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Senolytika - súčasný stav
Senolytics - current state

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Declaration

I declare that I have prepared the final work independently and that I have provided all the information sources and literature used.

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Abstract

Cellular senescence is a state of the permanent cell cycle arrest caused by different stresses or cell to cell fusion. Senescent cells, unlike naturally aged cells, exhibit a specific phenotype, referred to as senescence associated phenotype (SASP). It is characterized by the production of biologically active substances such as interleukins, chemoattractants or proteases that affect their surroundings. Long-term survival of these cells in the body is the cause of age-related diseases. Under normal circumstances, number of senescent cells is maintained in the body by the immune system. However, the age-related abrogation of immune system function per se (immunosenescence) contributes to accumulation of senescent cells in tissues and ageing of organism. This work describes origin, positive and negative effects of cell senescence, elimination of senescent cells by the immune system and current state of development of new substances causing specific lysis (killing) of senescent cells (senolytics).

Key words: senescent cells, senescence-associated secretory phenotype, DNA damage response, physiology and pathophysiology, age-related diseases, apoptosis, senolytics

Abstrakt


Kľúčové slová: senescenté bunky, fenotyp súvisiaci so senescenciou, odpoveď DNA poškodenia, fyziológia a patofyziológia, choroby súvisiace s vekom, apoptóza, senolytika
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1 Introduction

Ageing is the risk factor for chronic diseases, because senescent cells can accumulate with age and have negative effect on microenvironment, what is promote by their phenotype. These cells are most often arrested between G1-S phases of the cell cycle (Gire and Dulić, 2015). The cell cycle arrest is induced by the control mechanisms, which ensure the correct course of the cell cycle without disrupting gene integrity. DNA instability or damage can be induced by various means, such as ionizing radiation, depletion of the replication potential, drugs, toxins or increased expression of oncogenes. These stressors induce DNA damage response, whose permanent presence induce cellular senescence. Cellular senescence can also be induced by cytokines or cell fusion that plays a role in fetal development (Gioscia-Ryan et al., 2013).

Short-time presence of the senescent cells in an organism have positive effect on regeneration, wound healing and embryogenesis. However, long-time presence of senescent cells has detrimental impact on tissue microenvironment which is associated with age-related diseases such as, for example, atherosclerosis, osteoporosis or cancer. In the development of the chronic presence of senescent cells is also implicated immune system, which loses function in their clearance (Burton and Krizhanovsky 2014).

The healthspan and reduction of development chronic diseases can be enhanced by selectively killing of the senescent cells. The change in metabolism, cell surface and anti-apoptotic signalizing are targets for senolytics. The main purpose of this work is to provide current state of strategies directed to specifically remove senescent cells.
2 Cellular senescence

Cellular senescence was first described by L. Hayflick in 1961 as a state when cells (embryonal fibroblasts) cannot divide anymore after about 50 population doublings (Hayflick, Moorhead, 1961). This loss of proliferation potential (Sherwood et al., 1988) is due to inhibition of cyclin-dependent kinases (Cdks) by elevated levels of protein inhibitors of the Cdks (Cdki). The expression or stabilization Cdki can be activated by multiple mechanisms including DNA damage, oxidative stress (Passos, Saretzki and Von Zglinicki, 2007; Salama et al., 2014), cytokines (Scandura et al., 2004), or bacterial toxins (Blazkova et al., 2010). Trigger of oxidative stress are reactive oxygen species (ROS) that accumulate as a result of either impaired mitochondria, increased NADPH oxidase activity (Lener et al., 2009) or xanthine oxidase activity (Kuppusamy and Zweier, 1989).

Cdki comprise two families, INK4 (p16INK4a, p15INK4b, p18INK4c, p19INK4d) and Cip/Kip (p21\textsubscript{waf1/cip1}, p27Kip1, p57Kip2). The INK4 proteins inhibit the activity of the CDK4 in G1 phase of the cell cycle. The second family of Cdki decrease the activity of complex Cdks with A, D or E cyclins. However, p21\textsubscript{waf1/cip1} (p21) and p27Kip1 (p27) proteins stabilize the complex cyclin D-Cdk in G1 phase of the cell cycle (Sherr and Roberts, 1999). The p21 can be induced by the action of transcription factor p53, a tumour suppressor gene that is mostly stabilized in response to DNA damage (Chang et al., 2007; Brown et al., 2014). Cyclin D-Cdk4 complex phosphorylates inhibitor of the E2F transcription factors, retinoblastoma protein (pRB). This allows the transcription of cyclins E and A, and a transition to the S phase of the cell cycle (Sherr and Roberts, 1999). One of the downstream targets of E2F is protein p14\textsubscript{ARF}, which stabilizes pRB by the inhibition of its ubiquitin E3 ligase MDM2, and also the non-phosphorylated pRB allows to inhibit the cell cycle via the p53-p21 pathway (see Figure 1) (Chang et al. 2007).

Stopping the cell cycle in G1 phase is more frequent than in the G2 phase, because G1 checkpoint appear to be more efficient (Gire and Dunic, 2015). Furthermore, skipping cytokinesis in mitosis, as is frequently observed during development of cell senescence, results in formation of tetraploid senescent cells accumulating in the next G1 phase (Gire and Dunic, 2015).
Figure 1: Senescence response: triggers, pathways, features and markers. (Based on the diagram from Natalia Loaiza, Marco Demaria, 2016).

The characteristic feature of senescent cells is an increase of cell volume, elevated levels of proteins p16, p53, p21, elevated activity of senescence-associated β-galactosidase (SA-β-gal) and accumulation of lipofuscin (Kirkland et al., 2017). However, the p21 level decreases over a longer duration of senescence (Stein et al., 1999). Senescent cells have enlarged cell volume with an increased number of actin filaments (Kassem et al., 1997). The cause of cell growth and accumulation of proteins is deregulated mTOR activity, which promotes protein translation and facilitates interleukin 6 (IL-6) and interleukin 8 (IL-8) synthesis (Narita et al., 2012). Protein accumulation is also caused by decreased glycogen synthase 3 (GSK3) activity (Kim et al., 2010).

Genes responsible for proliferation are silenced by heterochromatinization forming senescence-associated heterochromatin foci (SAHF), but this is not typical for all senescent cell types as it is dependent on the type of senescence induction. For example, in keratinocytes, human dermal (BJ) and mouse embryonic fibroblasts (MEFs), the presence of SAHF was not found. p16 is likely to play a role in the formation of SAHF which together with hypophosphorylated pRB protects cells against possible malignant transformations (Kosar et al., 2011).

Senescent cells produce cytokines, chemokines, pro-thrombotic factors, extracellular proteases and other biologically active factors (Lasry and Ben-Neriah, 2015). This production of bioactive compounds is referred as senescence-associated secretory phenotype (SASP) and the quality and quantity of SASP depend on the type of senescent cells (Coppé, Desprez and Krtolica, 2014).
The expression of the SASP gene loci is enabled by high mobility group box 2 (HMGB2), which protects them from heterochromatinization (Aird et al., 2016). The major products are IL-6 and IL-8, which are activated by transcription factors NF-κB (nuclear factor-kappa B) and C/EBPβ (CCAAT/enhancer binding protein beta) (Guerrero and Gil, 2016). The upstream signal for their production are interleukin 1α (IL-1α) signalizing (Orjalo et al., 2009) and p38 mitogen-activated protein kinase (p38MAPK) pathways triggered by DNA damage response (DDR) cascade (Bredeson et al., 2014).
3 Mechanisms of cellular senescence

In general, the cell cycle arrest can be caused by various stress factors that interfere with cellular integrity during cell cycle progression. Exhaustion of replication potential, attrition of telomeres together with their irreparable damage and so-called end-replication problem (Olovnikov, 1973) are thought as causes of replicative senescence. Note that epithelial and endothelial cell senescence have different mechanisms and characteristics compared to replicative senescence of fibroblasts. Two senescent states are described for epithelial cells, the first one in keratinocytes and mammary epithelial cells is DDR-independent, unlike senescence of fibroblasts. The second is caused DDR pathway or p16/Rb pathway (Brenner et al., 1998; Nassour et al., 2016; Abbadie et al., 2017a).

Besides replicative senescence, other types of senescence caused by various stress stimuli were described. These types of senescence are referred to as Stress-Induced Premature Senescence (SIPS) and can be further divided according to type of stimulus as oncogene-induced senescence (OIS) induced by hyperactivation of oncogenes (Serrano et al., 1997), drug-induced senescence induced by various chemicals including chemotherapeutics (Petrova et al., 2016), bacterial toxin-induced senescence induced by some bacterial toxins with genotoxic activity (Hassane et al., 2003), cytokine-induced senescence triggered by autocrine or paracrine action of some cytokines (Frippiat et al., 2001).

3.1 Replicative senescence

3.1.1 Replicative senescence of fibroblast

As mentioned above, in the Hayflick production of fibroblasts in vitro, the cells had a limited number of divisions. This number of doublings depends on cell culture conditions. Experiments with mouse embryonic fibroblasts (MEF) grown in high or low oxygen concentration, or whose antioxidant status has been modified, have shown that hyperoxia caused shortened cell culture lifespan in cell populations grown in 20% oxygen whereas cell cycle arrest was not observed in cultures grown at 3% oxygen. Addition of hydrocortisone and α-tocopherol or bovine serum albumin into growth medium increased population doubling (Lu and Finkel, 2008).

Human telomeres are composed of DNA repetitive sequences 5’-TTAGGG-3’ with a single-stranded 3’ extension strand. This overhang creates the t-loop and d-loop structures that close the telomere end (Boeck and Forsyth, 2010). Telomeric repeat-binding factors TRF1 and TRF2F are important for formation of t- and d-loops, which protect double-strands DNA (dsDNA) segments of the telomeres (Martínez and Blasco, 2015). They are a part of multiple telomere-specific binding proteins, which make up the structure called "shelterin" (Boeck and Forsyth, 2010) (see Figure 2). These proteins inhibit the activity of checkpoint kinases ATM (ataxia telangiectasia mutated), ATR
ataxia telangiectasia and Rad3 related) in DDR involved in DNA repair (Karlseder et al., 2004). This pathways are activated by shortened and/or deprotected telomeres, DNA double-strand breaks (DSBs), and stalled or collapsed replication forks (Bekker-Jensen and Mailand, 2010).

During cellular lifespan the telomeres can be damaged, the telomeric damage can be repaired by telomerase activity in proliferating and tumour cells (Harley et al., 1990; Olovnikov, 1973). The enzymatic activity of human telomerase reverse transcriptase (hTERT) prolongs the 3´ends of telomeres, and these allow the DNA polymerase to elongate the second strand DNA of the chromosome terminals (Wu et al. 2006). It is thought that too short or uncapped and unrepaired telomeres can result in cellular senescence (Blackburn, 2001; Herbig et al., 2004) (see Figure 3.).

Damage in telomeric repeats may be caused with elevated levels of reactive oxygen species (ROS) because guanine is prone to oxidation (Oikawa et al., 2001). Consequently, the single-strand DNA break (SSB) or DSBs arising in consequence of DNA repair of oxidative DNA damage can trigger the DDR. In addition, ROS decline the activity of telomerase and this can lead again to cell cycle arrest (Passos et al., 2007). Mechanistically, high intracellular levels of ROS induced by the RAS–RAF–MEK–ERK cascade activate the p38MAPK, which leads to increased transcriptional activity of p53 and upregulation of p21 (Sun et al., 2007).
3.1.2 Replicative senescence of epithelial and endothelial cells

Endothelial cells are the specialized cells that line the vascular lumen in a single layer. Senescence in endothelial cells is manifested by elevated SA-β-gal activity and p53/p21 pathway. In the vein smooth muscle cells (VSMC) is senescence associated with reduced TRF2 expression and its loss in telomere, what is caused DDR (Bennett et al., 2015). The main feature of cells that, after the shape change described as VSMC-like, endothelial-like, foamy macrophage-like, is the increase of the expression of p16. Early foam macrophage cells produce chemotactants for leukocytes and monocytes, which together with them support development of atherogenic plaques (Childs et al., 2016). These senescent cells also degrade elastic fibers and plaque calcification leads to atherosclerosis. The removal of the foam macrophage-like cells results in the suppression of atherogenic plaque formation (Min et al., 2007; Childs et al., 2016).

In epithelial cells, such as keratinocytes and mammary epithelial cells, senescence is telomere and DDR-independent. Activation of senescence by the p16 pathway was also detected in other cells with or without the presence of telomere damage, which differs from senescence in fibroblasts. In the cell cycle arrest of fibroblast are present both pathways, p53/p21 and p16/RB, but in the epithelial cells only p16/RB (Abbadie et al., 2017). The p16 protein is induced by p38MAPK activity, which is activated in the presence of DNA damage, oxidative stress and other stress conditions. The senescence in epithelial cells is primarily caused by SSB and p16/p38MAPK pathway (Abbadie et al., 2017b).

Epithelial senescent cells have a distinct SASP. In keratinocytes and epithelial colon cells was detectable maspin, which has tumour-suppressor and anti-angiogenic effects. It was not detected in the senescent fibroblasts, nevertheless maspin affects their proliferation. With increasing age the level of
the maspin is increased and plays role as an antagonistic factor for malignant fibroblast transformation. With increasing age the level of the maspin increased (Nickoloff et al., 2004).

3.2 Premature types of cellular senescence

3.2.1 Oncogene-induced senescence

Oncogenes are mutant versions of normal genes that have the potential to transform cells in conjunction with additional mutations. Normal cells respond to many oncogenes by undergoing senescence. This phenomenon was first observed when an oncogenic form of RAS, a cytoplasmic transducer of mitogenic signals, was expressed in normal human fibroblasts (Collado, Blasco and Serrano, 2007), which was later shown to be telomere-independent type of senescence triggered by onco-proteins (We et al., 1999).

Cell cycle arrest occurs after the deregulation of oncogenes, which is mainly accompanied by persistent DDR signalling in response to DSBs or unprotected telomeres. These damages on DNA can be immunofluorescent detected for the phosphorylated histone H2A.X (γH2AX) and the adapter protein tumour suppressor p53-binding protein 1 (53BP1). It is referred to as DNA damage foci or DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS) and marker of the senescence (Rodier et al., 2011).

The effect depends on the type of the oncogene and on the cellular context. For example, activated oncogene Ras increases p53 and ARF, which have preventive effects on the oncogene-induced transformation (Serrano et al., 1997). Senescence-associated heterochromatin foci caused by Ras, suppress the expression of the E2F-induced genes (Narita et al., 2003).

A general feature of oncogene-induced senescence is the derepression of the CDKN2A locus (Kim and Sharpless, 2006). In addition, this type of senescence may also induce robust DDR owing to the DNA damage that is caused by aberrant DNA replication (Bartkova et al., 2006) and/or ROS. The relative importance of these mechanisms (p16, ARF or DDR-induced p53) varies across cell types (Alimonti et al., 2010).

3.2.2 Drug-induced senescence

Premature senescence can be induced in normal and cancerous cells by various chemicals depending on their dosage and mechanism of action. Drugs that interact directly with DNA or affect chromatin remodelling often cause SIPS. For example, trichostatin A, which inhibits histone deacetylases and triggers the p53 protein pathway can induce SIPS (Rebbaa et al., 2006). Topoisomerase inhibitors such as doxorubicin, camptothecin, amsacrine, and etoposide also trigger p53/p21 pathway by induction of DSBs (Rebbaa et al., 2006; Sabisz and Skladanowski, 2014).
Cell cycle arrest can be also caused by DNA G-quadruplex stabilizers that inhibit, for instance, telomerase activity, leading to an accelerated onset of senescence (Huang et al., 2008). Another way how to reduce the number of cell divisions and stop the cell cycle is to use inhibitors that interfere with nucleotide metabolism or forming mutations of nitrogen bases. An example is cyclopentenyl cytidine, which acts as a noncompetitive CTP synthase inhibitor in the form of triphosphate. This leads to increase of level p53 protein and overexpression of p53 target genes (Huang et al., 2011).

3.2.3 Bacterial toxin-induced senescence

Another pathophysiological stimulus that induces premature senescence are several cytolethal distending toxins (CDTs) produced by facultative pathogenic strains of G- bacteria such as Escherichia coli, Campylobacter jejuni, Helicobacter hepaticus, Shigella dysenteriae and Actinobacillus actinomycetemcomitans have been described and shown to have genotoxic effects on cells in vitro (Blazkova et al., 2010). Mammalian cells exposed to these bacterial proteins undergo cell type-dependent cell cycle arrest or apoptosis. This characteristic phenotype included persistently activated DNA damage signalling (detected as 53BP1/γH2AX foci), enhanced SA-β-gal activity, expansion of promyelocytic leukaemia nuclear compartment, expression of several cytokines (especially interleukins IL-6, IL-8 and IL-24) and activation of the two major tumour suppressor pathways – the p16/RB and p53/p21 cascades, overall features shared by cells undergoing replicative or premature cellular senescence (Duane C Hassane, Lee and Pickett, 2003).

3.2.4 Cytokine-induced senescence

Cytokines are small signalling proteins that are involved in autocrine, paracrine and endocrine signalling as immunomodulating agents through membrane bound receptors. Senescence-inducing effect of cytokines on mouse cancer cells was described for cytokines, such as the T helper-1 (Th1)-cytokines interferon-γ (IFN-γ) and tumour necrosis factor alpha (TNF-α) (Braumüller et al., 2013) or transforming growth factor-β (TGF-β) (Untergasser et al., 2003).

Cytokines from the TGF-β family increase ROS in the cell by induction the expression NADPH oxidase Nox4 (Burdak et al. 2008). TGF-β induces the cell cycle arrest in cancer cells also by direct induction of cdki p21 and p15 (Senturk et al., 2010).

For the pro-inflammatory Th1 cytokines, the senescence signalling pathways in mouse beta cells have been partially deciphered: permanent growth arrest needs the simultaneous activation of TNF receptor 1, IFN-γ signalling, and downstream stabilization of the p16/RB pathway (Reimann et al., 2010).
4 Physiological roles of cell senescence

Recent evidence has pointed to beneficial effects of cellular senescence beyond tumour suppression, for instance in directing wound repair and in embryogenesis. To achieve this, senescent cells arrest their own proliferation, recruit phagocytic immune cells and promote tissue renewal. In these contexts, senescence serves a tissue remodelling role and the senescent cells have a relatively short half-life, presumably because they are efficiently cleared by immune cells (Storer et al., 2013).

4.1 Organism development

Developmental senescence is a physiologically programmed senescence pathway that has recently been described to actively contribute to embryonic patterning. This process is also accompanied by the SASP that attracts macrophages which in turn seem to be necessary to remove senescent cells in a coordinate manner in order to foster the physical development of the embryo (Gioscia-Ryan et al., 2013). In embryo, the occurrence of senescence was substantiated by SA-β-gal staining as well as absence of proliferation detected as negative Ki67 staining and 5-bromo-2′-deoxyuridine (BrdU) incorporation, increased heterochromatin markers such as histone 3 lysine 9 trimethylation (H3K9me3) and heterochromatin protein 1 homologue-γ (HP1γ), and increased expression of cell cycle inhibitors (p15, p21 and p27) (Storer et al., 2013).

DNA damage markers were absent in the structures undergoing developmental senescence. Altogether, cellular senescence seems to be common throughout the developing embryo, but it has distinctive features compared to damage-induced senescence.

4.2 Megakaryocytes and placental syncytiotrophoblasts

Apart from embryonic development, senescence also occurs in a physiologically programmed manner in adult organisms. In particular, normal megakaryocytes (Besancenot et al., 2010) and placental syncytiotrophoblasts (Chuprin et al., 2013) undergo senescence as part of their natural maturation programmes. In case of mouse and human megakaryocytes, senescence is characterized by SA-β-gal activity, DDR, induction of p21, proliferative arrest and accumulation of HP1γ. Megakaryocyte senescence, similar to developmentally programmed senescence, is dependent on p21 but is independent of p16, p53 or p27 (Besancenot et al., 2010). The human placenta shows marked SA-β-gal activity at the syncytiotrophoblast in association with DNA damage markers, p16, p21 and p53 (Chuprin et al., 2013).
4.3 Wound healing and tissue regeneration

It has been shown that many factors comprising SASP of senescent cells are important for tissue repair: growth factors and proteases that participate in wound healing, attractants for immune cells that kill pathogens, and proteins that mobilize stem or progenitor cells. Thus, the SASP may serve to communicate cellular damage/dysfunction to the surrounding tissue and stimulate repair, if needed. Upon acute liver injury in mice, hepatic stellate cells initially proliferate and secrete extracellular matrix (ECM) components, which produce a fibrotic scar. Shortly after the proliferative stage, stellate cells in the injured liver undergo senescence (Krizhanovsky et al., 2008). This senescence response is accompanied by a decline in ECM production and increased secretion of several matrix metalloproteinases (MMPs), which are known to degrade ECM proteins. This finding suggested that the senescence response helps to resolve the fibrotic scar. When stellate cells are compromised for their ability to undergo senescence, mice developed severe fibrosis after acute liver injury.

Jun and Lau (2010) demonstrated the role of senescence in limiting fibrosis in skin wound healing and showed the pivotal role of CCN1 (CYR61) in converting wound-activated fibroblasts into senescent fibroblasts. The extracellular matrix protein CCN1 is crucial for the induction of senescence in dermal fibroblasts, the associated expression of pro-inflammatory cytokines and antifibrotic MMPs (Jun and Lau, 2010).

Another important role is the senescence generated by cell fusion in the formation of syncytiotrophoblast. This cell-cell fusion-induced senescence (FIS) is provided by the protein ERVWE1 (Chuprin et al., 2013). This syncytium of the placenta likely play the role in resistance to apoptosis, which is necessary for development of the embryo. Secreted proteases and cytokines maintain feto-placental function. Next positive effects of senescence on tumour suppression have been demonstrated in OIS models (Burton et al., 2014) as described further.
5 Pathophysiology of cell senescence

5.1 Cellular senescence as primary tumorigenesis barrier

Cell senescence represent intermediate stage between normal proliferating cells and tumour cells (Serrano, 2011). Telomere shortening has been shown to be responsible for decreased tumour formation in telomerase-deficient mice crossed with p53R172P mutant which is unable to initiate apoptotic response (Cosme-Blanco et al., 2007). Using this model authors demonstrated that p53-mediated cellular senescence in the context of telomere shortening acts as a main mediator of tumour suppression in this mouse model. Other authors have shown that oncogene-induced senescence acts as a barrier to melanoma development in melanocytic nevi, which is associated with activation of oncogenic BRAF protein kinase and independent to telomere shortening (Michaloglou et al., 2005). RASV12 knock in mice develop lung adenomas characterized by a low proliferative index which was associated with the elevation of SA-β-gal activity and induction of other senescence markers (Collado et al., 2005).

In a mice model for p53-dependent liver cancer, re-expression of p53 in lymphomas and osteosarcomas provokes tumour regression by inducing senescence, in a tissue-dependent manner (Ventura et al., 2007). Using p53 restoration model showed that senescent cells activate an inflammatory program that leads to a dramatic regression of invasive hepatocarcinomas and their clearance by the innate immune system (Xue et al., 2015).

Altogether the literature data from in vivo studies increasingly support the concept that cellular senescence corresponds to a potent physiological mechanism protecting against oncogenic transformation which is consistent with results from in vitro studies.

5.1.1 Escape (bypass) of senescence

Various tumour suppressors and oncogenes have been shown to act as control mechanism regulating senescence in normal cells thus preventing uncontrolled cell proliferation. Escape from senescence (senescence bypass) resulting in cell immortalization, on the other side, appears to be an important step in the cancer development. Virtually all human cancers lack functional p53/pRB pathways (Sherr and Mccormick, 2002) and often carry mutations in sets of genes, which are known to collaborate in vitro in bypassing the senescence response. For example, almost all human pancreatic cancers suffer from an activating RAS mutation and a deficiency of the INK4A/ARF locus (Bardeesy et al., 2002).
5.2 Senescence-associated secretory phenotype

One of the main features of many senescent cells is production of a specific pro-inflammatory secretome referred as SASP (see chapter 2 above) The SASP is primarily a property of cells with genomic or epigenomic damage. In contrast, cells with detected DNA damage, dysfunctional telomeres, mitogenic signals, oxidative stress, and other senescence-inducing stimuli develop the SASP of varying qualities and robustness (Campisi, 2014).

As mentioned above SASP components can include several families of soluble and insoluble factors (Coppé, 2014), which can affect surrounding cells by activating various cell-surface receptors and corresponding signal transduction pathways that may lead to multiple pathologies, including cancer. Whereas some SASP factors are known to fuel the deleterious effects of senescent cells, other or even the same factors may have beneficial effects. SASP factors can be divided into the following major categories: soluble signalling factors (cytokines, chemokines and growth factors), secreted proteases, and secreted insoluble proteins/extracellular matrix components. SASP proteases can have three major effects: 1) shedding of membrane-associated proteins, resulting in soluble versions of membrane-bound receptors, 2) cleavage/degradation of signalling molecules, and/or 3) degradation or processing of the extracellular matrix. These activities provide potent mechanisms by which senescent cells can modify the tissue microenvironment. SASP components, most notably TGF-ß, can also trigger senescence in neighbouring cells in paracrine manner through mechanism that generates ROS and DNA damage (Hubackova et al., 2012; Acosta et al., 2013).

5.2.1 SASP in tumour promotion

There is mounting evidence that SASP of senescent cells can drive protumorigenic cell proliferation. Fibroblasts from the human prostate gland that undergo senescence in culture have been shown to create a local tissue environment that favours prostate epithelial cell hyperproliferation (Bavik et al., 2006). Matrix metalloproteinases secreted by senescent fibroblasts, in particular MMP3 (stromelysin) which also promotes tumour cell invasion, have been shown to be responsible for the higher tumorigenicity of breast epithelial cell xenografts in mice (Liu et al., 2007). Malignant melanocytes express high levels of CXCR-2 receptor and can be stimulated to grow by melanoma growth stimulatory activity/growth regulated protein (MGSA/GRO) and IL-8. The senescent microenvironment may therefore stimulate the proliferation of rare premalignant cells in nevi, thereby leading to the development of melanoma (Wang et al., 2009; Coppé et al., 2014).

Besides promoting cell proliferation an array of SASP factors can stimulate cell motility, i.e. cell migration, invasion and metastasis. In breast cancer, high levels of IL-6 and IL-8 secreted by senescent fibroblasts are responsible for a transition of epithelial cells into mesenchymal ones which is
an important morphological transition enabling epithelial cells to invade and migrate through tissues and is critical in the development of metastatic cancer (Coppé et al., 2008; Gioscia-Ryan et al., 2013).

Senescent cells that senesce in response to DNA-damaging radiation or chemotherapeutics secrete some factors (WNT16B, IL-6, tissue inhibitor of metalloproteinases-1) that can protect neighbouring tumour cells from being killed by the same chemotherapeutic agents (Xue et al., 2007).

5.2.2 SASP in immune system suppression

SASP includes proteins that can help senescent cells evade immune recognition and clearance (Coppé et al., 2010). For example, high secreted levels of matrix metalloproteinases by senescent cells can cleave both the cell surface ligands on natural killer target cells and the cell surface receptors on natural killer cells, thereby preventing natural killer cells from targeting and killing senescent cells.

On the other side, CD8+ T cells are suppressed by the action of IL-6 on myeloid cells. This suppression reduces tumour-suppressive immunity in tumours with chronic presence of senescence cells in the vicinity (Ruhland et al., 2016; Wang et al., 2018).

Another major immune modulator is the programmed cell death ligand 1 (PDL1), which permits cancer cell immune evasion by suppressing apoptosis in regulatory T cells and promoting death in effector T cells (He et al., 2015). CD4+ T cells, which are involved in senescent cells clearance, express the PDL1 receptor, but it is unknown to what extent this ligand is used by senescent cells for immune evasion.

5.2.3 SASP in degenerative diseases

Senescent cells have been shown to drive degenerative changes that can disrupt normal tissue structures essential for normal tissue function, largely through their secreted proteins (Rodier et al., 2011). Numerous degenerative diseases have been associated with cellular senescence, including atherosclerosis, osteoarthritis, Alzheimer disease, chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis (Bhat et al., 2012; Bar-Shai et al., 2014; Childs et al., 2016; Schafer, 2017).

Senescent vascular smooth muscle cells and endothelial cells accumulate at sites of atherosclerotic lesions and secrete several pro-atherogenic factors, such as IL-1α, monocyte chemotactic protein 1, MMP12 and MMP13. Selective elimination of these cells in a mouse model using different approaches blocked lesion growth and stabilized the plaque structure (Childs et al., 2016).
In a mouse model of traumatic osteoarthritis, a factor made by senescent chondrocytes has been shown to inhibit cartilage regeneration. Clearance of senescent cells in this system promoted the repair of damaged cartilage, possibly due to reduction of levels of SASP factors MMP13, IL-6 and IL-1β. Clearance of naturally occurring senescent cells in naturally aged mice also prevented age-related osteoarthritis (Jeon et al., 2017).

In fat tissue, levels of the SASP factors IL-6 and IL-1β increase with age, and these are known to cause insulin resistance when chronically high (Gao et al., 2014).

5.2.4 SASP in ageing

Ageing is the progressive loss of tissue and organ function over time (Flatt, 2012). Senescent cells accumulate in aged tissues with high SA-β-gal activity and increased expression of the senescence master regulator, p16 (Krishnamurthy et al., 2004). Keeping senescent cells with age is related to their reduction by removing immune mechanisms that become less effective (Burton and Stolzing, 2018).

In vitro studies suggest that cell senescence promote deterioration of tissue maintenance processes due to the SASP and disrupt reparative stem and progenitor cells from the proliferative pool. SASP factors that were described to have in vivo functions in ageing are cytokines IL-6 and TNF-α (Starr et al., 2015). Optimal function of stem cells depends on their highly specialized microenvironment (O’Connor, 2009) and therefore SASP may deleteriously affect stem cells by altering this microenvironment. For example, metalloproteinases which are prominent compounds of SASP could destroy the polarized extracellular matrix. The SASP could also affect parenchymal cell function and tissue composition without influencing stem cells. Structural changes caused by the secretion of matrix metalloproteinases could damage surrounding extracellular matrix, potentially leading to effects such as loss of skin or lung elasticity (Liu et al., 2007). The SASP of senescent cells can directly affect the endocrine-responsive intracellular signalling cascades through TNFα, IL-1β and/or IL-6, secretion of which cause resistance to IGF1 signalling (O’Connor, 2009).

In ageing skin, accumulation of senescent cells has been shown both in the epidermis and the dermis (Nassour et al., 2016). The generation of reactive oxygen species and the degradation of the extracellular matrix by overexpressed matrix metalloproteinases are common features of ageing skin (Toutfaire, 2017).
6 Removal of senescent cells by immune system

Induction of cellular senescence commonly coincides with an immunogenic phenotype that promotes self-elimination by components of the immune system, thereby facilitating tumour suppression and limiting excess fibrosis during wound repair (Sagiv et al., 2016). The mechanism by which senescent cells regulate their immune surveillance are not completely understood. Proinflammatory secretome of senescent cells attract immune cells of the innate and adaptive immune system (Xue et al., 2007; Kang et al., 2011) which kill and remove senescent cells. Among the cells that participate in the clearance of senescent cells are natural killer cells, macrophages and T cells (Xue et al., 2007; Krizhanovsky et al., 2008).

The effect of senescent cell on the organism is regulated by the immune system, which control their frequency, but long-lasting presence adversely affects neighbouring cells and tissues. The role of the immune system is to prevent the long-term presence of senescent cells in the body that is failing together with age and is probably the cause of age-related diseases (Burton et al., 2018). For example, the CD4$^+$ T cell, promote the anti-tumour role of immunity by eliminating premalignant senescent cells in liver by macrophages or monocytes (Kang et al., 2011). The senescent cells show changes in the membrane, such as modified vimentine (Frescas et al., 2017), oxidation-modified phospholipids (Ademowo, 2017), modified glycolipids (Itakura et al., 2016), and loss of CD47 phagocytosis inhibitory protein (Liu et al., 2017). These changes may have role in removal of senescent cell by the macrophages (see Figure 4).

Senescent cells or other stressed cells (e.g. tumour, virus-infected cells) have increased expression of the NKG2D ligands. NKG2D receptor is on the membrane of the natural killer (NK) cells, which recognize MICA and ULBP1-6 ligands. The type of ULBP ligand depend on the cell type and cause of cell senescence, but their expression is not based on DDR, unlike MICA. Their elimination is promoted by chemoattractants and cytokines, what facilitate faster recognition by the NK (Sagiv et al., 2016).

Cancer cells can escape programmed immune clearance through a combination of decoy receptor presentation, immunomodulatory cytokines and checkpoint ligands. Decoy receptors DCR2 and DCR3 expressed widely on cancer cells titrate away FAS ligand and TNF$\alpha$-related apoptosis-inducing ligand that are presented by cytotoxic T cells, thereby blocking apoptosis (Wu et al., 2014). Similarly, hepatic senescent cells produced by liver injury as well as senescent cells that result from other stimuli have upregulated levels of DCR2 which neutralizes activation by FAS-mediated extrinsic apoptosis pathway by natural killer cells (Collado et al., 2010; Sagiv et al., 2013).
Figure 4: Possible mechanisms of senescent cell recognition by macrophages (based on the diagram from Burton, Stolzing, 2018).
7 Strategies of pharmacological removal of senescent cells

The finding that killing senescent cells in vivo increases healthspan in mice and delays multiple age-related symptoms and pathology (Baker et al., 2011) has opened the door for the development of agents and strategies to specifically target senescent cells for the prevention and treatment of age-related diseases. These strategies comprise the selective elimination of senescent cells (senolysis), suppressing onset of senescence, immune-mediated clearance of senescent cells and SASP neutralization.

7.1 Specific features of senescent phenotype as target of their removal

Senescent cells are inherently diverse in various aspects: senescent cells of different origins secrete different SASP factors (Coppé et al., 2008), drive disease pathogenesis through varying mechanisms and can be triggered to enter apoptosis through distinct senolytic mechanisms (Sturmlechner et al., 2017). Some features of all senescent cells can potentially be exploited for senotherapy, e.g. the proliferation cessation (growth arrest) which is essentially irreversible, resistance to cell death signals (apoptosis resistance), widespread changes in gene expression and pro-inflammatory secretion profile.

7.1.1 Resistance to apoptosis

One of the most prominent features of senescent cells, at least in cell culture, is that they show alterations in apoptotic signalling which causes their relative resistance to programmed cell death (Burton et al., 2015). Unlike normal cells, senescent cells are protected from both intrinsic and extrinsic pro-apoptotic signals that allow them to persist and promote diverse biological processes under stress conditions (Sagiv et al., 2013). Targeting these apoptotic pathways preferentially in senescent cells can result in selective death of these cells and preventing them from exerting their detrimental effects (Ovadya et al., 2018).

One key determinant of the senescent versus apoptotic cell fate choice is signalling through the p53 stress response pathway. Recent studies highlight the p53/p21 axis as a promising target for development of novel senolytics (Baar et al., 2017; Yosef et al., 2017). Interfering in direct interaction of p53 with the transcription factor FOXO4 leads to the release of p53 from the nucleus and induction of cell-intrinsic apoptosis. Administration of a modified FOXO4/ p53-interfering peptide was able to
neutralize murine liver chemotoxicity of doxorubicin treatment and restore fitness, hair density, and renal function in progeroid and naturally aged mice (Baar et al., 2017).

p21 itself can block apoptosis and apoptotic cells actively silence p21 expression via p53-dependent DNA (cytosine-5)-methyltransferase 3A (DNMT3A) activity (Zhang et al., 2011). In mice, p21 knockout leads to a reduction of senescent cells in fibrotic livers and alleviates liver fibrosis (Yosef et al., 2017). Therefore, the development of drugs that can induce apoptosis in senescent cells by inhibition of p53 or p21 is a promising strategy to target senescent cells. For example quercetin (Zhu et al., 2015) and agmatine (Song et al., 2016).

Senescent cells are characterized by a state of permanent growth arrest and their prolonged survival. Several of the senescent cells pro-survival pathways that have been identified can be utilized for directed elimination of senescent cells. Currently, most identified senolytics are directed against members of the BCL-2 protein family. these anti-apoptotic proteins inhibit Bak / Bax polymerization on the outer membrane of the mitochondria. Their polymerization produces a cytotoxic cytochrome trap, the cytoplasmic cytoplasm triggers the apoptotic pathway of procaspases (Manuscript, 2012) (see Figure 5). Studies across different cell types have demonstrated an up-regulation of the BCL-2 family members BCL-2, BCL-W, and BCL-XL during senescence (Yosef et al., 2016). Silencing those proteins by substances as navitoclax (ABT-263) and TW-37 leads to the activation of programmed cell death (Zhu et al., 2016).

Figure 5. Apoptotic pathways. Bcl-2 family members act upstream of mitochondrial- mediated apoptosis (based on the diagram from the Zhu et al., 2016).

7.1.2 Metabolism of senescent cells

Senescent cells show altered gene expression leading to a specific metabolome and this feature can be utilized for their therapeutic targeting. One well-described feature of senescent cells is
senescence-associated β-galactosidase activity (Dimri et al., 1995). A targeted delivery system using mesoporous silica nanoparticles coated with galacto-oligosaccharides was developed based on this feature (Agostini et al., 2012). While the coated particles cannot be activated in nonsenescence cells, the coating is digested in senescent cells and the nanoparticle content can be released. Coated nanoparticles containing a cytotoxic drug could release it to the cytoplasm of senescent cells to induce apoptosis. Senescent cells also exhibit a secretory profile that is largely conserved between different senescent states and cell origins. Modulation of signalling pathways that lead to the proinflammatory secretome could therefore neutralize these negative effects of senescent cells. These signalling can be influenced by a broad spectrum of drugs (Laberge et al., 2015) For example rapamycin, metformin and ruxolitinib (Kirkland et al., 2017).

Targeting specific components of SASP could also provide a safer way to mitigate the deleterious effects of SASP. Cytokines such as IL-6, IL-8, and matrix-remodelling proteases such as ADAM17, could serve as possible targets. One method to block these molecules is the application of neutralizing antibodies, which can be developed based on available monoclonal antibodies (Ovadya et al., 2018). For example, simvastatin and anti-IL1α substances (Soto-Gamez et al., 2017).

Recently, a novel class of senolytic targeting of HSP90 proteins has been identified. HSP90 comprise a family of ubiquitously expressed molecular chaperones that can promote cell survival via stabilization of AKT and ERK which are members of signalling pathways that are up-regulated during senescence (Karkoulis et al., 2013). Disruption of the HSP90-AKT interaction inhibited the PI3K/AKT pathway, resulting in selective killing of senescent cells of different origins. For example geldanamycin, 17-AAG (tanespimic) and 17-DMAG (alvespimicin) (Fuhrmann-Stroissnigg et al., 2017). Another is mitochondria-targeted tamoxifen (MitoTam), which reduce the adenine nucleotide translocase-2 (ANT2) function in oxidative phosphorylation. Inhibition ANT2 is an effective and selective drug for senescent and tumour cells (Hubackova et al., 2018).

7.2 Cellular senescence specific drugs

There is growing interest to target senescent cells therapeutically as a part of anti-ageing and rejuvenation strategies. The most straightforward option to remove senescent cells is by their direct killing, either by apoptotic (senoptosis) or non-apoptotic means (senolysis) (Zhu et al., 2015). Other therapeutic strategies for removal of senescent cells comprise their immune-based clearance by antibodies or cytotoxic T-cells (Ovadya et al., 2018). Advantages of directed killing of senescent cells include permanent removal of the SASP, elimination of preneoplastic cells, and reducing cancer risk from senescent escape (Childs et al., 2015).

At present, the scientist is focused on the discovery of pharmacological agents that can induce cell death in senescent cells. Many of these agents target upregulated anti-apoptotic system of
senescent cells, such as signalizing through the BCL-2 family of proteins (BCL-2, BCL-XL, and BCL-W) (Chang et al., 2015; Yosef et al., 2016). The senolytic molecules ATB-737 and its next generation orally available analog ATB-263 (navitoclax) bind to BCL-2, BCL-XL and BCL-W, counteract their anti-apoptotic functions and permits senescent cells to initiate apoptosis (Ovadya et al., 2018).

ATB-737 efficiently eliminates senescent cells that were induced by DNA damage in lungs of γ-irradiated mice, as well as senescent cell formed by p14ARF induction in skin epidermis of transgenic mice (Yosef et al., 2016) and positively affected hair growth by inducing hair follicle steam cells proliferation.

Navitoclax has been shown to eliminate senescent cells from sublethally irradiated mice and naturally aged mice, including senescent muscle stem cells and senescent hematopoietic stem cells (Chang et al., 2016), which resulted in rejuvenation and partial restoration of hematopoietic function of mice. Navitoclax also showed the capacity to eliminate senescent foam cell macrophages from early atherosclerotic lesions and block senescent cells-dependent progression of atherosclerosis (Childs et al. 2016).

On the other side, targeting BCL-2 family of proteins with general inhibitors has been shown to be a cause several mechanism-based hematological toxicities, such as neutropenia and thrombocytopenia (Cang et al., 2015). Specific BCL-XL inhibitors, such as A1331852 and A1155463, are expected to cause less toxicity to nonsenescent cells (Zhu et al., 2017). These substances has been shown to induce apoptosis in senescent cholangiocytes and fibroblasts in a mouse model of biliary liver fibrosis and reduced liver injury and fibrosis (Moncsek et al., 2018).

Intraarticular injection of the BCI-2-targeting UBX0101 compound efficiently eliminated senescent cells in articular cartilage and synovium and reduced signs of osteoarthritis in aged mice model (Jeon et al., 2017). The combination of the pan-tyrosine kinase inhibitor dasatinib and a naturally occurring flavonoid quercetin has been shown to act in selectively killing senescent in tissue culture (Schafer et al. 2017). In addition to quercetin, other natural compounds, including fisetin and piperlongumine, have been suggested to have senolytic effects (Wang et al., 2016a; Zhu et al., 2017). Piperlongumine have good selectivity and pro-apoptotic potency in vitro and acts synergistically with navitoclax (Wang et al., 2016b). Targets for piperlongumine is oxidatition rezistence 1 (OX1) protein, which protect the senescent cell from reactive oxidation species accumulation. Piperlongumine binds to the protein, induces proteasome degradation and apoptosis (Zhang et al., 2018).

Other senescence-specific pathways could also be inhibited by small molecules to eliminate senescent cells. Disruption of the p53–FOXO4 interaction using a d-retro-inverso peptide (DRI-FOXO4) that corresponds to the reverse sequence of the FOXO4–p53-binding domain leads to the release of p53 from the nucleus and induction of cell-intrinsic apoptosis by catalysing cytochrome c release into the cytoplasm from mitochondria. In progeroid and naturally aged mice, short-term
treatment with DRI-FOXO4 has been shown to neutralize murine liver chemotoxicity after doxorubicin treatment and restore fitness, hair density and renal function (Baar et al., 2017).

As mentioned above, HSP90 proteins have been identified as novel target for senolysis. *In vivo*, administration of the HSP90 inhibitor 17-DMAG to progeroid mice reduced the senescence signature and extended health span (Fuhrmann-Stroissnigg et al., 2017).

Antibodies raised against senescence-specific surface antigens, such as CD44 in the senescent endothelium (Mun et al., 2010) can be utilised to killing by cytotoxic T cells or to deliver cytotoxic nanoparticles for indirect killing of senescent cells. Specific senescent cell antigens could be used to raise T cells *in vitro* armed with chimeric antigen receptors against senescent-specific surface antigens for infusion (Grupp et al., 2013).

### 7.3 Senolytics as anti-ageing drugs

Studies performed on a rapidly ageing mouse model with deficiencies in the mitotic checkpoint protein BUBR1 have demonstrated a causal link between senescent cells and ageing (Hanks et al., 2004). In this model, prematurely aged tissues accumulate high numbers of p16\(^{INK4A}\)-positive senescent cells which has been shown to trigger natural features of mouse ageing, including sarcopenia, cataracts and lipodystrophy. Baker et al. (2008) have demonstrated that genetic inactivation of p16\(^{INK4A}\) block the formation of senescent cells and attenuates these early-ageing phenotypes.

Further studies using transgenic mice with senescent cell-killing system INK-ATTAC (INK-linked apoptosis through targeted activation of caspase) have shown that removal of senescent cells from *Bub1b*-mutant progeroid mice mimicked the ageing phenotype-attenuating effects of p16\(^{INK4A}\) dysfunction (Baker et al., 2011). In a naturally aged non-progeroid mouse clearance of senescent cells extended the healthspan and blunted multiple age-related features, including glomerulosclerosis, cardiomyocyte hypertrophy, diminished cardiac stress tolerance, cataract formation and lipodystrophy as well as cancer (Baker et al., 2016) suggesting that elimination of senescent cells after they arise does not have the tumour-promoting side effects. These results spurred wide interest in exploring senolysis as a potential therapy to treat age-related symptoms and diseases.

Strategies targeting SASP of senescent cells are also under development. Several studies suggest that SASP inhibition can improve lifespan and healthspan. For instance the drug rapamycin which inhibits the mTOR pathway and effectively suppresses SASP (Laberge et al., 2015) has been shown to extend lifespan in a variety of model organisms (de Magalhães et al., 2012).

Inhibition of NF-\(\kappa\)B signalling (the main transcription factor regulating the SASP) both genetically and pharmacologically, has been shown to prevent age-related deterioration in progeroid mouse models (Osorio et al., 2012). Telomerase-based anti-ageing therapies are also being developed
and a natural product-derived telomerase activator called TA-65 has already been made available. One study reported that taking TA-65 may result in the decline of senescent immune system cells in patients (Harley et al., 2011). TA-65 can also increase telomerase levels in some mouse tissues and was reported to improve health indicators in mice but it did not increase mean or maximum lifespan (de Jesus et al., 2011).

7.4 Senolytics as adjuvants of anticancer therapy

The findings that senescent cells can fuel malignant phenotypes and tumour growth suggests that senotherapies aimed at their removal may be used as a potential supplementary anti-cancer therapy. The reactivation of senescence in cancer and the subsequent clearance of senescent cells are suggested as therapeutic intervention in the eradication of cancer (Malavolta et al., 2018). It has been shown that senescent cells, particularly those that senesce in response to DNA-damaging radiation or chemotherapeutic agents, secrete factors that can protect neighbouring tumour cells from being killed by those same chemotherapeutic agents (Gilbert and Hemann, 2010; Sun et al., 2012). These chemoprotective SASP factors include WNT16B, IL-6, and TIMP-1 (tissue inhibitor of metalloproteinases-1). In contrast, at least some SASP components can be chemosensitizing. For example, global suppression of the SASP (through NF-κB inhibition) promoted resistance to chemotherapy in a mouse lymphoma model (Chien et al., 2011).

Existing inhibitors of prosurvival pathways used in cancer therapy may have utility to block cell death-resistance pathways promoting senescent cell killing by inducing apoptosis and could be even more effective for this use because senescent cells do not proliferate. Therefore, no strong selective pressure for development of drug resistance can occur. An example is a cancer drug dasatinib which inhibits a broad spectrum of kinases and in combination with a plant flavonoid quercetin has been shown to have a pronounced senolytic effect in vitro (Schafer et al., 2017). Several natural compounds that activate Nrf2 (nuclear factor erythroid-derived 2-related factor 2) pathway, which is involved in complex cytoprotective responses, have been shown to induce cell death or senescence in cancer. Senolytic activity shown by some Nrf2-activating compounds could be used to target senescent cancer cells (particularly in aged immune-depressed organisms) that escape immunosurveillance. The examples of such bioactive compounds are tocotrienols, curcumin, epigallocatechin gallate, quercetin, genistein, resveratrol, silybin, allicin, berberine, piperlongumine, fisetin and others (Malavolta et al., 2018). Incorporation of these compounds into the therapeutic scheme still needs to be carefully tested.
8 Conclusion

Senescence in the body can be induced by various stimuli, can even arise from cell fusion, which is necessary for embryo development. Another beneficial effect is the suppression of tumour development, which however does not apply to chronically present cancer. On the other side, the long-term presence of senescent cells in tissues has negative effects associated with development of age-related diseases. By studying the mechanisms of formation and maintenance of senescent cell phenotype we can find ways to eliminate them from the body with benefits to rejuvenate organism by suppression of age-associated diseases or to improve the current anticancer strategies. New group of compounds have been developed to specifically kill senescent cells by reactivating apoptosis. The specific (energetic) metabolism of senescent cells can serve as target as well. Another approach is to utilize function of immune system to specifically remove senescent cells.

In animal models, senolytics seem to be effective against ageing and the diseases associated with them. Effective agents are anti-apoptotic agents or antioxidant protection such as piperlongumine. others are effective in the fight against cancer by stopping the cell cycle of tumour cells and inducing senescence. Testing these substances for senescent cells and monitoring their effect is a promising option to eliminate age-related disease and prolong the viability of the organism.
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