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Cellular aging and immunity

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Abstract

Science is constantly evolving and updated with current data on cell biology. The cellular aging phenomenon should be considered an evolutionary mechanism of the biological regulation of all living organisms. Factors initiating cellular aging are variable. Each cell type can respond differently to the activation factors of cellular aging. In recent decades, science has been supplemented with new data that provide a deeper understanding of cellular and molecular mechanisms of cellular aging and the formation of immune homeostasis. There is a real prospect of using effective means of its regulation. In recent years, scientists have come close to discovering the mechanisms of cellular aging. Factors and mechanisms of cell regeneration are more deeply revealed. Scientists are also better aware of the phylogeny and ontogenesis of immune processes and the role of immune factors in developing pathologies. Researchers are increasingly focusing on modern diagnostic methods and xenotherapy. However, the specific factors of immunoregulation and the interaction of microphages, macrophages, and lymphocytes with other body cells are not yet fully understood. Accordingly, this requires further in-depth study. This review reviews the current literature on cellular aging and its regulatory mechanisms. The authors also present the results of their research on the mechanisms of immune responses in reproductive pathology. They draw parallels with modern scientific theories and interpret research. We will also focus on the issues that need to be addressed in the near future for the progressive development of this field of science. Thus, the study of the mechanisms of cellular aging and the development of effective means of hay therapy today requires further painstaking work. Despite significant advances in preclinical studies, many questions remain about the practical use of the drugs. This is especially true in the medicine of oncology, neurology, and cardiology. Nevertheless, scientists will be able to use pharmacological agents to influence cell division, differentiation, and determination in the future. We also hope to have developed effective means of immunotherapy of diseases. The molecular mechanisms of cell aging and mediators involved in the mechanisms of cell aging and death are being studied in detail. The field of research contains countless fascinating studies that are sure to be discovered.

Keywords: cellular aging, phenotypes compose programmed cell death, immunity.

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1. Introduction

Since the 60s of the last century, scientists from different countries have attracted attention to studying the phenomenon of cellular aging. Initiated research by Hayflick and Moorhead (1961) allowed the researcher to rethink the biological cycle of cell division, the biology of cell differentiation, and aging (Hayflick & Moorhead, 1961).

The cellular aging phenomenon should be considered an evolutionary mechanism of the biological regulation of all living organisms. Factors initiating cellular aging are variable. Each cell type can respond differently to the activation factors of cellular aging (Di Micco et al., 2006; Kuilman et al., 2010; Passos et al., 2010; García-Prat et al., 2017; Mikuła-Pietrasik et al., 2021).

In recent decades, science has been supplemented with new data that provide a deeper understanding of cellular and molecular mechanisms of cellular aging and the formation of immune homeostasis, and there is a real prospect of using effective means of its regulation (Collado et al., 2007; Faget et al., 2019;Wang et al., 2020; Zhelavskyi et al., 2021).

The *aim of the review* of the study is the mechanisms of cellular aging and the associated state of innate immunity.

2. Literature review

Evolution aspect senescence

When considering why and how aging features might have evolved, the first thing to appreciate is that evolutionary logic does not support the idea that aging is due to a genetic program (Kirkwood & Melov, 2011). Although the

appeal of programmed aging is understandable, aging cannot be explained easily in this way, if at all (Kowald & Kirkwood, 2016). Biological old age is rarely attained in natural populations, and it, therefore, makes little sense to expect that evolution resulted in a process that is seldom seen. Furthermore, aging is deleterious to the individual, and natural selection should oppose rather than promote it. This conclusion, embodied in the "disposable soma" theory, is that aging results from the progressive accumulation of molecular and cellular damage due to evolved limitations in maintenance and repair. The same logic explains how the limitations on maintenance and repair would be tuned accordingly in different species, where the exposure to natural hazards is different. This is confirmed by evidence that cells from longer-lived species are generally better protected than cells from shorter-lived species (Kapahi et al., 1999; Teulière et al., 2020). It also explains why the age-incidence curves of damage-related diseases, such as cancer, scale with life span.

The fact that aging is not programmed in itself does not exclude the possibility that secondary consequences of the aging phenotype are the result of evolutionary programming. Damage is a ubiquitous threat to all living systems, and it is only to be expected that adaptations to deal with damage are fundamental. In multicellular organisms, the risk to the organism that arises from damage to individual cells is countered by regulated responses, particularly apoptosis and cellular senescence. The fundamental nature of cellular senescence as a damage response is also highlighted because associated genes are more highly conserved in mammals than would be expected by chance (Avelar et al., 2020). Damage also arises through wounding and infections, for which immune and inflammatory mechanisms provide protective responses. Although much interest currently focuses on the consequences that responses such as senescence, inflammation, and apoptosis may have for health at older ages, it is essential to appreciate that the origin of these responses needs to be sought in the benefit they confer at younger ages. The idea that evolution might have produced a good trait in youth but harmful in later life is known as "antagonistic pleiotropy" (Rose & Graves, 1989; Mitteldorf, 2019).

As we address how natural selection may have shaped the roles of cellular senescence, both of the above concepts–disposable soma and antagonistic pleiotropy–will be relevant. The concepts are complementary, not exclusive.

The most popular idea today is that cellular senescence is a mechanism that helps to suppress the development of cancer (Sager, 1991). According to this proposal, different types of stress and damage can lead to the generation of premalignant cells. Cellular senescence is the mechanism that senses this state and prevents further progression into a full-blown malignant state by permanently withdrawing the cell from the cell cycle.

Cellular senescence has been suggested to be an anticancer strategy. However, the senescence-associated secretory phenotype (SASP) has many negative consequences (shown in red), which are difficult to reconcile with this idea.

However, senescent cells have a property that is hard to reconcile with this picture. They display a senescence-associated secretory phenotype (SASP), consisting of a complex cocktail of chemokines, cytokines, growth factors, and proteases (Coppe et al., 2010; Baar et al., 2017). The SASP can have an extensive range of effects harmful on organismal health. The bystander effect, for instance, describes the fact that the paracrine action of the SASP can convert neighboring cells into new senescent cells, thus amplifying and spreading the original generation of senescent cells to affect non-damaged healthy cells. Although the composition of the SASP is somewhat heterogeneous, a general property is that it promotes inflammation (Franceschi & Campisi, 2014; Hernandez-Segura et al., 2018). Evolution theory would also predict that the link between the beneficial and detrimental effects is broken over evolutionary time, if possible. If the adverse effects that emerge as a consequence of the SASP can be separated (i.e., eliminated) from the positive, anti-tumorigenic effects of cellular senescence, we would expect this to happen since it would increase overall fitness. An obvious way to achieve this would be if senescent cells would not have the associated secretory phenotype. True, in this case, the beneficial effects of SASP on wound healing and tissue repair would also be affected, but this function could be delegated to other cell types (Acosta et al., 2008; Hinds & Pietruska, 2017), could also be converted into a purely intracellular signaling pathway.

Furthermore, there is an even more radical way to avoid the harmful effects of senescent cells and SASP while still providing an anticancer mechanism. That alternative is, of course, apoptosis. If a cell has suffered damage beyond repair, a cell can trigger a suicide program that results in the removal from the body without causing any inflammation. Apoptosis is an effective anticancer mechanism, and its deregulation is involved in many types of cancer (Pistritto et al., 2016). Apoptosis avoids the negative consequences of the SASP, but it also completely removes potentially premalignant cells instead of only rendering them post-mitotic. Apoptosis thus seems to be an anticancer strategy with much fewer problems than cellular senescence.

Factors influencing cellular senescence and immune cells

Cellular activation of apoptosis can be influenced by external and internal factors, various mitogenic signals, oncogenic activation, genotoxic and radiation stress, epigenetic changes, exposure to compounds and metabolites of oxidation, etc. (Ryu, 2014; Gorgoulis et al., 2019).

Scientists have proved that the most characteristic signs of cellular aging are a decrease in the size of telomeres, changes in chromosomes, and organoid dysfunction (Kuilman et al., 2010; Campisi, 2013).

Scientists share the evolutionary hypothesis of "antagonistic pleiotropy", which is that genes that affect more than one phenotypic outcome (pleiotropy) have a beneficial effect. Along with this, in the process of ontogenesis, genes trigger the mechanisms of aging.

From these positions in modern science, cell aging is considered a multistage process in a living organism, in which active changes occur, depending on the activation factors. Significant signs of aging are increased lysosomal activity, gene expression, chromatin remodeling, and DNA damage. The aging process is closely related to the secretory phenotype (SASP) (Coppé et al., 2010; Saleh et al., 2019).

The critical step was discovering that replicative senescence could be caused by the erosion of telomeres – the protective structures capping the ends of linear chromosomes. Telomere erosion occurs because of the inability of DNA polymerases to copy the very ends of the chromo-

somes. In germ cells and certain other specialized cell types, this limitation is overcome by the actions of telomerase. However, telomerase expression is switched off in fibroblasts and many other differentiated cell types. This suggested initially that senescence might be a programmed process in which the telomeres acted as a form of a molecular clock. Against the idea of a simple clock was the finding that replicative senescence exhibits marked heterogeneity in the division potential of the individual cells within the population and even in clonally derived sub-populations.

Furthermore, evolutionary considerations argued against aging being programmed and against the idea of it having a single molecular cause (Kirkwood, 2005). Theoretical modeling of the interactions between different candidate mechanisms of molecular aging (somatic mutations, mitochondrial dysfunction, telomere erosion) indicated that the observed heterogeneity in cell division potentials could be explained by multiple mechanisms acting together (Sozou & Kirkwood, 2001). This led to experimental tests of this possibility, which revealed that the random effects of mitochondrial mutations (resulting in intracellular oxidative stresses, to which telomeres are particularly susceptible) could account for the stochastic heterogeneity in telomere-driven replicative senescence. At the same time, it was found that not only telomere attrition but also a diverse range of damaging conditions (oxidative stress, DNA damage, radiation, or the expression of specific oncogenes), all of which involve DNA damage in some form, could trigger cellular senescence (CS) (Coppe et al., 2010; Davalos et al., 2010; Gorgoulis et al., 2019).

In response to the evidence that pathways leading to the establishment of senescence were proving to be more complex than previously envisaged, efforts were made to combine the power of bioinformatics and systems modeling with functional analysis of gene regulation. This revealed a dynamic feedback loop triggered by a DNA damage response (DDR) and which, after a delay of several days, locks the cell into an actively maintained state of "deep" cellular senescence. The essential feature of this discovery was that cellular senescence was a regulated process offering an alternative response to damage than the option of cellular "suicide", known as apoptosis. Although apoptosis provided a means to remove a damaged cell altogether, senescence allowed the cell to remain but permanently removed its potential for further division. Furthermore, many senescent cells are highly resistant to the induction of apoptosis (Childs et al., 2014).

At first sight, senescence and apoptosis could simply be seen as complementary alternatives to managing the potentially harmful effects of acquired cellular damage (Childs et al., 2014), especially concerning cancer risk. If the cell type is high-risk, such as a stem cell, apoptosis will eliminate it. However, some indicated that boosting apoptosis resulted in faster aging by accelerating the age-related loss in tissue cellularity (Barbé-Tuana et al., 2020). Therefore, it was conceivable that there might be circumstances in which the damaged cell would better be preserved while being locked out of the possibility of further division. However, of course, things are never as straightforward as they seem at first sight. It was already clear that apoptosis had more roles than protection against cancer. It is essential, for example, during morphogenesis and in managing the risk of autoimmune reactions during hematopoiesis. With cellular senescence, an important discovery was that most senescent cells undergo alteration to produce the "senescence-associated secretory phenotype" (SASP) (Coppe et al., 2010; Birch & Gil, 2020). The SASP involves the production of a complex array of chemokines, cytokines, growth factors, and proteases, which cause significant effects on neighboring cells, even including conversion into new senescent cells by way of the so-called "bystander" effect (Zheng et al., 2017; da Silva et al., 2022). Many of the impacts of the SASP appear to be negative: it promotes chronic inflammation, which is an essential contributor to a wide range of age-related diseases. However, as with apoptosis, senescent cells turn out to have beneficial effects on development, wound healing, and tissue repair (Demaria et al., 2015; Lewis-McDougall et al., 2019).

Given the complexity of what is known already about cellular senescence, it seems prudent to consider why and how natural selection might have shaped the roles of senescent cells in our bodies in the hope that this might also deliver new insights into future therapeutic possibilities.

Senescent cells are characterized by stable cell cycle arrest and morphological and metabolic changes, chromatin reorganization, altered gene expression, and acquisition of the SASP. It is important to note that not all senescent cells display all senescence biomarkers.

Lysosomes are organelles at the interplay between cell function and metabolism. In particular, they are signaling platforms to sense the nutritional state of the cells, which impacts overall energy metabolism and cell growth. Activation of mTORC1 on the lysosomal surface induces anabolic pathways while repressing catabolic processes such as autophagy (Klionsky et al., 2021). Therefore, the lysosome is a crucial organelle involved in fine-tuning the overall metabolic state of the cell. In oxidative stress-induced senescence, lysosomal dysfunction is associated with autophagy impairment (Sun et al., 2021). However, a previous report suggested that the acquisition of senescent phenotype is driven by autophagy (Narita & Narita, 2017).

A common outcome of the injury process is cellular senescence, an irreversible but stable form of cell cycle arrest, defined by an altered transcriptome, which occurs in proliferating cells when they have reached the end of their replicative lifespan or when subjected to stress. Senescent cells are often characterized by an enlarged and flattened shape (Cho et al., 2004; Zhelavskyi, 2008) and exhibit hallmarks of senescence, including DNA and chromatin alterations and gene expression changes (Martínez-Zamudio et al., 2017); mitochondrial dysfunction and the subsequent release of reactive oxygen species (ROS) (Yang et al., 2019); protein modifications and accumulation of lipofuscin granules, expression of SA-β-galactosidase, and the release of SASP factors (Hardeland, 2019). Moreover, while often found in injured tissues (Le Roux et al., 2015), senescent cells can also be present in uninjured organs, especially those that have previously experienced damage or disease (Chiche et al., 2020), and particularly in older individuals. Cellular senescence was first discovered in primary cell culture, where cells grown for long periods, akin to aging, reached a state where they could no longer replicate. For many years following this, senescence was solely viewed as a result of organismal aging; however, in the last decade, our understanding has dramatically evolved, indicating that cellular senescence can occur in response to a range of stimuli, including cellular damage, oxidative stress, oncogenic signaling, telomere attrition, ionizing radiation, and some cancer drugs and is even seen during development (Muñoz-Espín et

al., 2013; Wang et al., 2020). The transient induction of senescence followed by senescent cell elimination promotes tissue remodeling and regeneration (Zhelavskyi, 2009; Regulski, 2017; Rhinn et al., 2019).

Our many years of research (Zelavskyi, 2009; Zelavskyi et al., 2020; 2021) in animal immunopathology have also traced certain features in the formation of immune homeostasis. In the study of local immunity of the mammary gland of cows with the development of subclinical mastitis, changes in the reactivity of Oxygen-independent and Oxygen-dependent mechanisms of phagocytic cells of mammary gland secretion were found. This was especially evident in the intense increase in the percentage of reactive phagocytes in response to intraleukocyte lysozyme and lysosomal cationic proteins. The intracellular reactivity of myeloperoxidase and the metabolic reactivity of oxidases (NBT test) of phagocytic cells were also involved in the pathogenetic mechanisms of inflammation, which indicated the activation of antimicrobial potential of phagocytes in the development of subclinical inflammation. The percentage of induced apoptosis of epithelial cells and phagocytes increased. In serial experiments on the pyometra of cats and bitches, it was found that the formation of extracellular protective traps (NETs) also ends with the potentiation of apoptosis of phagocytes.

While coupling mTOR activity and autophagy contributes to SASP production in senescence (Gonçalves et al., 2021), the exact role of autophagy in senescence might depend on multiple factors. In mononuclear phagocytes, the role of autophagy in effector functions is more clearly defined. For instance, autophagy is part of the antimicrobial response to infection in macrophages (Visvikis et al., 2014). Furthermore, mTORC1 is essential for antigen presentation by dendritic cells and the subsequent regulation of T cell functions. Autophagy induction by inhibitors of the mammalian target of rapamycin enhances antigen presentation in dendritic cells (Jagannath et al., 2009).

In many cell types, including macrophages, iron transit through the lysosomal compartment is essential for oxidative phosphorylation and synthesis of tricarboxylic cycle metabolites in human macrophages (Zhelavskyi & Shunin, 2017; Labib et al., 2019). These observations suggest the existence of a lysosome–iron–mitochondria pathway in senescent cells that could be connected to SASP induction.

Identifying mechanisms underlying SASP induction and regulation is an active area of research. Compounds inducing senescence could be used therapeutically to complement. Recent reports have shown overlapping pathways between senescent cells and activated macrophages.

Assembly of the inflammasome complex initiates the release of IL-1β and preferentially occurs in myeloid cells with phagocytic activity, such as monocytes/macrophages (Onufe et al., 2019; Zhelavskyi et al., 2020). Inflammasome activation is a two-step process that includes priming and activation steps under transcriptional and posttranscriptional regulation, respectively (Schroder & Tschopp, 2010). In macrophages, the priming step triggers NF-κB and transcription of pro-inflammatory genes such as IL-1β after a pro-inflammatory stimulus such as LPS. The induction of NF-κB has been reported in senescent cells, even though the priming signal was either oxidative stress (Gu et al., 2020) or oncogenic activation (Jiang et al., 2017). A recent study has found de novo cohesin peaks enriched in SA genes during oncogene-induced senescence.

An example is de novo cohesin binding involved in new loop formation in the IL1B locus. Interestingly, this organization is commonly observed in oncogene-induced senescence cells (Olan & Narita, 2021). When it comes to the activation step, stimuli such as nigericin toxin, extracellular ATP, silica, or cholesterol crystals cause inflammasome activation in primed macrophages. This implicates NLRP3 polymerization with adaptor protein ASC, pro–caspase-1, and subsequent cleavage of pro–IL-1β and pro–IL-18, together with gasdermin-D, which facilitates the release of mature cytokines through lytic pores (Zhang et al., 2020). This is a well-established innate immune response in macrophages. Recent advances in phenotyping tissue macrophages could help us contextualize senescent cell phagocytosis and its potential link with SASP. Phagocytic macrophages showed blunted expression of Il1b and supported tissue homeostasis (Nicolás-Ávila et al., 2020). These results are exciting and suggest that a tissue-resident, nonphagocytic macrophage has a phenotype distinct from those that participate in the clearance of senescent or apoptotic cells. When considered in the context of senescence, one can thus wonder whether senescent cells display different activation states on them, an additional phenotype with anti-inflammatory effects.

Future studies to understand senescent cell phagocytosis should also consider the surface receptors involved in the regulation of phagocytosis. Phagocytic cells present nonopsonic receptors, apoptotic cell receptors, and opsonic phagocytic receptors, and the investigation of these in senescent cells could facilitate our understanding of the mechanisms underlying phagocytosis in senescent cells. CD36 plays an essential role in the clearance of apoptotic cells in vivo by macrophages (Kim & Nair, 2019; Zhelavskyi et al., 2020), so investigating its role in senescent cell phagocytosis could be of potential interest.

p53/p21$^{WAF1/CIP1}$ and p16^{INK4A}/pRB Pathway

Only cells with stable cell cycle arrest are considered senescent. Unlike a quiescent cell, a senescent cell will not reenter the cell cycle in response to any known physiological stimuli. Cell cycle arrest is mediated by the p53/p21^{CIP1} and p16$^{INK4A/pRb}$ tumor suppressor pathways described below. Expression of p16^{INK4A} is frequently observed in senescent cells, serving as a useful biomarker.

p53/p21$^{WAF1/CIP1}$ is activated in response to DNA damage caused by telomere attrition and oxidative or oncogenic stress. Activation of p53 is dependent on various post-translational modifications such as phosphorylation, methylation, acetylation, sumoylation, ubiquitination, and neddylation (Kruse et al., 2021). As p53 performs different functions within a cell, it is regulated at multiple levels by different factors. MDM2, an E3 ubiquitin ligase, regulates the levels of p53 in conjunction with MDM4. Interaction of p53 with FOXO4 during cellular senescence plays a crucial role in regulating its transcriptional activity and localization (Baar, 2017).

p21$^{WAF1/CIP1}$, a 21 KDa protein encoded by the CDKN1A gene, is a member of the Cip/Kip family of CDKIs in addition to p27 and p57. It can inactivate all CDKs, thereby inhibiting cell cycle progression (Wang et al., 2021). It inhibits the kinase activity of cyclin-CDK complexes by interacting with cyclins through the two cyclin binding motifs (Cy1 and Cy2). This leads to inhibition of phosphorylation of the RB family of proteins and subsequent association with E2Fs and formation of the DREAM

complex, thereby leading to a cell cycle arrest (Leontieva & Blagosklonny, 2017; Ferreira et al., 2020).

Induction of $p21^{WAF1/CIP1}$ is crucial for initiating senescence-mediated growth arrest by different stimuli (Hernandez-Segura et al., 2018). Upregulation of $p21^{WAF1/CIP1}$ plays a crucial role in developmental senescence as mice lacking it show defects in embryonic senescence. Developmental senescence is a transient programmed cellular senescence that occurs during mammalian embryonic development (Di Micco et al., 2021).

p53 dependent and independent mechanisms are also regulated at the post-translational level. Newly synthesized $p21^{WAF1/CIP1}$ is stabilized by WISp39, an Hsp90 binding tetratricopeptide repeat protein that prevents its proteasome-mediated degradation. $p21^{WAF1/CIP1}$, a 21 KDa protein encoded by the CDKN1A gene, is a member of the Cip/Kip family of CDKIs in addition to p27 and p57. It is capable of inactivating all CDKs, thereby inhibiting cell cycle progression. It inhibits the kinase activity of cyclin-CDK complexes by interacting with cyclins through the two cyclin binding motifs (Cy1 and Cy2). $p21^{WAF1/CIP1}$ plays a conflicting dual role in cell cycle progression depending on its expression level (Liu et al., 2020). High levels of $p21^{WAF1/CIP1}$ inhibit the kinase activity of cyclinD/CDK4,6 complexes leading to inhibition of cell cycle progression, whereas low levels of $p21^{WAF1/CIP1}$ act as an assembly factor for cyclinD/CDK4,6 complex and promote its activation resulting in cell cycle progression (Mohs et al., 2021). $p21^{WAF1/CIP1}$ can also be activated by p53 independent mechanisms by other stimulators such as nuclear receptors, including androgen, vitamin D, and retinoid receptors. Members of the Krüppel- like factor (KLF) transcription factor (TF) family can activate the *CDKN1A* gene by cooperating with p300-CREBBP (Gu et al., 2011).

The expression of p16(INK4A) was significantly increased, whereas CDK4, CDK6, and p-Rb expression levels were decreased in the MSCs from both untreated and treated SLE patients. Knockdown of p16(INK4A) expression reversed the senescent features of MSCs and upregulated TGF-β expression. In vitro, when purified CD4+ T cells were incubated with p16(INK4A)-silenced SLE MSCs, the percentage of regulatory T cells was significantly increased. Further, we have found that p16(INK4A) promotes MSC senescence via suppressing the extracellular signal-regulated kinase (ERK) pathway. p16(INK4A) knockdown upregulated ERK1/2 activation. Our results demonstrated that MSCs from SLE patients were senescent and that p16 (INK4A) plays an essential role in inhibiting ERK1/2 activation (Gu et al., 2012).

Senescent cells influence macrophages and senotherapy

A possible explanation for $p16^{INK4a}$ macrophages in tumors or aged tissues is the induction of paracrine senescence in these macrophages. Nevertheless, it seems that tissue macrophages, in certain conditions, can have high $p16^{Ink4a}$ expression, but this should not necessarily be interpreted as senescence. Using the same experimental setup, the authors had previously reported that senescent cells preferentially attract macrophages characterized by $p16^{Ink4a}$ gene expression and β-galactosidase activity (Hall et al., 2016), raising the possibility of an interplay between senescent cells and macrophages that contributes to the shared high $p16^{Ink4a}$ expression in each cell type.

In recent decades, science has been enriched with the latest knowledge about the mechanisms of cell determination. According to the new scientists began to understand gene expression in the regulation of cell division and differentiation. This opened behind the scenes of the unknown and gave impetus to developing methods for diagnosing various pathologies and clinical use of treatments.

Those strategies often take advantage of senescent cells' characteristics, such as targeting the increased β-galactosidase activity present on senescent cells (e.g., galactooligosaccharides–encapsulated nanoparticles containing cytotoxic or senolytic drugs and galactose-derived pro-drugs (González-Gualda et al., 2021). Since macrophages and senescent cells share many phenotypic characteristics, a double question arises.

As covered in previous sections, the role of macrophages in maintaining tissue homeostasis, clearing senescent cells, and promoting regeneration are beginning to be better established. Due to the innate role of macrophages in these processes, an emerging idea of treating diseases/age-related pathologies related to senescent cell accumulation falls within the realm of manipulating macrophage function. With macrophage therapy, such ideas include treating Alzheimer's disease, myocardial infarction, cancer, and inflammatory diseases such as rheumatoid arthritis.

One method of achieving a therapeutic approach is by engineering macrophages to ignore “do not eat me” signals found on the surface of senescent cells. As previously mentioned, the CD47–SIRP*α* axis allows senescent cells to avoid removal by the immune system; therefore, blocking this axis may be an effective treatment against the accumulation of senescent cells in aging and injury. Molecules that target this axis have already been developed and include those that target CD47, as well as SIRPα specifically, alongside bispecific targeting agents. These agents have been well characterized in cancer, where anti-CD47 antibodies are currently in clinical trials. Considering the role of macrophages in blocking the CD47–SIRPα axis, engineering macrophages to target CD47 may be effective. For example, similar to CAR-T cells, Chimeric Antigen Receptors for Phagocytosis (CAR-Ps) are designed to phagocytose specific targets. It has been suggested that engineering macrophages to target CD47 may be an effective anti-tumor therapy (Xia et al., 2020). Indeed, these macrophages may prove effective in the clearance of chronic senescent cell accumulation following injury. However, there remain concerns over the manipulation of the CD47–SIRPα axis for therapeutic use; including, but not limited to, the consensus that the CD47–SIRPα interactions demonstrated in mouse studies may not wholly or as efficiently translate to humans; the discovery that the clustering of CD47 can also influence the interaction between CD47 and SIRPα, and that the anti-CD47 non-blocking antibody 2D3 increases CD47 clustering, and the considerable functions of CD47 that are independent of SIRPα, which instead act upon SIRPγ or Thrombospondin 1 (TSP1), leading to potential off-target effects on T cells. Another possibility is to increase the phagocytic capability of macrophages by increasing “eat me” signals. Defective expression of the “eat me” signal calreticulin, a ligand required to activate engulfment receptors on phagocytic cells, results in cellular resistance to efferocytosis, and apoptotic cells fail to be cleared by neighboring macrophages. A proportion of aged and cancerous cells are susceptible to being “labeled” by macrophage-secreted calreticulin and are cleared from tissue (Feng et al., 2018; Hernández-Mercado et al., 2021).

The researchers are constantly faced with problems of interpretation of the obtained results. This is related to the dynamic changes in somatic cells, periods of lactation, and daily fluctuations in animals' number, breed, and individual characteristics. Thus, cytochemical studies will certainly give researchers a new search for the diagnosis of subclinical mastitis in cows.

The role of innate neutrophils in immunity and mastitis development is only the beginning of evaluating the physiological functions of apoptosis (Zhelavskyi, 2019). The investigation of the cascade of immune responses and the role of apoptosis is essential in determining the physiological constants of immunity. This fact confirmed that inflammatory reactions occur against the background of activation of neutrophil migration (Zhelavskyi, 2017).

Senotherapy is concerned with developing therapeutic strategies to slow the aging process and alleviate its associated diseases by preventing, eliminating, or reversing senescence in cells. Senolytic therapy is one of the more, if not the most, rapidly developing strategies for senotherapy. Senolytic agents are a class of small molecules that can selectively induce the apoptosis of senescent cells by interfering with the SCAPs. Most senolytic drugs that have been identified so far are repurposed anticancer drugs, such as the likes of dasatinib and quercetin (used in combination) – the first few senolytics to be discovered (Veret & Brondello, 2020; Kondoh & Hara, 2022).

3. Conclusions

The study of the mechanisms of cellular aging and the development of effective means of hay therapy today requires further painstaking work. Despite significant advances in preclinical studies, many questions remain about the practical use of the drugs. This is especially true in the medicine of oncology, neurology, and cardiology. Nevertheless, scientists will be able to use pharmacological agents to influence cell division, differentiation, and determination in the future. We also hope to have developed effective means of immunotherapy of diseases.

Prospects for further research. The molecular mechanisms of cell aging and mediators involved in the mechanisms of cell aging and death are being studied in detail. The field of research contains countless fascinating studies that are sure to be discovered.

Conflict of interest.

The authors state that there is no conflict of interest.

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