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Cancer and Aging: A Complex Biological Association

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ABSTRACT

Cancer is one of the leading causes of death in both developing and developed countries. It is also a particularly significant health problem in older populations since half of all malignancies occur in patients aged 70 years or older. Cancer is a disease of aging, and as such there is a strong biological association between the mechanisms of aging and carcinogenesis. During the past few decades, mechanisms of aging exerting pro- and anti-oncogenic effects have been described, and the role of these mechanisms in cancer treatment and prognosis is currently being investigated. In this review we describe the different theories of aging and the evidence on the biological link between these mechanisms and carcinogenesis. Additionally, we review the implications of the biology of aging on the treatment and prognosis of older adults with cancer, and the opportunities for translational research into biomarkers of aging in this patient population. (REV INVES CLIN. 2016;68:17-24)

Key words: Cancer. Elderly. Oncogenesis. Cancer treatment.

INTRODUCTION

One of the main risk factors for the development of chronic disease is increasing age, and cancer represents no exception to this finding. Biologically speaking, aging is a process of progressive decline of morphological and physiological traits, whereas, from a biochemical point of view, aging is characterized by the accumulation of age-related molecular changes. During the past few decades, aging mechanisms exerting pro- and anti-oncogenic effects have been described, and this has helped clarify between the gradual functional decline seen with aging and the process of carcinogenesis. The aim of this review is to describe the current evidence on the biological links between aging, carcinogenesis, and cancer treatment, and to understand the importance of considering these links when designing research into cancer in older adults.

THEORIES ON AGING

A number of theories on aging have been proposed, although none of them is universally accepted. Several

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of them will be described below to provide a theoretical framework for the association between cancer and the aging process.

Mutation accumulation theory

The mutation accumulation theory is based on the assumption that natural selection does not have an effect on mutations without detrimental effects until later in life. It also proposes that mutated genes accumulate through time and finally express themselves as aging[1]. Other theories on aging, such as the oxidative stress theory, may be understood as part of the mutation accumulation theory since both of them attribute aging to the damage produced by reactive compounds. Frequently found molecular modifications associated to reactive compounds include general hypomethylation[2], hypermethylation of CpG islands, and accumulation and/or mislocalization of heterochromatin.

In response to the accumulation of molecular modifications, mechanisms of damage response are needed. One such mechanism is the DNA damage response (DDR), which aids in maintaining the integrity of the genome and epigenome as a response to toxic stimuli. The DDR may lead to programmed cell death or cell cycle arrest, but also to DNA and chromatin repair. Repair may induce mutations and epimutations favoring cellular degeneration and uncontrolled cell proliferation, both of which may subsequently increase the risk of cancer[3].

Antagonistic pleiotropy

This theory states that genes that favor reproduction may be selected as a priority, even though they could be associated with disadvantages later in life[4]. Both p53 and $p16^{INK4a}$ may provide useful examples; these proteins have anticancer effects throughout the life of an individual, but since their expression in different tissues is upregulated with age[5-7], they may be more easily affected by mutations and epimutations. In human cells, both mutations and epimutations of the $p16^{INK4a}$ locus have been proven to favor carcinogenesis[8].

Disposable soma

The theory of disposable soma was proposed in 1977, and is based on the assumption that organisms should optimize the allocation of resources between somatic maintenance, growth, and reproduction. Too high an investment in somatic maintenance, when the probabilities of dying from extrinsic mortality are elevated, would represent a waste. Inversely, too low an investment would probably result in premature death. The theory suggests that organisms develop differential accuracy promoting mechanisms in somatic and germ cell lines. In somatic cells, reduced accuracy allows energy saving, accelerated development and reproduction, while leading to eventual deterioration and death. On the other hand, in germ cells, high levels of accuracy are maintained so that defective cells can be eliminated[9].

TELOMERE BIOLOGY

Replicative senescence is a mechanism that leads to irreversible growth arrest after a number of cell divisions during serial cultivation[10]. Replicative senescence belongs to a more complex process characterized by permanent cell cycle arrest, induction of cyclin-dependent kinase inhibitors, expression of senescence-associated beta galactosidase, morphological and metabolic alterations, significant chromatin and nuclear remodeling, changes in the transcriptional program of the cell, and the secretion of a specific set of factors. Cellular senescence increases with age and can be induced by molecular damage, leading to the development of aging-associated phenotypes. This set of phenomena is closely associated with telomeric function. Telomeres are nucleoprotein structures containing repetitive DNA sequences located at the end of each chromosome[11]. Each cell division induces the loss of part of these telomeres. When a critically short telomere length is reached, a cascade of events leading to the inhibition of proliferation through replicative senescence or apoptosis ensues[12,13]. In some cell populations, telomere shortening can be reversed through the synthesis of a specific enzyme (telomerase). It is worth noting that to maintain adequate tissue development and regeneration, normal telomere function is required. Nevertheless, cellular senescence has other critical executors including retinoblastoma 1 (regulated by $p16^{INK4a}$) and p53, both of which are also implicated in tumorigenesis. The levels of $p16^{INK4a}$ have been described to increase progressively with the proliferation of cells[14,15], and depression of the CDKN2a locus (the locus encoding for p16INK4a and p19ARF) can be accelerated through the expression of certain oncogenes (oncogene-induced senescence)[16,17].

SENESCENCE AND CANCER

Some studies have demonstrated the presence of a high number of senescent cells in pre-neoplastic lesions, suggesting that cellular senescence has an anti-oncogenic role[18-20]. It is believed that oncogene-triggered senescence represents an early protective mechanism against excessive proliferation of oncogene-expressing cells. Additionally, cellular senescence may induce oncolytic effects through the immune system[21,22]. However, it is still unknown whether it is replicative senescence, stress-induced senescence, or both that induce antiproliferative mechanisms.

Senescence may also promote cancer through extrinsic and intrinsic pathways. Previous work shows that reactive oxygen species, due to their high mutagenicity, promote the molecular switches needed for the emergence of post-senescent cells with tumorigenic characteristics[23]. Another view holds that cancer represents a metabolic rebellion against host aging. It has been shown that cancer cells amplify oxidative mitochondrial metabolism, which contrasts with the classically described aging-induced metabolic changes such as a switch towards aerobic glycolysis. As a result, a two-compartment metabolic scenario is established; cancer cells are oxidative, and the aging host is glycolytic[24]. The amplification of oxidative mitochondrial metabolism likely involves the expression of genes such as PCG1a/b (positive transcriptional regulator of mitochondrial genes) and the activation of its downstream target nuclear respiratory factor 1 (NRF1). It has been proposed that the loss of telomerase activity during the aging process induces activation of p53, which in turn decreases the expression of PCG1a/b and its targets[25].

In addition, senescent cells express a secretory profile characterized by the presence of increased amounts of proinflammatory cytokines and growth factors. These changes can induce proliferative or degenerative defects in the neighboring non-senescent cells with a dual role in oncogenesis, contributing to tumor clearance by activating immunity[21,22], but also inducing mesenchymal transition and invasiveness of premalignant cells[26].

In tissues with a high rate of cellular turnover, adult stem cells are largely responsible for the maintenance of tissue homeostasis. Stem cells are capable of self-renewal, but as an organism ages this process may become impaired, resulting in a diminished stem cell pool. This reduction may not be only quantitative in nature, but also qualitative, with the remaining stem cells showing impaired abilities to proliferate and differentiate. These changes may, in turn, contribute to impaired immune function, as happens in the context of hematopoietic stem cells, for example[27,28].

The fate of stem cells is determined by an intricate balance between proliferation, cell arrest, self-renewal, and differentiation. Since stem cells have long lives, their risk for acquiring mutations is increased. Therefore, specific genome protection mechanisms are put into action. Hematopoietic stem cells are able to maintain cellular cycle arrest, minimizing errors and mutations induced by DNA replication. However, this cellular cycle arrest also leads to the inability to use homologous recombination to repair DNA damage since this mechanism can be only activated in the S phase of the cell cycle. Alternate mechanisms (non-homologous end joining) are more error prone and must be used when the hematopoietic stem cells are quiescent, which increases the risk of mutations.

It is noteworthy that stem cells in different organs use distinct mechanisms to maintain genomic integrity, although the reasons for this phenomenon remain unclear. Nevertheless, it is known that there is an age-dependent accumulation of DNA damage in stem cells across different organs[29,30], even in the presence of these protective mechanisms.

During DDR, p53 is phosphorylated and G1 cell arrest is induced. Deletion of p53 increases stem cell proliferation, cell renewal capacity, and de-differentiation in animal models[32-34]. The DNA damage may lead to premature differentiation of stem cells, as in the myeloid skewing of hematopoietic stem cells.

Senescence and apoptosis have different implications, depending on the cell type, amount of DNA damage, and degree of telomere dysfunction. A popular view is that functional changes of stem cells through time are a byproduct of cancer-suppressing mechanisms, where p53 and $p16^{Ink4A}$, both regulators of tumor suppression and senescence, illustrate the antagonistic pleiotropy theory.

There are also several extrinsic regulators of stem cell fate, including the microenvironment as well as paracrine and systemic factors[27,35,36]. At least two extrinsic cell mechanisms have been described to promote oncogenic changes: (i) loss of proliferative competition, and (ii) impaired immune clearance of senescent cells. Proliferative competition is the process by which the selection of undamaged cells from the stem cell pool is carried out[37]. As we age, more stem cells with DNA damage accumulate, restricting the cell pool and contributing to the selection of premalignant clones[38]. On the other hand, immune-mediated depletion is a process favoring tissue integrity[21,22]. Some mutations associated with the extension of lifespan have been discovered in recent years, most of them involved in the balance of energy intake, storage, and use in response to the environmental conditions. For example, insulin, insulin-like growth factor 1 (IGF-1), and target of rapamycin pathways can be activated by the availability of food, whereas food restriction activates AMP-activated protein kinase and sirtuins. Lifespan extension-associated pathways inhibit the former or stimulate the latter, decreasing degenerative diseases, including cancer. Examples of extrinsic stimuli for these pathways include caloric restriction without malnutrition and exercise[39-42]. One of the most important downstream targets of IGF-1 signaling is the mammalian target of rapamycin (mTOR), which is a conserved serine/threonine kinase that regulates cell growth, aging, and metabolism[43]. It is a key modulator of aging and age-related disease, and its inhibition extends the lifespan of organisms and confers protection against a growing list of aging-related pathologies[44]. The mTOR pathway is frequently activated in human cancers, and its inhibition has been hypothesized as a potential mechanism to counter both the aging process and carcinogenesis[45]. One such inhibitor, metformin, has recently received a considerable amount of attention due to its potential as a regulator of the growth of cancer cells. At the cellular level, metformin has a profound effect on mitochondrial respiration rate and on the production of ATP[46]. Metformin affects multiple cellular pathways via the activation of AMP-activated protein kinase (AMPK) by liver kinase 1, which leads to a decrease in growth factor signaling and proliferation via mTOR inhibition[47]. However, until now epidemiological studies have shown discordant results, and trials exploring the role of metformin in different cancer types are currently recruiting participants[46,48].

On the other hand, inhibiting IGF-1 as well as growth hormone-dependent pathways may further impair muscle mass maintenance in elderly patients, contributing to frequent clinical phenomena in this age group, such as frailty (a state characterized by diminished strength, gait speed, and physical activity, and poor endurance). Table 1 summarizes the implications of the different theories of aging on terms of aging and oncogenesis.

AGING AND ANTINEOPLASTIC THERAPY

As we have seen, although there is no accepted unified explanation for aging, there are several mechanisms that may be responsible for this process. Additionally, most of these mechanisms share common characteristics with carcinogenesis, representing a physiological link between cancer and aging. Since chemotherapy was used for the first time in the first half of the 20th century[49], there has been an exponential growth in the quantity and quality of available treatments for cancer patients. This, in turn, has led to an increase in the number of cancer survivors, most of which have been exposed to some form of systemic antineoplastic therapy. In fact, in the USA alone, as of 2014 there were nearly 14.5 million people with a history of cancer, of which 46% were 70 years of age or older (Cancer Treatment and Survivorship facts and figures 2014-15)[50]. Some of the long-term issues cancer survivors face include an early onset of decreased cognition, chronic fatigue, skin changes, and others, which are commonly seen with aging. This has led to research into chemotherapy and other cancer treatments as causes of accelerated aging, and to the search for biomarkers of aging in patients undergoing curative-intent antineoplastic treatments.

CHEMOTHERAPY AND ACCELERATED AGING

Chemotherapy can potentially cause or accelerate several of the cellular stressors that have been implicated in the process of normal aging, including free radical damage, direct DNA damage, telomere shortening, and neuroendocrine/immunologic dysfunction[51]. Recently, there has been increasing interest in measuring such an effect in order to guide clinical decision making and to better understand the way in which

Table 1. Summary: Implications of different theories of aging in terms of aging and oncogenesis

Theories of aging	Implications in terms of aging	Implications in terms of oncogenesis
Mutation accumulation theory	Proposes that mutated genes accumulate through time and finally express themselves as aging. Theories such as the oxidative stress theory and the DNA damage theory may be understood as aspects of this theory. Progeroid syndromes, genetic diseases that appear to be accelerated aging, frequently originate in genes that are related to DNA repair or metabolism.	In response to the accumulation of molecular modifications, mechanisms of damage response are put into action. Repair of molecular modifications may induce mutations and epimutations, favoring cellular degeneration and uncontrolled cell proliferation and subsequently increasing the risk of cancer.
Antagonistic pleiotropy	It states that genes that favor reproduction might be selected as a priority even though they could be associated with disadvantages later in life.	p53 and $p16^{INK4a}$ are proteins providing anticancer effects throughout life; however, since their expression in different tissues is upregulated with age, they may be more easily affected by mutations and epimutations. These epimutations have proven to favor carcinogenesis.
Disposable soma	It is based on the assumption that organisms should optimize the allocation of resources between somatic maintenance, growth, and reproduction.	In germ cells, high levels of accuracy are maintained so that defective cells can be eliminated.
Telomere shortening	Closely associated with cellular senescence. In order to maintain adequate tissue development and regeneration, normal telomere function is required.	High number of senescent cells is found in pre-neoplastic lesions suggesting that cellular senescence has an anti-oncogenic role. However, senescence may also promote cancer. Reactive oxygen species, due to their high mutagenicity, promote the molecular switches needed for the emergence of post-senescent cells with tumorigenic characteristics. Senescent cells express a secretory profile that may induce proliferation and degeneration in neighboring cells.

treatment may affect cancer survivors in the long term. As we have already seen, a final common mechanism for all stressors is the induction of cellular senescence, which is in turn strongly associated with the activation of the INK4/ARF (CDKN2a) locus on chromosome 9p21.3, which encodes the $p16^{INK4a}$ and ARF tumor-suppressor proteins[52]. These two proteins play major roles in senescence by controlling the retinoblastoma (in the case of $p16^{INK4a}$) and p53 (in the case of ARF) tumor-suppressor pathways[53]. Because they are not only passive biomarkers of aging, but also play a fundamental role in the aging of organisms, changes in the expression of these proteins in response to different stressors have been studied both in preclinical models and in cancer patients. $P16^{INK4a}$ has been shown to be a good *in vivo* marker of cellular senescence, as well as a marker of tissue insults that may not represent cellular senescence such as tissue wounding caused by various stressors[54]. Activation of $p16^{INK4a}$ and induction of senescence in murine models has been related to cellular exposure to gerontogenic compounds such as arsenic, high-fat diet, UV light, and cigarette smoking[55]. These findings were recently translated to human aging by Sanoff, et al., who studied the expression of both $p16^{INK4a}$ and ARF mRNA in $CD3^+$ lymphocytes of 33 women with stage I-III breast cancer before and at three different time points after receiving anthracycline-based adjuvant chemotherapy[52]. Both $p16^{INK4a}$ and ARF were increased by the administration of adjuvant chemotherapy, with an absolute increase of 75% (equivalent to 14.7 years of chronological aging). The authors also studied a cross-sectional cohort of previously treated breast cancer survivors (median time after completion of chemotherapy of 3.4 years), finding a similar increase in both markers, which was equivalent to 10.4 years of chronological aging. Chemotherapy has also been shown to cause telomere dysfunction both in healthy and neoplastic cells by directly interfering with the shelterin nucleoprotein complex, particularly with

the binding of the telomeric repeat-binding factor 2[56]. While these studies show that chemotherapy may indeed have gerontogenic properties, the translational possibilities of the observed results are still unknown, and trials studying the implications of this accelerated aging in the prognosis and functional status of cancer survivors are ongoing (NCT01472094).

RADIOTHERAPY AND ACCELERATED AGING

Radiation therapy plays a fundamental part in the treatment of solid and hematological malignancies, and up to 50% of cancer patients require it for the treatment of localized disease, local control, and palliation[57]. Although the goal of modern radiotherapy is to destroy cancer cells while sparing adjacent tissues, it still has the potential of causing damage to normal cells. Radiation causes cell death by several mechanisms (apoptosis, autophagy, and loss of clonogenic survival, among others), which ultimately induce necrosis or senescence[58]. Additionally, ionizing radiation may also induce significant biological changes in tissues that are widely separated from the irradiated area, causing non-targeted side effects, which may be detrimental for long-term cancer survivors[59]. Studies measuring different biomarkers have shown that exposure to ionizing radiation has the potential to increase molecular aging. For instance, a study in 10 acute lymphoblastic leukemia survivors found that the expression of $p16^{INK4a}$ was 5.8-times higher in scalp biopsies than in biopsies of non-irradiated skin[60]. Radiation therapy is also capable of damaging telomeres, either directly via ionization events or indirectly through post-irradiation alteration of the telomere maintenance mechanisms[59]. This telomeric DNA damage may theoretically induce accelerated telomere shortening in normal cells, which in turn could lead to accelerated aging. However, a clinical study performed on 25 patients with solid tumors undergoing treatment with radiation therapy showed that telomere length was not affected by ionizing radiation[61].

BIOMARKERS OF AGING AND CLINICAL DECISION MAKING IN ONCOLOGY

Measuring and quantifying the gerontogenic properties of specific drugs or combinations of drugs by employing biomarkers of aging could be particularly useful for deciding whether to administer chemotherapy, especially when the absolute benefits of treatments are small[62]. An ideal biomarker of aging should predict the rate at which a person is aging, should monitor a process central to the biology of aging, should be able to be repeatedly tested, and should have an animal model in which to be replicated[63]. Several candidate biomarkers have been studied (Table 2), all of which have strengths and limitations. The measurement of telomere length, which has been shown to decrease with aging, has fallen out of favor due to its variability,

Table 2. Potential biomarkers of aging

Biomarker of aging	Advantages	Disadvantages
Proinflammatory markers (IL-6, C-reactive protein)	Identification of "aging phenotype"[65] Easily measured in the clinical setting	Unspecific, may be increased in inter-current illnesses Lack of a causal role in aging
Advanced glycation end products	Associated with increased mortality in older adults[66] Easy to measure in blood and urine samples	Lack of a causal role in aging Different AGEs available for measurement, lack of data regarding the most useful one
Senescence-associated beta galactosidase	Direct staining in tissues, highly reproducible *in vitro*[67]	Lack of specificity for aging Lack of a causal role in aging
Telomere length	Inversely correlated with chronological age[68] Can be measured from peripheral blood	High interindividual variability[62] Assays are expensive and cumbersome
p16iNK4a mRNA in T-lymphocytes	High correlation with chronological age Validated *in vitro* in animal models and in human cells	Difficult to perform in the clinical setting due to problems with the sorting of T-cells

IL: interleukin; AGE: advanced glycation end product.

low reproducibility, and high costs. More recent markers, such as the aforementioned $p16^{INK4a}$, appear to be more promising in human studies, but have been hampered by the fact that its measurement requires the isolation of peripheral blood T lymphocytes in order to obtain good quality RNA[62].

Obtaining a high-quality biomarker of aging would potentially represent a shift in the way many decisions are made in the treatment of cancer patients, and perhaps especially in older adults. Even though recent research has shifted the focus from chronological to physiological age when planning treatment for an older cancer patient[64], there are still gaps to be filled regarding the appropriate choice of therapy in this population. Establishing the patient's "molecular age" could help in providing patients with tailored treatments aimed at achieving optimal results while minimizing toxicities. This represents an exciting field of research, and changes in biomarkers of aging should be considered as relevant translational endpoints when designing therapeutic clinical trials in oncology.

CONCLUSIONS

In conclusion, aging and cancer have an incredibly complex relationship, not only from the biological point of view, but also because of the ways in which aging can influence the outcome of both cancer and its treatments. In the future, translational and clinical researchers should take into account this complex relationship when designing clinical trials across the whole spectrum of cancer treatment. Including biomarkers of aging into clinical studies could potentially improve our understanding of the effect of both cancer and its treatment on the senescence of normal cells. Additionally, the inclusion of these translational endpoints in clinical trials would give us further insight into the long-term toxicity of cancer treatments. Finally, being able to correlate these markers of aging with functional outcomes in patients undergoing treatment would potentially allow us to predict such outcomes and would perhaps open the way for preventive interventions. Without a doubt, research into the aging-related changes in cancer is necessary for directing future prevention strategies, understanding the way in which treatment can be tailored for older adults, and improving the outcomes of cancer in this patient population.

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