. Thus, up to 50% of the glioblastoma cell lines show a deletion in CDKN2a, coding for p16^{INK4A} and p14^{ARF} [51] and, according to the PanCancer atlas, 56% of glioblastoma display homozygous deletion of this gene (https://www.cbioportal.org/study/summary?id=gbm_tcga_pan_can_atlas_2018), which is the most frequently deleted gene in glioblastoma. On opposite, CDKN1A shows genomic alterations in only less than 1% of glioblastoma. Therefore, CDKN1A/p21 appears to play a key role in drug-induced senescence in glioblastoma. This is supported by the finding that p16^{INK4a} is not involved in topoisomerase I inhibitor irinotecan-induced senescence in glioblastoma cells [14].

Can Senescent Cells Escape and Become Reactivated for proliferation?

Senescent cells exhibit a specific gene expression profile and metabolic condition and are irreversibly blocked in the cell cycle. However, there is increasing evidence that there are exceptions to the rule and that therapy-induced senescent cells (TIS) can acquire the ability to be reactivated for proliferation. Thus, reactivation of tumor cells from a senescent stage induced by etoposide and dexamethasone has been demonstrated. This was associated with weakening of the SASP, an increase in the polyploidy level and aggressive tumor growth [52]. Reactivation from the dormant stage, in which the cells can remain for a long time (months,

years), is a rare event; in a lung carcinoma model, this was determined to be 1 in 10⁶ cells [53]. Although this low rate corresponds to gene mutation frequency, it is conceivable that clonal tumor regrowth can occur. If release from the dormant stage occurs months or years after radiation or chemotherapy (or biologicals inhibiting proliferation and causing CSEN), this would inevitably lead to late recurrence. In principle, one reactivated cell in the remaining senescent population would be sufficient to clonally form a tumor again. The reactivation of senescent cells for proliferation is a plausible explanation for recurrences, which can appear even years after treatment.

The finding that TMZ is an extremely efficient inducer of senescence in glioblastoma cells makes it very likely that this scenario applies to glioblastomas. Thus, it is reasonable to suppose that the induction of senescence occurs already after the first radiochemotherapy cycles, leading to a highly resistant tumor cell subpopulation (of note, TMZ needs replication and senescent cells are inherently resistant due to blocked apoptotic pathways). Furthermore, these cells likely exhibit the SASP [54], which is a hallmark of senescent glioblastoma cells after TMZ treatment [55]. Therapy-induced senescent cells secrete proinflammatory cytokines, can promote angiogenesis and modulate the immune system and thus can evade immune surveillance [56, 57]. They therefore worsen the clinical outcome. Overall, there is accumulating evidence that therapy-induced senescence plays a role in glioma treatment, recurrence and cancer progression [55, 58].

Given that the proinflammatory properties of senescent cells are a driving force in tumor progression and senescent cells can be reactivated for proliferation, three strategies are conceivable going along with genotoxic therapy: (a) prevention of induction of senescence, (b) suppression of SASP and (c) eradication of therapy-induced senescent cells. Combining senotherapeutics with radiation and chemotherapy is anticipated to enhance the effectiveness of genotoxic cancer therapies.

Senopreventics

This strategy aims at inhibiting the induction of senescence, driving cells into the apoptotic pathway. Since senescence induction occurs *via* activation of the DDR, classical inhibitors of ATM, ATR, CHK1 and CHK2 would fit this criterion. However, these drugs are not specific, since they do not only block senescence induction, but also efficiently kill both tumor and normal cells and therefore have significant side-effects. An alternative might be a specific p21 or p16 (for p16 non-mutated tumors) inhibitors, which would force damaged cells into the cell cycle and thereby induce

cell death. However specific p21 inhibitors or PROTACs that are clinically used are not available. In our previous experiments we observed that fisetin, a natural plant flavonoid, given together with TMZ enhanced the level of apoptosis and reduced senescence [59]. Similar findings were observed with artesunate, a TCM drug extracted from *Artemisia annua* L., which ameliorated the cytotoxicity of TMZ and reduced the CSEN level [60]. Thus, the natural compounds fisetin and artesunate might be considered senopreventive agents. A recent study showed that astemizole, a hERG/Eag1 K⁺ channel blocker, administered together with TMZ ameliorated apoptosis and reduced the yield of CSEN [61], thus being effective as senopreventives.

Senolytics

These drugs (compiled in Table 1) are defined being agents that specifically induce death of senescent, but not proliferating or resting non-senescent (differentiated) cells. The discovery of senolytic drugs was based on the observation that senescent cells are resistant to apoptosis, due to upregulation of specific senescent-cell anti-apoptotic pathways (SCAP) caused by senescence-associated mitochondrial dysfunction (SAMD) [64, 65]. Although it's early days and evidence is limited, senolytics targeting these SCAP have been tested in several clinical trials on aging and geriatric syndroms [66]. Among these, especially inhibitors of the antiapoptotic members of the BCL-2 family (BCL-2, BCL-W and

BCL-X_L) induce mitochondrial-mediated apoptosis in senescent cells. Thus, ABT-737 and ABT-263 (navitoclax) act predominantly by inhibiting BCL-2, and A-1331852 and A-1155463 by inhibiting BCL-W and BCL-X_L. In vitro, navitoclax was shown to be senolytic in HUVECs, IMR90 human lung fibroblasts and murine embryonic fibroblasts, but not human primary preadipocytes [67]. The compounds A1331852 and A1155463 proved to be senolytic also in HUVECs and IMR90 cells, but not in preadipocytes [68]. This data shows the existence of cell type specificities. In patient-derived GBM lines triggered into senescence by radiation and TMZ, navitoclax, A1331852 and A1155463 showed senolytic activity [63]. In vivo, treatment with A1331852 eliminated senescent cholangiocytes and thereby reduced liver fibrosis in mice [69].

Previously, we demonstrated that targeting either c-IAP1 and c-IAP2 using BV6, or Bcl-2 using venetoclax can eliminate senescent cells following TMZ [54]. BV6 was also effective in killing senescent GBM cells triggered by the anticancer drug irinotecan [70].

ABT-737 was originally shown in vivo to eliminate senescent cells induced by DNA damage in the lung as well as through activation of p53 in a transgenic p14^{ARF} mouse model [71]. Oral administration of ABT-263 to either sublethal irradiated or normally aged mice depleted senescent bone marrow hematopoietic stem cells and senescent muscle stem cells and thus rejuvenated aged mice [72]. Also, proteasomal degradation of BCL-2 provoked by the curcumin analog EF24 was shown to mediate senolytic effects [73] and the senolytic activity of cardiac glycosides like ouabain is, at least partially, caused by interfering with SCAP, activating the proapoptotic BCL-2 family member NOXA [74].

In glioblastoma cells some of the compounds were tested. In our hands, ABT-737 induced a robust and reliable senolytic response. However, the compound is not yet in the clinic, while ABT-263 (navitoclax), which was effective in eliminating senescent GBM cells [62], is clinically approved and being used for the treatment of some forms of leukemia, myelofibrosis and some solid cancers. The derivative venetoclax is more selective in inhibiting Bcl-2, does not show side effects like thrombocytopenia and thus is going to replace navitoclax [75]. Venetoclax was not yet tested on gliomas.

Some senolytics act by interfering with cell death-related signaling cascades involving tyrosine kinases, or the target of rapamycin (mTOR) pathway. This seem to be very effective strategies. Thus, the multi-kinase inhibitor dasatinib was shown to be senolytic in different cell systems, and the combination with quercetin showed superior senolytic activity both in vitro and in vivo, eliminating senescent cells in chronologically aged, radiation-exposed, and progeroid *Erccl^{-Δ}* mice. In old mice, this senolytic effect improved

Table 1 (A) senolytic drugs positively tested in TMZ-induced senescent GBM cell lines [62]. (B) senolytic drugs positively tested in radiation- and TMZ-induced senescent GBM cell lines [63]. (C) senolytics not yet tested in GBM cells (see text)

Senolytic agent Mechanism	
A	
ABT-737	Bcl-2/Bcl-xL/Bcl-w inhibitor
ABT-263 (Navitoclax)	Bcl-2/Bcl-xL inhibitor
Chloroquine	Autophagy inhibitor
PX-866	PI3K/autophagy inhibitor
BV-6	c-IAP/XIAP inhibitor
AZD1390	DNA damage response (ATM) inhibitor
VE-821	DNA damage response (ATR) inhibitor
Fisetin	Flavonoid; BCL-xL pathway modulation
Artesunate	Generates ROS; anti-malarial derivative
Curcumin	Anti-oxidative/apoptosis modulator
B	
A1331852	Bcl-xL inhibitor
A1155463	Bcl-xL inhibitor
ABT-263 (Navitoclax)	Bcl-2/Bcl-xL inhibitor
C	
Piperlongumine	Unknown
Gingerone A	Unknown

the cardiac function and delayed age-related symptoms like osteoporosis [76].

Using dasatinib and quercetin, a direct involvement of senescent cells in aging related disease and the effectiveness of their elimination for health was shown *in vivo*. Transplanting senescent cells into young mice induced senescence in the host tissue and led to persistent physical dysfunction. Transplanting senescent cells in old mice showed similar effects and reduced the lifespan of the animals. Under these conditions, combined treatment with dasatinib and quercetin eliminated senescent cells, reduced the physical dysfunction and increased the overall survival [77]. Also, in clinical phase I studies performed in patients with diabetic kidney disease [78] and idiopathic pulmonary disease [79], the combination of dasatinib and quercetin reduced the amount of p16^{INK4a}/SA- β -gal positive cells. The combination of dasatinib and quercetin has also been shown to be senolytic in senescent lung fibroblasts during idiopathic pulmonary fibrosis in mice [80], as well as in the medial layer of aorta from aged and hypercholesterolemic mice, which improved vasomotor function and blood flow in the animals [81]. Taken together, the combination of dasatinib and quercetin appears to be the most effective strategy in senolytic therapy. As dasatinib is well established as to therapeutic dose and side effects, its use as a repurposed drug should facilitate clinical trials with glioma patients. Of note, senolytic therapy means short-interval treatment, for which unwished side effects are negligible.

Of special interest are natural compounds with senolytic activity (Table 1). Quercetin and fisetin belong to this group. They are natural flavonoids present in fruits and vegetables. Quercetin was shown to be cytotoxic for GBM cells by inhibiting the AXL/IL-6/STAT3 signaling pathway without affecting Akt or MAPK [14, 48]. Quercetin notably in combination with resveratrol is pro-apoptotic and induces a senescence-like growth arrest in glioma cells [82]. In human lung fibroblasts treated with doxorubicin quercetin was effective in triggering senescent cell death [83]. Although the cytotoxic and senolytic activity of quercetin is well described, robust studies on the senolytic activity of the flavonoid given alone or in combination with dasatinib to GBM cells are not yet available.

Fisetin was shown to be senolytic in senescent human vascular endothelial cells (HUVECs), but not in human lung fibroblasts and primary human preadipocytes [68], indicating cell specificity. *In vivo*, fisetin was reported to exert marked effects. Thus, treatment of progeroid mice with fisetin reduced senescence markers in multiple tissues, reduced age-related pathology, and extended the median and maximum lifespan [84]. In our studies with TMZ-induced GBM cells, fisetin was clearly effective in inducing apoptosis in senescent cells, confirming its senolytic activity [62].

Interestingly, fisetin at high dose level (40–80 μ M) exerts genotoxic activity, inducing DNA damage (DSBs) and p53 activation. Furthermore it enhanced the cytotoxic effects of alkylating agents [59]. Therefore, the compound might be considered a reasonable supplement in GBM therapy.

Another senolytic compound is piperlongumine, a natural ingredient of the long pepper (*Piper longum*), which has been shown to eliminate human fibroblasts upon senescence-induction by radiation, replicative exhaustion, or ectopic expression of Ras [85]. The mechanism underlying the senolytic activity is not yet clear.

A screening study with several plant extracts and IR-induced human fibroblasts revealed ginger extract causing selective CSEN death. The active agent was identified gingerone A, which is able to elicit an apoptotic program in senescent cells [86].

Finally, our own screening study on TMZ-induced senescent GBM cells performed under standardized conditions should be summarized. It revealed that ABT-737, navitoclax, chloroquine, ATMi, ATRi, BV-6, PX-866 and the natural compounds fisetin and artesunate exhibit senolytic activity, inducing death in senescent GBM cells clearly more effectively than in the proliferating cell population [62]. Similar to fisetin, artesunate exhibited genotoxic activity, inducing oxidative DNA damage and DSBs [87, 88]. The drug seems to exert pleiotropic effects as it inhibits HR and ameliorates the therapeutic effect of TMZ upon coadministration [60]. The cytotoxicity on CSEN cells might be speculated to be due to excessive mitochondrial damage through sustained ROS formation.

In the study referred to above, no specific effect on CSEN was observed by inhibition of CHK1/CHK2, p21, NF- κ B, Rad51 and PARP. We concluded that these factors neither play a critical role in maintaining TMZ-induced CSEN nor can their inhibitors be considered as senolytics [62]. It should be noted that IR and CCNU, which are usually applied alone or in combination with TMZ or procarbazine (PCV scheme) were ineffective in killing senescent GBM cells [62].

There are other senolytic mechanisms reported. Thus, the histone deacetylases (HDAC) inhibitor panobinostat has been described as senolytic, which eliminates senescent cells that were accumulating during standard chemotherapy in lung cancer patients [89]. The FOXO4 peptide (proxofim), which perturbs the FOXO4/p53 interaction, induced apoptosis in senescent cells and restored fitness, fur density, and renal function in premature aging *Xpd*^{TTD/TTD} and naturally aged mice [90]. The compounds have not yet been tested on GBM model systems or in clinical settings.

Besides inhibitors of targets involved in the maintenance of CSEN, other senolytic strategies are under development. Thus, the increased SA- β -gal activity of senescent cells was

harnessed to deliver cytotoxic agents or senolytic drugs coated with galacto-oligosaccharide nanoparticles into lysosomes of senescent cells [91–93]. Another strategy is based on the finding that senescent cells are subjected to immunosurveillance [94, 95]. Based on the assumption that senescent cells might accumulate during aging due to declined immune response, it is currently tested whether restoration or activation of the immune system could specifically eliminate senescent cells (for review see [96]).

Senomorphics/senostatics

Senomorphics and senostatics are compounds that suppress the senescence-associated secretory phenotype (SASP) without causing death of senescent cells. The terms are often used synonymously. However, it would be prudent to reserve the term “senostatics” for treatments that aim to stabilize the senescent state and prevent the escape of senescent cells. An example is the CDK4 inhibitor palbociclib, which we have shown to stabilize TMZ-induced senescent cells in G2 [14].

The SASP is characterized by secretion of multiple immune factors, including interleukins, chemokines, growth factors and matrix metalloproteinases [56, 97]. Important SASP factors are the interleukins IL-6 and IL-8 as they seem to modulate CSEN [98]. Thus, depletion of IL-6 abolished oncogene-induced senescence [97] and IL-8 increased ROS production and DNA damage [99]. Beside IL-6 and IL-8, IL-1 α has been proposed as a general autocrine regulator of SASP [100]. The SASP can play both a tumor-promoting and a tumor-suppressing role [101]. As tumor-suppressing mechanism, SASP can reinforce the growth arrest by increasing ROS production and enhancing DDR, thus stabilizing the CSEN phenotype [97, 99]. In addition, SASP induces an inflammatory response and activates immune cells which can eliminate senescent tumor cells [102, 103]. On the other hand, SASP factors also act as potent tumor promoters, driving tumorigenesis. As an example, SASP can enhance the proliferation of neoplastic epithelial cells [104], and promotes EMT [105] as well as tumor growth in vivo [106, 107]. Of note, SASP factors do not only promote cancer, but also trigger multiple chronic and degenerative aging-related pathologies like neurodegenerative diseases and diabetes [98, 108].

Having said this, it follows that inhibitors of factors involved in SASP are useful for reducing the deleterious effects of senescent cells. Senomorphic activity was shown for several agents like the IL-1R antagonist anakinra, the IL1 antagonizing antibodies canakinumab and rilonacept, the THF antagonizing compounds etanercept and infliximab, as well as the IL-6R antagonizing antibody tocilizumab and

the IL-6 antagonizing antibody siltuximab (for further reading see [109]).

An important factor involved in the regulation of the SASP is NF- κ B. Thus, several compounds, which interfere with NF- κ B signaling (metformin, apigenin, kaempferol and BAY 11–7082) possess senomorphic activity [109]. The NF- κ B pathway shows crosstalk with p38K since the p38K inhibitor SB203580 reduced NF- κ B transcriptional activity and subsequently the transcription and secretion of IL-6 and IL8 in normal human fibroblasts, which underwent senescence following IR or oncogenic H-RAS^{V12} expression [110].

An important regulator of cytokine production is the JAK/STAT (Janus kinase/signal transducer and activator of transcription) pathway. In line with this, the JAK inhibitor ruxolitinib suppressed the mRNA levels of the SASP components IL-6, IL-8, and MCP-1 in human primary IR-induced senescent cells [111].

Already more than 10 years ago, it was shown that rapamycin, an inhibitor of the mTOR pathway, was able to delay several ageing-related dysfunctions and increase the lifespan of mice [112]. Newer studies showed that rapamycin significantly decreased IL6 secretion in normal human fibroblasts and immortal, but non-tumorigenic human breast epithelial cell lines, in which senescence was induced by ionizing radiation and other stimuli [113]. However, it did not reverse cellular senescence, but repressed the ability of senescent cells to stimulate cell proliferation and tumorigenesis in mice. Mechanistically, rapamycin suppresses the translation of the membrane-bound cytokine IL-1A, which normally stimulates NF- κ B transcriptional activity [113]. Delivery of rapamycin to senescent cells via CD9 monoclonal antibody-conjugated lactose-wrapped calcium carbonate nanoparticles induced anti-senescence effects (reduced β -galactosidase and reduced p53/p21/CD9/cyclin D1 expression) in old human dermal fibroblasts [114].

Although we know much about SASP and senomorphics, our knowledge regarding translation to brain cancers is very limited. These gaps in knowledge justify intensive research in this area addressing several questions: Do gliomas exhibit SASP in vivo? Which cytokines are released. Are there differences in CSEN level and SASP in tumor grading? Is SASP related in any way to MGMT, MMR and IDH1/2 status? Does the administration of senomorphics improve treatment and impacts patient's survival?

Conclusions and Summary

The signaling pathways triggered by TMZ and other methylating agents in GBM cells are well described. Although they provoke cell death through apoptosis, induction of

CSEN is clearly the major trait. CSEN cells are inherently resistant to genotoxic therapies. Therefore, complete elimination of the tumor through radiation and chemotherapy is strictly impossible.

Therapy-induced senescent GBM cells are arrested in G2 with incomplete DNA replication inhibition, resulting in endoreduplications. Whether this facilitates release of senescent cells into proliferating state needs to be clarified. It is clear, however, that senescent cells cannot only be reactivated, but also drive tumor progress through SASP.

In view of this, it is appropriate to include into the genotoxic therapy with radiation, TMZ, procarbazine and CCNU supportive treatments with senopreventics, senolytics and senomorphics administered either concomitantly (senopreventics), in a hit-and-run fashion (senolytics) or during maintenance therapy (senomorphics).

Senolytics active in GBM cells include natural substances such as fisetin, quercetin and artemisinin (artesanate). The natural compounds deserve special attention. Fisetin is a flavonoid and polyphenol found in smoke tree (fiset wood), various fruits (apples, strawberries, grapes, persimmons) and vegetables (cucumbers, onions). Like artesunate, it is genotoxic in glioblastoma cells at high doses. Fisetin has been shown to be an effective senolytic agent in various tumor cell systems [57] and in mice, oral administration of fisetin led to an extension of lifespan, most likely by selectively killing aged cells [58]. Fisetin has therefore gained popularity as a dietary supplement and senolytic agent in healthy individuals.

Overall, natural senotherapeutics are well tolerated and gained popularity as dietary supplements taken by a wide range of people. It is possible that these natural substances, together with the anticancer effect of curcumin, are effective in adjuvant therapy. Chloroquine has also been shown to exert a senolytic effect on glioblastoma cells. Case reports allow the conclusion that these natural senolytics have no adverse side effects (unpublished observations and [115]). Clinical studies will have to provide information on their effectiveness. Importantly, a first clinical phase I trial, which started in July 2025, will test the safety, side effects and effectiveness of combinations treatment of glioma residual disease using dasatinib, quercetin, fisetin and TMZ (NCT07025226, <https://clinicaltrials.gov/study/NCT07025226>).

In addition to natural substances, well-known pharmaceuticals are also coming into focus, such as dasatinib, simvastatin and metformin. Simvastatin has been shown to enhance TMZ-induced apoptosis by inhibiting autophagy [59] and, together with metformin, inhibits glioblastoma growth through a senolytic effect, which has been shown in vitro, in animal models and in initial clinical studies [60].

Key References

- Kandhaya-Pillai, R.; Miro-Mur, F.; Alijotas-Reig, J.; Tchkonja, T.; Schwartz, S.; Kirkland, J. L.; Oshima, J., Key elements of cellular senescence involve transcriptional repression of mitotic and DNA repair genes through the p53-p16/RB-E2F-DREAM complex. *Aging* 2023, 15, (10), 4012–4034.
 - This paper of importance provides evidence that the DREAM complex is a general repressor in senescent cells, downregulating also DNA repair genes.
- Di Micco, R.; Krizhanovsky, V.; Baker, D.; d'Adda di Fagagna, F., Cellular senescence in ageing: from mechanisms to therapeutic opportunities. *Nature reviews. Molecular cell biology* 2021, 22, (2), 75–95.
 - This review is a comprehensive review of importance on senescence.
- Bujarrabal-Dueso, A.; Sendtner, G.; Meyer, D. H.; Chatzinikolaou, G.; Stratigi, K.; Garinis, G. A.; Schumacher, B., The DREAM complex functions as conserved master regulator of somatic DNA-repair capacities. *Nat Struct Mol Biol* 2023, 30, (4), 475–488.
 - This is a highly important paper describing the role of DREAM as master regulator of DNA repair in senescent cells.
- Schmidt, A.; Allmann, S.; Schwarzenbach, C.; Snyder, P.; Chen, J. X.; Nagel, G.; Schoneis, A.; Rasenberger, B.; Beli, P.; Loewer, A.; Hofmann, T. G.; Tomicic, M. T.; Christmann, M., The p21CIP1-CDK4-DREAM axis is a master regulator of genotoxic stress-induced cellular senescence. *Nucleic Acids Res* 2024, 52, (12), 6945–6963.
 - This paper describes the p21-CDK4-DREAM axis to be activated after genotoxic stress induced by environmental carcinogens like benzo(a)pyrene.
- Schwarzenbach, C.; Rinke, J.; Vilar, J. B.; Sallbach, J.; Tatsch, L.; Schmidt, A.; Schoneis, A.; Rasenberger, B.; Kaina, B.; Tomicic, M. T.; Christmann, M., Therapy-induced senescence of glioblastoma cells is determined by the p21(CIP1)-CDK1/2 axis and does not require activation of DREAM. *Cell Death Dis* 2025, 16, (1), 357.
 - This paper of importance shows that in glioblastoma cells defective in p16 the p21-CDK1,2 axis regulates

senescence and DREAM without the activation of functional DREAM.

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Declarations

Competing Interests The authors declare no competing interests.

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References

- Kaina B. Temozolomide, procarbazine and nitrosoureas in the therapy of malignant gliomas: update of mechanisms, drug resistance and therapeutic implications. *J Clin Med*. 2023. <https://doi.org/10.3390/jcm12237442>.
- Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, Ludwin SK, Allgeier A, Fisher B, Belanger K, Hau P, Brandes AA, Gijtenbeek J, Marosi C, Vecht CJ, Mokhtari K, Wesseling P, Villa S, Eisenhauer E, Gorlia T, Weller M, Lacombe D, Cairncross JG, Mirimanoff RO, European Organisation for R; Treatment of Cancer Brain, Radiation T, Oncology G, National Cancer Institute of Canada Clinical Trials., G., Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol* 2009, 10, (5), 459–66.
- Weller M, van den Bent M, Preusser M, Le Rhun E, Tonn JC, Minniti G, Bendszus M, Balana C, Chinot O, Dirven L, French P, Hegi ME, Jakola AS, Platten M, Roth P, Ruda R, Short S, Smits M, Taphoorn MJB, von Deimling A, Westphal M, Soffietti R, Reifenberger G, Wick W. EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. *Nat Rev Clin Oncol*. 2021;18(3):170–86.
- Danson SJ, Middleton MR. Temozolomide: a novel oral alkylating agent. *Expert Rev Anticancer Ther*. 2001;1(1):13–9.
- Knizhnik AV, Roos WP, Nikolova T, Quiros S, Tomaszowski KH, Christmann M, et al. Survival and death strategies in glioma cells: autophagy, senescence and apoptosis triggered by a single type of temozolomide-induced DNA damage. *PLoS One*. 2013;8(1):e55665.
- Strik HM, Marosi C, Kaina B, Neyns B. Temozolomide dosing regimens for glioma patients. *Curr Neurol Neurosci Rep*. 2012;12(3):286–93.
- Beltzig L, Stratenwerth B, Kaina B. Accumulation of Temozolomide-Induced Apoptosis, senescence and DNA damage by metronomic dose schedule: A Proof-of-Principle study with glioblastoma cells. *Cancers*. 2021;13:24.
- He Y, Roos WP, Wu Q, Hofmann TG, Kaina B. The SIAH1-HIPK2-p53ser46 damage response pathway is involved in Temozolomide-induced glioblastoma cell death. *Mol Cancer Res*. 2019;17(5):1129–41.
- Christmann M, Kaina B. Transcriptional regulation of human DNA repair genes following genotoxic stress: trigger mechanisms, inducible responses and genotoxic adaptation. *Nucleic Acids Res*. 2013;41(18):8403–20.
- Christmann M, Boisseau C, Kitzinger R, Berac C, Allmann S, Sommer T, Aasland D, Kaina B, Tomicic MT. Adaptive upregulation of DNA repair genes following benzo(a)pyrene diol epoxide protects against cell death at the expense of mutations. *Nucleic Acids Res*. 2016;44(22):10727–43.
- Matt S, Hofmann TG. The DNA damage-induced cell death response: a roadmap to kill cancer cells. *Cell Mol Life Sci*. 2016;73(15):2829–50.
- Roos WP, Thomas AD, Kaina B. DNA damage and the balance between survival and death in cancer biology. *Nat Rev Cancer*. 2016;16(1):20–33.
- Tomicic MT, Meise R, Aasland D, Berte N, Kitzinger R, Kramer OH, Kaina B, Christmann M. Apoptosis induced by Temozolomide and nimustine in glioblastoma cells is supported by JNK/c-Jun-mediated induction of the BH3-only protein BIM. *Oncotarget*. 2015;6(32):33755–68.
- Schwarzenbach C, Rinke J, Vilar JB, Sallbach J, Tatsch L, Schmidt A, et al. Therapy-induced senescence of glioblastoma cells is determined by the p21(CIP1)-CDK1/2 axis and does not require activation of DREAM. *Cell Death Dis*. 2025;16(1):357.
- Chien Y, Scuoppo C, Wang X, Fang X, Balgley B, Bolden JE, Premisrur P, Luo W, Chicas A, Lee CS, Kogan SC, Lowe SW. Control of the senescence-associated secretory phenotype by NF-kappaB promotes senescence and enhances chemosensitivity. *Genes Dev*. 2011;25(20):2125–36.
- Aasland D, Gotzinger L, Hauck L, Berte N, Meyer J, Effenberger M, Schneider S, Reuber EE, Roos WP, Tomicic MT, Kaina B, Christmann M. Temozolomide induces senescence and repression of DNA repair pathways in glioblastoma cells via activation of ATR-ChK1, p21, and NF-kappaB. *Cancer Res*. 2019;79(1):99–113.
- Buccarelli M, Marconi M, Pacioni S, De Pascalis I, D'Alessandris QG, Martini M, Ascione B, Malorni W, Larocca LM, Pallini R, Ricci-Vitiani L, Matarrese P. Inhibition of autophagy increases susceptibility of glioblastoma stem cells to Temozolomide by igniting ferroptosis. *Cell Death Dis*. 2018;9(8):841.
- de Souza I, Monteiro LKS, Guedes CB, Silva MM, Andrade-Tomaz M, Contieri B, Latancia MT, Mendes D, Porchia B, Lazarini M, Gomes LR, Rocha CRR. High levels of NRF2 sensitize temozolomide-resistant glioblastoma cells to ferroptosis via ABCC1/MRP1 upregulation. *Cell Death Dis*. 2022;13(7):591.
- Kaina B, Christmann M, Naumann S, Roos WP. MGMT: key node in the battle against genotoxicity, carcinogenicity and apoptosis induced by alkylating agents. *DNA Repair Amst*. 2007;6(8):1079–99.

20. Kaina B, Fritz G, Mitra S, Coquerelle T. Transfection and expression of human O⁶-methylguanine-DNA methyltransferase (MGMT) cDNA in Chinese hamster cells: the role of MGMT in protection against the genotoxic effects of alkylating agents. *Carcinogenesis*. 1991;12:1857–67.
21. Stratenwerth B, Geisen SM, He Y, Beltzig L, Sturla SJ, Kaina B. Molecular dosimetry of temozolomide: quantification of critical lesions, correlation to cell death responses, and threshold doses. *Mol Cancer Ther*. 2021;20(10):1789–99.
22. Agnihotri S, Gajadhar AS, Ternamian C, Gorlia T, Diefes KL, Mischel PS, Kelly J, McGown G, Thorncroft M, Carlson BL, Sarkaria JN, Margison GP, Aldape K, Hawkins C, Hegi M, Guha A. Alkylpurine-DNA-N-glycosylase confers resistance to Temozolomide in xenograft models of glioblastoma multiforme and is associated with poor survival in patients. *J Clin Invest*. 2012;122(1):253–66.
23. Christmann M, Nagel G, Horn S, Krahn U, Wiewrodt D, Sommer C, Kaina B. MGMT activity, promoter methylation and immunohistochemistry of pretreatment and recurrent malignant gliomas: a comparative study on Astrocytoma and glioblastoma. *Int J Cancer*. 2010;127(9):2106–18.
24. Weller M, Stupp R, Reifenberger G, Brandes AA, van den Bent MJ, Wick W, Hegi ME. MGMT promoter methylation in malignant gliomas: ready for personalized medicine? *Nat Rev Neurol*. 2010;6(1):39–51.
25. Christmann M, Verbeek B, Roos WP, Kaina B. O(6)-Methylguanine-DNA methyltransferase (MGMT) in normal tissues and tumors: enzyme activity, promoter methylation and immunohistochemistry. *Biochim Biophys Acta*. 2011. <https://doi.org/10.1016/j.bbcan.2011.06.002>.
26. Margison GP, Povey AC, Kaina B, Santibanez Koref MF. Variability and regulation of O⁶-alkylguanine-DNA alkyltransferase. *Carcinogenesis*. 2003;24(4):625–35.
27. Christmann M, Kaina B. Epigenetic regulation of DNA repair genes and implications for tumor therapy. *Mutat Res*. 2019;780:15–28.
28. Dosch J, Christmann M, Kaina B. Mismatch G-T binding activity and MSH2 expression is quantitatively related to sensitivity of cells to methylating agents. *Carcinogenesis*. 1998;4:567–73.
29. Hickman MJ, Samson LD. Role of DNA mismatch repair and p53 in signaling induction of apoptosis by alkylating agents. *Proc Natl Acad Sci U S A*. 1999;96(19):10764–9.
30. McFaline-Figueroa JL, Braun CJ, Stanciu M, Nagel ZD, Mazzucato P, Sangaraju D, Cerniauskas E, Barford K, Vargas A, Chen Y, Tretyakova N, Lees JA, Hemann MT, White FM, Samson LD. Minor changes in expression of the mismatch repair protein MSH2 exert a major impact on glioblastoma response to Temozolomide. *Cancer Res*. 2015;75(15):3127–38.
31. Roos WP, Nikolova T, Quiros S, Naumann SC, Kiedron O, Zdzienicka MZ, Kaina B. Brca2/Xrcc2 dependent HR, but not NHEJ, is required for protection against O(6)-methylguanine triggered apoptosis, DSBs and chromosomal aberrations by a process leading to SCEs. *DNA Repair (Amst)*. 2009;8(1):72–86.
32. Quiros S, Roos WP, Kaina B. Rad51 and BRCA2—new molecular targets for sensitizing glioma cells to alkylating anticancer drugs. *PLoS One*. 2011;6(11):e27183.
33. Roos WP, Frohnapfel L, Quiros S, Ringel F, Kaina B. XRCC3 contributes to temozolomide resistance of glioblastoma cells by promoting DNA double-strand break repair. *Cancer Lett*. 2018;424:119–26.
34. Roos WP, Batista LFZ, Naumann S, Wick W, Weller M, Menck CFM, et al. Apoptosis in malignant glioma cells triggered by the temozolomide-induced DNA lesion O⁶-methylguanine. *Oncogene*. 2007;26:186–97.
35. Kaina B, Ziouta A, Ochs K, Coquerelle T. Chromosomal instability, reproductive cell death and apoptosis induced by O⁶-methylguanine in Mex⁻, Mex⁺ and methylation-tolerant mismatch repair compromised cells: facts and models. *Mutat Res*. 1997;381:227–41.
36. Gunther W, Pawlak E, Damasceno R, Arnold H, Terzis AJ. Temozolomide induces apoptosis and senescence in glioma cells cultured as multicellular spheroids. *Br J Cancer*. 2003;88(3):463–9.
37. Hirose Y, Berger MS, Pieper RO. p53 effects both the duration of G2/M arrest and the fate of temozolomide-treated human glioblastoma cells. *Cancer Res*. 2001;61(5):1957–63.
38. Beltzig L, Schwarzenbach C, Leukel P, Frauenknecht KBM, Sommer C, Tancredi A, et al. Senescence is the main trait induced by temozolomide in glioblastoma cells. *Cancers*. 2022. <https://doi.org/10.3390/cancers14092233>.
39. Hubackova S, Krejčíková K, Bartek J, Hodny Z. IL1- and TGFβ₂-Nox4 signaling, oxidative stress and DNA damage response are shared features of replicative, oncogene-induced, and drug-induced paracrine ‘bystander senescence’. *Aging*. 2012;4(12):932–51.
40. Storzynsky QT, Han X, Komant S, Agopsowicz KC, Potts KG, Gamper AM, et al. Radiation-induced cellular senescence reduces susceptibility of glioblastoma cells to oncolytic vaccinia virus. *Cancers*. 2023. <https://doi.org/10.3390/cancers15133341>.
41. Jinno-Oue A, Shimizu N, Hamada N, Wada S, Tanaka A, Shinagawa M, Ohtsuki T, Mori T, Saha MN, Hoque AS, Islam S, Kogure K, Funayama T, Kobayashi Y, Hoshino H. Irradiation with carbon ion beams induces apoptosis, autophagy, and cellular senescence in a human glioma-derived cell line. *Int J Radiat Oncol Biol Phys*. 2010;76(1):229–41.
42. Lee JJ, Kim BC, Park MJ, Lee YS, Kim YN, Lee BL, Lee JS. PTEN status switches cell fate between premature senescence and apoptosis in glioma exposed to ionizing radiation. *Cell Death Differ*. 2011;18(4):666–77.
43. Fishman H, Monin R, Dor-On E, Kinzel A, Haber A, Giladi M, Weinberg U, Palti Y. Tumor treating fields (TTFields) increase the effectiveness of Temozolomide and lomustine in glioblastoma cell lines. *J Neurooncol*. 2023;163(1):83–94.
44. Giladi M, Munster M, Schneiderman RS, Voloshin T, Porat Y, Blat R, Zielinska-Chomej K, Haag P, Bomzon Z, Kirson ED, Weinberg U, Viktorsson K, Lewensohn R, Palti Y. Tumor treating fields (TTFields) delay DNA damage repair following radiation treatment of glioma cells. *Radiat Oncol*. 2017;12(1):206.
45. Eich M, Roos WP, Nikolova T, Kaina B. Contribution of ATM and ATR to the resistance of glioblastoma and malignant melanoma cells to the methylating anticancer drug Temozolomide. *Mol Cancer Ther*. 2013;12(11):2529–40.
46. Schmidt A, Allmann S, Schwarzenbach C, Snyder P, Chen JX, Nagel G, Schoneis A, Rasenberger B, Beli P, Loewer A, Hofmann TG, Tomicic MT, Christmann M. The p21CIP1-CDK4-DREAM axis is a master regulator of genotoxic stress-induced cellular senescence. *Nucleic Acids Res*. 2024;52(12):6945–63.
47. Kandhaya-Pillai R, Miro-Mur F, Alijotas-Reig J, Tchkonja T, Schwartz S, Kirkland JL, Oshima J. Key elements of cellular senescence involve transcriptional repression of mitotic and DNA repair genes through the p53-p16/RB-E2F-DREAM complex. *Aging*. 2023;15(10):4012–34.
48. Bujarrabal-Dueso A, Sendtner G, Meyer DH, Chatzinikolaou G, Strati K, Garinis GA, Schumacher B. The DREAM complex functions as conserved master regulator of somatic DNA-repair capacities. *Nat Struct Mol Biol*. 2023;30(4):475–88.
49. Frey Y, Haj M, Ziv Y, Elkon R, Shiloh Y. Broad repression of DNA repair genes in senescent cells identified by integration of transcriptomic data. *Nucleic Acids Res*. 2025. <https://doi.org/10.1093/nar/gkae1257>.
50. Stein GH, Drullinger LF, Souillard A, Dulic V. Differential roles for cyclin-dependent kinase inhibitors p21 and p16 in the

- mechanisms of senescence and differentiation in human fibroblasts. *Mol Cell Biol*. 1999;19(3):2109–17.
51. Hartmann C, Kluwe L, Lucke M, Westphal M. The rate of homozygous CDKN2A/p16 deletions in glioma cell lines and in primary tumors. *Int J Oncol*. 1999;15(5):975–82.
 52. Saleh T, Tyutyunyk-Massey L, Murray GF, Alotaibi MR, Kawale AS, Elsayed Z, Henderson SC, Yakovlev V, Elmore LW, Toor A, Harada H, Reed J, Landry JW, Gewirtz DA. Tumor cell escape from therapy-induced senescence. *Biochem Pharmacol*. 2019;162:202–12.
 53. Roberson RS, Kussick SJ, Vallieres E, Chen SY, Wu DY. Escape from therapy-induced accelerated cellular senescence in p53-null lung cancer cells and in human lung cancers. *Cancer Res*. 2005;65(7):2795–803.
 54. Schwarzenbach C, Tatsch L, Brandstetter Vilar J, Rasenberger B, Beltzig L, Kaina B, et al. Targeting c-IAP1, c-IAP2, and Bcl-2 eliminates senescent glioblastoma cells following temozolomide treatment. *Cancers*. 2021. <https://doi.org/10.3390/cancers13143585>.
 55. Chojak R, Fares J, Petrosyan E, Lesniak MS. Cellular senescence in glioma. *J Neurooncol*. 2023;164(1):11–29.
 56. Coppe JP, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu Rev Pathol*. 2010;5:99–118.
 57. Demaria M, O'Leary MN, Chang J, Shao L, Liu S, Alimirah F, Koenig K, Le C, Mitin N, Deal AM, Alston S, Academia EC, Kilmarx S, Valdovinos A, Wang B, de Bruin A, Kennedy BK, Melov S, Zhou D, Sharpless NE, Muss H, Campisi J. Cellular senescence promotes adverse effects of chemotherapy and cancer relapse. *Cancer Discov*. 2017;7(2):165–76.
 58. Ordóñez-Rubiano EG, Combata A, Baldoncini M, Payan-Gomez C, Gomez-Amarillo DF, Hakim F, Camargo J, Zorro-Sepulveda V, Luzzi S, Zorro O, Parra-Medina R. Cellular senescence in diffuse gliomas: from physiopathology to possible treatments. *World Neurosurg*. 2024;191:138–48.
 59. Beltzig L, Christmann M, Dobreau M, Kaina B. Genotoxic and cytotoxic activity of fisetin on glioblastoma cells. *Anticancer Res*. 2024;44(3):901–10.
 60. Berte N, Lokan S, Eich M, Kim E, Kaina B. Artesunate enhances the therapeutic response of glioma cells to temozolomide by inhibition of homologous recombination and senescence. *Oncotarget*. 2016;7(41):67235–50.
 61. Haas B, Roth I, Sacker L, Wos-Maganga M, Beltzig L, Kaina B. Apoptotic and senolytic effects of hERG/Eag1 channel blockers in combination with temozolomide in human glioblastoma cells. *Naunyn Schmiedeberg's Arch Pharmacol*. 2025. <https://doi.org/10.1007/s00210-025-03955-w>.
 62. Beltzig L, Christmann M, Kaina B. Abrogation of cellular senescence induced by temozolomide in glioblastoma cells: search for senolytics. *Cells*. 2022. <https://doi.org/10.3390/cells11162588>.
 63. Rahman M, Olson I, Mansour M, Carlstrom LP, Sutiwisesak R, Saber R, et al. Selective vulnerability of senescent glioblastoma cells to BCL-XL inhibition. *Mol Cancer Res*. 2022;20(6):938–48.
 64. Kirkland JL, Tchkonja T, Zhu Y, Niedernhofer LJ, Robbins PD. The clinical potential of senolytic drugs. *J Am Geriatr Soc*. 2017;65(10):2297–301.
 65. Kirkland JL, Tchkonja T. Cellular senescence: a translational perspective. *EBioMedicine*. 2017;21:21–8.
 66. Zhang L, Pitcher LE, Prahalad V, Niedernhofer LJ, Robbins PD. Recent advances in the discovery of senolytics. *Mech Ageing Dev*. 2021;200:111587.
 67. Zhu Y, Tchkonja T, Fuhrmann-Stroissnigg H, Dai HM, Ling YY, Stout MB, Pirtskhalava T, Giorgadze N, Johnson KO, Giles CB, Wren JD, Niedernhofer LJ, Robbins PD, Kirkland JL. Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. *Aging Cell*. 2016;15(3):428–35.
 68. Zhu Y, Doornebal EJ, Pirtskhalava T, Giorgadze N, Wentworth M, Fuhrmann-Stroissnigg H, et al. New agents that target senescent cells: the flavone, fisetin, and the BCL-XL inhibitors, A1331852 and A1155463. *Aging*. 2017;9(3):955–63.
 69. Moncsek A, Al-Suraih MS, Trussoni CE, O'Hara SP, Splinter PL, Zuber C, et al. Targeting senescent cholangiocytes and activated fibroblasts with B-cell lymphoma-extra large inhibitors ameliorates fibrosis in multidrug resistance 2 gene knockout (Mdr2(-/-)) mice. *Hepatology*. 2018;67(1):247–59.
 70. Sallbach J, Woods M, Rasenberger B, Christmann M, Tomicic MT. The cell cycle inhibitor p21(CIP1) is essential for irinotecan-induced senescence and plays a decisive role in re-sensitization of temozolomide-resistant glioblastoma cells to irinotecan. *Biomed Pharmacother*. 2024;181:117634.
 71. Yosef R, Pilpel N, Tokarsky-Amiel R, Biran A, Ovadya Y, Cohen S, et al. Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL. *Nat Commun*. 2016;7:11190.
 72. Chang J, Wang Y, Shao L, Laberge RM, Demaria M, Campisi J, Janakiraman K, Sharpless NE, Ding S, Feng W, Luo Y, Wang X, Aykin-Burns N, Krager K, Ponnappan U, Hauer-Jensen M, Meng A, Zhou D. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. *Nat Med*. 2016;22(1):78–83.
 73. Li W, He Y, Zhang R, Zheng G, Zhou D. The curcumin analog EF24 is a novel senolytic agent. *Aging*. 2019;11(2):771–82.
 74. Triana-Martinez F, Picallos-Rabina P, Da Silva-Alvarez S, Pietrocola F, Llanos S, Rodilla V, et al. Identification and characterization of cardiac glycosides as senolytic compounds. *Nat Commun*. 2019;10(1):4731.
 75. Roberts AW, Huang D. Targeting BCL2 with BH3 mimetics: basic science and clinical application of venetoclax in chronic lymphocytic leukemia and related B cell malignancies. *Clin Pharmacol Ther*. 2017;101(1):89–98.
 76. Zhu Y, Tchkonja T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, Palmer AK, Ikeno Y, Hubbard GB, Lenburg M, O'Hara SP, LaRusso NF, Miller JD, Roos CM, Verzosa GC, LeBrasseur NK, Wren JD, Farr JN, Khosla S, Stout MB, McGowan SJ, Fuhrmann-Stroissnigg H, Gurkar AU, Zhao J, Colangelo D, Dorransoro A, Ling YY, Barghouty AS, Navarro DC, Sano T, Robbins PD, Niedernhofer LJ, Kirkland JL. The achilles' heel of senescent cells: from transcriptome to senolytic drugs. *Aging Cell*. 2015;14(4):644–58.
 77. Xu M, Pirtskhalava T, Farr JN, Weigand BM, Palmer AK, Weivoda MM, et al. Senolytics improve physical function and increase lifespan in old age. *Nat Med*. 2018;24(8):1246–56.
 78. Hickson LJ, Langhi Prata LGP, Bobart SA, Evans TK, Giorgadze N, Hashmi SK, Herrmann SM, Jensen MD, Jia Q, Jordan KL, Kellogg TA, Khosla S, Koerber DM, Lagnado AB, Lawson DK, LeBrasseur NK, Lerman LO, McDonald KM, McKenzie TJ, Passos JC, Pignolo RJ, Pirtskhalava T, Saadiq IM, Schaefer KK, Textor SF, Victorelli SG, Volkman TL, Xue A, Wentworth MA, Wissler Gerdes EO, Zhu Y, Tchkonja T, Kirkland JL. Senolytics decrease senescent cells in humans: preliminary report from a clinical trial of dasatinib plus Quercetin in individuals with diabetic kidney disease. *EBioMedicine*. 2019;47:446–56.
 79. Justice JN, Nambiar AM, Tchkonja T, LeBrasseur NK, Pascual R, Hashmi SK, Prata L, Masternak MM, Kritchevsky SB, Musi N, Kirkland JL. Senolytics in idiopathic pulmonary fibrosis: results from a first-in-human, open-label, pilot study. *EBioMedicine*. 2019;40:554–63.
 80. Schafer MJ, White TA, Iijima K, Haak AJ, Ligresti G, Atkinson EJ, et al. Cellular senescence mediates fibrotic pulmonary disease. *Nat Commun*. 2017;8:14532.
 81. Roos CM, Zhang B, Palmer AK, Ogrodnik MB, Pirtskhalava T, Thalji NM, Hagler M, Jurk D, Smith LA, Casaclang-Verzosa G, Zhu Y, Schafer MJ, Tchkonja T, Kirkland JL, Miller JD. Chronic

- senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. *Aging Cell*. 2016;15(5):973–7.
82. Zamin LL, Filippi-Chiela EC, Dillenburg-Pilla P, Horn F, Salbego C, Lenz G. Resveratrol and Quercetin cooperate to induce senescence-like growth arrest in C6 rat glioma cells. *Cancer Sci*. 2009;100(9):1655–62.
 83. Bientinesi E, Ristori S, Lulli M, Monti D. Quercetin induces senolysis of doxorubicin-induced senescent fibroblasts by reducing autophagy, preventing their pro-tumour effect on osteosarcoma cells. *Mech Ageing Dev*. 2024;220:111957.
 84. Yousefzadeh MJ, Zhu Y, McGowan SJ, Angelini L, Fuhrmann-Stroissnigg H, Xu M, Ling YY, Melos KI, Pirtskhalava T, Inman CL, McGuckian C, Wade EA, Kato JI, Grassi D, Wentworth M, Burd CE, Arriaga EA, Ladiges WL, Tchkonina T, Kirkland JL, Robbins PD, Niedernhofer LJ. Fisetin is a senotherapeutic that extends health and lifespan. *EBioMedicine*. 2018;36:18–28.
 85. Wang Y, Chang J, Liu X, Zhang X, Zhang S, Zhang X, Zhou D, Zheng G. Discovery of Piperlongumine as a potential novel lead for the development of senolytic agents. *Aging*. 2016;8(11):2915–26.
 86. Moaddel R, Rossi M, Rodriguez S, Munk R, Khadeer M, Abdelmohsen K, et al. Identification of gingerenone A as a novel senolytic compound. *PLoS One*. 2022;17(3):e0266135.
 87. Li PC, Lam E, Roos WP, Zdzienicka MZ, Kaina B, Efferth T. Artesunate derived from traditional Chinese medicine induces DNA damage and repair. *Cancer Res*. 2008;68(11):4347–51.
 88. Berdelle N, Nikolova T, Quiros S, Efferth T, Kaina B. Artesunate induces oxidative DNA damage, sustained DNA double-strand breaks, and the ATM/ATR damage response in cancer cells. *Mol Cancer Ther*. 2011;10(12):2224–33.
 89. Samaraweera L, Adomako A, Rodriguez-Gabin A, McDaid HM. A novel indication for panobinostat as a senolytic drug in NSCLC and HNSCC. *Sci Rep*. 2017;7(1):1900.
 90. Baar MP, Brandt RMC, Putavet DA, Klein JDD, Derks KWJ, Bourgeois BRM, Stryeck S, Rijkse Y, van Willigenburg H, Feijtel DA, van der Pluijm I, Essers J, van Cappellen WA, Houtsmuller IWF, Pothof AB, de Bruin J, Madl RWF, Hoeijmakers T, Campisi JHJ, de Keizer J. Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging. *Cell*. 2017;169(1):132–e14716.
 91. Munoz-Espin D, Rovira M, Galiana I, Gimenez C, Lozano-Torres B, Paez-Ribes M, et al. A versatile drug delivery system targeting senescent cells. *EMBO Mol Med*. 2018. <https://doi.org/10.15252/emmm.201809355>.
 92. Guerrero A, Guiho R, Herranz N, Uren A, Withers DJ, Martinez-Barbera JP, et al. Galactose-modified duocarmycin prodrugs as senolytics. *Aging Cell*. 2020;19(4):e13133.
 93. Gonzalez-Gualda E, Paez-Ribes M, Lozano-Torres B, Macias D, Wilson JR 3rd, Gonzalez-Lopez C, et al. Galacto-conjugation of Navitoclax as an efficient strategy to increase senolytic specificity and reduce platelet toxicity. *Aging Cell*. 2020;19(4):e13142.
 94. Sagiv A, Krizhanovsky V. Immunosurveillance of senescent cells: the bright side of the senescence program. *Biogerontology*. 2013;14(6):617–28.
 95. Sagiv A, Burton DG, Moshayev Z, Vadai E, Wensveen F, Ben-Dor S, Golani O, Polic B, Krizhanovsky V. NKG2D ligands mediate immunosurveillance of senescent cells. *Aging*. 2016;8(2):328–44.
 96. Ovadya Y, Krizhanovsky V. Strategies targeting cellular senescence. *J Clin Invest*. 2018;128(4):1247–54.
 97. Kuilman T, Michaloglou C, Vredeveld LC, Douma S, van Doorn R, Desmet CJ, et al. Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell*. 2008;133(6):1019–31.
 98. Campisi J. Aging, cellular senescence, and cancer. *Annu Rev Physiol*. 2013;75:685–705.
 99. Acosta JC, O’Loughlen A, Banito A, Guijarro MV, Augert A, Raguz S, Fumagalli M, Da Costa M, Brown C, Popov N, Takatsu Y, Melamed J, Fagagna F, Bernard D, Hernando E, Gil J. Chemokine signaling via the CXCR2 receptor reinforces senescence. *Cell*. 2008;133(6):1006–18. d’Adda di.
 100. Orjalo AV, Bhaumik D, Gengler BK, Scott GK, Campisi J. Cell surface-bound IL-1alpha is an upstream regulator of the senescence-associated IL-6/IL-8 cytokine network. *Proc Natl Acad Sci USA*. 2009;106(40):17031–6.
 101. Velarde MC, Demaria M, Campisi J. Senescent cells and their secretory phenotype as targets for cancer therapy. *Interdiscip Top Gerontol*. 2013;38:17–27.
 102. Kang TW, Yevsa T, Woller N, Hoenicke L, Wuestefeld T, Dauch D, Hohmeyer A, Gereke M, Rudalska R, Potapova A, Iken M, Vucur M, Weiss S, Heikenwalder M, Khan S, Gil J, Bruder D, Manns M, Schirmacher P, Tacke F, Ott M, Luedde T, Longrich T, Kubicka S, Zender L. Senescence surveillance of premalignant hepatocytes limits liver cancer development. *Nature*. 2011;479(7374):547–51.
 103. Xue W, Zender L, Miething C, Dickins RA, Hernandez E, Krizhanovsky V, Cordon-Cardo C, Lowe SW. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature*. 2007;445(7128):656–60.
 104. Bavik C, Coleman I, Dean JP, Knudsen B, Plymate S, Nelson PS. The gene expression program of prostate fibroblast senescence modulates neoplastic epithelial cell proliferation through paracrine mechanisms. *Cancer Res*. 2006;66(2):794–802.
 105. Laberge RM, Awad P, Campisi J, Desprez PY. Epithelial-mesenchymal transition induced by senescent fibroblasts. *Cancer Microenvironment: Official J Int Cancer Microenvironment Soc*. 2012;5(1):39–44.
 106. Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. *Proc Natl Acad Sci USA*. 2001;98(21):12072–7.
 107. Liu D, Hornsby PJ. Senescent human fibroblasts increase the early growth of xenograft tumors via matrix metalloproteinase secretion. *Cancer Res*. 2007;67(7):3117–26.
 108. Palmer AK, Tchkonina T, LeBrasseur NK, Chini EN, Xu M, Kirkland JL. Cellular senescence in type 2 diabetes: a therapeutic opportunity. *Diabetes*. 2015;64(7):2289–98.
 109. Di Micco R, Krizhanovsky V, Baker D, d’Adda di Fagagna F. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. *Nat Rev Mol Cell Biol*. 2021;22(2):75–95.
 110. Freund A, Patil CK, Campisi J. p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. *EMBO J*. 2011;30(8):1536–48.
 111. Xu M, Tchkonina T, Ding H, Ogrodnik M, Lubbers ER, Pirtskhalava T, et al. JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. *Proc Natl Acad Sci U S A*. 2015;112(46):E6301–10.
 112. Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, Nadon NL, Wilkinson JE, Frenkel K, Carter CS, Pahor M, Javors MA, Fernandez E, Miller RA. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature*. 2009;460(7253):392–5.
 113. Laberge RM, Sun Y, Orjalo AV, Patil CK, Freund A, Zhou L, Curran SC, Davalos AR, Wilson-Edell KA, Liu S, Limbad C, Demaria M, Li P, Hubbard GB, Ikeno Y, Javors M, Desprez PY, Benz CC, Kapahi P, Nelson PS, Campisi J. MTOR regulates the pro-tumorigenic senescence-associated sec