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REVIEWS

To Find and Destroy: Identification and Elimination of Senescent Cells

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Abstract—"Our oldness is a disease that has to be treated like any other one,"—this statement formulated about a hundred years ago seems to be of current interest in the context of modern investigations. Recently, it has been established that accumulation of senescent cells in various organs and tissues is one of the main causes for the organismal aging, as well as for the progression of multiple age-related pathologies. On the one hand, this observation brings us one step closer to the desired goal—reversal or slowing down of aging. On the other hand, this raises a number of complicated questions: in what essentially lies the difference between senescent and normal cells and how they can be identified; whether senescent cells can be eliminated from the body and can this elimination stop/reverse aging; can such a targeted removal of senescent cells be accompanied by negative consequences, in particular, by an increase in the cancer incidence? This review summarizes the main features of senescent cells, surveys the existing approaches of targeted elimination of senescent cells in vivo, and highlights their advantages and disadvantages.

Keywords: cell senescence, identification of senescent cells, targeted elimination of senescent cells

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INTRODUCTION

Various geriatric diseases of wide range, including Alzheimer's and Parkinson's diseases, sarcopenia, osteoarthritis, atherosclerosis, diabetes mellitus, obeseness, etc., have a common risk factor: senescence [1]. In recent years there is an increasing evidence that age-related physiologic failures of organs and tissues the same as progression of the above-mentioned age-associated diseases are linked with the accumulation of senescent cells in a body [2]. At the same time, it was found that cell senescence (CS) is an inherent element of the normal body development since an embryonic stage [2, 3]. What is the way to involve the same phenomenon into two processes of opposite senses: healthy development and aging of an organism? First of all, we should understand what CS is. The main point of the phenomenon is that damaged cells irreversibly loose proliferative activity, while retaining their metabolic activity [4]. On the one hand, CS prevents expansion of damaged cells and thus serves as an effective antitumor mechanism [5, 6]. On the other hand, as the functional activity of senescent cells is impaired, their accumulation may cause organ and tissue dysfunctions [7]. A young healthy organism avoids these adverse effects, because cells of its immune system eliminate the senescent cells from the population [8]. However, with aging the efficacy of all systems, including the immune one, is decreased, and this leads to the accumulation of senescent cells within the body and can in turn promote both progression of various age-associated diseases and aging of the organism [9].

In spite of the established view on irreversibility of the aging process, scientific community more and more frequently addresses the idea that aging is flexible, and a certain delicate modulation can reverse it [10, 11]. It is clear that a targeted modulation of such a complex process as aging implies the detailed examination of the mechanisms mediating its progress. First of all, we should reveal the cause–effect relations between aging of a cell and of an organism. In particular, it is necessary to understand whether the targeted elimination of the senescent cells from the body can prevent the aging progression or preclude consequences of aging that are already manifested. To solve this issue, a targeted therapy eliminating senescent cells and not affecting normal cells of the microenvironment should be developed. Revealing of features exclusive to senescent cells, which will then ultimately identify such cells in vivo, will become the molecular base of the selective elimination of the senescent cells. In this context, this review contains two complementary parts: the first one surveys the main CS progress-associated alterations that may facilitate identification of senescent cells, and the second one describes the current strategies in targeted elimination of the senescent cells in vivo.

TO FIND THE SENESCENT CELLS

The idea of cell senescence (CS) was formulated half a century ago [12]. However, the term "CS" has been essentially modified since then. For example, now we consider not only a replicative senescence triggered by shortening of telomeres but a premature one, too. Premature CS can occur independently of the telomere length in response to oncogene overexpression, treatment with chemotherapeutic agents, and various stress factors, such as UV- and γ-irradiation, heat shock, and oxidative stress [13–17]. According to the modern concepts, the term "CS" implies a proliferation block of metabolically active cells in response to the DNA damage; in the case of replicative senescence, damages are associated with the telomere regions, while in the case of premature senescence, they have stochastic locations [4]. The main signs of CS are the same in various CS types and in various types of proliferating cells, in spite of differences in the nature of their inductors [4]. It should be emphasized that CS is a dynamic process, and most of associated alterations evolve in time, complicating the revealing of their ultimate cause–effect relationships. Within the frames of the review, we shall headline the intracellular changes, nominally distributed to the nuclear and cytoplasmic events, and to provide a special description of alterations in cell membrane and in secretory profile of the senescent cells (Fig. 1).

1. Events in a Cell Nucleus

1.1. DNA damage. DNA damages, namely, double breaks, whose occurrence activates DNA damage response (DDR), present one of the most important characteristics of the senescent cells. In most types of cells, DDR begins from the activation of three sensor kinases: ATM (Ataxia telangiectasia mutated), ATR (ATM and Rad-related), and DNA-PK (DNA-dependent protein kinase) that recognize DNA damages and phosphorylate the downstream members of this signal response [18, 19]. Phosphorylation of H2AX causes formation of γ-H2AX foci, essential to the following recruitment and anchoring of other DDR members, such as MRE11/RAD50/NBS1 (MRN-complex), MDC1, BRCA1, RAD51, and 53BP1 in the sites of the DNA damage [18]. It is of importance that DDR should be continuously active to establish an irreversible cell cycle arrest in the senescent cells, and the result of this condition is the formation of large complexes referred as DNA-SCARS (DNA segments with chromatin alterations reinforcing senescence) or TIF (telomere dysfunction-induced foci) in the case of the localization of a damage in telomere regions [20, 21].

1.2. Senescence associated growth arrest, SAGA. According to classical definition, CS is characterized by the irreversible cell cycle arrest in the G1 phase [4]. At the molecular level, cycle arrest in senescent cells is mediated by activation of signaling pathways p53/$p21^{Waf1/Cip1}$/Rb and/or $p16^{INK4a}$/Rb. It is well recognized that $p21^{Waf1/Cip1}$ and $p16^{INK4a}$ inhibit the activity of cyclin-dependent kinases, causing the hypophosphorylation of retinoblastoma (Rb) protein, and preventing DNA replication [13, 14, 16]. Information on the important role of the G2/M arrest in realization of the aging program is more and more abundant [22, 23]. For example, damage of DNA and subsequent activation of p53 in G2 cells mediate the cyclin B1 retention in the nucleus and induce senescence [24]. It could be expected that the G2/M arrest serves as some protection mechanism preventing the formation of polyploid cells in the case when damaged cells were not arrested in the G1 phase and entered the S phase [24].

1.3. Epigenetic modifications of DNA and histones. Selective DNA methylation underlies differential gene expression and is the best characterized epigenetic modification, which is essentially a covalent attachment of methyl groups to the cytosine residues in the CpG islands. Age-dependent inhibition of DNA methylation was first described in 1967 [25]. Today, global genomic hypomethylation is considered as a common characteristic of replicative and premature CS [26]. It was shown that hypomethylation mainly occurs in regions of repetitious sequences and may cause reactivation of retrotransposons in the course of aging [27]. In addition, in terms of aging we usually speak about local hypermethylation of separate DNA regions that inhibits transcription of certain genes, for example, target genes of the Polycomb family proteins [26]. It is worth mentioning that along with DNA hypermethylation, CS is associated with hypermethylation of H3 histone by lysine residues in positions 9 and 27 (H3K9me3 and H3K27me3) [28].

The occurrence of specific senescence-associated heterochromatin foci (SAHF) is one more sign of senescent cells [4, 29]. SAHF consist of the special facultative heterochromatin, different from other facultative heterochromatin regions (inactive X chromosome in female cells) and from constitutive heterochromatin (centromeres and telomeres) [30]. At the molecular level the formation of such foci is mediated by recruiting of various proteins, for example, retinoblastoma protein, to the promoters of E2F-controlled genes playing the key role in the G1- to the S-phase transition and controlling the cell proliferation [30]. Condensation of chromatin and inhibition of the E2F target gene expression are the consequences of these molecular events. It is noteworthy that accumulation of the foci requires stable inhibition of the E2F target genes expression, and hence, formation of SAHF is inherent only to the senescent cells and cannot be found in the reversibly arrested quiescent cells.

1.4. Alterations in the morphology of nuclear structures. In the course of CS, the most nuclear structures, including nuclear envelope, matrix, nucleolus, and nuclear bodies (PML bodies) undergo certain alterations. First, a nucleus size in senescent cells

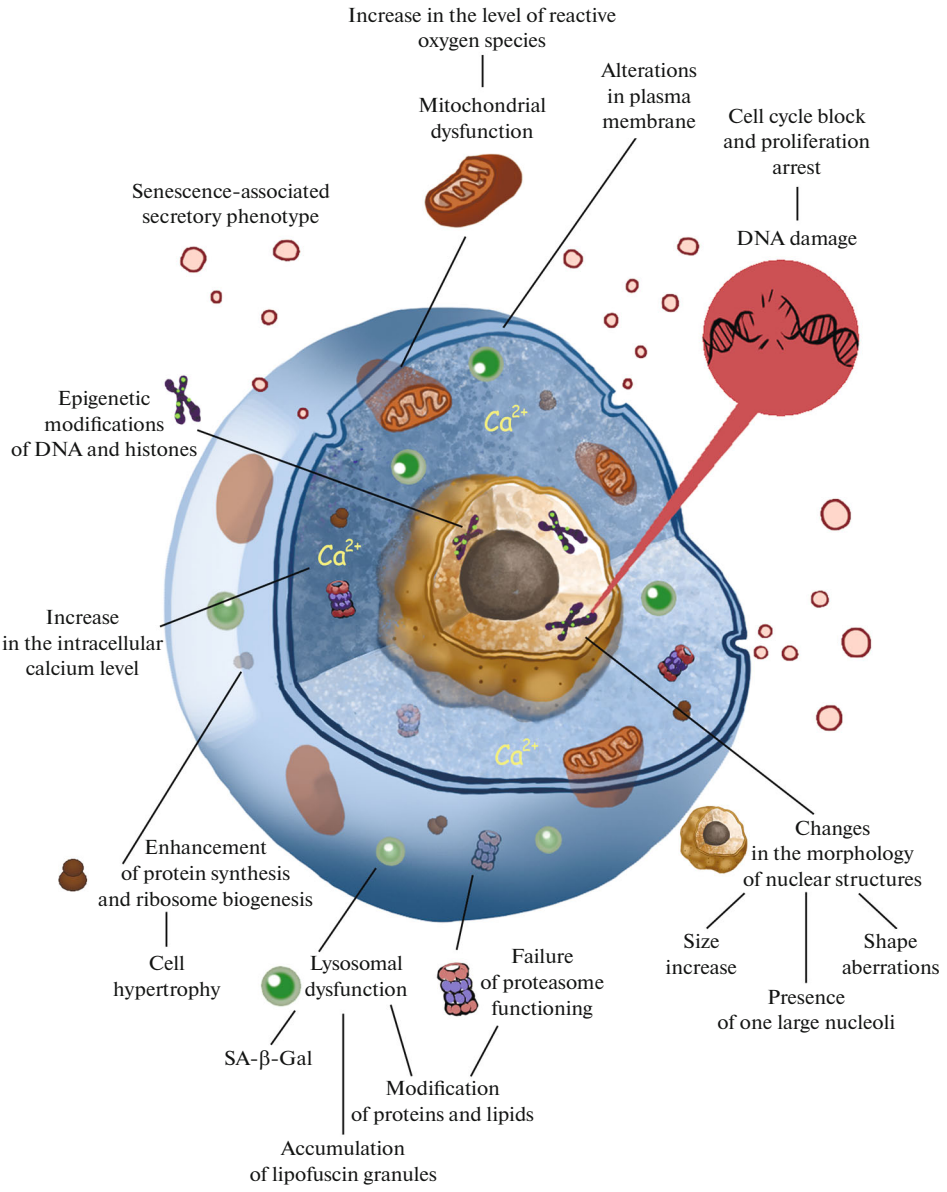

Fig. 1. Attributes of senescent cells. The scheme presents main changes accompanying the CS progression.

becomes essentially greater [29]. Second, senescent cells can be characterized by the presence of one large nucleolus, while normal proliferating cells usually contain many small nucleoli [29]. Third, the expression of the nuclear matrix proteins along with their ability to bind to telomeric DNA regions is altered, and this may affect progression of the cell cycle and mediate its arrest in senescent cells [31]. Fourth, PML bodies can affect cell movement through cell cycle and CS [29]. The integrity of the PML bodies is shown to be necessary for acetylation and stabilization of p53, and for the progression of the oncogene-induced senescence [32]. Moreover, overexpression of the PML protein, a key component of PML bodies, can initiate senescence in the p53-dependent manner. And, finally, it was found that in the course of the CS, a fraction of cells with impaired nucleus shape increases [33–35]. Specifically, it was shown that in nuclei of senescent cells spatial alterations of nucleus envelope coincide with the lamina A redistribution and local decrease in the protein mobility [35].

1.5. Senescence-associated non-coding RNAs. According to the current knowledge, various non-coding RNAs (ncRNAs) are involved in the CS regulation [36]. It was shown that ncRNAs UCA1, MEG3, and ANRIL are involved in the enhancement of the expression of proteins p53, $p21^{Waf1/Cip1}$ and $p16^{INK4a}$ responsible for establishment of the cycle arrest in the senescent cells [36]. Moreover, let-7, miR-34, miR-519, and lincRNA-p21 can suppress synthesis of the prolif-

eration mediating proteins, thus promoting the CS progression [36]. Additionally, the authors found ncRNAs, affecting the mitochondria functioning and telomere integrity in course of senescence.

2. Events in the Cytoplasm

2.1. Alterations in cell morphology, modulation of ribosome functioning and alterations in protein synthesis. It is known that the senescent cells are flattened, abundantly vacuolated, and have essentially greater sizes [4, 16]. In the course of senescence proliferation is blocked, while the cell growth remains unchanged, and this causes their hypertrophy [37]. An important result of such an "unbalanced growth" is accumulation of protein macromolecules within the cell that promotes an increase of the osmotic pressure and intake of water and/or ions. At the molecular level, cell hypertrophy during senescence is mediated by two interrelated processes: modulation of the protein synthesis and ribosome biogenesis [38].

mTOR signaling pathway plays a key role in the regulation of protein synthesis [30]. Ribosome S6-kinase, the main mTOR target, is known to induce mRNA translation via phosphorylation of the ribosome protein S6 and control of various factors of translation initiation, for example, of eukaryotic factor 4B (elF4B). Moreover, inhibiting the specific elF4E-binding protein (4E-BP), mTOR releases factor elF4E that also triggers translation. It is important that the activity of the mTOR pathway considerably increases in senescent cells, while inhibition of this pathway can prevent the CS progress [30, 39].

Besides, mTOR takes part in the synthesis of ribosome components, including transcription and processing of pre-RNA, expression of ribosome proteins, and synthesis of 5S rRNA [38]. More and more papers published nowadays indicate that some ribosome proteins, specifically, ribonucleoprotein complex 5S, can activate p53 and promote CS [40]. However, specific alterations in ribosome biogenesis process depend on the CS form. For example, the oncogene-induced senescence accelerates the rRNA transcription, while the replicative senescence delays the rRNA processing [40]. In both cases, it causes accumulation of ribosome proteins not bound with the ribosomes that interact with MDM2—down-regulator of p53—and promote p53 activation and initiation of CS.

2.2. Dysfunction of the degradation system. A finely adjusted process of intracellular degradation is presented in a cell by two systems, lysosomal and proteasomal. According to the current data, activity of both systems is significantly lowered in the senescent cells [41, 42].

Disturbance of lysosomal degradation of damaged organelles and macromolecules results in accumulation of numerous cytoplasmic aggregates. These aggregates, reacting with various cell components, form fluorescent lipofuscin granules involved into generation of reactive oxygen species (ROS) [43]. Yet another consequence of the lysosome dysfunction is deteriorated autophagy [44]. Autophagy is the most significant catabolic process responsible for utilization of organelles and macromolecules in specific cell compartments formed by fusion of autophagosomes and lysosomes. Lysosome dysfunction accompanying CS leads to deterioration of the autophagy cycle and accumulation of damaged molecules [45].

Besides, it was found that CS is associated with a decrease in the activity of 26S-proteasomes [41]. It is supposed that functional degradation of proteasomes may lead to a temporary arrest of degradation and accumulation of various proteins, including p53, resulting in initiation of the p53/$p21^{Waf1/Cip1}$-mediated arrest of cell cycle. Partial inhibition of proteasomes promotes induction of premature senescence in young cells [46].

2.3. Increase in the senescence-associated β-galactosidase activity (SA-β-Gal). Increase in β-galactosidase activity is a recognized senescence marker in vitro and in vivo [4, 47]. β-Galactosidase is known as the key lysosome enzyme cleaving lipids and glycoproteins; its activity can be detected in normal cells at pH 4.0. However, β-galactosidase activity of senescent cells increases essentially, and it can be found at a pH 6.0 [47]. In such a case, β-galactosidase is referred to as senescence-associated β-galactosidase (SA-β-Gal).

2.4. Mitochondrial changes and increased ROS level. It is known that senescent cells contain a large number of mitochondria and that their functioning in the course of CS is impaired [48]. Most recognized CS-associated alterations of mitochondria include: oxidative modifications of some mitochondrial enzymes, such as aconitase, adenine nucleotide translocator, and cytochrome-*c*-oxidase; peroxidation of phospholipids present in a mitochondrial membrane; decreased mitochondria membrane potential, and accumulation of the mitochondrial DNA (mtDNA) damages [48, 49]. Accumulation of oxidative damages and mtDNA mutations in the course of CS may be mediated by the impairments in the mtDNA reparation system [48].

Alterations in the mitochondrial physiology may cause an increase in ROS formation in senescent cells [48]. Now we know that ROS are involved both into the initiation and into the progress of replicative, oncogene- and stress-induced senescence [37, 49]. On the one hand, it was shown that ROS promote DNA breaks, thus initiating DDR activation, cell cycle arrest and senescence. On the other hand, the main DDR effectors mediate ROS generation, finally leading to the formation of the so-called positive feedback loop [49].

2.5. Increase in the intracellular calcium content. CS process is associated with alterations in cell ion homeostasis, especially in cell calcium control. For example, an increased cytosol Ca^{2+} at replicative, oncogene- and stress-induced types of senescence is a common opinion [50–54]. Examination of molecular

mechanisms mediating calcium participation in the CS control showed that an increase in the intracellular Ca^{2+} concentration leads to accumulation of Ca^{2+} in mitochondria and, as a consequence, to the impairment of normal mitochondrial metabolism and increased generation of ROS [52]. It is of note that knockout of genes encoding proteins of mitochondrial calcium channels makes it possible to prevent the CS progress [52].

Interrelation between the intracellular calcium and the activity of the p53 transcription factor is considered as an alternative mechanism. It was found that alterations in the cytosol calcium concentration can control interactions between calcium-binding proteins and p53 and increase the p53 transcription activity and stability [55]. According to our data, increase in intracellular calcium concentration can control the CS initiation and progression due to induction of DDR and activity of the p53/$p21^{Waf1/Cip1}$/Rb pathway, while calcium chelation can prevent the CS progress [54].

2.6. Protein and lipid modification. Malfunctions of various organelles taking place at CS lead to different chemical modifications (oxidation, glycation, bridging, etc.) in cell macromolecules [56]. For example, oxidation of polyunsaturated fatty acids in cell membranes and organelles is a consequence of an increase in the endogenous ROS level [57]. Oxidized lipids can, in turn, damage DNA and promote senescence. Protein carbonylation is another CS-associated modification [58]. It was found that the CS progression both in vivo and in vitro is followed by an essential enhancement of carbonylation in such a way that finally every third protein becomes carbonylated. Carbonylation leads to suppression or total loss of protein catalytic function and formation of high molecular cell toxic protein aggregates. Under normal conditions, carbonylation tags damaged or aberrant proteins and directs them to the proteasome degradation. However, a considerable increase in carbonylated proteins in the course of senescence can compromise proteasome functioning [59]. So, damaging effects of the above-mentioned modifications in the senescent cells occur because modified molecules are accumulated in these cells much faster than degrade, though all the described modifications can occur in healthy cells as well.

3. Alterations in Plasma Membrane of the Senescent Cells

CS progress is followed not only by various nuclear and cytoplasmic alterations but also by modifications in the plasma membrane (PM). In senescent cells, lipid composition of PMs, as well as their biophysical, chemical, and electrical properties, differs from the corresponding parameters of normal cell membranes. In particular, PMs of senescent cells are more rigid and less elastic [60–62]. Moreover, replicative senescence of keratinocytes is associated with a decrease in the level of sphingomyelin-rich domains [63]. Examination of fibroblast senescence demonstrated a decrease in the number of caveoli and, respectively, in the amount of caveolin at the PM surface [64]. Along with changes in PM properties, expression of the PM-associated proteins also changes; for example, the expression of ICAM-1 increases [65]. It was recently found that in the course of the fibroblasts senescence, expression of vimentin modified with malondialdehyde by cysteine 328 (C328) increases [66]. Candidate identifiers of senescent cells, in form of PM-associated proteins expressed by these cells, include: DEP, NTAL, EBP50, STX4, VAMP3, ARMX3, B2MG, LANCL1, VPS26A, and PLD3 [67].

It is worth mentioning that the data on CS and PM modification are insufficient nowadays. Most of these studies are rather descriptive and not specifically focused on the mechanisms mediating changes observed in the course of CS. Japan scientists Kono et al. formulated an interesting hypothesis. According to the unpublished data of these authors, a damage of PM can initiate a cycle arrest in mammalian cells. For example, they found an interrelation between a "scar" formation in the point of a membrane damage and replicative cell senescence and formulated a new concept of plasma membrane damage-dependent senescence.

4. Senescence-Associated Secretory Profile

For a long time, CS was studied only in view of the nuclear-cytoplasmic modifications. Later, scientists noticed that as the senescence develops, the profile of cell-secreted factors alters [68–71]. For example, the production of cytokines, chemokines, and matrix metal proteases increases notably; in addition, the production of most growth factors changes qualitatively and quantitatively [68]. A profile of molecules secreted by the senescent cells was termed as Senescence Messaging Secretome (SMS), or Senescence-Associated Secretory Phenotype (SASP) [68, 70]. It is expected that molecules secreted by senescent cells, upon entering the extracellular space, can affect the adjacent cells via autocrine/paracrine ways [68–70]. SASP is supposed to mediate the engagement of senescent cells in various processes, such as reparation, senescence propagation, immune clearance, embryogenesis, and tumorogenesis [3, 72–75].

DESTROY SENESCENT CELLS

The previous section shows that the CS progression is associated with a great number of modifications involving virtually all intracellular systems. It should seem that at such a wide range of alterations, picking of an appropriate marker for elimination of senescent cells by targeted therapy should not be difficult. However, unambiguous identification of senescent cells in vivo presents a great number of challenges. It is noteworthy that not all of the above-mentioned markers

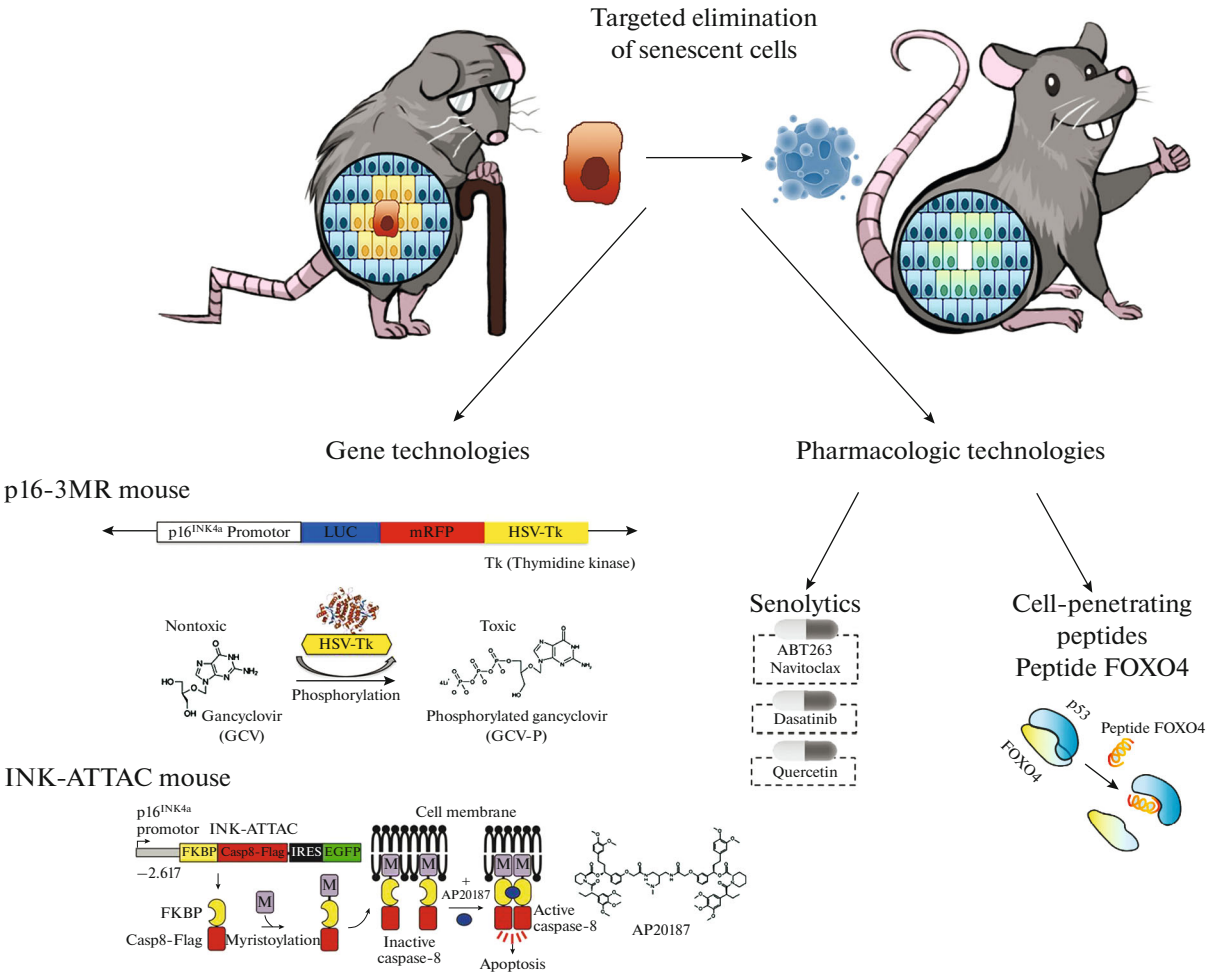

Fig. 2. Approaches to targeted elimination of senescent cells from a body.

are universal and not all of them are peculiar to senescent cells. For example, all senescent cells are irreversibly arrested but not all of them express $p21^{Waf1/Cip1}$ or $p16^{INK4a}$ [13, 76–78]. Interestingly, SA-β-Gal, the most common and quiescent marker of senescent cells, not detectable in quiescent or terminally differentiated cells, can be found in cells forming a dense monolayer [79]. The specific list of factors secreted by senescent cells depends significantly on a cell type and on a senescence inducer [68, 80–82]. DNA condensation following the cell cycle arrest occurs mainly in cells fraction expressing $p16^{INK4a}$ [83]. Thus, no specific marker enabling the robust identification of senescent cells in vivo exists nowadays. Nevertheless, targeted elimination of senescent cells from a body is considered as a promising strategy of life prolongation [11, 84, 85]. Further, we describe the most common markers used for identification of the senescent cells in vivo and approaches for targeted elimination of such cells based on these markers (Fig. 2).

Historically, SA-β-Gal was the first attribute enabling identification of senescent cells in vivo. Age-correlated activity of the enzyme was found in biopsy specimens of human skin [86]. However, as the detection of the SA-β-Gal activity requires a preliminary fixation and staining of cell or tissue samples, the use of this marker for targeted elimination of the senescent cells from an organism does not seem possible [87].

Expression of cyclin-dependent kinase inhibitor $p16^{INK4a}$ is recognized today as a key attribute of senescent cells [88]. The presence of $p16^{INK4a}$ in senescent cells provided a basis for the development of the first mouse model INK-ATTAC enabling targeted elimination of senescent cells from a senescent body [89]. The main idea of the model is the establishment of a transgenic mouse line: a Fabp4 promotor that mediates the expression of a special fused protein containing an inactive caspase-8 is replaced in the cells of these animals with a fragment of the $p16^{INK4a}$ gene promoter (Fig. 2). In such a case, initiation of the $p16^{INK4a}$ expression is immediately followed by the expression of inactive caspase-8. Subsequent application of a special agent AP20187 causes activation of

caspase-8 and hence targeted elimination of the $p16^{INK4a}$-positive cells from the body.

More recently, one more mouse model p16-3MR enabling targeted elimination of senescent cells in vivo was developed on the basis of the $p16^{INK4a}$ expression [73]. In this model, a sequence encoding a three-domain fused protein containing a truncated thymidine kinase of the simple herpes type 1 (HSV-TK) is placed under the control of the *p16^INK4a^* gene promoter (Fig. 2). Application of a special anti-herpes agent ganciclovir, whose affinity to the virus enzyme is two orders of magnitude higher than that to a host-cell thymidine kinase, triggers the death of cells expressing $p16^{INK4a}$. The molecular mechanism of their death relies on the ability of HSV-TK to phosphorylate ganciclovir, which, due to the activity of cell kinases, is converted into triphosphate and is incorporated into a synthesized DNA chain, thus terminating the replication process.

Targeted elimination of $p16^{INK4a}$-positive cells from a body gave quite striking results. It was found that elimination of senescent cells prevents the progression of age-associated pathologies in young animals and inhibits it in senescent organisms. For example, an increase in muscle fiber diameter, endurance rise, and thickening of the subcutaneous fat layer was shown, as well as a delayed occurrence of such senile changes as spinal curvature and cataract [89]. In addition, it was found that a local elimination of senescent cells prevents the progression of posttraumatic arthritis and creates a regeneration-favorable microenvironment [90]. Thus, elimination of senescent cells in mice favors their health improvement and a prolongation of active life [84, 89, 90].

The data obtained with the use of genetic constructs inspired researchers to develop alternate ways of elimination of senescent cells in the organism by means of pharmaceutical agents [85, 91–94]. Death tolerance of senescent cells is one of the key consequence of CS. Analysis of signaling pathways ensuring viability of senescent cells and, respectively, their resistance to apoptosis created the basis for the development of a special class of substances referred to as senolytic drugs [92]. These substances include, for example, agents ABT-263 (Navitoclax) and ABT-737, specific inhibitors of anti-apoptotic proteins BCL-2 and BCL-XL that selectively kill senescent cells by the induction of apoptosis [93, 94]. It turned out that oral administration of ABT263 in old or irradiated mice leads to an effective elimination of senescent cells, including senescent hematopoietic stem cells of bone marrow, and thus promotes rejuvenation of the organism, improvement of the cardio-vascular system functioning, an increase in endurance, and a decrease in osteoporosis [91]. Moreover, other senolytic drugs, including kinase inhibitor from a Scr family, dasatinib, and inhibitor of the PI3K signaling pathway, quercetine, also exert positive effects [91, 92]. Combination of these substances prolongs an active life of mice, improves cardiac function, promotes rehabilitation of the carotid activity, inhibits osteoporosis, and prevents the loss of the intervertebral disc proteoglycans [92].

Triggering of controlled death of senescent cells by substances of another type, cell penetrating peptides, along with senolytic drugs, is now in the focus of drug developers. These peptides can arrest certain protein–protein interactions, without effects on the proteins themselves, thus enabling very fine adjustment of the specific signaling events. A key role of FOXO4 in survivability of senescent cells was recently disclosed [85]. These data were used to develop a special peptide preventing FOXO4–p53 interaction that leads to the p53 accumulation in senescent cells and triggers apoptosis. In vivo use of this FOXO4 peptide prevents hair loss and improves the performance status of $Xpd^{TTD/TTD}$ mice with premature senility syndrome [85].

Targeted elimination of senescent cells appears quite successful; nevertheless, each approach has its essential restrictions. For example, attempts to increase the lifespan of INK-ATTAC mice failed, though the quality of their life was improved [89]. Moreover, targeted elimination based on the $p16^{INK4a}$ expression failed in tissues, where CS developed independently of $p16^{INK4a}$, for example, in liver [89]. On mouse model p16-3MR it was found that elimination of senescent cells prevents normal wound healing [73]. Administration of senolytic drugs has its own disadvantages. A substance that can effectively eliminate senescent cells in all body tissues is not found yet. Dasatinib destroys senescent cells mainly in the adipose tissue, while quercetin is more effective against senescent cells of human umbilical cord [92]. When using Navitoclax and other inhibitors of the BCL family members, it should be born in mind that these proteins are expressed not only in senescent cells but in most other cell types of an organism. Therefore, these substances are not selective and their administration may exert adverse effects. For example, Navitolax can cause a severe thrombocytopenia [85]. Finally, one more problem remains unsolved: will targeted elimination of senescent cells increase the risk of tumor occurrence, given that CS is an effective antitumor mechanism? The results of targeted elimination of senescent cells entail the possibility of the directed modulation of the senescence process; however, the above-mentioned restrictions call for further studies in this area.

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