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Roles of Apoptosis and Cellular Senescence in Cancer and Aging

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Abbreviations: APAF-1, apoptotic protease activating factor-1; ARF, alternative reading frame; Bak, Bcl-2 homologous antagonist killer; Bax, Bcl-2-associated X protein; Bcl-2, B cell lymphoma-2; Bcl-xL, B-cell lymphoma extra-large; BH-3, Bcl-2 homology domain-3; BID, BH-3-interacting protein; CICD, caspase-independent cell death; COX-2, cyclooxygenase-2; CDK4/CDK6, cyclin-dependent kinase 4/6; CML, chronic myeloid leukemia; DAC, 5-aza-2'-deoxycytidine; DISC, death-inducing signaling complex; E2F, E2 transcription factor; Fas, apoptosis stimulating factor; γ H2AX, phosphorylated form of the histone H2AX; HAT, histone acetyltransferase; HDACs, histone deacetylases; HMTs, histone methyl transferases; IAPs, inhibitors of apoptosis; IL-1RA, interleukin-1 receptor antagonist; IL-1, interleukin-1; JAK2, Janus-activated kinase 2; Mcl-1, myeloid cell leukemia-1; MAPK, mitogen-activated protein kinase; MBD, methyl-CpG-binding domain; MDM-2, murine double minute-2; MOMP, outer mitochondrial membrane permeabilisation; mTOR, mammalian target of rapamycin; NF- κ B, nuclear factor- κ B; PI3K, phosphatidylinositol kinase; pRb, retinoblastoma protein; PS, phosphatidylserine; PTEN, phosphatase and tensin homolog deleted on chromosome 10; ROS, reactive oxygen species; SA β G, senescence-associated β -galactosidase; SAHF, senescence-associated heterochromatin foci; SASP, senescence-associated secretory pathway; sFASL, soluble FASL; sFAS, soluble FAS; STAT, signal transducer and activator of transcription; TNFR, tumor necrosis factor receptor; Trail, TNF-related apoptosis inducing ligand.

Abstract

Cancer and aging are two similar processes representing the final outcome of time-dependent accumulation of various irreversible dysfunctions, mainly caused by stress-induced DNA and cellular damages. Apoptosis and senescence are two types of cellular response to damage that are altered in both cancer and aging, albeit through different mechanisms. Carcinogenesis is associated with a progressive reduction in the ability of the cells to trigger apoptosis and senescence. In contrast, in aging tissues, there is an increased accumulation of senescent cells, and the nature of apoptosis deregulation varies depending on the tissue. Thus, the prevailing model suggests that apoptosis and cellular senescence function as two essential tumor-suppressor mechanisms, ensuring the health of the individual during early and reproductive stages of life, but become detrimental and promote aging later in life. The recent discovery that various anticancer agents, including canonical inducers of apoptosis, act also as inducers of cellular senescence indicates that pro-senescence strategies may have applications in cancer prevention therapy. Therefore, dissection of the mechanisms mediating the delicate balance between apoptosis and cellular senescence will be beneficial in the therapeutic exploitation of both processes in the development of future anticancer and anti-aging strategies, including minimizing the side effects of such strategies. Here, we provide an overview of the roles of apoptosis and cellular senescence in cancer and aging.

1. Introduction

The ability to block mutated cells, which may be hazardous to the health of the body as a whole, is a crucial feature allowing multicellular organisms to cope with stress conditions and preserve their integrity. Several protective mechanisms have evolved to prevent the harmful consequences of accumulating functionally aberrant cells/tissues, including mechanisms to block the proliferation of mutated cells and to activate cell death programs within these cells. Alternatively, the deleterious effects of damaged cells may be restrained by activating forms of “proliferative death” e.g., mitotic catastrophe and cellular senescence [1-3]. While these mechanisms act as potent tumor-suppressor strategies against damaging conditions, they also come at a cost; indeed, the same cellular responses that protect individuals from cancer may contribute to aging. This evolutionary theory implies that certain anticancer strategies, while ensuring the most suitable health conditions during the early fertile, reproductive phase of life, may promote the progressive decline of various systemic functions later in life, becoming a gateway for multiple pathologies, including diabetes, cardiovascular diseases, degenerative disorders, and even cancer [4, 5].

Many studies suggested that apoptosis and cellular senescence are two key events contributing to this dual antagonistic effect of the anticancer response. Both cancer and aging are related to accumulating dysfunctions in apoptosis and cellular senescence. For example, the gradual resistance to apoptosis and evasion of senescence-related programs accompanies the progression from preneoplastic growth towards neoplastic growth during carcinogenesis [6]. In contrast, during aging, apoptosis rate is either increased or decreased depending on the tissues/organs. Degenerative pathologies occurring during aging are typically associated with increased rates of apoptosis, while alterations in gene expression promoting resistance to apoptosis are frequent [7, 8]. In addition to changes in apoptosis during aging, senescent cells also tend to accumulate in various tissues during aging [9, 10].

Major advancements in cancer research have shown that many treatments generally thought to induce apoptosis in cancer cells actually trigger various additional responses in cancer tissues, including cellular senescence and non-apoptotic modalities of cell death [11, 12]. These outcomes may be part of a complex stress-induced regulatory network in response to treatment, contributing to the eradication of cancer (Figure 1). Animal studies have excluded the possibility that cellular senescence is a phenomenon merely occurring under *in vitro* conditions [13]. Moreover, there is evidence that senescence and apoptosis may occur concomitantly in cancer tissues during anticancer treatment [14]. On one hand, this type of heterogeneity may be a normal phenotype associated with anticancer cytotoxic therapies as a consequence of drug accessibility to the cancer tissue and therefore graded exposure of cancer cells to chemotherapeutic agents. Consequently, the level of stress would promote varying degrees of damage and cellular reactions in the same tumor. On the other hand, there could be a major tendency to trigger certain stress responses rather than others, depending on the cellular context. This second scenario might depend on defects developed in cancer cells that may in fact compromise their ability to trigger apoptosis in response to damaging stress. Alternative forms of cell death or senescence may therefore represent forms of backup mechanisms, thus allowing targeting of cancer cells.

Experimental manipulations aimed at protecting cancer cells from apoptosis, e.g., *via* the ectopic overexpression of anti-apoptotic proteins, do not appear to effectively modulate the clonogenic survival of treated cancer cells [1, 15]. These findings suggest that cell death and senescence may be essential outcomes of the anticancer activities of therapeutic agents, wherein cell death may function to reduce overall tumor burden and senescence may be relevant in the prevention of the expansion of potential cell death-resistant cells, thereby modulating the formation of metastases. In this scenario, escape from senescence would contribute to tumor relapse. Accordingly, the development of strategies promoting an effective immune-mediated clearance of senescent cells is expected to be another important area of anti-cancer therapeutic intervention in the future.

Intriguingly, apoptosis and senescence functions seem to overlap as recent findings prove the existence of cellular senescence during embryonic development [16, 17]. These recent studies demonstrate the requirement of senescence, besides apoptosis, in both physiological and pathological processes. Furthermore, the analysis of the involved regulatory pathways suggests that stress-mediated senescence occurring in adults corresponds to an evolutionary derivation of embryonic senescence [16-18].

There is no doubt that the activation of senescence is a potent tumor-suppressor mechanism [2]. Accordingly, the aberrant expression of oncogenes (e.g., RAS) or the loss of tumor suppressors (e.g., phosphatase and tensin homolog deleted on chromosome 10 [PTEN]) may induce senescence [19]. Moreover, additional approaches to cancer treatment besides induction of apoptosis or other cell death mechanisms should be feasible. The development of more effective anticancer therapies may require the implementation of therapeutic approaches enabling cancer cells to activate senescence [20] while limiting any risks of escaping this program and promoting aging as well. One challenge to future research on this topic will be the identification and limitation of senescence-induced downstream molecular events that promote aging and are dispensable for anticancer activity.

2. Apoptosis in cancer and aging

2.1. Apoptosis: morphological features and molecular mediators

Apoptosis is a highly regulated program of cell death. It was first identified in 1972 by Alastair Currie, John Kerr, and Andrew Wyllie, who observed the acquisition of peculiar morphological features in cells [21]. These features include global cell shrinkage, cell blebbing, and chromatin condensation accompanied by nuclear and DNA fragmentation into specific fragment sizes (i.e., DNA laddering). These early alterations may eventually culminate in subcellular division into fragments, namely apoptotic bodies, which maintain the plasma integrity and may be recognized and phagocytosed by macrophages. Clearance of apoptotic cells *via* macrophage phagocytosis prevents the occurrence of inflammation.

Two main signaling pathways, *i.e.*, the extrinsic or physiological pathway and the intrinsic or mitochondrial pathway, converge to determine the particular apoptotic phenotype. Both pathways rely on the activation of a family of aspartate-specific cysteine proteases called caspases [22].

The extrinsic or physiological pathway is activated upon binding and activation of plasma membrane cell death receptors (e.g., apoptosis stimulating factor [Fas]; tumor necrosis factor [TNF] receptor [TNFR]; and TNF-related apoptosis-inducing ligand [Trail]) by their specific ligands. This event then leads to the formation of a multiprotein intracellular complex (the death-inducing signaling complex [DISC]), recruiting pro-caspase-8 and determining its activation after processing (cleavage).

The intrinsic or mitochondrial pathway is triggered by various damaging conditions and evolves through mitochondrial alterations, resulting in permeabilisation of the outer mitochondrial membrane (MOMP) and the loss of the mitochondrial membrane potential. MOMP is orchestrated by the mutual interactions established between antagonistic members of the B cell lymphoma-2 (Bcl-2) family of proteins. Any modulation disabling the function or expression of anti-apoptotic members (*i.e.*, Bcl-2, B-cell lymphoma extra-large [Bcl-xL] and myeloid cell leukemia-1 [Mcl-1]) frees the pro-apoptotic Bcl-2-associated X protein (Bax) or Bcl-2 homologous antagonist killer (Bak) to interact with other mitochondrial components, thus allowing the release of cytochrome c and additional pro-apoptotic factors into the cytosol. Once released, cytochrome c forms a complex with apoptotic protease activating factor-1 (APAF-1) and pro-caspase-9, thereby promoting the cleavage and activation of this caspase.

The activation of effector caspases (caspase-8 and -9) facilitates the activation of the common pool of executor caspases (caspase-3, -7, and -6), which eventually carry out the cell death process. Crosstalk between the intrinsic and extrinsic apoptotic pathways exists, ensuring the completion of the apoptotic program. In the extrinsic apoptotic pathway, once activated, caspase-8 truncates Bcl-2 homology domain-3 (BH-3)-interacting protein Bid (BID), which in turn interacts with mitochondria and the pro-apoptotic Bcl-2 family members Bak and Bax to promote MOMP. Alternatively, caspase-8 may be also activated downstream of the mitochondrial pathway being a substrate of caspase-3.

2.2. Apoptosis in cancer

Apoptosis is a form of cell death that plays crucial roles in several physiological processes, including tissue remodeling during embryonic development or immune tolerance through the selective elimination of T cells in the thymus. Additionally, apoptosis represents an important tumor-suppressor mechanism contributing to the eradication of mutated cells, which may otherwise progress into preneoplastic cells [3]. The ability of this process to protect against cancer development is underlined by the observation that evading cell death is one of the earliest aberrations acquired during carcinogenesis. Indeed, this is a typical hallmark of cancer that universally occurs in tissues of diverse histological origins [23].

Multiple factors contribute to the escape of cell death. Cancer cells may accumulate defects in important components of the apoptotic machinery, thus compromising the execution of the apoptotic program. Although several studies have provided examples of this situation, the identification of other mechanisms of cell death and the elucidation of crosstalk among these mechanisms clearly indicates that inefficient apoptotic machinery is rarely the determining factor conferring cells with the ability to avoid death in response to constant cell damage, even in the context of aberrant cells [12, 22]. Many *in vitro* and *in vivo* studies performed in different cancer cell lines have clearly shown that exposure of

cancer cells to several cytotoxic treatments, including canonical inducers of apoptosis, may cause the cells to promptly switch from an apoptotic program to autophagic cell death or one of several forms of regulated necrosis [22]. Additionally, alterations in cell cycle progression may cause cell demise through mitotic catastrophe [24]. Interestingly, the existence of backup mechanisms of cell death for both intrinsic and extrinsic apoptotic pathways has been proposed. Regulated necrosis, called necroptosis, may be activated in the context of defects in the initiation of the extrinsic apoptotic pathway. A form of caspase-independent cell death (CICD) has been proposed as a mechanism of backup cell death for the intrinsic apoptotic pathway, operating downstream of mitochondrial alterations [25, 26].

The existence of backup cell death mechanisms has two important implications. First, these mechanisms may represent an important evolutionary protective system to properly cope with severe damaging stress and to ensure the removal of damaged cells. Secondly, both *in vitro* and *in vivo* studies have suggested that cancer cells may respond heterogeneously to inducers of apoptosis, with a fraction of the cells undergoing these other processes rather than apoptosis [27]. This assumption also implies that backup cell death pathways actually occur concomitantly with apoptosis, but may be masked by the massive induction of apoptotic cell death; consequently, failures in these backup pathways may be an unidentified determinant of chemoresistance and consequent tumor relapse.

More frequently, cancer cells develop alterations in intracellular mediators and signaling events, which interfere with the progression of cells through the apoptotic pathway. The multistep nature of apoptotic signaling also requires a multifaceted regulatory network. Many mechanisms have been shown to prevent apoptosis, and we will not discuss all of these mechanisms here. Instead, we will present only the mechanisms that are most relevant to therapeutic interventions.

Alterations in the expression levels of Bcl-2 family proteins is commonly found in cancer cells [28]. Upregulation of at least one anti-apoptotic Bcl-2 family protein is an important hallmark in cancer cells. In addition to modulation of expression, post-translational modifications, including phosphorylation at one or more sites, also control the stability and activity of Bcl-2 family proteins [29]. Moreover, oxidative stress may alter the status of protein thiols and affect the ability of the protein to establish protein-protein interactions [30, 31]. Furthermore, cancer cells may also exhibit dysfunctions in pro-apoptotic proteins of the Bcl-2 family. In summary, regardless of the mechanism involved, dysfunctional mutual interactions between pro-apoptotic and anti-apoptotic Bcl-2 family members reduces the susceptibility of cancer cells to apoptosis, resulting in poor prognosis and/or insufficient response to anticancer therapies.

Although the delicate balance between anti-apoptotic and pro-apoptotic Bcl-2 family members plays a central role in the resistance to cell death, Bcl-2 proteins are also involved in many other regulatory processes, including the regulation of autophagy, the cell cycle, and calcium homeostasis [32]. All these processes may also be deregulated in cancer. Recent studies of cancer cell metabolism indicated that Mcl-1 protein stabilization is an important factor promoting the survival of cancer cells and may represent a novel target for anticancer therapy [33]. Interestingly, calorie restriction, which improves lifespan, also suppresses Mcl-1 expression through inhibition of the mammalian target of rapamycin (mTOR) pathway [34].

Cancer cells may develop addiction towards high expression levels of anti-apoptotic Bcl-2 family proteins; besides, these alterations are often causes of chemoresistance. One strategy is to implement combinational treatments to enhance the efficacy of conventional chemotherapeutic agents [28]. Major efforts are currently underway to identify small molecule inhibitors for anti-apoptotic Bcl-2 family members and/or compounds that mimic the action of pro-apoptotic BH-3 proteins (BH-3 mimetics) [35]. ABT-737 is a prototypic example. While ABT-737 efficiently binds Bcl-2, thus blocking its function, this molecule cannot bind Mcl-1 and is therefore not effective for cancers that overexpress Mcl-1 spontaneously or secondary to anticancer therapies [33]. Accordingly, derivatives of this molecule have been generated with the intent of targeting Mcl-1. However, these approaches are also associated with common side effects of chemotherapies, including thrombocytopenia, thereby limiting their therapeutic applications [36]. Elucidation of the roles of cell metabolism in mediating Mcl-1 expression is important for testing combinational therapies based on the concomitant use of lower doses of Bcl-2 and mTOR inhibitors [37].

Many studies strongly supported the pro-carcinogenic role of chronic inflammation. Aberrant constitutive expression of pro-inflammatory mediators occurs at early stages of carcinogenesis, and the expression of such mediators correlates with the activation of prosurvival mechanisms, directly affecting the efficiency of the apoptotic machinery. Two major pro-inflammatory mediators, nuclear factor- κ B (NF- κ B) and cyclooxygenase-2 (COX-2) [38-40], are deeply involved in cancer progression.

Promoters of the major anti-apoptotic proteins contain binding sequences for the transcription factor NF- κ B. Constitutive activation of NF- κ B leads to the upregulation of Bcl-2, Bcl-xL, and Mcl-1 proteins. Additionally, the inhibitor of apoptosis (IAP) proteins are also transcriptionally modulated by NF- κ B [22].

Despite the importance of NF- κ B, its role in apoptosis is not clearly defined. Indeed, NF- κ B may play pro-apoptotic or anti-apoptotic roles depending on the cellular context and the pathway leading to NF- κ B activation. In mice and colon cancer cell models exposed to potent carcinogens, canonical activation of NF- κ B promotes Fas transcription and physiological apoptosis, whereas non-canonical activation of NF- κ B acts as a transcriptional repressor of the Fas cell death receptor, thereby playing an inhibitory role on the apoptotic pathway [41]. These results imply that NF- κ B may act as a tumor promoter or a tumor suppressor through its effects on apoptosis. Notably, many natural compounds, including phytochemicals and compounds isolated from marine plants/animals, are inhibitors of the NF- κ B pathway [22, 42]. In many instances, these compounds are associated with important anti-proliferative and cytotoxic activities in cancer cells and have minimal toxic effects in healthy cells [43, 44]. This ideal combination makes a number of these compounds promising as chemopreventive and anti-cancer agents.

COX-2 is aberrantly expressed in several forms of cancer [40]. A prototypic example is colorectal cancer. There is a direct correlation between the constitutive overexpression of COX-2 and specific forms of colorectal cancer, including hereditary polyposis. Moreover, elevated levels of COX-2 correlate with the most aggressive and poorly responsive forms of this disease. Because of the druggability of COX-2 with preferential or selective inhibitors already available on the market, the enzymatic modulation of this protein may represent a candidate chemopreventive or therapeutic

intervention. Moreover, many studies have highlighted the effects of these compounds on the sensitization of cancer cells to extrinsic and intrinsic apoptotic pathways induced by canonical chemical agents or radiation [40]. However, the side effects associated with the chronic use of these agents limit their application, suggesting that alternative interventions are required. Thus, studies are currently underway to investigate alternative strategies for cancer treatment based on the aberrant modulation of COX-2 in cancer cells through the use of natural compounds [45].

In addition to genetic alterations, epigenetic modifications also play a crucial role in all steps of carcinogenesis [46, 47] and their involvement in cell death and senescence mechanisms cannot be excluded.

2.3. Apoptosis in aging

Many studies suggested that alterations in apoptosis are associated with aging. The nature of the modulation, however, is not well defined; some studies have shown that apoptosis is elevated during aging, while other authors showed that resistance to apoptosis increases during aging. Despite this inconsistency, both elevated apoptosis and increased resistance to apoptosis may have deleterious effects on health and survival. For example, while elevated rates of apoptosis are correlated with deterioration of the immune system and the development of several degenerative diseases, thereby reducing lifespan, decreased rates of apoptosis (*i.e.*, from increased resistance to apoptosis) are associated with the upregulation of anti-apoptotic or pro-survival genes, which may increase the risk of cancer and affect longevity.

Elevated apoptosis is thought to be a response to the reduced ability of cells to maintain genomic integrity and repair damage, as is commonly observed during aging. Cells sense telomere attrition, a defined hallmark of aging [48], as an event associated with DNA damage and accordingly adapt their gene expression patterns to block further proliferation. Induction of apoptosis is one of the possible outcomes of this process. This strategy is clearly protective, thus preventing the entry of dysfunctional cells into the cell cycle. Importantly, induction of apoptosis has been shown to exist in aged hematopoietic stem cells [49]. However, this mechanism may also contribute to the limited regenerative potential of stem cells that occurs during aging. Aging is also characterized by reduced efficiency of the immune system [48]. Indeed, in addition to its effects on stem cells, apoptosis may also affect the reduction of specific differentiated immune cell subpopulations. A comparative microarray analysis of the gene expression profiles of CD8⁺ T cells from aged and young healthy human subjects showed the complex age-associated changes in gene expression [50]; some of the major functional groups of genes over-expressed during aging included genes related to apoptosis signaling, and oxidative stress response. In contrast, genes encoding factors involved in maintenance of genomic integrity and in protein and nucleic acid synthesis were down-regulated. Therefore, this study suggested that apoptosis is promoted to eliminate damaged immune cells, possibly due to defects in DNA/protein synthesis resulting from increasing oxidative stress.

Oxidative stress is increased in aged subjects [51], and formation of reactive oxygen species (ROS) may further promote the risk of DNA damage, thereby inducing apoptosis in the damaged cells. Recently, an alternative hypothesis has been proposed to explain the role of oxidative stress in aging

and may also implicate apoptosis. During aging, there is a gradual increase in ROS produced by mitochondria (mtROS) [48]. This phenomenon appears to be associated with accumulating defects in mitochondria. Additionally, mtROS may be part of a stress response and can extend cell longevity in nematodes [52]. A recent study on *Caenorhabditis elegans* showed that the pro-longevity effect of mtROS is mediated by components of the apoptotic machinery, specifically involved in the mitochondrial apoptotic pathway [52]. mtROS induced changes in gene expression by upregulating genes that promote genome stability (e.g., genes related to DNA repair or DNA synthesis). Modulation of human analogs of Bcl-2, APAF-1, and caspase-9, together with a species-specific BH-3 only protein, has been observed in response to mtROS production in *C. elegans*. Mutations of these factors alter the ability of mtROS to induce gene expression and accordingly reduce longevity in nematodes. Based on these previous results, components of the apoptotic machinery may play a signaling role rather than triggering apoptosis. A similar mechanism may occur in vertebrates; however, evidence indicates that this signaling function is not dissociable from pro-apoptotic activity.

Although evidence suggests that apoptosis plays a protective role by removal of dysfunctional and potentially dangerous cells, hyperactivation of apoptosis will generally have negative effects on lifespan. A recent screening in *Drosophila melanogaster* identified a list of genes whose abnormal expression reduces lifespan [53]. Among these genes, several are initiators of the apoptotic program. Strategies aimed at inhibiting these factors also prolong longevity of adult flies.

Aging may be associated with a decline in the apoptotic response. As discussed in section 3.3, the accumulation of senescent cells may be a determinant factor in aging. Damaged cells that accumulate in aged tissues may undergo apoptosis or senescence. Senescent cells remain metabolically active and continue to interact with neighboring cells or tissues. **Although resistance to apoptosis is not a general feature of senescent cells, there are documented examples of the ability of some types of senescent cells to evade apoptotic programs.** Senescent cells may synthesize various factors that modulate tissue function via paracrine signaling. This phenomenon, called the senescence-associated secretory pathway (SASP), also may lead to the secretion of anti-apoptotic and pro-survival factors [54]. Evidence has shown that the synthesis of these factors is correlated with the up-regulation of p21 following p53 activation, an event observed in most senescent cells. Furthermore, the use of conditioned media from cells induced to produce p21 protects cells from apoptosis. These findings suggest that senescence-induced factors may promote aging-related processes rather than events associated with *de novo* carcinogenesis. The lack of clearance of senescent cells in aged tissue may contribute to this negative modulation [10]. A recent study involving 204 healthy subjects found a gradual age-dependent decrease in the levels of specific circulating apoptotic serum markers, such as cytochrome c and soluble FAS ligand (sFASL), concomitant with a gradual increase in the soluble form of FAS (sFAS), which generally has an inhibitory effect on apoptosis [55]. Interestingly, the age-dependent elevation in expression of the inhibitory factor sFAS is more pronounced in men than in women, while the levels of pro-apoptotic factors are much lower. Thus, these results suggest that secretory events play a major role in aging. However, further studies are required to fully elucidate the secretory mechanisms associated with aging.

3. Cellular senescence in cancer and aging

3.1. Cellular senescence: different modalities and molecular determinants

Cellular senescence is a state of terminal proliferation. Its discovery dates back to 1961, when Leonard Hayflick and Paul Moorhead described the irreversible growth arrest of cultured fibroblasts and considered this event a form of cell aging [56]. Currently, we know that the determinants of this form of senescence, called replicative senescence, are associated with the gradual attrition and consequent shortening of telomeres progressively occurring during cell division [48]. Telomere erosion is caused by the inability of the DNA replication machinery to properly copy the linear DNA sequence. Over a certain threshold, cells sense this erosion as an event of DNA damage, thereby triggering the canonical DNA damage response (DDR) pathway [57]. Therefore, senescence represents a type of counting system for cell division [10].

Interestingly, a number of oncogenes may also induce cellular senescence. RAS and other factors related to the RAS pathway are a prototypic example. Furthermore, the loss of tumor-suppressor genes, such as PTEN, or the inhibition of MYC may drive senescence [19, 20]. Studies in several mouse models confirmed the importance of senescence in the prevention of tumorigenesis *in vivo*. Taken together, these findings indicate that cellular senescence is a tumor-suppressor mechanism and an alternative to apoptosis, thus blocking damaged cells from proliferating and thereby preventing tumor progression. Additionally, other stress-inducing conditions may stimulate the senescence response. Several types of DNA damage, pro-oxidant conditions, and agents perturbing the microtubular network are other examples of senescence inducers [1]. Moreover, epigenetic mechanisms may induce cellular senescence [58]. Possible triggers include altered patterns of gene methylation and chromatin organization as well as altered expression of proteins that modify histones and DNA [59].

One factor limiting the study of cellular senescence is the lack of available markers of senescence. The most common system used to identify cellular senescence relies on the detection of elevated cellular levels/activity of a lysosomal form of the enzyme β -galactosidase (namely, senescence-associated β -galactosidase or SA β G) by X-Gal colorimetric assays. Other additional molecular alterations (e.g., the presence of foci positive for the phosphorylated form of histone H2AX, γ H2AX) are not ubiquitously detectable in all types of senescence. The lack of common markers also reflects intrinsic differences in the intracellular signaling pathways that mediate senescence. Nevertheless, some intracellular molecular mediators appear to play active and causative roles in senescence.

Upregulation and/or activation of p53 occur in most of the forms of cellular senescence identified to date [20, 60]. The activation of this tumor suppressor can be explained by the occurrence of DNA damage (or conditions resembling DNA damage) during the induction of cellular senescence. In addition to telomere attrition, which can be sensed as DNA damage, oncogene-induced senescence (OIS) is frequently associated with the generation of ROS because of excessive mitogenic stimulation [10]. ROS may then produce DNA damage. These events typically induce p53 expression by affecting transcription *via* DDR. In addition to transcriptional activation, upregulation of p53 may occur through sustained translation; indeed, loss of PTEN or other related conditions leading to hyperactivation of the mTOR pathway has been shown to induce continual translation of p53, resulting in its increased

expression [20]. The control of p53 expression is associated with other mechanisms modulating p53 activity, such as phosphorylation of p53 at specific residues *via* RAS-dependent activation of the phosphatidylinositol kinase (PI3K) or mitogen-activated protein kinase (MAPK) pathways [61].

One of the main executors of p53 is p21, a potent cell cycle inhibitor. p53-dependent transcriptional activation of p21 interferes with the activity of cyclin/cyclin-dependent kinase (CDK) complexes, thereby determining growth arrest. Additionally, p21 can interfere with other transcription factors and cofactors, such as E2 transcription factor (E2F), c-Myc, signal transducer and activator of transcription (STAT) family members, and p300/CBP [62], thereby affecting the gene expression profiles of cells.

The p16 (also known as p16INK4)/alternative reading frame (ARF) locus is another central player in the induction of cellular senescence. p16 and ARF are generally expressed at low levels in young cells and exhibit increased expression during aging [10]. This event promotes the arrest of cells in the G₁ phase of the cell cycle due to the inhibitory activity of p16 on the CDK4/CDK6 complex while ARF stabilizes p53 via inactivation of the E3 ubiquitin ligase murine double minute-2 (MDM-2). Progressive accumulation of p16 relies on the inactivation of Polycomb family repressors [63]. Alternatively, DNA damage promotes p16 up-regulation *via* p53-mediated transcriptional activation [9]. Up-regulation of p16 promotes senescence even in presence of long telomeres; this ability is lost in cells deficient in the p16/ARF locus [64]. Thus, these findings suggest that the p16/ARF locus may work as a “clock” in cells, independent of telomere attrition [10].

3.2. Cellular senescence in cancer

Cellular senescence is a tumor-suppressor mechanism activated in response to pro-carcinogenic conditions, such as DNA damage, oxidative stress, and aberrant expression of oncogenes or tumor suppressors. Additionally, senescence may be activated in cancer cells in response to various anticancer treatments, thus contributing to their therapeutic efficacy. The implementation of pro-senescence strategies is therefore an attractive alternative in cancer research in terms of its chemopreventive and therapeutic potential [20].

The transition from the preneoplastic to the neoplastic phase is associated with reduced frequencies of senescent cells. This phenomenon may be the consequence of two events, namely, that mutated cells are insensitive to stimuli inducing senescence or that senescent cells may re-enter the cell cycle under certain circumstances. This latter scenario implies that cellular senescence is a reversible phenomenon. However, regardless of the mechanisms involved, many studies have suggested that the dysfunction of pathways important in the induction and maintenance of senescence may be one of the initiators of this transition.

The disruption of the p53 and/or 16/retinoblastoma protein (pRb) pathway prevents the induction of senescence both *in vitro* and *in vivo* [65-68]. Similarly, changes in the expression or function of ARF or abnormalities in RAS-dependent pathways play the same inhibitory roles [68]. Loss of PTEN induces senescence *via* a mechanism requiring p53; if p53 is deficient, cells are more susceptible to undergoing malignant progression [69]. Taken together, these findings confirm that the bypass of cellular senescence is probably critical for carcinogenesis.

The evasion of senescence may occur through accumulating defects in crucial senescence mediators. Approximately 50% of tumors bear mutations in the *p53* gene [70]. Micro-injection of p53 antibodies and suppression of p53 expression in senescent fibroblasts reversed senescence [71, 72]. Alternatively, ablation of pRb, the downstream effector of p16, in RAS-expressing senescent cells reactivates proliferation [67]. Similarly, mutation or deletion of components acting downstream of these pathways may reverse senescence [73]. These findings provide evidence supporting the reversible nature of senescence and highlight causative roles of specific factors in the senescence process. Even if not undergoing cell cycle progression and proliferation, cells are not protected from accumulating mutations. Exposure to chemical or physical mutagens as well as infective agents [74, 75] may therefore revert the proliferative arrest. The development of chemoresistance in response to certain types of targeted therapies further supports the reversibility of senescence. Targeted therapies leading to downregulation of c-Myc cause cancer cells to undergo proliferative arrest, accompanied by a regression of the malignant phenotype. This condition is reversed when c-Myc expression is reactivated [76, 77]. This phenomenon has been described as tumor dormancy and it reflects the ability of a subset of cancer cells to initiate rather a quiescent-like state following oncogene inactivation. Depending on the cellular context, recently intersections between senescence and tumor dormancy were hypothesized (see [77, 78] for recent reviews). Interestingly, the inflammatory response associated with COX-2 overexpression is suspected to promote the reversal of senescence; therefore, COX-2 inhibitors may prevent cell cycle progression by preventing c-Myc expression [40].

In addition to the intrinsic properties of preneoplastic and neoplastic cells, the environment may dramatically influence the avoidance or evasion of cellular senescence. Indeed, tumor-infiltrating immune cells may avoid senescence, and a recent study in mice bearing PTEN-null prostate tumors showed that the presence of a specific tumor-infiltrating subpopulation (*i.e.*, CD11b⁺ Gr1⁺ myeloid cells) inhibits senescence *via* paracrine signaling [79]. These cells produce and secrete interleukin-1 receptor antagonist (IL-1RA), which counteracts the activity of interleukin-1 (IL-1) released by PTEN-null tumor senescent cells. These findings confirm the influence of SASPs in the maintenance of their senescent status and support the relevance of the tumor microenvironment in determining senescence; furthermore, these results suggest that inhibiting the recruitment of selective tumor-infiltrating immune cells may prevent the evasion of senescence [79]. The same group also demonstrated that the antagonistic role of tumor-infiltrating cells in tumors counteracts the efficiency of chemotherapy [79]. The secretory activity of senescent cells, however, plays pro-tumorigenic roles. Indeed, senescent cells produce various immune modulators and inflammatory cytokines that help in the clearance of senescent cells but also stimulate mitogenic and pro-survival signals in neighboring tumor cells. Secretory mechanisms mediated by the Janus-activated kinase 2 (JAK2)/STAT3 pathway promote immunosuppression in PTEN-null tumors, thus contributing to tumor growth and chemoresistance [80]. The identification and targeting of pro-tumorigenic pathways in the SASPs will be required to minimize the potential side effects of pro-senescent therapies. Importantly, genetic backgrounds may play important roles in this dualistic effect. Additionally, the NF- κ B pathway plays dual functions in modulating immune surveillance. While the p65 subunit promotes the induction of senescence in

preneoplastic cells, concomitant expression of RAS inhibits the cytotoxic activity and clearance of recruited macrophages, thereby promoting cancer [81].

Senescence constitutes a robust physiological antitumor response, acting together with cell death programs. Thus, senescence-inducing drugs, alone or in combination with classical therapeutic approaches, could represent an attractive alternative approach to treating cancers that are resistant to apoptosis-based therapies [20].

A variety of common anticancer agents have been shown to promote cellular senescence. For example, DNA-damaging agents, such as the topoisomerase inhibitors etoposide and adriamycin, as well as the alkylating agent cisplatin have been shown to drive senescence. Additionally, ionizing radiation and common microtubule agents (vincristine and vinblastine) may also induce senescence [82]. These findings support the primary role of the p53/p21 axis in triggering this response. Accordingly, strategies aimed at impairing p53/p21 have been shown to prevent senescence while enhancing apoptosis [83, 84]. Generally, DNA damage is considered the primary cause of this phenomenon. Telomere elongation does not prevent senescence, whereas telomere attrition may enhance the susceptibility to various DNA-damaging treatments [85, 86]. This result is in line with the ability of cells to perceive telomere attrition as a synergistic DNA damaging event.

Considering the importance of epigenetic mechanisms in the senescence pathway, reactivation of the senescence program in cancer cells through modulation of the epigenetic mechanisms regulating this pathway could provide insights facilitating the improvement of cancer treatment [87]. In this context, our group has recently reported that 5-aza-2'-deoxycytidine (DAC), a highly specific and potent inhibitor of DNA methylation that is currently undergoing preclinical and clinical trials, is able to induce senescence in leukemia cells [88, 89]. DAC stimulates the rapid down-regulation of c-Myc, thereby triggering telomere-dependent senescence in the context of chronic myeloid leukemia (CML). Therefore, DAC may have applications as a senescence-inducing drug in the treatment of cancer.

3.2. Cellular senescence in aging

Cellular senescence is now classified as a hallmark of aging [48]. Many studies have shown that there is an inverse correlation between the induction of senescence and longevity. Several investigations also supported the role of p53/p21 and p16 in reducing lifespan [90-92]. Interestingly, genetically modified mice expressing constitutively active p53 are resistant to cancer; however, they exhibit features of accelerated aging [90, 91]. This condition is associated with an increased frequency of senescent cells. On the other hand, the deletion of p53 prolongs the lifespan of telomerase-deficient mice, but also confers an increased risk of developing cancer [93]. Studies in telomerase-deficient zebrafish have confirmed that inhibition of the p53 pathway rescues cells from the pro-aging effects of telomere shortening [94]. This evidence also suggests that the mechanisms controlling aging are highly conserved among different organisms.

Other findings have suggested that p53 activity may alter the cellular outcome. Indeed, moderate levels of p53 activation may result in an anti-aging phenotype by controlling the adverse effects of DNA damage; extensive DNA damage or mutations leading to the hyperactivation of p53 may produce damaging pro-aging effects, leading to impaired tissue regeneration [10]. Moreover, upregulation of

p21 appears to be a critical determinant of pro-aging effects. Telomere-deficient mice exhibit prolonged lifespans when the *p21* gene is deleted but without accelerated rates of tumorigenesis [95]. These findings suggest that p21 promotes aging, which has prompted researchers to investigate the use of drugs inducing p21-independent senescence for therapeutic purposes. However, this scenario is quite complicated as other evidence indicates that p21 plays an essential dualistic (pro-aging versus anti-aging) effect downstream of p53 in the same cellular context [96].

p16 expression is increased in aged tissues; this phenomenon is associated with cellular senescence. Furthermore, p16 expression is upregulated in response to gerontogens, including arsenic, UV light, and cigarette smoke [97]. These findings suggest that p16 is a valid marker of aging. In further support of this, mice deficient in p16 show increased regenerative potential [98], whereas enhanced expression of p16 is correlated with tissue degeneration [99]. Additionally, mice deficient in *Polycomb* genes show alterations in stem cell functions [100]. These findings imply a critical modulatory role of p16 in stem cell biology and may predict a correlation between senescence-associated aging pathways and accumulating dysfunctions of stem cells.

4. Concluding remarks

Apoptosis and cellular senescence are two important tumor-suppressor mechanisms. However, there is no unique interpretation about the nature of their interplay [101]. On one side, these two processes may be simultaneously activated as part of a stress response but depending on the cellular context and/or extracellular environment. One pathway might be favored compared to the other. In this view, conditions inhibiting one pathway (e.g., apoptosis) might promote the switch towards the other one (e.g., cellular senescence). This model assumes that these two events are competing but possibly also act as compensatory (backup?) mechanisms in case of stress. On the other side, activation of both apoptosis and senescence may be simultaneously required to play complementary essential roles. This alternative view might be supported by physiological examples. The occurrence of senescence during embryonic development suggests that this phenomenon may play instructive functions on the adjacent cells/tissues through its secreted factors [18]. As adult stress-dependent senescence is suspected to be an evolution of this physiological event, it is conceivable to hypothesize a major role of senescence in influencing, or even dictate, the biology of the microenvironment. The determinants causing these differential roles in the tissue remain to be elucidated.

One ideal strategy in cancer prevention and therapy might be the triggering or reactivation of both of these processes, thus allowing the targeting of malignant cells by cell demise or proliferative death. Cellular senescence is also thought to promote aging and can induce pro-tumorigenic effects. While the mechanisms mediating these processes are still unclear, again, the secretory and paracrine activities of senescent cells in the tumor microenvironment are thought to be essential. Therefore, researchers are aiming to develop pro-senescent therapies that may be applied as anticancer interventions; however, studies must achieve the uncoupling of anti-carcinogenic versus pro-carcinogenic effects and of anticancer versus pro-aging activities in senescent cells. Dissection of the molecular mechanisms involved in these processes will pave the way for combinational therapies exhibiting dual cytocidal and

cytostatic effects while avoiding accelerated aging. Elucidation of these same mechanisms will also be beneficial for preventing the negative consequences of aging while avoiding accelerated tumorigenesis.

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Figure legend

Figure 1. Different cellular outcomes following damaging stress. Cells may trigger apoptosis, characterized by phosphatidylserine (PS) exposure, chromatin condensation, nuclear fragmentation, cell shrinkage, and fragmentation into apoptotic bodies. Alternatively, cells may undergo one of several forms of regulated necrosis, characterized by the rapid loss of plasma membrane integrity and cell swelling. Caspase-independent autophagic cell death may occur, accompanied by intense vacuolization of the cell and the formation of autophagolysosomes. Autophagy, as a primary stress response, may also precede apoptosis or necrosis. In conditions of aberrant cell division, cells may activate a program of mitotic catastrophe (characterized by the presence of micronuclei, uncondensed chromosomes, and cell enlargement), which eventually may lead to apoptosis. Cells may cope with this stress by blocking proliferation while maintaining metabolic activity, a state called senescence, characterized by the increased activity of the senescence-associated β -galactosidase (SA β G), senescence-associated heterochromatin foci (SAHF), senescence-associated secretory phenotype (SASP), most of the time paralleled by a cell cycle arrest in G1. Evidence indicates that this latter process may be reversible.

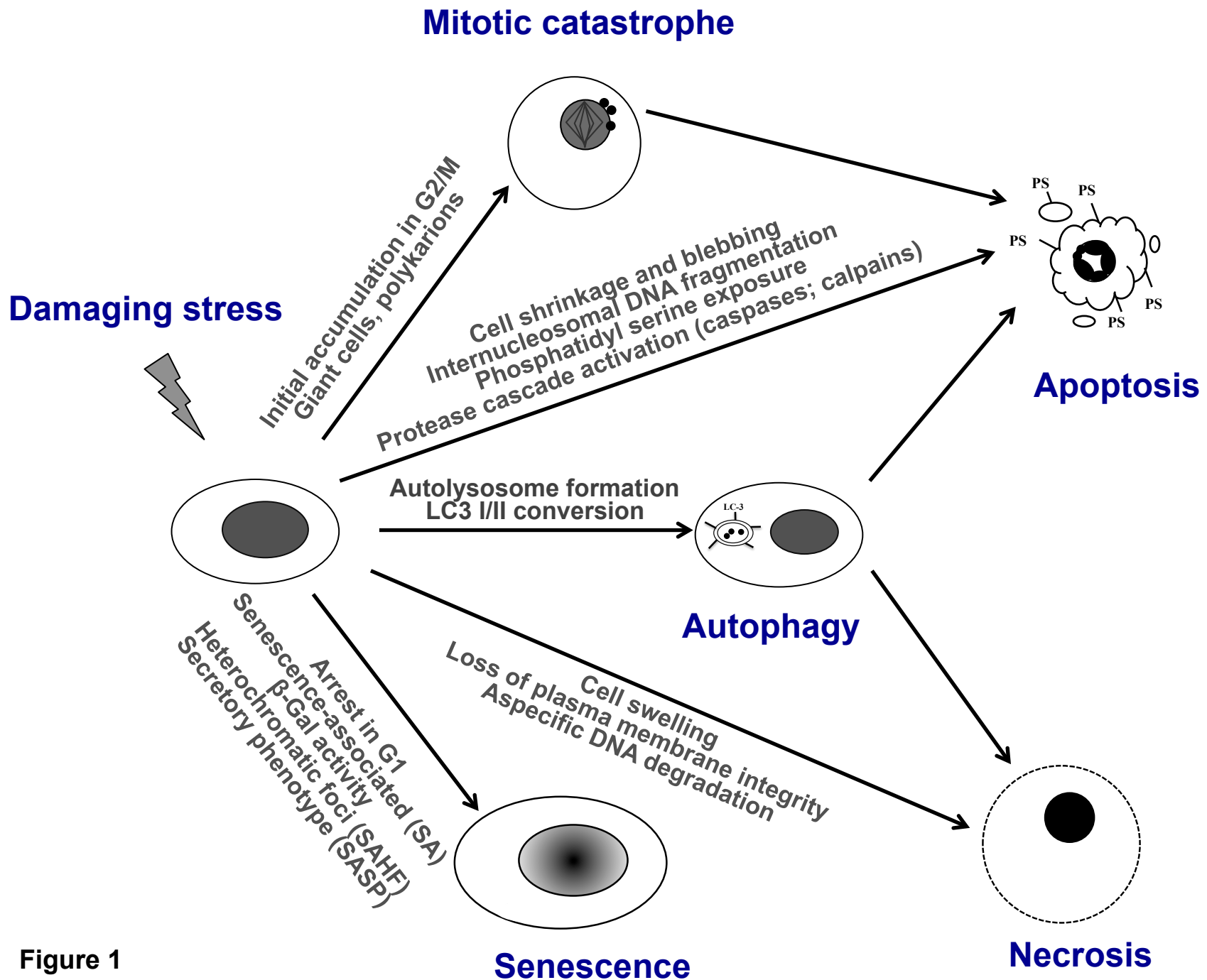


Figure 1