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Aging and Disease

Part I

Guest Editors

José A. Ávila-Funes

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Cover figure: Senile amyloid plaques in the temporal cortex from a patient with Alzheimer's disease. Staining with Thioflavin S and acquired in a Olympus BX41 fluorescence microscope (400x). Courtesy of Braulio Martínez-Benítez, MD, Department of Pathology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico.

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Preface

José Alberto Avila-Funes[1,2]* and Silvestre Frenk-Freund[3]

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The elderly population is increasing worldwide. Never before in history have there been so many persons aged 60 years or older. This has multiple consequences and challenges for health systems and governments. Therefore, the promotion of research on aging is imperative, not only from a biological viewpoint but also from social, economic, and political standpoints. A better understanding of these phenomena will allow the formulation of new recommendations focused on the aging population and based on a solid scientific background, far from obsolete stereotypes.

There is no unique phenotype for the elderly: the heterogeneity of their health status is not only determined by a genetic basis, but also by the positive and negative effects of the cumulative events of a lifetime. Therefore, the identification of those potentially modifiable risk factors through a lifetime approach could promote a healthy and active aging process, with less disability and comorbidities[1].

The development of knowledge on human aging is an ideal and strategic field for translational research. Since the goals of translational research are to combine disciplines, expertise, and techniques for the advancement in prevention, diagnosis, and therapies, the title of this special issue of the Revista de Investigación Clínica – Clinical and Translational Investigation – "Aging and Disease" is very pertinent[2]. Aging, the action of getting older and the result of metabolic processes initiated at birth, if not at gestation, includes the concept of "development": the paradigm of differentiation and the natural delimitation of every species. It is understood that the concept of development makes up the governing motive of thought and paidopsychiatric action, as well as the conceptual basis of what in the past was defined as "pediatric judgment."

From time to time, almost every other periodic publication, including those of scientific character, produces monographic issues. The reason is that they include mainly or exclusively articles focused on particular themes from specific areas of knowledge. Often issues of this nature, due to the high quality of their content, reach permanent prestige in the literature. The present issue of the renowned-from-birth journal Revista de Investigación Clínica is indeed monographic and is published in English to ensure that the issue is available to a greater number of readers.

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The content of this issue ranges from basic science to clinical aspects and epidemiology. The biology of healthy aging, immunosenescence, neuroinflammation as well as sarcopenia and frailty and all the way to the epidemiology of cognitive aging are included. Other contributions are related to frequent pathologies that develop during aging, and the extensive experimental work in the field of geriatrics currently in progress. Distinguished Mexican researchers in the field of aging have participated in this issue of the journal and have also invited foreign colleagues who share the interest in studying the processes that take place in the elderly and how to improve the quality of aging itself.

Research in Mexico has accepted a new challenge: to contribute to a better understanding of human aging and to use this knowledge to benefit our population, which is not immune to this outstanding and unprecedented demographic transition. In this sense, we hope that this special number of the Revista de Investigación Clínica – Clinical and Translational Investigation – becomes an excellent testimony to the current state of the art in the gerontological sciences.

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Biology of Healthy Aging and Longevity

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ABSTRACT

As human life expectancy is prolonged, age-related diseases are thriving. Aging is a complex multifactorial process of molecular and cellular decline that affects tissue function over time, rendering organisms frail and susceptible to disease and death. Over the last decades, a growing body of scientific literature across different biological models, ranging from yeast, worms, flies, and mice to primates, humans and other long-lived animals, has contributed greatly towards identifying conserved biological mechanisms that ward off structural and functional deterioration within living systems. Collectively, these data offer powerful insights into healthy aging and longevity. For example, molecular integrity of the genome, telomere length, epigenetic landscape stability, and protein homeostasis are all features linked to "youthful" states. These molecular hallmarks underlie cellular functions associated with aging like mitochondrial fitness, nutrient sensing, efficient intercellular communication, stem cell renewal, and regenerative capacity in tissues. At present, calorie restriction remains the most robust strategy for extending health and lifespan in most biological models tested. Thus, pathways that mediate the beneficial effects of calorie restriction by integrating metabolic signals to aging processes have received major attention, such as insulin/insulin growth factor-1, sirtuins, mammalian target of rapamycin, and 5' adenosine monophosphate-activated protein kinase. Consequently, small-molecule targets of these pathways have emerged in the impetuous search for calorie restriction mimetics, of which resveratrol, metformin, and rapamycin are the most extensively studied. A comprehensive understanding of the molecular and cellular mechanisms that underlie age-related deterioration and repair, and how these pathways interconnect, remains a major challenge for uncovering interventions to slow human aging while extending molecular and physiological youthfulness, vitality, and health. This review summarizes key molecular mechanisms underlying the biology of healthy aging and longevity. (REV INVES CLIN. 2016;68:7-16)

Key words: Aging. Sirtuin. Calorie restriction. Acetylome. Genome. Epigenetics.

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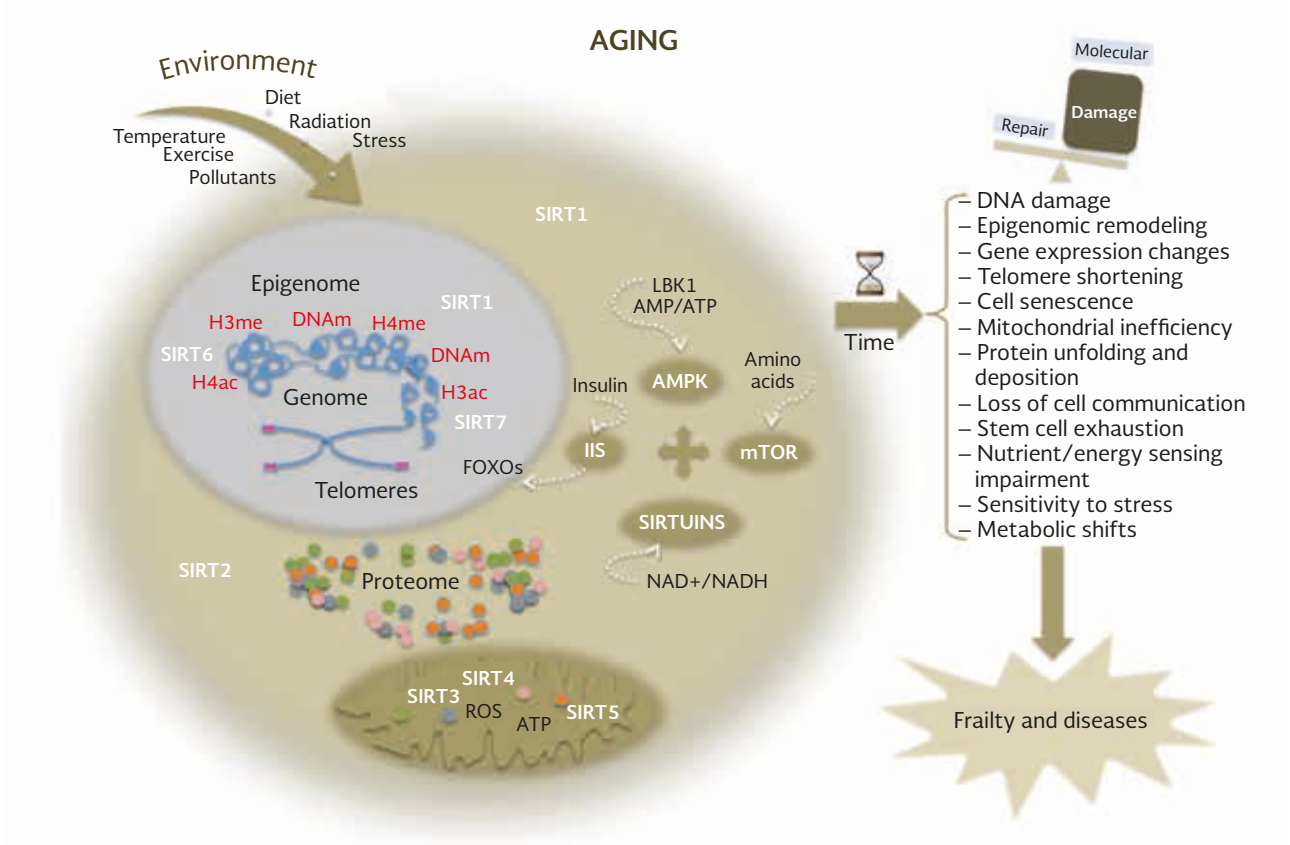
INTRODUCTION

Aging is a process of gradual physiological deterioration that all living beings experience with time. It is a heterogeneous and heterochronic process. As a heterogeneous process, aging may occur at different rates across diverse organisms, and even organisms of the same species can age at variable rates. Furthermore, the asynchrony by which various cells and tissues age within a single organism highlights the heterochronic nature of aging. At the biological level, aging is characterized by the accumulation of molecular and cellular damage, which leads to structural and functional aberrancies in cells and tissues, such as loss of mitochondrial homeostasis, impaired intercellular communication, senescence (cell arrest that hampers growth and division), and decreased regenerative capacity[1]. The dynamic interaction between a living being and its environment defines the rate and fate of aging, as is shown in figure 1. The ability of organisms to overcome stress and respond to external environmental challenges/insults is blunted within aged individuals when compared to younger counterparts[2,3]. Healthy aging, however, refers to the warding off of molecular and cellular decline for the longest length of the lifespan. Not surprisingly, healthy aging

Figure 1. Molecular mechanisms of aging. Homeostasis of the genome, telomeres, epigenome and proteome, all contribute to molecular integrity and healthy aging. Biological pathways that have the dual ability of sensing nutrients and/or energy levels, while also regulating cellular processes like epigenomic remodeling, gene expression, protein activity and organelle integrity –*i.e.*, mTOR, IIS, AMPK, sirtuins (SIRT1 in nucleus and cytoplasm; SIRT2 in the cytoplasm; SIRT3, SIRT4 and SIRT5 in the mitochondria; and SIRT6 and SIRT7 in the nucleus)– each play a key role in aging. Moreover, dynamic interaction between a living being and its environment also impacts the rate and fate of aging. Loss of molecular homeostasis leads to the cellular hallmarks of aging, ultimately contributing to frailty and diseases. Epigenetic marks such as methylation (me) or acetylation (ac) of histones H3 and H4 and DNA methylation (DNAm) are shown in red in the nucleus. The mitochondrion is the major source of energy production (ATP) and reactive oxygen species (ROS) generation. Mitochondrial fitness is thus an essential feature of healthy aging.
SIRT: sirtuin; FOXO: forkhead box protein; ATP: adenosine triphosphate; AMPK: AMP-activated protein kinase; IIS: insulin/insulin-like growth factor-1 signaling; mTOR: mammalian target of rapamycin; NAD+: nicotinamide adenine dinucleotide (oxidized); NADH: nicotinamide adenine dinucleotide (reduced form); ROS: reactive oxygen species.



has been associated with increased longevity. This claim is substantiated by the fact that genetic, dietary, and/or pharmacological interventions that promote cellular homoeostasis, stress resistance, and protection against age-related diseases also tend to extend lifespan and *vice versa*[4-6]. Overwhelming scientific evidence supports the claim that there is no single cause of aging. Indeed, notable advancements in the biology of aging, especially during the last few decades, have contributed to the identification of multiple mechanisms that modulate the aging process[1,7-9]. Despite this progress, uncovering interventions that can achieve healthy aging in humans is challenging. The conserved molecular and cellular mechanisms that underlie aging, especially how these pathways interplay and how complex lifestyles and environments to which humans are exposed modify them, are not completely understood.

Biological models such as yeast, worms, flies, and mice have contributed greatly towards understanding the biological mechanisms of aging as they offer many advantages over humans: e.g., they (i) have much shorter lifespans; (ii) are easier to manipulate genetically; and (iii) allow for better experimental control and study design, given the reduced environmental variability while studied. Diverse approaches can be achieved with the use of animal models such as the appealing contributions to the aging field of heterochronic parabiosis experiments in mice, which implies the fusion of an old and young mouse through their capillaries to share blood circulation. Heterochronic parabiosis has shown that systemic administration of young blood plasma into aged mice improves diverse aging features like cognitive impairments, neurogenesis, skeletal muscle regeneration, and cardiac hypertrophy. It also has allowed identifying circulating aging/pro-aging factors such as the growth differentiation factor 11 (GDF11) that acts in muscle and the cytokine CCL11 in the brain[10-12]. Experiments with animal models, especially rodents, have recently challenged the older paradigm of aging as an immutable process. Today, a growing body of evidence suggests that external/environmental manipulations related to diet, exercise, and changes in blood composition (due to the introduction of circulating factors from young animals into older ones) may rejuvenate diverse aspects of the physiology, including those associated with the aged central nervous system as reviewed by Buchard, et al., like the restoration of regenerative capacity and improvement of neuronal synaptic and cognitive function, besides the potential to extend health and lifespan[13].

In humans, most aging studies are performed using peripheral blood samples, biopsies, postmortem tissues, and various types of cells that can be propagated *in vitro* using artificial culturing conditions. In all of these cases, it is difficult to control for individual-level lifestyle/environmental factors between people, which adds substantial experimental variability. Furthermore, additional aging models are being reported in the literature, which hope to elucidate more about the multifactorial process of aging across species. Some of these emerging models include: the shortest-lived vertebrate killifish (~ four months), the longest-lived rodent mole nude rat (30 years), the bowhead whale (presumed to be the longest-lived mammal at 200 years), and bivalve mollusks (survive up to 500 years)[2,14,15]. Across all of these species, diverse experimental approaches have been used to study the biological signals of aging, ranging from the discovery of aging-associated biomarkers, genes/polymorphisms, regulatory proteins, hormones, compounds/metabolites, and various diets (from starvation and reduced calorie intake to intermittent fasting), which may modify lifespan and the aging process, as well as delay the onset of age-associated diseases and/or confer resistance to environmental challenges[8,16-18].

THE LIFELONG EFFECTS OF CALORIE RESTRICTION

Calorie restriction (CR) has endured for more than 75 years as the single most robust method to increase longevity and delay aging and disease across diverse organisms. Clive McCay at Cornell University performed the first CR experiments in 1935, using rats fed with 30% less food than the regular chow given to control-fed (*ad libitum*) rats. The CR diet increased both mean and maximum lifespan by more than 30%[19]. Since these initial experiments, all species tested by feeding them with 20-40% less food, from yeast to rodents, show lifespan extensions of up to 50%[20]. Experiments in rodents and monkeys have shown that CR decreases basal metabolic rate and energy expenditure, while physical activity remains unaltered. In these same animals, CR reversed key physiological biomarkers of aging like endometriosis,

osteoporosis, sarcopenia, high blood pressure, body fat accumulation, and glucoregulation imbalance[21,22]. Molecularly, data show that CR may reverse the nine typical cellular features of aging: (i) telomere erosion; (ii) epigenetic alterations; (iii) stem cells depletion; (iv) cellular senescence; (v) mitochondrial dysfunction; (vi) genomic instability; (vii) proteostasis imbalance; (viii) impaired nutrient sensing; and (ix) abnormal intercellular communication[23].

The beneficial effects of CR have also been documented in experiments using genetic and/or chemical models of disease linked to various cancers, neurodegeneration, cardiac disease, and inflammation[24-26]. Whether CR is sufficient to retard aging and extend longevity in humans is still unknown. Recent studies on a regimen of intermittent CR, induced by subjecting mice and humans to only a few days of fasting per week, have shown rejuvenation in the endocrine, immune, and nervous systems of mice and improvement in biomarkers of diseases (diabetes, cardiovascular disease, and cancer) and regeneration in humans, all without major adverse effects[27]. The "geroprotective" action of CR has led to a growing interest in searching for CR mimetics: i.e., small-molecule therapeutics or other chemicals/interventions that can recapitulate the rejuvenation effects attributed to CR. Examples of some of these compounds include resveratrol, rapamycin, and metformin, which will be discussed further below[28].

GENOME INTEGRITY, TELOMERES AND HEALTHY AGING

The genome contains the whole set of DNA, encoding the biological information (i.e., the genes) that determines the ability and extent to which organisms can develop within and respond, cope, and adapt to their environmental conditions. The genome not only includes genes but also non-coding sequences important for gene regulation. The major portion of the human genome –highly structured and ordered in the nucleus– is comprised of ~3 billion base pairs of linear DNA (called the "nuclear genome"); about less than 2% of these billion base pairs encode approximately 25,000 human genes. Lastly, a very small amount of circular DNA containing only 37 genes is confined to the mitochondria (called the "mitochondrial genome").

In the nucleus, the DNA is tightly looped around histone proteins organized into fundamental units named nucleosomes. Nucleosomes then package into the chromatin that is further ordered into discrete chromosomes. The ends (or "tips") of the chromosomes are protected by telomere "caps," which are repetitive sequences of DNA that conform to a highly specific motif (TTAGGG), spanning 8,000-15,000 base pairs in tandem[29]. Aging is classically associated with shortening of telomeres and high levels of DNA damage, including mutations, DNA breaks, and chromosomal rearrangements. Although the DNA experiences anywhere from 10,000 to 1,000,0000 molecular lesions per day, cells are equipped with a repair machinery that detects DNA lesions and repairs them[30]. With age, genome damage surpasses DNA repair capacity, causing genome instability. In line with this, studies have shown that DNA damage accumulates in old human tissues[31]. Strikingly, experiments in mice have demonstrated that accumulation of mutations in the mitochondrial genome also lead to premature aging[32]. As for the telomeres, 20-200 base pairs are lost per cell division. Once the telomeres reach a critically short length, the cells stop replicating, become senescent and die. Conversely, fetal tissues, stem cells, adult germ cells, and tumor cells –all with the capacity to propagate indefinitely– have some specialized machinery that allows them to maintain telomere length[33]. Several studies have shown that short telomeres in human leukocytes or peripheral blood mononuclear cells correlate with aging, unhealthy lifestyle habits, and diverse diseases like atherosclerosis, inflammation, and neurodegeneration[33]. It is widely documented that failures in telomere maintenance and/or DNA repair are associated with premature aging phenotypes, including cellular senescence, inflammation, cardiac disease, cancer, and neurodegeneration[30,34]. Thus, preservation of genomic integrity during the lifespan is essential for protecting against senescence, diseases, and for promoting healthy longevity. Importantly, CR favorably impacts the DNA repair and telomere machinery to enable these beneficial ends[35].

The genomics revolution continues to foster technological advancements that allow the sequencing of thousands of genomes from diverse organisms and facilitates their study simultaneously in an unprecedented manner. Genome-level analyses and comparisons, within the same or across different species,

continue to inform all branches of science, especially aging research[36]. To this end, comparative genomic studies of mammals that live longer than humans and that are more resilient to environmental challenges, senescence, and/or the development of age-related diseases may help to better elucidate the genes and molecular mechanisms that preserve health. For instance, the genome of the bowhead whale, which lives twice as long as the average person and has a body comprised of a thousand times more cells than us, shows striking modifications in the content of genes involved in DNA repair, cancer, cell cycle pathways, and aging[14]. Similarly, studies in the mole rat, a rodent resistant to senescence and cancer and that maintains elevated fecundity rates until death, show that this animal has powerful genomic traits such as anti-tumorigenic gene arrangements, which correlate with its resilience to cancer as it ages[37].

In the human genome, two canonical syndromes of accelerated aging, Werner syndrome (WS) and Hutchinson-Gilford progeria syndrome (HGPS), are caused by mutations in genes involved in DNA repair and mechanisms vital to preserving the nuclear envelop that protect the genome. Accordingly, cells derived from WS and HGPS patients exhibit higher levels of DNA damage, genomic instability, and telomere shortening[38,39].

EPIGENETIC INFLUENCE ON LONGEVITY VIA THE ENVIRONMENT

Although it is well accepted that the genome plays a key role in aging, and that experimental genetic manipulations in various species can increase or decrease their lifespans[40], studies in genetically identical human twins have shown that they do not necessarily develop similar diseases/phenotypes or age synchronously[41]. Data from twins suggest that the genome does not solely account for physiological traits or disease risk, but that additional layers of biological information may also shape cellular homeostasis, health, and aging. A new field has thus emerged: "epigenetics," which literally means "above the genes." The "epigenome" then is the entire collection of mechanisms that contribute to the modulation of gene expression, which has direct impact upon disease/phenotypes without changing the underlying sequence of the DNA.

Epigenomic regulation may occur at different levels: (i) at the DNA level itself (DNA methylation, abbreviated DNAm, which is the addition of a methyl group to cytosines); (ii) at the histone level (modifications of histones by methylation, acetylation, or phosphorylation); and (iii) at the nucleosome level (ATP-dependent chromatin remodelers regulate nucleosome positioning). Collectively, these epigenetic mechanisms regulate the genome's topology via chromatin structure to affect gene expression. For instance, tight and compact chromatin, packed densely by DNAm and specific histone modifications, may assemble into "closed" chromatin that can limit the accessibility of the machinery needed for gene expression. While a looser or "open" form of the DNA (usually facilitated by un-methylated DNA and a different set of histone modifications) is associated with active gene transcription[42]. The ability of various environmental signals (e.g., ambient toxins/pollutants, temperature, diet, etc.) to alter the genome via epigenetic alterations is well established in the literature[42,43]. As such, environmental signals can modulate chromatin remodeling and gene expression by adding/removing epigenetic marks on histones or the DNA itself[42-44].

Epigenetic alterations and gene expression changes are known to occur during human aging and disease. Studies in mice and in humans have shown that tissues experience gene expression changes, possibly due to epigenetic mechanisms across time in the young versus the old. For example, analysis of brains from 26- to 106-year-old humans shows that gene expression changes involved in neuronal function, mitochondrial fitness, DNA repair, antioxidant activity, and stress response occur after age 40[45]. In addition, Peters, et al. (2015) recently identified 1,497 genes that were differentially expressed with age in the whole blood of 14,983 individuals. These gene-expression profiles were used to determine the "transcriptomic age" of individuals. Interestingly, differences between transcriptomic age and chronological age were associated with key physiological hallmarks of aging such as cholesterol levels, body mass index, blood pressure, and fasting glucose[46]. Yet, it remains unclear how environmental factors orchestrate epigenetic and gene transcription changes in the brain, blood and/or in other tissues to affect health and the aging process itself. Disease susceptibility as a consequence of aging, therefore, is likely due to the combination of environmental "programming" via epigenetic

marks and predetermined factors, namely genetic mutations/polymorphisms, operating together to shape individual human health trajectories[44,47].

Human studies are inherently difficult to design and perform since they have to account for variations in lifestyle/environmental factors across people and time (e.g., changes in the type of diet, smoking, exercise, alcohol consumption, sleep, etc.), which are all self-reported measures subject to recall bias. Therefore, it is critically important to continue gaining biological insights from model organisms, where conserved genetic pathways relating to aging can be more easily disentangled from environmental/epigenetic factors, given better control of experimental variability in the laboratory setting. Despite these issues, human epigenetic research using robust, well-characterized cohorts has produced seminal contributions to the aging field. For example, accumulation of DNAm at specific loci across tissues can serve as a novel type of "biological clock" to inform key questions pertaining to development, cancer, and aging research[48]. Indeed, a recent application of this work shows that DNAm-calculated age from human blood samples could accurately and sufficiently predict all-cause mortality later in life[49]. Nevertheless, recent studies by Peters, et al., suggest that a combination of gene expression, epigenetic and telomeric data should be considered to refine age prediction[46].

Moreover, other epigenetic markers, such as methylation and/or acetylation in specific lysine residues of certain histone tails, have been shown to change with age[43]. Therefore, newer technological platforms and biocomputational methods are constantly being developed to better detect and map epigenetic changes (DNAm, histone modifications, transcriptomic-level profiles) to help elucidate more about the role of epigenetics and the environment within human aging and disease risk[42,43,46].

ENHANCED PROTEIN STABILITY ASSOCIATES WITH EXTREME LONGEVITY

Proteins are the factors that directly perform or enable cellular function and taken together they comprise the "proteome". Proteins build up diverse intracellular structures, establish metabolic networks through a diverse set of enzymatic activities, and integrate all physiological pathways to act in concert. Protein stability or proteostasis refers to the cellular capacity to protect protein structure and function against ambient stressors like changes in temperature, pH, oxidative stress, radiation, and aging. Vulnerability in proteostasis correlates with changes in aging and longevity rates among species[50]. Studies show that long-lived species are highly resistant to protein unfolding and to several environmental stressors, thereby maintaining endogenous enzymatic activities compared to short-lived organisms with less effective/robust proteostasis[15]. Cells contain an elaborate proteostasis network that involves: protein synthesis, chaperones, autophagy, the unfolded-protein response, and the ubiquitin-proteasome pathway. These network components collectively are aimed at maintaining protein turnover, counteracting protein misfolding, clearing-up unfolded proteins, and recycling of long-lived products[51,52]. Studies show that with age, this network can be compromised, leading to protein accumulation and the aggregation of anomalous unfolded and/or damaged proteins. Indeed, age is the major risk factor for cytotoxic deposition of protein aggregates. For instance, tau and beta-amyloid protein deposition are hallmarks in Alzheimer's disease and alpha-synuclein in Parkinson's disease[53]. In addition, differences in protein abundance and features that alter protein function, like protein cellular localization and gain/loss of protein marks or posttranslational modifications, can all occur with aging and at different rates in tissues. For example, protein alterations show that the brain ages more rapidly than the liver[54], thus allowing us to gaining deeper insights into the molecular basis of heterochrony during aging. Also, a set of proteins that are posttranslationally modified by reversible acetylation (known as the "acetylome") change with aging, among which is found the tau protein associated with Alzheimer's disease. Interestingly, CR as well as other types of diets including high fat diet, which alter aging, also impact the acetylome[55,56].

Various pathological states due to aging-related nutrient deregulation are also linked to protein damage. Aging markers at the organismal level, like hyperglycemia or hyperinsulinemia, enhance the generation of damaged proteins via glycosylation or oxidation, respectively. Molecules that are non-enzymatically modified by carbohydrates known as advanced glycosylation end products (AGEs) are linked to accelerated

aging, inflammation, and chronic diseases. Accordingly, untreated diabetic patients that maintain elevated glucose levels experience several physiological features consistent with accelerated aging, including osteoporosis, obesity, cataracts, altered wound healing, and vascular and microvascular deterioration[57].

MOLECULAR PATHWAYS THAT MODIFY THE RATE OF AGING

Some molecular pathways have the dual ability of sensing nutrients and/or energy levels while also regulating cellular processes like epigenome remodeling, gene expression, protein activity, and organelle integrity. Not surprisingly, such pathways have been found to act as key regulators of aging and diseases. For instance, the mechanistic target of rapamycin (mTOR) and insulin/insulin-like growth factor-1 (IGF-1) signaling (IIS) pathway are sensitive to nutrients, while AMP-activated protein kinase (AMPK) and the sirtuin enzymes sense energy levels[58-62] (Fig. 1). All of these pathways, to varying degrees, have been implicated in mediating the beneficial effects of CR in aging and a variety of age-related disorders. Accordingly, small molecules that target these pathways have been identified as CR mimetics, including resveratrol, rapamycin, and metformin[26,63-65].

Insulin/insulin-like growth factor-1 signaling pathway

The first pathway to alter the rate of aging, IIS, was identified in the worm model *C. elegans*[66]. The IIS pathway couples growth/survival signals with glucose nutrient status. In worms, flies, and mice, a reduction in IIS signaling increases longevity. In mice, a decrease in IGF-1 also delays the aging processes, including the onset of cancer and immune decline, as well as helping to maintain youthful cognitive ability[64]. The gene expression regulatory factors known as forkhead box proteins or FOXOs are key components of the IIS pathway. Nutrient conditions define FOXOs' cellular localization, which may be either in the cytoplasm or in the nucleus. Although the role of the IIS pathway in humans is poorly understood, genetic variants and/or combinations of small-nucleotide polymorphisms (SNP) in the human components of the IIS pathway correlate with low IGF-1 plasma levels in centenarians. Strikingly, in some human populations, various FOXO SNPs are associated with extreme longevity, thereby suggesting that this pathway may play a role in human lifespan extension[62].

Mammalian target of rapamycin and rapamycin

Rapamycin is a type of antibiotic produced by the bacteria *Streptomyces hygroscopicus* that has long been used as an immunosuppressant and in cancer therapy. Studies in yeast cells allowed the field to identify the TOR genes as important mediators of the antiproliferative effects of rapamycin. Later on, the mTOR gene in mammals was identified as the physical target of rapamycin. The mTOR acts as a serine/threonine protein kinase that responds to insulin, amino acids, and hormones to regulate a large range of cellular functions, including protein and lipid synthesis, autophagy, inflammation, mitochondrial function, and glucose metabolism[67]. In yeast cells, it was also first discovered that mTOR plays an important role in aging. Subsequently, a collection of data from different species, including worms, flies, and mice, have demonstrated that either genetic or pharmacological inhibition of mTOR (or components of the pathway) increases lifespan[61,68]. In zebra fish, mice, and humans, depletion of the mTOR pathway improves age-related disorders like cognitive decline, cancer, Alzheimer's disease, and kidney and heart diseases[69]. It has also been described, paradoxically, that mTOR inhibition may cause adverse effects *in vivo*, such as glucose intolerance, insulin resistance, and dyslipidemia[61]. In addition, genetic evidence shows that functional integrity of the mTOR pathway is necessary for conferring the effects of CR-mediated lifespan extension[70]. Thus, mTOR stands out as a key albeit complex modulator of longevity and health.

AMP-activated protein kinase

AMP-activated protein kinase is a kinase that senses changes in energy status. Both low levels of adenosine triphosphate (ATP) and phosphorylation by the liver kinase B1 (LKB1) activate AMPK to regulate a large number of physiological processes via modulation of molecular cascades that involve protein phosphorylation. The AMPK activation leads to a decrease in ATP utilization and an increase in energy production. Studies in rodents have shown that CR activates the AMPK pathway in heart, liver, and skeletal muscle[71]. Yet, data have

also shown that chronic CR fails to activate this pathway[26]. Overexpression of AMPK increases the lifespan of worms, flies, and mice prone to dying of cancer. Increased levels of LKB1 also promote longevity in flies. Interestingly, FOXO factors that are key regulators of the IIS pathway (as mentioned above), may also participate in mediating the longevity effects of AMPK[72]. Studies in worms demonstrate that metformin (a drug that disrupts mitochondrial function) requires AMPK to cause a 50% increase in worm lifespan. Pro-longevity effects of metformin have also been reported in both normal and cancer-prone mice. In humans, metformin has been widely used for the treatment of type II diabetes and also shows anti-tumorigenic effects. Although it is well substantiated that metformin activates AMPK, it is still unclear whether all the beneficial health effects of this drug depend entirely on the AMPK pathway[60].

Sirtuins

The sirtuin pathway was originally identified in yeast as a regulator of lifespan[73]. In humans, there are seven members of these proteins expressed ubiquitously in tissues, yet each of them is localized to distinct or shared cellular compartments. Three sirtuins regulate mitochondrial functions (SIRT3, SIRT4 and SIRT5), while SIRT1, the most comprehensively studied of the seven, modulates gene expression and the function of proteins involved in diverse cellular pathways. Both SIRT6 and SIRT7 are mainly confined to the nucleus to regulate gene expression, and SIRT2 functions in the cytoplasm. A unique aspect of sirtuins is that they require nicotinamide adenine dinucleotide (NAD^+) for their enzymatic activity, which is a coenzyme essential for metabolic homeostasis. By removing acetylation marks from the lysine residues of histones or non-histone proteins, sirtuins regulate the epigenome and the acetylome, respectively. Also, sirtuins modify the proteome by removing diverse types of acyl groups, including succinyl, malonyl, or fatty acids, or by adding an ADP-ribose moiety onto targets[55,65]. Thus, sirtuins have the remarkable ability to directly detect changing energy levels and orchestrate an enzymatic response to maintain cell homeostasis[59]. A large body of work has demonstrated that sirtuins play an important role in mediating aging and age-related diseases like cancer, inflammation, cardiac function, and cognition through the regulation of diverse molecular/cellular process, including genomic stability, senescence, DNA repair, mitochondrial function, metabolic homeostasis, and stem cell exhaustion. Using genetically engineered mouse models that express altered levels of SIRT1 in the brain, we have demonstrated that the integrity of this pathway is essential for normal learning and memory[74]. In addition, high levels of SIRT1 in the villi of the intestine protect against colon cancer[74,75]. In other experiments, ubiquitous overexpression of SIRT2 and SIRT6 has been reported to promote longevity in a mouse prone to cardiac disease and in normal mice, respectively[76,77]. Decreased levels of sirtuins, however, may also cause beneficial effects. For instance, genetic or pharmacological inhibition of SIRT2 protects against neurodegeneration in mouse models[78]. In humans, a variety of SNPs associated with sirtuin genes have been identified to correlate with healthy aging and longevity. For example, SNPs linked to the *SIRT1* and *SIRT3* genes have been found in long-lived populations of Chinese and Italian people, respectively[79,80]. Metabolic markers of aging, such as atherosclerosis, obesity, type II diabetes, and neurodegeneration (Alzheimer's and Parkinson's disease), also correlate with sirtuin polymorphisms[81,82]. Accordingly, small molecules that have been identified as activators of the sirtuin pathway, such as the natural polyphenol resveratrol[83], recapitulate CR-like effects in obese mice like improvements in insulin sensitivity, motor function, and endurance. At the molecular level, this polyphenol reduces IGF-I levels, increases AMPK enzymatic activity, and boosts mitochondrial number. However, resveratrol has so far failed to extend lifespan in lean mice[84].

CONCLUSIONS

Despite the impressive advancements made towards understanding more about the molecular basis of aging, there is still no definitive intervention for ensuring healthy aging in humans. To uncover new therapeutic avenues, we need to gain deeper knowledge about how different internal and external factors regulate the cellular hallmarks of aging, and how their regulation changes across time and individuals. All molecular pathways exhibit complex communication known as "crosstalk." The genome, epigenome, organelles, proteome, and pathways such as those involving sirtuins, mTOR, AMPK, and IIS-all integrate and process signals that must act coordinately to promote homeostasis

in cells and tissues (Fig. 1). It is unclear, however, how these complex molecular networks are affected by diverse environmental challenges and how they become impaired with aging. Lastly, in an effort to find beneficial interventions to delay aging-linked deterioration, the search for small molecules that can mimic CR –and the dissection of their pharmacological modes of action *in vivo*— is a growing area of research that merits more attention. Collectively, through all of these scientific efforts, we may someday achieve the longstanding human dream of living a long and healthy life.

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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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Frailty and Vascular Cognitive Impairment: Mechanisms Behind the Link

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ABSTRACT

The relationship between frailty and cognitive impairment has been recognized for decades, but it was not until a few years ago that the interest in this relationship increased and is now being understood. Epidemiological evidence suggests that physical frailty may be linked to cognitive impairment since both conditions share pathophysiological mechanisms at the cellular and systemic levels. Aging itself promotes multiple vascular changes, making the brain susceptible to cognitive decline through mechanisms such as thinning of blood vessels, increased collagen accumulation, rupture of the blood-brain barrier, inflammation, and oxidative damage. The prevalence of frailty and cognitive decline increases as individuals become older, and cognitive impairment attributable to cerebrovascular disease has become a major public health problem since vascular dementia is now the second most common subtype of dementia. However, full understanding of the mechanisms underlying the relationship between frailty and vascular cognitive impairment remains fragmented. This review examines the link between frailty and vascular cognitive decline and also explores the role of vascular changes in the genesis of both conditions. (REV INVES CLIN. 2016;68:25-32)

Key words: Frailty. Vascular cognitive impairment. Molecular vascular risk.

INTRODUCTION

The frailty syndrome is characterized by the loss of physiological reserves and resilience, leading older adults towards an elevated risk of declines in health and function. In the last two decades, the concept has gained general interest although its relationship with various biomarkers of aging remains debatable[1]. The frailty phenotype has been operationally defined by the presence of at least three of the five following criteria: unintentional weight loss, weakness, exhaustion, slowness, and low level of physical activity. Identifying frailty in older adults is relevant as it helps clinicians recognize those at increased vulnerability for adverse health-related outcomes, including premature death, hospitalization, and disability[2]. Several studies have shown that frailty is associated with impaired cognitive performance and has been proposed

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as a risk factor for both dementia and mild cognitive impairment (MCI)[3,4]. Pathophysiological mechanisms, such as subcellular disturbances (e.g., oxidative stress and protein misfolding), systemic disorders, as well as impaired maintenance of mitochondrial function (failures in chaperone proteins, autophagy) are common features in frail and cognitively impaired people as well[5]. Disturbances in hematological and inflammatory pathways leading to a catabolic status and endothelial dysfunction could also be responsible for the association between frailty and multiple cardiovascular diseases including vascular dementia[6]. On the other hand, cerebrovascular disease (CVD) is a well-known determinant of cognitive decline in older adults. Cerebrovascular disease is a marker of atherosclerosis; for example, an increased thickness of the carotid intima-media has been associated with a greater presence of silent cerebral infarcts, higher white matter hyperintensity (WMH), and further decline of cognition[7,8].

Despite being biologically plausible, the relationship between frailty and cognitive impairment has been inconsistently reported, and the pathophysiological bases remain under consideration[9,10]. Recent evidence suggests that CVD, via vascular endothelial dysfunction (e.g., impaired signaling molecules), is present among both cognitively impaired and frail persons. In this regard, the altered synthesis of nitric oxide (NO) by the endothelium has been implicated as a potential factor in the genesis of CVD[11]. Even though the relationship between frailty and Alzheimer's disease (AD) is yet to be clarified, the one between frailty and vascular dementia has proven more consistent[12,21]. In fact, a cognitive domain has been proposed as another criterion of frailty; as it adds cognition to the conventional frailty phenotype, it improves its predictive value for adverse health-related outcomes. Furthermore, frail individuals have a higher risk of cognitive decline and dementia in comparison to their non-frail counterparts and *vice versa*[13]. The purposes of this review are to examine the mechanisms underlying the relationship between frailty and cognitive decline and to explore the role of vascular changes in these conditions.

FRAILTY AND COGNITIVE IMPAIRMENT

A number of epidemiological studies have reported that frailty increases the risk of cognitive decline and that cognitive impairment in turn elevates the risk of being frail, suggesting that both conditions interact within a cycle of an aging-associated decline[13]. It is biologically plausible that both conditions share physiopathological mechanisms, but previous research remains inconclusive around this issue[14].

The concept of a frailty phenotype is relatively well understood in the context of aging, and has been used in studies conducted over the past two decades. However, how the cognition domain should be included within this concept is still under debate. The term "cognitive frailty" has emerged and is attractive as a suggestion of an existing parallel between aging, physical frailty, and cognitive function. Although cognitive frailty may seem useful in common practice, its actual use remains controversial. Kelaiditi, et al. state that cognitive frailty must be considered as being independent of dementia or pre-existing brain disorders, even if both conditions share several pathophysiological mechanisms and risk factors[15,16].

The important role of the central nervous system in frailty manifestations has been postulated since the central components of frailty, such as problems in gait or balance, can be related to neurological disturbances. Previous investigation has shown that impaired physical performance (measured by walking speed or the Short Physical Performance Battery [SPPB]) is an independent risk factor for cognitive decline[17]. Execution of the SPPB test (including walking speed, balance, and chair stands) requires the complex interplay of sensory, cognitive, and motor functions, which could be impaired early in the pathway to cognitive decline[18] and be expressed as frailty. A secondary analysis of the Rush Memory and Aging Project (a cohort study of aging and dementia) showed a postmortem association between common age-related brain disorders (including cerebral infarctions, Lewy body pathology, and AD pathology) and the frailty phenotype. Their results showed that subjects who were frail before death had more postmortem evidence of AD pathology compared to non-frail participants, an association independent of a previous dementia diagnosis[19].

Considering the cognition domain (in the frailty phenotype) has improved the identification of frail persons at risk of adverse health-related outcomes. Avila-Funes, et al. proved that cognitively impaired frail individuals a had higher risk of disability and incidental hospitalization compared with their non-frail counterparts, even

after adjusting for potentially confounding variables[20]. Nevertheless, it seems that a frail status is more strongly associated with the risk of incident vascular dementia even after adjustment for many potential confounders[21]. Within the same line of study, Gray, et al. in the Adult Changes in Thought (ACT) Study explored the association between frailty and incident AD and non-AD dementia type. In this population-based study, frailty was associated with a 2.6-fold increased risk for non-AD dementia subtypes, but the authors are inconclusive about the underlying mechanisms[22]. The relationship between frailty and cognitive impairment could be bidirectional, but in light of these studies, the frailty phenotype probably represents a physiological state that occurs before non-AD cognitive impairment, suggesting that frailty is a prodromal state of non-degenerative dementia.

On the other hand, cognitive performance probably plays a role in the prognosis of frail individuals. A recent study conducted in Hong Kong aiming to establish the transition between the different frailty states showed that at baseline, among pre-frail older adults (Fried's criteria 1 or 2), 23.4% of men and 26.6% of women improved to non-frail status after two years of follow-up, and 11.1% of men and 6.6% of women worsened to frailty[23]. In this study, among pre-frail men, a higher Mini-Mental State Examination (MMSE) score was inversely associated with frailty, suggesting that a lower cognitive performance could be a marker for future frailty. Another study suggests a low cognitive performance as a risk factor for dementia rather than frailty *per se,* since the risk was seen only in the subject subgroups that presented both frailty syndrome criteria in addition to low cognitive performance, and the risk was not present in those showing only frailty phenotype[20].

VASCULAR COGNITIVE IMPAIRMENT

Vascular pathology of the aging brain and AD includes cerebral amyloid angiopathy, which leads to lobar mass hemorrhages, small or recurrent bleeds and ischemic infarcts, microvascular degeneration, disorder of the blood-brain barrier, white matter lesions, microinfarcts, lacunes, and cerebral hemorrhages[24]. Beyond the possible role of vascular risk factors and vascular-related diseases, there are several potential pathways by which frailty could contribute to cognitive decline.

Cognitive impairment attributable to CVD has been termed "vascular cognitive impairment" (VCI), which is related not only with cortical or subcortical infarcts, but also with small vessel disease (WMH, lacunar infarcts, and microbleeds) inducing ischemic and hemorrhagic brain injury[25]. Recently, hypertension[26], diabetes[27], and dyslipidemia[28], among others, have been identified as risk factors of CVD as well as cognitive impairment, explaining a large proportion of cases of small vessel disease, but not all. Although several monogenic forms in early onset of small vessel disease have been described among patients with VCI, most are sporadic cases with an increased frequency in the familial aggregation, but with no clear Mendelian inheritance pattern[29,30].

The most widely investigated genetic variants are the single nucleotide polymorphisms (SNP), which are bi-allelic variants in the human genome involving a nucleotide exchange. Certain SNPs have been associated with lesion development in the cerebral white matter. A meta-analysis of Paternoster, et al. identified 46 genetic studies of polymorphisms in 19 genes in a total of ≈ 19,000 subjects[31]. The CHARGE (Cohorts for Health and Aging Research in Genomic Epidemiology) consortium is an investigator-initiated collaboration to facilitate genome-wide association studies (GWAS) meta-analyses among multiple large and well-phenotyped cohort studies, with cerebral magnetic resonance imaging (MRI) and genome data[32]. Seven community-based cohorts included in this study performed a GWAS for WMH burden in 9,361 stroke-free European descent individuals; results identified six novel risk-associated SNPs in one locus of chromosome 17q25 all encompassing six known genes: WBP2, TRIM65, TRIM47, MRPL38, FBF1, and ACOX1. The most significant association with cerebral white matter lesion was rs3744028. Other polymorphisms of a single nucleotide were rs9894383, rs936393, rs3744017, and rs1055129. Variant alleles at these loci conferred a small increase in WMH burden (4-8% of the overall mean WMH). This study provides the first characterization of this new locus on chromosome 17 as a possible factor contributing to pathophysiology associated with WMH burden of individuals of European descent[33].

Apolipoprotein E (*APOE*) is a gene that has been linked to the vascular and amyloid metabolism. The homozygosity for *APOE* ϵ4 allele has been associated with the presence of small vessel disease, higher volumes of WMH, and lacunar infarcts. In addition, the results

of the Austrian Stroke Prevention study suggest an association between *APOE* ε2 expression and the presence of WMH and lacunar infarction, while the Rotterdam scan study also reported higher prevalence of cerebral microbleeds among *APOE* ε4 allele carriers[34-36].

On the other hand, the genes involved in VCI must be of two non-reciprocal exclusive classes: (i) those which predispose to CVD, and (ii) those which determine the tissue response to CVD (e.g., genes conveying tolerance or susceptibility to ischemia or the ability to recover from ischemia). The two best-studied monogenetic forms of CVD are the cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), and the hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D). In contrast, little is known about the second class of genes, but evidence of their existence is seen in patients with a similar vascular pathology load (type of injury, number, and location), differing in the severity of cognitive impairment[37].

It is also possible that some genetic factors contribute to the development of conventional cardiovascular risk factors (such as hypertension, diabetes, or hyperhomocysteinemia), which may also interact with environmental factors or contribute directly to an intermediate phenotype[38].

The *NOTCH3* gene has been associated with development of CVD. The *NOTCH3* gene (a heterodimer composed of a large extracellular fragment and a smaller transmembrane intracellular fragment) is normally expressed in vascular smooth-muscle cells and pericytes (including those of the cerebral vasculature). *NOTCH3* encodes a cell-surface receptor related to cellular proliferation and to differentiation and survival of vascular smooth muscle. Mutations in the *NOTCH3* gene have been associated with the development of CADASIL. About 95% of patients with CADASIL have missense mutations that cluster in exons 3-4 and consist in changes of cysteine residues, but the pathogenic mechanism associated with this mutation is still unknown[39].

CONTRIBUTION OF VASCULAR DISTURBANCE TO NEURODEGENERATION

Neuroinflammation is a key component in AD and CVD, involving the proliferation of microglia and astrocytes, transcription factor activation (nuclear factor kappa beta [NF-κβ]), and upregulation of inflammatory cytokines (tumor necrosis factor alpha [TNF-α], interleukin [IL]-1β, prostaglandin E2, reactive oxygen and nitrogen species)[40].

Based on epidemiological studies, Thiel, et al. propose two hypothesis linking AD and CVD. The first is the hypothesis of "independence", which assumes that multiple cortical or subcortical ischemic events cause neuronal loss, leading to a decrease in neuronal connectivity and a sudden decrease in cognitive function, from which patients could recover. In case of additional presence of AD pathology, these vascular ischemic processes decrease the cerebral reserve's capacity to compensate the ongoing neurodegeneration as well as to restore cognitive function[41]. The second is the hypothesis of "interaction", which states that patients with cognitive impairment at the time of a stroke have a higher risk of developing dementia, suggesting that ischemic stroke triggers additional pathways to degenerative processes and accelerates ongoing neurodegeneration[42,43].

THE RENIN-ANGIOTENSIN SYSTEM

The renin-angiotensin system (RAS) plays an important role in vascular regulation, inflammation, oxidative stress, and apoptosis. RAS contributes to the pathogenesis of several human diseases that have a clear association with advanced aging, including hypertension, myocardial infarction, congestive heart failure, atrial fibrillation, coronary artery disease, diabetes, nephropathy, stroke, dementia, and even frailty[44]. The RAS disturbances may be involved in the occurrence of cerebral vascular lesions. Hypertension induces damage to brain microcirculation, contributing to the development of dementia. However, evidence of benefit from RAS blockers on cognitive function has been controversial. RAS is a major regulator of systemic blood pressure and cerebral blood flow; therefore, gene polymorphisms in the RAS coding are excellent candidates for cerebral small vessel disease. The plasmatic angiotensinogen (AGT, GenBank ID183) synthesized by the liver is converted to angiotensin II (Ang II) by the serial action of renin and angiotensin-converting enzyme (ACE GenBank ID1636). The association between ACE I/D (insertion/deletion) polymorphism and WMH has been investigated in nine

studies, and the deletion-deletion genotype is a significant predictor of WMH. The most frequently studied SNP in the *AGT* gene is the M235T; it is possible, however, that this polymorphism *per se* is not the causal mechanism but a functional variant. One of them might be a haplotype (-6:a, -20:c, -153:g, -218:g, positions relative to transcriptional start site) at the AGT promoter. The Austrian Stroke Prevention Study described the association between this haplotype and greater severity of WMH, which was independent of hypertension, and the haplotype enhanced the basal transcriptional activity of the AGT promoter in astrocytes but not in hepatocytes, suggesting that this association is mediated by disturbances in activity of cerebral and not the systemic RAS[45]. Another study showed that the rate of progression of WMH in elderly males is influenced by polymorphisms in genes of angiotensinogen (*AGTR1* and *AGTR2*). Homozygous individuals for the 1166A allele in the *AGTR1* gene had less changes in cerebral white matter in comparison with carriers of the 1166C allele[46].

AGING AND NEUROVASCULAR UNIT

Pericytes, microglia, mast cells, oligodendroglia, and neurons respond to an ischemic event or an inflammatory stimulus by activation. While the individual features of specific cell responses are known, it is not understood how they respond as a whole. It is essential to understand these interactions because processes leading to injury in one compartment of the neurovascular unit (which includes perivascular neurons, astrocytes, endothelial cells, and vascular smooth muscle cells) probably affect other compartments, potentially irreversibly unless the initial event is limited. Furthermore, aging could modify these inflammatory responses in unclear ways. Interactions between components of the neurovascular unit and their responses to external stimuli are an open field for research[47].

Vascular disturbances may precede neuronal changes in dementia syndromes. A decline in cerebrovascular function includes a decrease in cerebral blood flow in different parts of the brain such as limbic and association cortex[48]. Studies have shown that in comparison with subjects without the *APOE* ϵ4 allele, carriers of the *APOE* ϵ4 allele without neurological disease have lower cerebral blood flow in several brain regions, rendering them more vulnerable to AD pathology[46].

NEUROVASCULAR PATHOLOGY AND AGING

Aging leads to normal vascular changes, including thinning of blood vessels, decreased capillary density, increased endothelial pinocytosis, decreased mitochondrial content, increased collagen accumulation, and rupture of the blood-brain barrier (BBB)[49]. Reduction in the BBB of GLUT1-mediated glucose transport across the plasmatic membranes decreases glucose uptake by the BBB, which predisposes to cerebral atrophy and impairment of cognitive function[50,51].

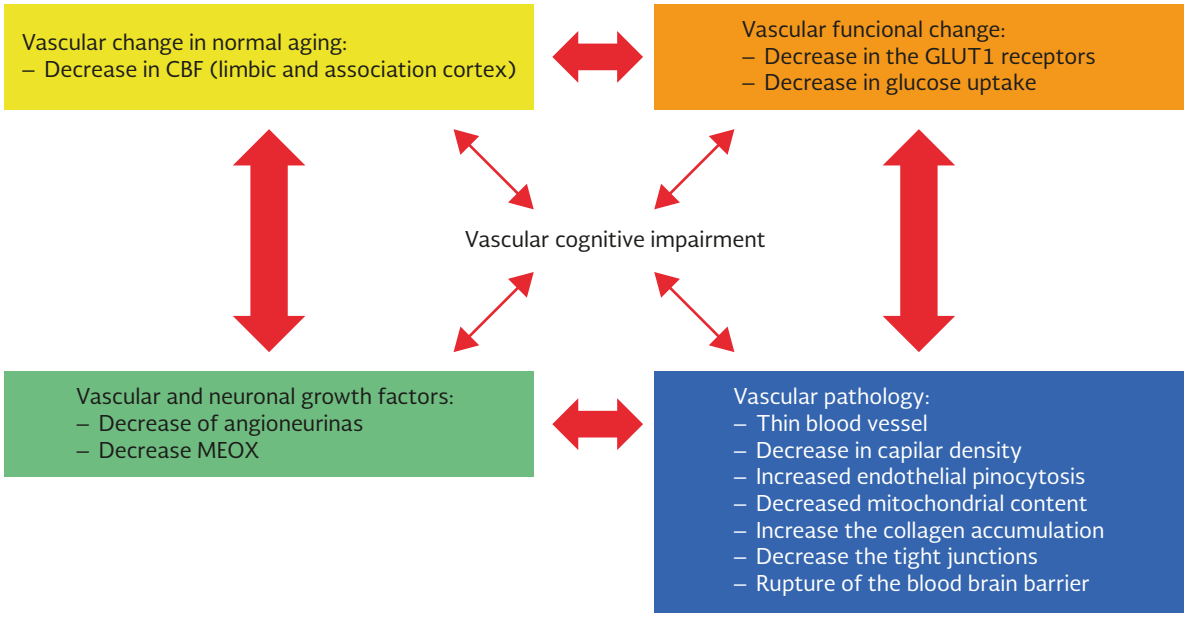
Furthermore, angioneurins are growth factors with neurotrophic properties. Vascular endothelial growth factor regulates vessel formation, neuronal survival, and axonal growth. Ephrin, semaphorin, and netrins are factors that regulate the function of axons as well as the development of the vascular system[52].

An important aspect in the pathophysiology of VCI is the role of inflammation: the incidence of VCI is influenced by gene polymorphisms of inflammatory mediators (IL-1, IL-6, TNF-α, toll-like receptor 4, E and P selectin, and C-reactive protein), lipid metabolism (APOE), NO, and extracellular matrix (matrix metalloproteinase)[53]. During a stroke, the microglia increases brain inflammation, releasing a wide variety of inflammatory mediators and free radicals until ultimately reaching its quiescent state. All of these mechanisms contribute to neuronal damage and eventually lead to cellular death. The development of VCI may then result from various pathological processes, including vascular damage, predisposition to cognitive impairment, as well as the number and type of vascular injuries (small or large vessel)[48]. Figure 1 shows neurovascular changes and their effect on VCI.

POSSIBLE PATHOPHYSIOLOGICAL MECHANISMS BETWEEN FRAILTY AND VASCULAR COGNITIVE IMPAIRMENT

The mechanisms linking cognitive impairment and frailty could be associated to endothelial dysfunction within a pro-inflammatory environment with increased oxidative stress[54]. Atherosclerosis could be a common biological pathway that explains how frailty and CVD are interrelated, as could also be the interaction between multiple factors such as aging, inflammation, and activation of the blood coagulation and fibrinolytic systems[55].

Figure 1. Neurovascular changes and their effect on vascular cognitive impairment.
Vascular cognitive impairment is a consequence of vascular aging. This determines changes in vascular function (such as decrease in GLUT 1 receptors, resulting in decrease in glucose uptake). Also, others factors reduce vascular and neuronal growth; furthermore, through the vascular aging, changes in the blood vessels (thinning, decrease in capillary density, increased accumulation of collagen, among others). All these changes are related to each other and converge in the pathophysiological process of the vascular cognitive impairment.
CBF: cerebral blood flow.

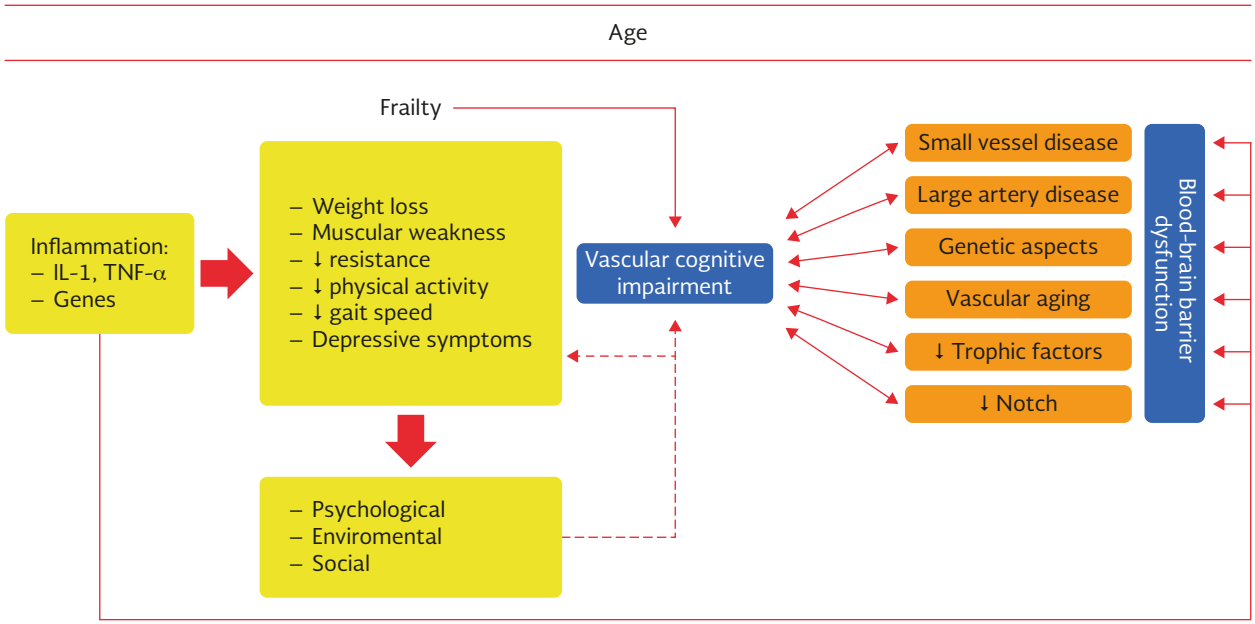


It is difficult, however, for only one mechanism to explain incident VCI or even frailty. Frailty is related with multiple chronic diseases and a functional decline, which requires a greater amount of energy; this condition could then explain why mitochondrial metabolism produces higher quantities of free radicals[56]. At the same time, this increased production of free radicals could also activate the NF-kB pathway, which in turn leads to inflammation[57]. Immune disturbances have a systemic impact. The accumulation of mitochondrial and nuclear DNA damage can compromise the integrity of the cell, leading to loss of myocytes and muscle wasting, both cornerstone features of frailty[58]. Chronic inflammation as a process of aging (inflammaging) has been associated with poor physical performance and weakness. A recent review also suggested that since the central nervous system and the immune system are in constant interaction, inflammation in one area of the body might promote inflammation in the brain. An inflammatory response in cerebrovascular areas may trigger another response in the blood-brain barrier and release inflammatory cytokines into the brain[59]. For example, IL-6 interrupts adult neurogenesis and, considering IL-6 receptors are expressed in the hippocampus and pre-frontal cortex, this constant inflammatory state could have serious consequences for cognitive function, particularly in memory and executive functions[60,61].

CONCLUSIONS

Frailty and cognitive impairment are closely related. Aging promotes cellular and molecular accumulative damage, which can be evidenced through laboratory measurements and histopathology. Hypertension, diabetes, and hypercholesterolemia are well-known risk factors for cognitive impairment such as AD and VCI in later-life. The increased permeability of the blood-brain barrier in people with WMH plays a causal role in the development of lacunar infarcts. VCI and frailty are closely inter-related. Understanding the relationship between cognition and frailty is useful because frail individuals have a higher risk of cognitive impairment and vice versa. In addition, understanding the link between frailty and VCI may lead to interventions aimed at preventing and treating both conditions. Clinical trials on any type of dementia should consider including frail

Figure 2. Possible interaction between frailty and vascular cognitive impairment.
IL: interleukin; TNF: tumor necrosis factor.



elderly subjects since frailty appears to lead to an expression of disease that may be the neuropathological key to expressing the deleterious effects in classic dementia (Fig. 2).

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Epidemiology of Cognitive Aging in the Oldest Old

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ABSTRACT

The proportion of persons aged 85 and over, the so-called "oldest old", is increasing dramatically worldwide. While a quarter of this population is affected by dementia, little is known about the specific features of cognitive functioning in the oldest old. In the presence of clinical specificities such as numerous comorbidities, multi-medication and visual and/or auditory loss, which are very frequent in extreme old age, neuropsychological assessment can be particularly challenging. This article presents an overview of the epidemiology of cognitive functioning in the oldest old, and discusses the issues regarding neuropsychological assessment and dementia in this specific elderly population. (REV INVES CLIN. 2016;68:33-9)

Key words: Oldest old. Epidemiology. Dementia. Cognition. Neuropsychological assessment.

INTRODUCTION

While persons reaching 85 years were only an anecdotal phenomenon in the past, nowadays they represent 13% of the population aged 65 and over in the world. This age group is the fastest growing segment of the older population. Indeed, the number of people aged 100 and over has doubled each decade since 1950 in the more developed countries[1]. Since the 1980s, people over 85 have been qualified by the demographers as the "oldest old" population. The exceptional rise in this segment of the population is explained on the one hand by the general improvement of living conditions and medical care, contributing to increased life expectancy; and on the other hand, the baby boomers born in the 1930s who are currently reaching very advanced ages. Such phenomenon has important consequences. First of all, oldest old people usually represent a high economic burden for health insurance systems. In addition, with age being the strongest risk factor for dementia[2,3], the oldest old are the more likely group to develop dementia. Yet, despite the high prevalence of dementia beyond 80, four out of five persons with dementia do not access recommended diagnosis procedures[4]. Reaching advanced ages is accompanied by substantial changes in morbidity, cognition, physical condition, and autonomy. Therefore, distinguishing normal aging from pathological aging is in some cases particularly challenging. Moreover, because of their global health condition, it seems inappropriate to provide oldest old individuals with the same neuropsychological evaluation as that used in the younger old. Unfortunately, there is an obvious lack of tests and norms adapted

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to this population. In the present article, we present an overview of the epidemiology of cognitive functioning in the oldest old, and discuss the issues regarding neuropsychological assessment and dementia in this specific elderly population.

COGNITIVE FUNCTIONING

Little is known about the specificities of cognitive functioning in very advanced age. First of all, it is important to remember that cognitive functioning in the oldest old is the result of the long-term decline that progressively occurrs with advancing age in several cognitive functions, in particular episodic memory, executive functions, and cognitive speed[5]. As in the younger old, there seems to be a sex difference in cognitive performances as women have better performances in verbal memory and cognitive speed tasks than men, even after controlling educational level[6]. In addition to the long-term progressive age-related decline, van Exel, et al. report a "terminal decline" characterized by an acceleration of the decline preceding the death[7]. Indeed, this study showed that the proximity to death is associated with an accelerated decline of crystalized knowledge and verbal abilities in a non-demented oldest old population.

CHALLENGES IN THE NEUROPSYCHOLOGICAL ASSESSMENT

General characteristics of the oldest old

As we all know, age is a major risk factor of numerous health concerns affecting not only cognitive, but also physical and functional abilities. Indeed, the oldest old are often affected by multiple morbidities or at least two or more chronic diseases[8]. The more frequent pathologies in this population are: dementia[9,10], depression[11], frailty syndrome[12], osteoporosis, diabetes, osteoarthritis, chronic kidney disease, cancer, cardiovascular diseases including hypertension and strokes[13]. The prevalence of hearing impairment is estimated at 70-90% for people aged over 85[14] and the prevalence of visual impairment in the USA is more than 23% in persons aged 80 and over[15]. They usually have multimedication[13] and more than 44% of women and more than 35% of men aged 90 and more consume psychotropic drugs[16]. Multimorbidity weakens the global health condition of the oldest old and leads to functional disabilities, which are extremely common among the oldest old[17]. The incidence of functional disability increases with age from 8.3% for people aged 90-94 years to 25.7% for those aged 95 years and over[18]. Moreover, fatigability is a common feature in the oldest old, especially in the everyday mobility[19].

In this context, neuropsychological assessment is often a challenge for clinicians. How can we diagnose cognitive deficits in a person aged 95 exhibiting multimorbidity, consuming multiple medications including psychotropic drugs for several decades, and exhibiting significant visual and auditory loss? This is a question that many clinicians have to solve when it comes to assessing neuropsychological functions in their oldest old patients.

The need for normative scores

The numerous sources of variability of cognitive performances in this specific population lead to questioning the issue of normality in the fourth age. If a large number of articles provide normative scores for elderly population, those presenting norms for elders aged 85 and over are much scarcer. These studies are indexed table 1. Such studies are tremendously important for clinicians. Nonetheless, the characteristics of the samples from which they are derived have to be carefully considered. Indeed, the selection bias of the sample can affect the representativeness of the population and requires us to use norms with caution. For instance, the norms provided by the Georgia Centenarian Study are based on a sample including both demented and non-demented octogenarian and centenarian subjects[20]. In the 90+ Study, norms are based on a sample of middle-upper class and highly-educated oldest old participants[21]. The WISE Study provides norms only for women[22]. An interesting project lead by the National Institute of Aging of United States[23] provides an online z-score calculator estimating the percentile ranges for an individual according to sex, age, and/or education for several tests of the Uniform Data Set[24]. Finally, the PAQUID Study provides normative scores for seven widely used neuropsychological tests from a population-based sample of French oldest old[25]. Furthermore, for most of these tests, the specific cutoff scores with best sensitivity and specificity for dementia diagnosis remain to be

assessed. As an alternative, Kahle-Wrobleski, et al. have shown that the Mini-Mental State Examination has acceptable sensitivity and specificity in oldest old populations[26].

The need for adapted tools

It is not only important to have validated norms for commonly used tests. It should be also necessary to make special efforts to adapt neuropsychological tools to the clinical specificities of such populations. In particular, the multiple sensory impairments justify the development of new tools of assessment, combining verbal and visual modalities and providing the opportunity for clinicians to choose the appropriate test modality according to patients' sensory deficits. Moreover, due to increased fatigability, the duration of the tests to be developed or adapted has to be relatively short.

DEMENTIA IN THE OLDEST OLD

Prevalence and incidence

It is important to underline that aging does not necessarily mean developing neurodegenerative disorders. In most of the studies, the proportion of dementia-free cases and healthy individuals among centenarians is far from marginal[27-29]. However, prevalence rates of dementia in the oldest old are rather high. Inhomogeneous prevalence rates are reported in the studies according to sample selection procedures and methods used in the screening of dementia[30-37]. Nonetheless, the study by Ferri, et al.[38] based on the Delphi consensus method[38] reports estimates for prevalence of dementia in the American continent from 28.1 to 33.2% and around 25% in Europe for the age group of 85 and over.

Incidence rates of dementia in the oldest old are also controversial in the literature. For most studies, incidence of dementia increases exponentially with age in men and women[2,39-44]. In the 90+ Study, dementia incidence increases exponentially with age between 65 and 90 and doubles approximately every five years with an incidence of 41% in centenarians[2,45]. By contrast, in the Monzino 80-plus Study, the largest population-based study conducted in the oldest old, dementia increases linearly. These authors question the plausibility of an exponential increase in dementia, arguing that if the exponential model was verified, everyone should have dementia around the age 100[30]. Finally, in the Bronx Aging Study[40], the increase in incidence slows relatively from 65 to 85 years old.

Risk factors of dementia in the oldest old

Several studies have examined the risk factors for dementia in the oldest old. Besides the major risk factors existing whatever age, some specific factors seem to have slightly different effects in the very old age. Most of these factors are still controversial in the literature. The main factors identified are the following:

Conventional risk factors

As for the younger old, Poon, et al. have shown an effect of age, gender, race, and education on the risk of developing incidence in the Centenarian Study[27]. Among these factors, education level seems to have a strong impact[31], with a lower risk of dementia in highly educated individuals, suggesting that the benefit of cognitive reserve[46] seems to exist throughout the aging process including very old age. Depressive symptomatology[47], delirium[48] and multimorbidity[49] were also associated with incident dementia. Furthermore, the association between family history of dementia and the risk of developing Alzheimer's disease (AD) would be stronger in the younger old than in the oldest old[50].

Cardiovascular factors

– Diabetes: Diabetes is known to be associated with increased risk of dementia in the younger old. In the Vantaa 85+ Study, diabetes was associated with incident dementia[51]. Conversely, in the WISE cohort, diabetes was not associated with increased dementia in the oldest old. The authors explained the absence of association by the reduction of life expectancy of persons presenting both diabetes and cognitive impairment[31].

– High-density lipoprotein (HDL) cholesterol: As for the younger old, low HDL cholesterol was shown to be associated with cognitive impairment and dementia in the oldest old[52].

Table 1. Studies providing normative neuropsychological scores for oldest old populations

Studies	Neuropsychological tests
PAQUID Study[25]	Mini Mental State Examination (Folstein, et al., 1975); Benton Visual Retention Test (Benton, 1965); Digit Symbol Substitution Test (Wechsler, 1981); Verbal Fluency test: Isaacs's Set Test (Isaacs and Kennie, 1973); WAIS-III Digit Span Test (Wechsler, 1997); Wechsler Paired-Associates Test (Wechsler, 1945); Wechsler Similarities Test (Wechsler, 1981); Zazzo's Cancellation Task (Zazzo, 1974)
Uniform Data Set project[23]	Mini Mental State Examination (Folstein, et al., 1975); Boston Naming Test (BNT) (30 item-odd numbered) (Kaplan, et al., 1983); Digit Span Forward and Backward; Digit Symbol Coding subtest (Wechsler, 1987); Trail Making Test A and B (Delis, et al., 2001); Verbal Fluency Test: Semantic Fluency (animals and vegetables) (Morris, 1989); Wechsler Adult Intelligence Scale-Revised (WAIS-R); Wechsler Memory Scale-Revised (WMS-R) subtests Logical Memory IA and IIA (Wechsler, 2008).
Georgia Centenarian Study[20]	Mini Mental State Examination (Folstein, et al., 1975); Behavioral Dyscontrol Scale (Grigsby, et al., 1992; 1996); Fuld Object Memory Evaluation (Fuld, 1981); Severe Impairment Battery (Saxton, et al., 1990)
90+ Study[21]	Mini Mental State Examination (Folstein, et al., 1975); 3MS (Teng and Chui, 1987); Boston Naming Test (15-items) (Kaplan, et al., 1978); CERAD Constructions (Morris, et al., 1992); Clock Drawing Test (Freedman, et al., 1994; Rouleau, et al., 1992); Letter Verbal Fluency; Trail Making Test A and B (Delis, et al., 2001); Verbal Fluency test: Category (Animal); WAIS-III Digit Span Test (Wechsler, 1997)
Mount Sinai - Alzheimer's disease research center[72]	Mini Mental State Examination (Folstein, et al., 1975); Boston Naming Test (15-items) (Kaplan, et al., 1978); CERAD Constructions (Morris, et al., 1992); Trail Making Test A and B (Delis, et al., 2001); Verbal Fluency test: Category (Animal); Word list memory; Word list recall; Word list recognition
Cambridge city over-75 cohort[73]	Mini Mental State Examination (Folstein, et al., 1975)
Framingham heart study[74]	Wide Range Achievement Test-Third Edition (WRAT-III) (Wilkinson, 1993)

– Hypertension: In the Vantaa 85+ Study, both diabetes and incident strokes were associated with increased probability to develop dementia. On the contrary, a history of hypertension was associated with a low probability to develop dementia[51].

Genetic and inflammatory factors

– APOE: The Apolipoprotein E ε4 allele (APOE4) is a well-established risk factor for AD, whereas the APOE ε2 allele (APOE2) was reported as protective in the younger old. The role of APOE4 is debated in the oldest old. Some studies concluded that APOE4 was a risk factor of dementia in the oldest old as in the younger old[30,32], whereas others like the Vantaa 85+ Study reported that the incidence of dementia in carriers of APOE4 was not increased compared to non-carriers[53]. In the 90+ Study, the authors conclude that APOE4 no longer plays a role in dementia and mortality at very old ages[54]. Regarding APOE2, it was associated with preserved cognition in the 90+ Study[55]. Surprisingly, in the Monzino 80-plus Study, carriers of the APOE ε2/ε2 or the APOE ε2/ε3 presented a higher risk of dementia[56].

– Inflammatory markers: While in the 90+ Study inflammatory markers such as C-reactive protein (CRP) was associated with increased dementia prevalence and mortality[57], in the Leiden 85-plus Study, the association between cognitive decline and inflammatory markers was only moderate[58]. However, in both studies the associations tended to be stronger in APOE4 carriers.

Clinical specificities of dementia

Dementia occurring after 85 years is called "very late-onset dementia". Clinical manifestations as well as the diagnosis procedures of late-onset dementia are highly

controversial. However, we know that AD and mixed dementia are the most common types of dementia in the oldest old[31]. Compared to the younger old, the progression of cognitive decline associated with AD is slower in the cases of late-onset dementia[59]. On the contrary, the decline in functional capacity would be faster after 85 years[60].

Physiopathology of dementia in the oldest old

The study of the oldest old brains also provides rich lessons. Neuropathological markers of dementia, such as neuritic plaques and neurofibrillary tangles, in oldest old seem to have different clinical consequences[61-63]. Savva, et al.[64] autopsied 456 donated brains of five age groups (under 80, up to 94 years, and older). The association between AD neuropathological lesions and diagnosis of dementia before death was stronger in younger old than in the oldest old. More precisely, when comparing persons who died at 75 years to persons who died at 95 years, the presence of neuritic plaques and neurofibrillary tangles was less strongly associated with dementia at age 95. In the same vein, in a large cross-sectional study of 2,014 subjects aged 70 and over at death, Middleton, et al. have shown that even if the risk of clinical AD diagnosis was associated with neurofibrillary tangles for each age group (70-74; 75-84; 85 and above), the strength of the association was weaker among the oldest old[65]. Furthermore, there are no clear differences in the neuropathological damages evidenced in demented and cognitively normal oldest old individuals[66,67]. According to Haroutunian, et al., the lack of differences in the lesion density between demented and non-demented individuals could be due to a low density of lesions in the brains of oldest old individuals with dementia rather than an augmentation of lesions in the brains of non-demented oldest old[61]. In the Cambridge City over-75s Cohort, where a post-mortem study has been conducted, a particularly high prevalence of vascular lesions, including micro-infarcts and vascular dementia, was found in the brains of oldest old individuals. Alzheimer-type lesions and cerebrovascular pathology were very common. The greater burden of these lesions and pathologies, but also of Lewy bodies, and hippocampal atrophy, were associated with a higher risk of late-onset dementia, but were not sufficient to define clinical dementia[68]. In an ancillary study of the 90+ Study (i.e. 90+ autopsy study), 137 brain autopsies were performed. Interestingly, the results showed that the subjects with high and low AD neuropathology (neuritic plaques, diffuse plaques, and neurofibrillary tangles) presented a similar cognitive trajectory three years before death[69]. Finally, compared to the younger old, the oldest old seem to have less salient morphometric specificities of AD such as reduced hippocampal volume and cortical grey matter thickness[70]. Taken together, these findings suggest that the underlying physiopathology leading to clinical dementia would be slightly different in the oldest old and the younger old.

CONCLUSIONS

Being the fastest growing segment of the elderly population and exhibiting specific clinical issues, the oldest old individuals cannot be considered either as an anecdotal phenomenon or as a group presenting similar issues as the younger ones. Further researches are necessary in many fields. Defining dementia in the very old people calls for international debate in order to lead to consensual definition. Neuropsychological tools of assessment have to be set up along with validated norms. Better characterizing the impact of social representations is also necessary. The fourth age goes often with representations of "senility" and general reduction of activity so dementia is often perceived as normal. In this context, the oldest old are rarely oriented to specific consultations for memory disorders. Better understanding the neuropathology leading to dementia at very advanced age is also important since outstanding differences seem to exist in this specific group. Undoubtedly, in the next decades, the study of the oldest old, sometimes called the "exceptional survivors"[71], will offer many challenges in the understanding of aging.

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The Role of Neuroinflammation in Age-Related Dementias

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ABSTRACT

The most common dementias such as Alzheimer's disease, vascular dementia, Lewy body dementia, and frontotemporal dementia are associated with a decline in cognitive and social abilities. Although the molecular mechanisms of tissue damage in these dementias are not completely understood, these neurodegenerative illnesses share certain alterations such as neuroinflammation and gliosis. Increasing evidence suggests that microgliosis and astrogliosis play a key role in neuroinflammation observed in these dementias. Here we provide an overview of the participation of microglia and astrocytes in the neuroinflammatory response in common dementias. (REV INVES CLIN. 2016;68:40-8)

Key words: Neuroinflammation. Dementia. Aging. Gliosis.

INTRODUCTION

Neuroinflammation is a complex inflammatory reaction in the central nervous system (CNS). It is a fundamental response triggered to isolate damaged tissue from uninjured areas, and clean and repair the extracellular matrix[1]. Inflammation is orchestrated by the immune system, which is divided in two functional categories called innate and adaptive immunity[2]. The innate immune response is the first line of defense against insult stimulus. In the CNS it is formed primarily by the blood-brain barrier (BBB), glial cells, and chemical mediators and constitutes the response mechanism to reset a baseline state following clearance of pathogens and tissue repair[3,4]. The adaptive or acquired immune response is based on specific recognition of foreign antigenic substances by white blood cells, leukocytes, which can produce both humoral responses by synthesizing and secreting antibodies (B lymphocytes), and cellular responses (T lymphocytes) mediated by the secretion of immune-regulatory factors[2].

Following any damage, the CNS may set off and develop a complex, local and rapid immune response, resulting in the activation of glial cells (mainly microglia and astrocytes) and the release of inflammatory mediators (cytokines) to clear pathogens and cell debris[5]. In general, acute inflammation is beneficial because it curbs the damage and promotes

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regeneration. However, excessive and prolonged inflammation as well as stable low-grade damage are detrimental and can lead to the onset or exacerbation of cell injury. Moreover, if a tissue is unable to overcome inflammation, this response becomes a chronic condition that results in its continuous damage[3,5].

On injury in the CNS, cells of the innate immune system recognize molecules present in pathogens (called pathogen-associated recognition patterns) through receptors known as pattern recognition receptors (PRR). These can also recognize endogenous molecules released by damaged or dead cells that are generally known as damage-associated molecular patterns, such as heat-shock proteins (HSP), high-mobility group box 1 (HMGB-1) protein, and uric acid, a component of the extracellular matrix[6,7]. Stimulation of these PRRs triggers intracellular signaling that induces phagocytosis of damaged or dead cells to promote tissue healing[6,7]. There are different receptor families of PRRs such as toll-like receptors (TLR), nod-like receptors, scavenger receptors, complement receptors, and C-type lectins that upon stimulation can respond by differentiating phagocytes and/or secreting several factors that act against the pathogen[6,7]. Other ways of responding to pathogens are by synthesizing and secreting proteins called cytokines, which are directly or indirectly involved in the elimination of pathogens. Cytokines are small proteins that act locally (paracrine or autocrine) to control numerous aspects of the cells, including proliferation, differentiation, and migration. They are divided into families such as interleukins (IL), interferons (IFN), tumor necrosis factors (TNF), chemokines (CC), and growth factors[6,7].

CELLULAR COMPONENTS OF THE CENTRAL NERVOUS SYSTEM INNATE IMMUNE SYSTEM

The CNS innate immune system includes barriers that are formed mainly by the BBB, which consists of a tight barricade of endothelial cells, astrocytes and pericytes. Besides participating in blood flow regulation, structural and metabolic support, the BBB restricts the dispersal of both pathogens and large hydrophilic molecules to maintain homeostasis[8,9]. Endothelial cells are immunologically quiescent under physiological conditions[10]. However, when injured these cells can sense a pathogen and release pro-inflammatory interleukins (IL-1b), adhesion factors such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin-1 (ELAM-1) that can alter their tight junction to facilitate blood cell migration[11-13]. Moreover, endothelial cells also express functional levels of several TLRs[14]. Pericytes are the contractile cells that wrap the endothelial cell layer in vessels and regulate endothelial functions[15]. Similar to endothelial cells, pericytes are immunologically quiescent under physiological conditions, but when there is tissue damage activation of TLR4, they can induce a macrophage-like activity and produce cytokines, chemokines, and nitric acid[16,17]. Microglia constitute approximately 10% of glial cells; they are resident macrophages of the CNS and the primary responders to any kind of damage[18,19]. In the healthy CNS, microglial cells are known as resting microglia, to differentiate them from activated or reactive microglia seen after brain insult. Resting microglia has a characteristic morphology: a small cell body with several fine processes extending in all directions. Resting microglia are very active cells that move constantly to detect and remove any cellular debris and toxic metabolites in their microenvironment that might produce alterations in the CNS homeostasis[20,21]. Microglial cells express receptors for different neurotransmitters (e.g., dopamine, glutamate, GABA), neurohormones (e.g., somatostatin, angiotensin), neuromodulators (e.g. histamine, opioids), cytokines (e.g., IL-1, IL-4, IL-10), and chemokines (e.g., CCR1, CCR5)[20]. Microglia also participate in refining synapses by phagocyte dysfunctional synapses and release neurotrophic factors to modulate neuron networks[22].

During an injury event, microglial cells change both their physiology and morphology to become active. Activated microglia display different phenotypes; however, in general, they present an enlarged cell body with short thick processes, and in the final stage of activation show an amoeboid shape[21]. Additionally, there are two phenotypes similar to those identified in macrophages, known as M1 and M2. The M1 state (classical activation or microglial "priming"), shows a phagocytic phenotype associated with the activation of mitogen-activated protein kinase and transcription factor nuclear factor kappa B (NFκB),

and the production and release of proinflammatory cytokines (IL-1b, IL-6, IL-12, IL-23, and TNF-α), cytotoxic substances such as quinolinic acid and reactive oxygen species[6,19,23]. In addition to the proinflammatory cytokines, M1 phenotype microglia also secrete several chemokines such as CXCL9, CXCL10, CXCL11, CCL2, CCL3, CCL4, CCL5, and CXCL8[6,19,23]. Because the M1 phenotype releases proinflammatory compounds that might be toxic for the cells, it has been suggested that this phenotype can increase neurotoxicity in neurodegenerative diseases[24]. In contrast to the M1 state, the M2 state (alternative activation) is neuroprotective, showing a phagocytic phenotype and release of anti-inflammatory interleukins IL-10, and transforming growth factor beta (TGF-β). The M2 state is induced by anti-inflammatory cytokines (e.g., IL-4 and IL-13)[6,19,23]. Microglial activation may start with an M1 phenotype and later adopt an M2 phenotype to mediate repair by releasing growth factors and phagocyte cell debris[6,19,23]. Based on evidence from aging animals, it has been proposed that microglia in the aging brain mainly presents an M1 phenotype that may result in an exaggerated immune response that can trigger age-related cognitive damage[25]. The M1 phenotype can be toxic due to the production of cytokines (IL-6, IL-2, TNF-α), reactive oxygen species, and release of glutamate[26]. Moreover, there is yet another microglial phenotype related with cognitive decline and impairment in aging: dystrophic microglia. This was observed in postmortem human brains and shows cytoplasmic degeneration. It has been suggested that this phenotype experiences replicative senescence, which can result in the generation of senescent and/or dysfunctional cells[27]. Dystrophic microglia has also been found in mice models of aging, where this phenotype precedes neurodegeneration, as well as in Huntington's disease in mice models and Alzheimer's disease[28-30].

Astrocytes are specialized glial cells that perform several important functions to maintain homeostasis of the CNS such as release neurotransmitters (glutamate, ATP); express neurotransmitter receptors (glutamate, GABA, glycine); up-take and clear neurotransmitters (glutamate and GABA); participate in homeostasis preservation of extracellular ions, pH and water; supply energy metabolites to neurons (lactate); and modulate blood flow and synapses[31,32].

Astrocytes react to all kinds of CNS damage through a complex process known as reactive astrogliosis, which is a histopathological hallmark of CNS lesion. Reactive astrogliosis is a context-regulated process produced by specific signaling molecules that can induce reversible changes from gene expression and cell hypertrophy, to long-lasting ones such as glial scars[33]. Under physiological and pathological conditions astrocytes express receptors to several cytokines and inflammatory mediators such as IL-1β, IL-6, interferon-γ (IFN-γ), TNF-α, TGF-β, CXCL12 (SDF-1), thrombin, and endothelin-1, TLR-2, TLR-3 and TLR-4[34]. In the context of neuroinflammation, reactive astrocytes can release different mediators that may exert either protective or toxic effects. Reactive astrocytes can release growth factors (NGF, GDNF, BDNF, IGF), interleukins (IL-1β, IL-6, IL-11), chemokines (CXCL1, CXCL10, CCL2, CCL7), tumor necrosis factors (TNF-α) and thrombospondins[35].

Various lines of evidence suggest that in the aging process, several brain regions show astrogliosis and increased expression of proinflammatory cytokines such as IL-1β and TNF-α[36-39].

Oligodendrocytes are glial cells that produce myelin to insulate axons and permit saltatory conduction in the CNS[40]. Oligodendrocytes express several ionic channels and cytokines (IL-1β, IL-6, IL-8, IL-17A, IL-18), chemokines (CCL2, CCL3, CCL5, CXCL5, CXCL10), and antigen presentation molecules (MHC class I, MHC class II, CD274, PDCD1LG2) that prove that these cells may be immunologically active[40,41].

Neurons also produce cytokines (IL-6), chemokines (GRO-α) and express several receptors to these mediators, such as TLR3, TLR7, TLR8, TLR9, CCR1, CCR3, CCR4, CCR5, CXCR3 and CXCR4[42-44].

AGE-RELATED DEMENTIAS

Aging is a complex process that involves several alterations, of which the most well known is dysregulation of the immune system, possibly resulting from deficiencies in both initiation and resolution of the immune response[45]. This age-related dysregulation of the immune system, or immunosenescence, can be explained by alterations in the inflammatory and anti-inflammatory networks, resulting in a low-grade

chronic status known as inflammaging[46], which leads to tissue damage and degeneration[47]. Evidence from both human and experimental models suggests that immunosenescence also takes place in the CNS and promotes dysfunction in different cellular populations[48]. Immunosenescence probably results from lifelong exposure to pathogens and antigens, intrinsic changes in immune cells, and possibly genetic predisposition[47]. Both microglia and astrocytes are cellular components of the CNS innate immune system that present altered physiology in aging and neurodegeneration[28,49,50]. Certain age-related illnesses show brain degeneration and dementia. Dementia is a syndrome characterized by memory, cognitive, and behavior impairments as well as the inability to perform everyday activities[51], in which both genetic and environmental factors participate. The latest estimation of people suffering from dementia amounted to 44.5 million worldwide and the most common dementing illnesses associated with aging is Alzheimer's disease (AD) accounting for 60-70%, followed by vascular dementia (VaD), dementia with Lewy bodies (DLB), and frontotemporal lobar degeneration (FTLD)[51].

ALZHEIMER'S DISEASE

Alzheimer's disease is a progressive brain disorder that damages and eventually destroys brain cells, leading to memory decline and cognitive dysfunction. It is characterized by the accumulation of amyloid-β (Aβ), neuritic plaques and intraneuronal neurofibrillary tangles, in addition to widespread synaptic loss, inflammation and oxidative damage, and neuronal death[52]. Most cases of AD are late-onset and the prevalence of the disease increases with life expectancy, affecting more than one-third of people over the age of 90[53]. Pre-clinical, genetic, and epidemiological evidence have shown that neuroinflammation is an important contributor to AD pathogenesis[54]. In addition, nonsteroidal anti-inflammatory drugs have been reported to reduce the risk of developing AD[55]. Moreover, several lifestyle factors and events known to increase the risk of developing AD have an associated inflammatory component; such is the case of obesity, severe infections, and chronic periodontitis, among others.

There is evidence that immune system activation mediated mainly by glial cells such as microglia and astrocytes follow Aβ deposition. However, recent studies have identified various novel alterations in immune system molecules, pathways, and genes in AD and have shifted our understanding of the timing of immune system changes in the course of this disease[56-59]. Ongoing neuroinflammation can be seen in patients by using positron emission tomography (PET) ligand [^{11}C](*R*)-PK11195, and this helps to identify patients who are likely to progress from experiencing mild cognitive impairment to developing clinical AD[60,61]. These observations imply that immune processes may drive AD pathology independently of Aβ deposition and sustain increased soluble Aβ levels, thus exacerbating pathology and culminating in a vicious, pathophysiological cycle[62]. The inflammatory response in AD is primarily driven by microglia and is the most intimately associated with tissue changes observed in AD. Soluble Aβ oligomers and Aβ fibrils can bind to various receptors expressed by microglia, including CD14, CD36, CD47, α6β1 integrin, class A scavenger receptors, receptors for advanced glycosylation end products, and toll-like receptors (TLR)[63-66]. Such binding results in the production of inflammatory cytokines and chemokines[67-69], which are known to alter the expression and processing of β-amyloid precursor proteins[70,71]. Postmortem studies of AD brains reveal the presence of intense inflammatory markers in senile plaques and neurofibrillary tangles[72,73]. Analysis of gene regulatory networks involved in late-onset AD has identified genes associated with innate immune pathways and microglial cells. Remarkably, these findings reveal a set of genes that point to a pathogenic role for neuroinflammation in AD, including several pathways involved in phagocytosis and therefore, presumably, Aβ clearance[54].

Microglia possess the machinery to degrade soluble Aβ species via extracellular proteases such as neprilysin and insulin-degrading enzyme, and it has been shown that Aβ is cleared by microglia *in vitro* through receptor-mediated phagocytosis and degradation[74]. However, there is now strong evidence for a progressive, Aβ-dependent impairment of microglial function, as shown by morphological and detrimental phagocytic functional changes, as well as reductions in the levels of Aβ-binding scavenger receptors and Aβ-degrading enzymes in mice models of AD[75]. Most importantly, efficient phagocytosis has recently been shown to involve a component of the

autophagy pathway, namely beclin 1, the levels of which were found to be markedly reduced in microglia derived from people with AD[76]. Microglia from AD patients show an increased expression of CD33, a receptor expressed at the surface of myeloid cells; its activation was shown to suppress the production of proinflammatory cytokines and prevent microglial cell-mediated removal of Aβ *in vitro* and *in vivo*[77]. These findings support the idea that impaired clearance mechanisms of Aβ may be responsible for most sporadic, non-hereditary cases[78]. Microglia impairment is also accompanied by a loss of trophic functions (brain-derived neurotrophic factor production)[79], eliminating certain protective properties, which may impact neuronal integrity in the course of AD. Paradoxically, microglial impairment might be sustained by inflammatory cytokines such as TNF, IL-1, IL-12, and IL-23[62], suggesting that AD pathology could be accelerated through this negative feedback loop, which may begin in the early stages of AD. Although the role of astrocytes is less well known than that of microglia, there is solid evidence of their participation in the pathology of AD. Postmortem samples from AD patients and animal models show that generalized astrogliosis and reactive astrocytes are associated with some amyloid plaques[80-82] and that astrocytes release pro-inflammatory cytokines[83]. Moreover, experimental evidence shows that APP production and apolipoprotein E (APOE) are related with astroglioisis[84,85]. These data suggest that reactive astrocytes participate in the neuroinflammatory response and may contribute to aggravate the damage.

VASCULAR DEMENTIA

Vascular dementia (VaD) is a disease which involves ischemia and/or vascular brain lesions with variable etiology, pathogenesis, location and extent, resulting in progressive impairment of memory and other cognitive functions[86]. Vascular dementia is the second most common dementia after AD and represents nearly 17% of all dementias[87]. It has been recently referred to as part of a wider concept called vascular cognitive impairment that includes a heterogeneous group of cognitive disorders with an alleged vascular cause, including cognitive impairment with or without dementia[88]. The most common subtypes of VaD are multi-infarct dementia (multiple small strokes), single infarct dementia (single major stroke with hippocampal damage), small vessel disease, and mixed dementia[88]. Together with aging, other risk factors such as vascular (hypertension, hyperlipidemia, diabetes), behavioral (obesity, physical inactivity), and genetic (APOE ε4) are involved in VaD[89]. Despite the lack of validated neuropathological criteria for pure VaD due to a high variability in the cerebrovascular pathology, a histopathological exam would show evidence of cerebrovascular disease without Alzheimer-type lesions exceeding those expected for age and other conditions causing dementia[86]. A wide range of vascular brain lesions can lead to VaD, and these include multiple large and small infarcts due to atherosclerosis, thromboembolism or hypoperfusion, lacunes or microinfarcts and microbleeds, mainly involving central white matter and subcortical structures such as the thalamus, basal ganglia, and brainstem as well as hemorrhagic stroke[89]. Ischemic and hemorrhagic events occurring in VaD can activate several cell mechanisms that damage brain tissue such as excitotoxicity and ionic imbalance, oxidative/nitrosative stress, apoptosis, and neuroinflammation[90]. Experimental evidence suggests that after a stroke, the microglia shows an M2 phenotype, which gradually transforms into a proinflammatory M1 phenotype in the peri-infarct area[91]. The pathologic mechanisms such as oxidative/nitrosative stress and apoptosis can stimulate the release of a proinflammatory mediator by reactive glial cells (microglia and astrocytes), and this effect can be exacerbated by an increase in BBB permeability, thus enabling the infiltration of proinflammatory factors such as interleukins (IL-1, IL-6) and TNF-α and lead to neurodegeneration and cell death in different cerebral regions, including those involved in cognitive functions such as the hippocampus[92,93].

MIXED DEMENTIA

Mixed dementia is recognized as a subtype of VaD[88] and the pathologic diagnosis is based on the presence of a combination of AD and VaD including multiple ischemic lesions comprising multiple strokes, white matter lesions, amyloid plaques, and neurofibrillary tangles[94]. This dementia is observed in approximately 50% of all dementia cases[89]. Cognitive impairment in this mixed neuropathology

depends on the location of vascular lesions and AD pathology[95]. In general, for every given level of cognitive deficit, patients with cerebrovascular lesions show no difference or lower densities of plaques and tau pathology compared to those with only AD. However, this is not true for certain areas of the brain, such as the temporal lobe and hippocampus, which show higher densities of plaques and tau pathology[96-98]. Though neuroinflammation and gliosis (microglia and astrocytes) play an important role in both AD and VaD pathology, there is no evidence of a synergistic neuroinflammation in mixed dementia.

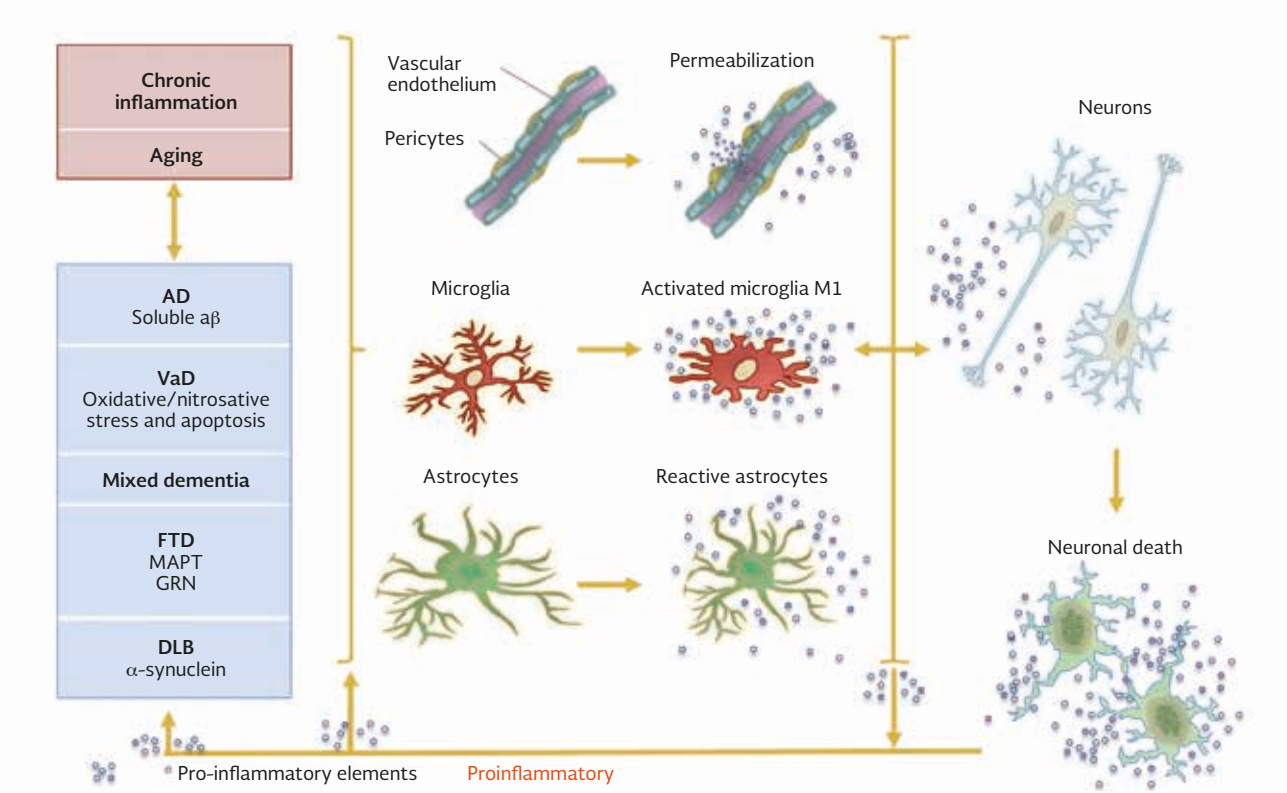
LEWY BODY DEMENTIA

Lewy body dementia (DLB) is a type of dementia characterized by changes in thinking and reasoning, confusion and alertness that vary significantly from one time of day to another or from one day to the next. There is an extreme reaction to narcoleptic drugs, visual hallucinations, REM sleep disorders, and sometimes Parkinson's symptoms due to abnormal microscopic deposits of a-synuclein that gradually destroy certain brain cells. The a-synuclein protein is a major component of Lewy bodies and is found extensively in the brain; however, its normal function is as yet unknown[99,100]. The level of neuroinflammation observed in patients with DLB seems to be lower than that of patients with other dementias[101]. Nevertheless, a higher number of activated microglia have been found in patients with DLB[102]. Alpha-synuclein is itself a potent activator of microglia and an increased expression of IL-1α and TNFa has been observed in microglia in close proximity to neurons bearing inclusions[103]. In DLB, there is a progressive association of microglia with degenerating Lewy body-containing neurons[104]. What DLB has in common with other dementias like AD is increased neuroinflammatory states driving progression of the disease.

FRONTOTEMPORAL DEMENTIA

Frontotemporal dementia (FTD) is a genetically and pathologically heterogeneous disorder characterized by personality changes, language impairment, and deficits in executive functions, associated with frontal and temporal lobe degeneration[105]. It is a form of progressive neuronal atrophy characterized by the loss of cells from the frontal and temporal cortices. Histopathologically, most patients show intraneuronal inclusions of the cytosolic phosphorylated TAR DNA binding protein-43 (TDP43) also known as TARDBP[105]. Different phenotypes have been recognized based on clinical symptoms, namely the behavioral variant of FTD, the agrammatic variant of primary progressive aphasia, and the semantic variant of PPA[106,107]. Moreover, some patients present associated parkinsonism, as in progressive supranuclear palsy and corticobasal syndrome, or motor neuron disease (FTD-MND)[108]. Genetic studies have identified several genes associated with monogenic FTD. The first mutations identified in families with FTD and parkinsonism were in the microtubule-associated protein tau (MAPT) gene in chromosome 17[109]. Forty-four pathogenic mutations in the *MAPT* gene have been identified[110], causing the accumulation of hyperphosphorylated tau protein in neurons and glial cells[109]. About 69 distinct pathogenic mutations have been identified in the *GRN* gene, accounting for up to 20% of familial and 5% of sporadic FTD cases[110]. Progranulin is expressed in many cell types; expression of GRN in the brain is restricted to microglia and neurons under physiological conditions, but it is selectively upregulated in microglia after excitotoxic activation[111], and is a secreted growth factor known for its role in biological processes, including cellular and tissue development, inflammation, wound healing, and cancer, and for its neurotrophic properties. Several findings suggest that progranulin acts as a mediator of the inflammatory response. It is proteolytically processed into peptides called granulins, of which their function in the nervous system is still largely speculative[112]. Some granulin peptides are able to attract and activate microglia in the brain and increase their phagocytic function[113]. Deficient production of progranulin leads to high levels of proinflammatory cytokines and low levels of anti-inflammatory cytokines, thus promoting neuronal cell death, according to studies using progranulin knockout mice and conditioned media from progranulin-deficient microglia[114,115]. These results suggest that the loss of progranulin may result in a dysregulated inflammatory response in microglia that could have detrimental effects on neuronal cell survival and promote the development of FTD.

Figure 1. The interrelationship between chronic inflammation and dementias in aging.
AD: Alzheimer's disease; VaD: vascular dementia; FTD: frontotemporal dementia; MAPT: microtubule-associated protein tau; GRN: granulin; DLB: dementia with Lewy bodies.



CONCLUSIONS

It is well established that in aging there is a loss of immune homeostatic regulations. Primary lesions in dementias may exacerbate and prolong dysregulation of the innate immune system in the CNS and cause a chronic condition that is recognized as a common characteristic in the dementias related with aging (Fig. 1). The most prominent sign in neuroinflammation is the gliosis in which persistent microglia of the M1 phenotype and astrogliosis release proinflammatory mediators, which can result in neurotoxicity to all kinds of CNS cells, contributing to an exaggerated and prolonged inflammatory response that could be conducive to the development of neurodegeneration observed in dementias. However, the relevance of neuroinflammation in aging-related dementias may be underestimated, as suggested by the negative results of anti-inflammatory therapy in clinical trials to evaluate the cognitive decline in the development of AD[116,117]. Nevertheless, the relevance in the various aging-related dementias may be different. Moreover, knowledge on the modulatory effects of anti-inflammatory drugs on gliosis is partial, and more studies are needed to identify new molecular targets that can be used to regulate the gliosis.

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