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Review Article

Significance of Cellular Senescence in Aging and Cancer

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Cellular senescence is a mechanism that induces an irreversible growth arrest in all somatic cells. Senescent cells are metabolically active but lack the capacity to replicate. Evolutionary theories suggest that cellular senescence is related to the organismal decline occurring in aging organisms. Also, such theories describe senescence as an antagonistically pleiotropic process that can have beneficial or detrimental effect on the organism. Cellular senescence is believed to be involved in the cellular changes observed as aging progresses. Accumulation of senescent cells appears to occur widely as the organism ages. Furthermore, senescence is a key element of the tumor suppressor pathways. Therefore, it is part of the natural barrier against the uncontrolled proliferation observed in cellular development of malignancies in multicellular organisms. Activation of the senescence process guarantees a limited number of cellular replications. The genetic network led by p53 is responsible for activation of senescence in response to DNA damage and genomic instability that could lead to cancer. A better comprehension of the genetic networks that control the cell cycle and induce senescence is important to analyze the association of senescence to longevity and diseases related to aging. For these reasons, experimental research both *in vitro* and *in vivo* aims to develop anticancer therapies based on senescence activation. The last decade of research on role and function of senescence in aging and cancer are discussed in this paper.

Key words
Aging, Neoplasms, Pleiotropy, Telomere, Genetic pathways

Introduction

Cellular senescence is an irreversible arrest of cell proliferation that occurs in all somatic cells within multicellular organisms and causes the cells to exhaust the potential of division. Senescent cells are metabolically active but they lack the capacity to replicate, therefore are unable to perform DNA synthesis and continue to grow (1-3). Hayflick and Moorhead (1961) first referred cellular senescence as "replicative senescence" because after a certain number of cell divisions in culture, human diploid fibroblasts would stop the growth process. In general, primary normal cells, cells freshly removed from an organism to be grown in culture, initially tend to divide rapidly and then the rate of division tend to be slower until it reaches a division arrest (4). The number of cell divisions prior to the state of senescence is often referred to as Hayflick limit, approximately 50 cumulative population doublings (5). Senescent cells manifest specific changes in morphology such as flatness, enlarged size and lack of vacuoles (6-8). Other characteristics that distinguish senescent cells are a different range of gene expression and transcription factors and the capacity to secrete degradative enzymes and cytokines (7). Senescent cells can also be detected by the presence of senescent associated beta-galactosidase biomarker which is not present in normal cells (9-11). The phenomenon of senescence is not limited to human fibroblasts since many cell types such as endothelial cells, epidermal keratinocytes, T lymphocytes, glial cells have been observed to manifest a finite number of divisions in culture and then undergo such proliferation arrest (12). Cellular senescence has been associated to the process of aging in various organisms due to the mechanisms that are believed to lead to the irreversible loss of proliferation in the cell populations. The last decade the main goal of researchers has been to comprehend the aging process by replicating *in vitro* the collection of cellular changes that occur *in vivo* as the organism ages (5,13,14). Because research focuses on primary cells grown in culture, usually a single cell type such as human diploid fibroblasts, genetic and epigenetic

variations can affect the results by unbalancing and disrupting gene expression and cellular behavior (15-17). The question that these studies attempt to answer is whether the lack of ability to proliferate infinitely observed in cultured cells corresponds exactly to the mechanisms occurring in organisms as they age. It has been noticed that normal cells manifest senescence while certain cell lines, germ cells and tumor cells both in human and other animals, never enter the senescent stage and are therefore described as immortal (18,19). Studies performed on senescent cells *in vitro* and *in vivo* have been fundamental to establish the existence of genetic pathways involved in the process of aging in humans and in other organisms and the association of the decline of growth potential with the aging of the whole organism (12).

To that end, the purpose of our review article is to summarize the last decade of research on cellular senescence and analyze the evidence that supports the linkage of senescence to aging process and cancer. While senescence is known to occur in all metazoans, it has been observed that few of these organisms do not undergo cellular senescence as they age. Among these organisms Hydra, which belongs to the phylum Cnidaria, lacks any sign of senescence and manifests a high degree of tissue renewal, low mortality rate, characteristics that indicate a potential immortality (20,21). The longevity of hydra and its ability to skip the senescent state at a cellular level might indicate that the decline in renewal capability of cells as they reach an irreversible arrest is a feature associated to the process of aging. It is important to emphasize that the senescent state is not equal to the quiescent state often observed in cells. Quiescence is a reversible process involving the capacity of the cells to participate again to the cell cycle while senescence involves a lack of response to mitogenic stimuli such as growth factors (22). The senescent state can be activated by different stimuli such as changes in physiological conditions and cellular stress that appear to increase as the organism is aging (21,23,24). While induction of senescence is considered a major natural barrier against the uncontrolled proliferation characteristic of cancer, accumulation of senescent cells contributes to the process of aging and might promote tumor development. Senescent cells that appear to be resistant to apoptosis might be involved to the general organ dysfunction associated to aging and eventually promote cancer (2,16,25). It is widely accepted that the decline of organs and tissue function observed to occur with aging is associated to the accumulation of senescent cells. Evidence of different expression of cell cycle regulatory genes between young cells and from senescent cells emerged from comparisons between differential screenings of RNA (1). Furthermore, increased genomic instability is associated to inefficiency in DNA double strand repair ability of presenescent and senescent cells accumulating with aging (26,27). In addition, senescent cells might favor a pro-oncogenic tissue environment by secreting growth factors, extracellular matrix components and inflammatory cytokines that disrupt tissue integrity (14). Therefore, it has been suggested that senescence contributes to the process of aging and protects cells from uncontrolled growth makes it a cellular process beneficial or detrimental depending on the age of the organism. This suggests that senescence is an antagonistically pleiotropic phenomenon. For this reason senescence has been ironically defined as the Dr Jekyll and Mr. Hyde of aging (28).

Evolutionary Theories of Aging

One of the goals of research on the mechanism of cellular senescence has been to understand why cells reach a proliferation limit. To investigate the underlying reasons of cellular senescence and the associated organismal decline observed, researchers have formulated different evolutionary theories based on the reduced force of natural selection in older organisms, which could provide insight on the process of aging (29). The theories most accepted are antagonistic pleiotropy and mutation accumulation. Both theories revolve around the concept of a decline in fitness and reproductive success observed in aged organisms. According to the theory of antagonistic pleiotropy there are genes with pleiotropic alleles which in early stages of life favor and increase the reproductive and survival success of an organism. However, in later stages of life the same pleiotropic alleles have the opposite effect since they contribute to a decline of reproductive and survival success and allow a faster senescence (30,31). Based on antagonistic pleiotropy theory the alleles of specific genes can affect multiple age specific features of an organism and can end up damaging the overall fitness as the organism is aging. It is believed that natural selection would favor and select for these genes with antagonistic effects only because the advantages in young organisms outweigh the detrimental effects in old ones. The idea that natural selection allows the accumulation of such genes within a population could be interpreted as a trade off between reproduction and longevity. Such trade off would occur to favor a high reproductive success when the organism is young and therefore to favor the transfer of genes of a fit individual to the next generation at the expense of an early decline in life. Evidence to support the theory of antagonistic pleiotropy and the existence of genes capable of exerting effects on organism longevity is difficult to gather due to the unsure consistency of data observed in the manipulated environment of laboratory compared to the conditions in the natural environment. It has been observed that classes of genes associated to a longevity assurance might exist and be involved in complex signaling pathways that control growth and fecundity of an organism (32). Also, organisms who become reproductively mature very early in life display the lack of equal reproductive success in later stages of life.

Several experiments with artificial selection in *Drosophila melanogaster* and quantitative genetics revealed the possibility of a genetic tradeoff between reproductive success, lifespan and other life history traits providing support for the theory of antagonistic pleiotropy (33). Such trade off would be responsible for a negative

correlation between high fitness in young individuals and reduced fitness in old ones based on analysis of genetic variance and covariance within a population (34). Yet, particular genes and chromosomal loci that are directly involved in the control of age specific survival and reproductive capacity that leads to senescence have not been identified or tested (35). Nevertheless, quantitative trait loci have been found to occur with antagonistic effects in both sexes in different environments of *Drosophila melanogaster* (29). Other studies on *Drosophila melanogaster* have provided insight on which genes might display antagonistic pleiotropy, in particular the gene hsp70, which expresses heat shock proteins that regulate certain characteristics of the individual fitness, has been observed to have a negative pleiotropic effect on age specific reproduction and longevity (34).

The theory of mutation accumulation is based on the accumulation and higher frequency of mutated alleles at several loci characterized by detrimental effects in late stages of life, an occurrence that is allowed by weak selection (33). Such mutated alleles have neutral effect on the fitness of young organisms but an increase in frequency leads to deleterious effects on the fitness and longevity of the aging organism. Therefore these mutations are believed to cause aging (29). According to this theory the genes affecting aging and senescence have small effects individually on the longevity, a fundamental quantitative genetic trait and life history trait, of organisms and by escaping the strength of natural selection are maintained in the genome of a population (30). Mutation accumulation involves an inbreeding depression that has an effect on age specific mortality and an increase in genetic variance maintained by mutation pressure in late life. This has been observed within populations of *Drosophila melanogaster* in which random mating occurs (36,37).

The genes that will display detrimental effects on fecundity and survivorship are generated constantly within a population gene pool but selection against these genes grows weaker as the organism is aging causing their effects to increase. Organisms with a short lifespan reproduce early in life and the decline in fitness and fertility observed in late life could be due to the increasing deleterious effects of such genes. The maintenance of these genes within a population leads to an accelerated senescence dependent on a mutation selection balance (38). It has been observed that long lived vertebrates who produce many offspring early in life are subject to an accelerated senescence and therefore a decline in longevity. Yet, the possibility of studies on breeding performance and senescence patterns is difficult in vertebrates due to variation in individual quality (39). In general, experiments and predictions have addressed preferentially the theory of antagonistic pleiotropy and the data collected favored it. Nevertheless, these studies do not exclude mutation accumulation theory since both could be involved in the activation of the senescent state (30). Overall, there is no true explanation of the causes underlying the evolution of senescence and about the validity of a model of mutational effects and fitness dependent on the age of the organism (40).

Role of Telomeres Length in Cellular Senescence

Telomeres are guanine rich repeating sequences, six nucleotides 5'-TTAGGG-3' in mammals, and associated proteins that cap the end of chromosomes in eukaryotes. Telomeres have the critical function of protecting the DNA sequence of genes by capping the end of chromosomes. The ends of linear chromosomes are known to be susceptible to degradation by DNA nuclease enzymes at each round of DNA replication. The activity of the enzyme telomerase maintains the length of telomeres in germ cells and stem cells but in most somatic cells this enzyme is not active. As a result, the length of telomeres gradually decreases during each replication. The activity of telomerase is believed to be under genetic control. In particular, several genes including TLC1 and four EST have been identified as components and regulators of telomerase proper functioning *in vivo* (41). Active telomerase is a requirement for immortalization to occur and its expression is accompanied by simultaneous loss of p16 and Rb, key elements of cell cycle regulation (42). The progressive shortening of telomeres is connected to the process of aging in the organism as a whole and to the activation of cellular senescence. By restoring and maintaining the telomere length, telomerase is believed to act as a molecular clock that functions to regulate the replication activity of cells and the onset of senescence (43). For each population doubling, telomeres tend to lose approximately 100 base pairs and when their length is drastically reduced the cells enter senescence assuring a finite number of replications. Experimental data suggests that the total cellular life span can be measured by the progressive loss of telomere repeats and that transcriptional silencing of telomeres adjacent genes mediates senescence initiation (44). The critical length of telomeres is less than 5 kb and represents the threshold at which p53 and Rb pathways are activated and can trigger senescence (45).

A strong telomerase activity has been detected in immortalized and tumor cells leading to the capacity to replicate infinitely and to a possible loss or inactivation of expression of specific genes involved in the senescence program and regulation of the normal cell cycle (46). By restoring the activity of telomerase in normal skin fibroblast it has been demonstrated that this enzyme prevents the shortening of telomeres and eventually leads to immortalization. The increase in cellular lifespan appears to be directly proportional to the increased length of telomeres (18). The limited number of cell replications guaranteed by the gradual shortening of telomeres might protect the organism from the occurrence of cancer. Loss or dysfunction of telomeres might lead to genomic instability and DNA damage. Therefore, the onset of senescence as a response to short dysfunctional telomeres represents a mechanism of tumor suppression *in vivo* that arrests division and growth of damaged cells at risk of malignant transformation (21,47). Telomere length or telomerase activity alone does not necessarily lead to immortalization or

senescence. Experimental data have reported a negative correlation between entry of senescent state or continuous growth and telomerase activity in somatic cell hybrids (12).

A telomeric sequence specific DNA binding protein called TRF2 has been observed to have a protective function on telomeres by forming t loops and to play a role in the rate of telomere shortening leading to suppression of senescence and consequently to extended cellular lifespan. In particular overexpression of TRF2 triggers a more rapid telomere shortening while its loss triggers chromosomal aberrations and senescence (48). It has been proposed that senescence induced by replicative exhaustion due to telomere shortening resembles the senescence induced by the activation of the DNA damage checkpoint pathway. The dysfunction detected in short telomeres activate a response similar to the one activated by broken DNA double strand (49). Telomere dysfunction and impairment in DNA damage signaling in mouse models, deficient for telomeric proteins or with very short telomeres, are associated to premature aging, aging associated diseases and development of tumors (50,51). The dysfunction of telomeres seems to occur due to individual drastically shortened telomeres rather than to average length of telomeres of cells measured after a certain number of divisions in culture (52). Dysfunctional telomeres are responsible of initiating senescence through the activation of DNA damage checkpoint pathways. Experimental inactivation of the protein kinases involved in such pathways lead to restoration of the normal cell cycle and favors the progression of the cell to the S phase (49).

Studies on the role of telomeres shortening associated to aging and cancer focus also on the genetic pathways, in particular p53 and its downstream target p21, that are believed to mediate dysfunctional telomeres and lead to the consequent senescence. The status of the length of telomeres affects their function. Critically short telomeres without telomerase activity lead to genome instability that promotes development of tumors. Telomerase catalytic activity has been shown to extend cellular life span *in vitro* (53). The elevated level of the enzyme telomerase in human cancer is characterized also by intrinsic templating RNA moiety, hTER, and hTERT, the core protein human telomerase reverse transcriptase. Such catalytic components are found to be necessary for upregulation of telomerase activity and therefore immortalization. Immortalization promoted by hTERT is known to down-regulate expression of p16INK4a and therefore bypass cellular senescence (54,55). Keratinocytes grown in culture to express hTERT have been incompletely rescued from senescence suggesting that other factors, such as p16INK4a expression, induce senescence independently of telomeres status (56). By activating DNA damage response checkpoints dysfunctional telomeres initiate the p53-p21 pathway that triggers the arrest of cell proliferation (47). Ongoing research on the role of these genetic pathways can lead to therapeutic solutions for cancer. Potential therapies attempt to exploit the possibility that inhibition of the activity of telomerase can initiate tumor suppressor pathways associated to p53 and reduce tumor growth through initiation of p53 induced senescence (57).

Mediators of Premature Senescence

The progressive shortening of telomeres by triggering DNA damage responses can induce cellular senescence in cells that have exhausted the capacity to proliferate. However, premature senescence can be induced regardless of the number of cell divisions or telomere length. There are different types of premature senescence independent of telomeres length. Premature senescence can be induced by several factors such as disruption of heterochromatin, overexpression of oncogenes and in general by stressors that can elicit a DNA damage response (11,58). It is remarkable that, like replicative senescence, premature senescence telomeres dependent or independent, involves a DNA damage response that is triggered by single strand or double strand lesions. Due to the lack of repair of such lesions the damaged cells are induced to the senescent state. Senescent cells are characterized by different morphology and by the formation in the nucleus of senescent associated heterochromatin foci which are involved in the repression of genes that promote cell division (59). Furthermore it has been suggested that the changes in the organization of heterochromatin in senescent cells are associated to mechanisms of a stable process of gene silencing that maintains the permanent growth arrest that distinguishes senescence from quiescence (60).

Dysfunction in mitochondria can lead to a premature senescence regardless of the number of replications. Such dysfunctions are known to cause high production of superoxide and reactive oxygen species (ROS), normal byproducts of mitochondrial respiration (61). It has been observed that generation of reactive oxygen species can lead to accumulation of oxidative damage to cellular components and physiological deficit that might accelerate aging (62). Analysis of prematurely senescent cells from young proliferating cultured cells has shown to have high levels of reactive oxygen species caused by mitochondrial dysfunction or by altered activity of the enzymatic antioxidant pathway (63). The activity of proteasome, an enzyme that degrades oxidized proteins, has been observed to decline during senescence suggesting that oxidative stress increases due to a rapid accumulation of oxidative proteins that cannot be efficiently degraded (13). ROS are believed to be responsible of DNA double strand lesions, often double strand breaks that remain unrepaired, which tend to accumulate in senescent cells indicating a possible cause of aging in mammals (27). The role of ROS in the induction of senescence is supported by experimental results in which cells treated with antioxidant have a longer lifespan while

cells treated with sublethal concentrations of hydrogen peroxide manifest senescence (64).

Overexpression of oncogenic ras in primary human cells can induce a state of proliferation arrest that is phenotypically similar to replicative senescence. The premature senescence induced by oncogenic ras arises as a response to aberrant mitogenic signaling and is a mechanism to protect cells from malignant transformations leading to tumors that involves the genetic pathways of p53 and p16 through MAPK, mitogen activated protein kinase, signaling cascade observed in replicative senescence (65-67). Both DNA damage response checkpoint and oncogene induced senescence are tumor suppressor mechanism. DNA damage response pathways when triggered causes the arrest of proliferation by initiating senescence in cells with damaged or unstable genome. Inactivation of DNA damage checkpoint response results in the development of transformed cells while its activation leads to senescence and to maintenance of oncogene induced senescence (68). It is remarkable that oncogenic ras can lead to either senescence or tumorigenesis depending on the intensity of Ras signaling, low levels of activation involved in the formation of tumors and high levels of activation involved in senescence (69). Oncogenic Ras induced senescence is an example of organismal natural defenses against genome instability and uncontrolled proliferation that might lead to tumorigenesis. Another oncogene, RAF, is known to counteract oncogenic transformation and consequent neoplastic transformation similarly to oncogene RAS. In normal cells RAF have effects on cell division cycle arrest and apoptosis by initiating a protein kinase signaling cascade that mediates senescence in response to activation of Ras (70). Independently of the signal or event that promotes the senescent state, the p53, p16 and p21 elements of the tumor suppressor pathways are required for the initiation and maintenance of senescence.

Genes and Genetic Pathways in Cellular Senescence

Cellular senescence is a phenomenon associated to both the process of aging and tumor suppression on a genetic basis. While the correlation between aging and senescence is still controversial the role of senescence as protection from tumorigenesis is supported by molecular and cellular *in vivo* data (71). It is widely accepted that senescence serves as a mechanism of protection at least in preneoplastic lesions consequent to a DNA damage check point and DNA replication stress (72). The permanent growth arrest observed in cellular senescence requires the activation of tumor suppressor pathways, p53 and p16 which is a regulator of the retinoblastoma pathway. Rb and p53 share similar functions and are regulated by two proteins, p16INK4a and p19ARF, encoded by a single genetic locus (73). p53 is a transcriptional activator responsible of a complex network and is the most investigated to elucidate the mechanism of aging and tumor suppression. Functioning as a transcriptional activator, p53 activates a series of proteins that are present at low levels in normal cells but are known to participate in cell cycle regulation and senescence once activated (74). Both p53 and p16, a protein of the retinoblastoma pathway, are characterized by loss of function mutation in cancer and failure to initiate and maintain senescence. When properly functional, p53 and Rb (retinoblastoma) are known to be required for regulation of the cell cycle while when disrupted they play a role in cancerous cells development. p53 restricts cellular proliferation in response to DNA damage, genomic instability and deregulation of mitogenic oncogenes by inducing a variety of cell cycle checkpoints, cellular senescence or apoptosis (75). It has been observed that in humans and rodents cells exposure to replicational stress, such as DNA strand breaks and DNA adducts, triggers a state of senescence dependent on p53 and its target p21 (76).

In normal cells p53 prevents the loss of genomic integrity but it is also the most commonly mutated gene in cancer and its loss of function appears to be required for maintenance of aggressive carcinomas (25,75). When cellular stress is absent p53 is inactive and maintained at low levels through degradation via ubiquitin proteasome pathway (77). Another function of p53 is the regulation of DNA double strand breaks repair mechanisms, which represents another approach to prevent genomic instability. It is remarkable that tumor suppressor p53 coordinates several cellular responses including the senescence like cell cycle arrest initiated by oncogenic activation of MAP kinase cascade (78). The two possible consequences of cell cycle regulation by p53, senescence or apoptosis depend on integration of signals that can antagonize cell proliferation. The type of response is affected by cell type, oncogenic status, survival stimuli, intensity of stress signals and level of p53 expression (79). According to the type of stress stimuli, distinct combinations of phosphorylation events modulate the p53 response for a specific cellular outcome (77). Furthermore, the cellular response produced by p53 pathway activation is dependent on transcriptional activation of p53 target genes including miR-34, small RNA frequent in certain tumors, in a context depending mode (80). This would suggest a role for miRNA in tumor suppression and oncogenic activity that could be further analyzed to elucidate opportunities for cancer treatment and diagnosis. Expression of p16INK4a and ARF, which are potent tumor suppressors, is considered to be associated to the type of oncogene induced senescence observed in precancerous lesions (72). The regulation of senescence by RB/p16INK4a has been observed both in cultured human melanocytic naevi and *in vivo* as a response to chromatin modifications accompanied by expression of typical markers of the senescent state (81). Expression of p16INK4a has been found to increase during replicative senescence and aging by inhibiting cyclin dependent kinases CDK4 and CDK6 to maintain pRb in a hypophosphorylated state (82). Studies suggest that p16 functions as a second barrier against unlimited proliferation by inducing a state

of senescence irreversible, while senescence induced by p53 can be reversed upon its inactivation if p16 expression is absent (83). The involvement of p53 in protection from tumorigenesis is established by observation that abrogation of proliferation arrest induced by p53 results in immortalization, a requirement for formation of cancerous cells (84). Spontaneous immortalization requires not only loss of function of p53 but also the loss of p16 expression which appears to contribute to initiation of senescence by inhibiting the phosphorylation of pRB (85). Furthermore, cellular senescence has been observed to suppress tumor development *in vivo* by activation of p53 and pRB pathways, which through germ line inactivation produce senescence defective cells and cancer prone organisms (2). Years of intense research have suggested that p53 and retinoblastoma pathways are indeed crucial in the initiation of senescence as a tumor suppressor mechanism both *in vitro* and *in vivo*.

In the last decade, experimental data confirmed the complexity of tumor suppressor pathways, genetic regulation of cell cycle and their multiple interactions. Another gene involved in cell cycle regulation is Bcl-2. Bcl-2 is a major regulator of apoptosis but its expression is also involved in initiation of a senescent like phenotype characterized by increased activity of β-galactosidase, a typical feature of senescence, in human carcinoma cells (86). The senescent phenotype activated by Bcl-2 has been suggested as an additional natural barrier against uncontrolled cellular proliferation and cancer development. Also there is evidence of a link between cell cycle regulation, protection against oxidative stress and counteraction against the effects of oxygen radicals mediated by Bcl-2 (87). Since oxidative stress is one of the causes of premature senescence Bcl-2 was proposed to delay the onset of senescence but experimental observation confirmed that Bcl-2 can initiate a senescent state. Recent findings suggest that loss of p63, a p53 related protein, might induce senescence and accelerated aging. In particular, p63 might repress positive regulators of senescence, such as p53 and p16INK4a, and have several roles in maintenance of epithelial cells and stem cells proliferation (88). Yet further investigation is needed regarding the role of p63 in aging and cancer. p21 is a cyclin dependent kinase inhibitor that can trigger senescence by selectively inhibiting genes involved in mitosis and DNA replication and repair (89). Also senescent cells are known to contain high levels of p21 protein. p21 is therefore considered to play a role in aging related diseases and cancer that is under research. Another player in tumor suppression is the gene PML. PML, promyelocytic leukemia gene, is known to control cell proliferation and to induce senescence predominantly upon requirement of an intact Rb pathway but not necessarily p53 pathway (90). It has been suggested that PML functions as a regulator of the p53 response and its expression is in turn unregulated by oncogenic Ras indicating that p53 acetylation upon Ras expression is a fundamental event in PML induced senescence (67).

In general, many genes and the proteins encoded have been discovered to be involved in the mechanism of senescence associated to aging and cancer. A database that catalogs all the genes so far identified as "aging genes" based on experimental measurements of life span is currently available. It has been suggested that genes involved in the process of aging might exert effects on tumor suppression. In particular, a large number of "aging genes" have been identified on molecular basis in *Drosophila melanogaster*, such as superoxide dismutase (SOD), that can extend the lifespan by up to 85% and increase stress resistance in the lab strain of origin (15). Among the cataloged "aging genes", many are involved in the tumor suppressor pathway regulated by p53. On this basis, the mechanisms of aging and senescence appear to be intrinsically connected to cancer development and increased cancer incidence. Overall, the mechanism of tumor suppressor pathways remains unclear. Difficulties arise from the complexity of the network of genes that appear to cooperate and interact in the regulation of the cell cycle. While the crucial role of p53 and pRb is widely accepted and subject to further analysis in order to gain knowledge about the process of aging and the possibility of efficient cancer therapies, the identification of other genes and knowledge regarding their involvement is still incomplete.

Cellular Senescence and Cancer

The knowledge acquired so far about the mechanism of senescence in relation to aging and cancer might lead to development of effective cancer therapies. The link between aging and cancer is based on the synergic occurrence of genetic events, oncogene mutations, and epigenetic events, cellular senescence (2). Since tumor suppressor genes are critical players in cell protection against tumorigenesis, they are a major target for the development of cancer therapies, in particular p53 based therapies. Such genes are usually found to be inactive in tumors due to mutational processes. It has been suggested that individuals carrying a germ-line mutated allele of one of these genes are more susceptible to cancer, therefore by increasing the gene dosage of tumor suppressor genes it could be possible to decrease the chances of inactivation and decrease the incidence of cancer (91). Increased dosage of the tumor suppressor locus Ink4a/Arf has been associated to a genetically inherited resistance to cancer without the occurrence of premature aging suggesting a model that results in a beneficial cancer resistant phenotype (92).

Another genetic approach involves the use of phosphorylation proteins associated with activation of p53 to suppress oncogene induced tumorigenesis *in vivo* (77). Anticancer agents have been shown to activate p53 and p16INK4a pathways which naturally suppress tumorigenesis by initiating a senescent program that contributes to chemotherapy drug action and treatment outcome *in vivo* (93). In fact, p53 is believed to be an important prognostic factor to determine treatment outcome. Drug induced senescence is

a possible anticancer therapy based on a forced induction of an irreversible proliferation arrest in cancerous cell. For example, cytotoxic drugs have been used to induce DNA damage in tumor cells leading to p53 induced senescence in tumor tissue *in vivo* (94). Yet, it has to be taken in consideration that senescent cells often acquire novel functions that could have a side effect on neighboring cells (95). Overall, anticancer therapies rely on the irreversibility of the growth arrest in senescent cells but it has been observed that senescence can be reversed. Reversal of the senescent state has been accomplished by inactivation of p53 or oncogenic Ras which results in proliferation (83). In sarcomas pharmalogical restoration of endogenous p53 expression has been shown to lead to tumor regression and cell growth arrest by initiating senescence (96).

Conclusion

The possibility of reversing senescence raises doubts about the possibility to block permanently cancerous cells growth by inducing senescence. It appears to be more feasible to focus on the development of drugs that can rescue p53 function following loss of function due to mutation. Additionally, the development of diagnostic tests to establish the status of p53 in cancer could result useful to predict the level of mutation (97). On the same line, identification of the genetic mutations and defects that result in inactivation of oncogenic Ras, therefore lack of oncogenic induced senescence as a tumor suppressive program is important towards optimal treatment strategies (9). So far, the current research on potential therapies to fight cancer is focusing on the organism natural barrier against malignancies that is p53 induced cellular senescence. Further investigation of the mechanisms underlying the tumor suppressor pathways and the senescent state is directed to exploit the possibility to use senescence inducing agents as a safe and efficient anticancer therapy. Research focused on each target genes roles and function in the p53 and pRb pathways could provide insight on the natural tumor suppressor mechanisms in organisms and the aging process. In particular, additional research *in vivo* is necessary to analyze the mechanisms underlying the linkage between senescence and aging. Such knowledge could lead to full restoration of the genetic pathways that are inactivated by the development of cancerous cells. Mutation and inactivation of these genetic pathways favors cell proliferation that is required for tumors to grow regardless of cell checkpoints. Therefore, if tumor suppressor pathways could regain their original function it would be possible to naturally suppress tumor growth through activation of senescence. Cellular senescence and loss of function of these genes contributes also to aging and the consequent accumulation of DNA damage. Thus, genetic research could elucidate if the detrimental effects of antagonistic pleiotropy typical of tumor suppressor pathways and aging genes can be reduced in order to increase longevity and if senescence can be used as protection against cancer without accelerating the aging process.

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