บทความปริทัศน์

Cellular aging

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Abstract

Aging is associated with the gradual accumulation of irreversible physiological changes that ultimately result in susceptibility to death. To date, the most widely accepted theories of aging that explain cellular aging are categorized into structural damage and programmed theories. A wide range of aging causes can be classified into four major groups: free radicals, glycation, telomere shortening, and accumulation of toxic and non-toxic garbage. These extrinsic and intrinsic factors collectively stimulate physiologic stresses to all types of cells. Postmitotic and mitotic cells conferring different proliferative capacity, undergo aging in response to these stresses via distinct mechanisms of cell death and cellular senescence, respectively. The progressive accumulation of these aged cells eventually contributes to dysfunction of aged tissues.

Key words: Cellular aging, Cellular senescence, Apoptosis

Introduction

Aging is characterized by a gradual decline in the capacity of physiologic systems, eventually leading to failure of a critical system, then death. Considering the aging process, it is surprising that there remain many unanswered questions about how aging happens at the cellular level. This review article has therefore summarized the current state of knowledge regarding some particular aspects of cellular aging, including the most widely accepted theories, the major causes, the cellular responses, and anti-aging interventions.

Cellular theories of aging

Scientists have tried to develop theories of aging for centuries, which in turn help them formulate the questions that drive research. Some theories have fallen out of favor over time. At present, two groups of cellular theories of aging become more widely accepted: structural damage theories and programmed theories. The first group states that aging is caused by accumulated damage to cellular components over time whereas the second group describes aging as a direct consequence of genetic programming. The two most widely accepted theories of each group are listed below.

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Structural damage theories: The free radical theory of aging

The free radical theory of aging (FRTA) was first proposed in 1957[1], stating that aging is the result of cumulative oxidative damage to biomolecules, e.g. lipid, protein, and nucleic acid (Fig. 1A). Such damage is indeed caused by increased production of free radical-containing reactive oxygen species (ROS) (e.g., $O_2^{\cdot -}$, $OH^{\cdot}$) and reactive nitrogen-oxygen species (RNOS) (e.g. nitric oxide, and NO), decreased antioxidant levels, and the inability to repair oxidative damage. Evidence to support the FRTA comes from the inverse correlation between basal metabolic rate and maximum lifespan of mammals and from the accumulation of oxidative damaged DNA, proteins, and lipids in aged organisms.[2] Additionally, increased expression of antioxidant enzymes can slow aging and increase the lifespan of flies and worms, but such a beneficial effect did not occur in mammals.[3]

Structural damage theories: The mitochondrial theory of aging

The mitochondrial theory of aging (MTA) was first proposed in 1972.[4] It is a variant of free radical theory of aging[2], treating aging as the result of damage to mitochondrial DNA (mtDNA). Mitochondria are the energy generators of the cell, which produce 90% or most of the ATP in the body and generate ROS through increased electron leakage in the respiratory chain.[5] Also, these organelles have limited capacity for DNA repair and mtDNA is not protected by a sheath of histones. Thus, mtDNA is especially sensitive to mutations (e.g., deletions, point mutations, gross DNA rearrangements, etc.) and its damage leads to defective functions of mitochondria, eventually resulting in aging. This theory has been supported by the observations that mtDNA mutations increase with age in mammals, especially in post-mitotic highly aerobic tissues such as brain, heart, skeletal muscle.[6]

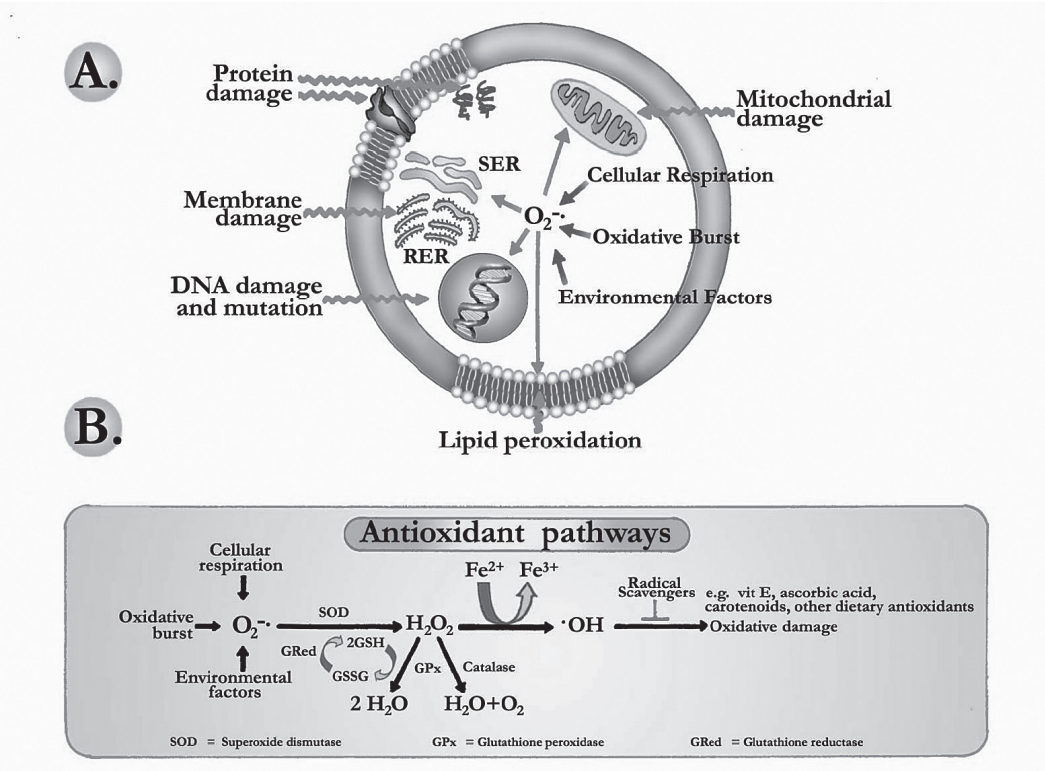

Fig. 1 (A) Free-radical-mediated cellular injury (B) Antioxidant pathways against oxygen toxicity

Programmed theories: The genetic theory of aging

In contrast to structural damage theories, the genetic theory of aging, one of the most widely accepted programmed theories formulated in 1981, proposes that lifespan is largely determined by the effect of genetics.[7] There are at least 30 genes that have a significant effect on human life span. However, such effect accounts for only 20-50% of lifespan, the other 50-80% being attributed to environment and developmental variations. The genetic theory of aging is apparent in animal studies. For example, mutations in genes of the insulin/insulin-like growth factor I (IGF-I) signaling network can significantly extend lifespan in diverse species ranging from worms[8] to rodents.[9] In humans, this network has been shown to be involved in the control of aging and longevity.[10] Recently, Jewish centenarians have been revealed to have more mutations in the IGF-1R gene.[11] Interestingly, the effects of mutations in genes of the insulin-like signaling network on longevity are likely associated with reduced oxidative damage and increased stress resistance. Besides, there are other genes associated with increased longevity in humans, e.g. variants in Apolipoprotein E (ApoE)[12] and cholesteryl ester transfer protein (CETP).[13] However, these genes seem to increase mean lifespan, probably by helping a person metabolize cholesterol, rather than increase longevity.

Programmed theories: The cellular senescence theory of aging

The cellular senescence theory of aging was formulated in 1965, describing cellular senescence as a biological program that limits the ability of normal human cells to proliferate in culture.[14] As mitotically competent cells normally increase in number of divisions (maximum ~ 50 to 70 times), they gradually lose proliferative capacity and their telomeres shorten slightly each time they divide. This phenomenon is termed replicative senescence. At the end of the replicative lifespan, all cells stop their proliferation, but remain viable. A prime cause of replicative senescence is progressive telomere shortening. Telomeres are the repeats of a specific 6-nucleotide DNA sequence with a loop-like structure at the end of a chromosome (Fig. 2A). Some experimental data indicate a link between telomere length and aging and lifespan[15], suggesting that telomere length could serve as a biomarker for aging in human somatic cells that continue to divide, e.g. hematopoietic stem cells, skin cells, epithelial cells.

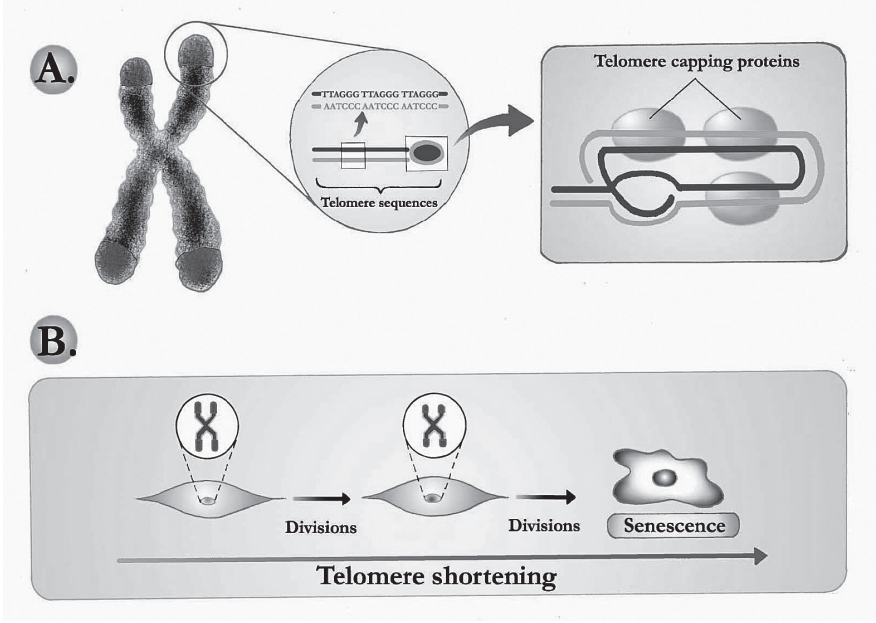

Fig. 2 (A) Telomere structure (B) Telomere shortening determines the proliferative lifespan of mitotically competent cells

The causes of cellular aging

Although aforementioned aging theories cover some causes of aging, many of which are still not included. Moreover, new aspects of some particular causes are later discovered besides the old one referred by the theories. Accordingly, this review categorizes various causes of aging into four major groups as follows.

Free radicals

Free radicals (oxidants) can originate from many external sources such as air pollution, radiation, domestic chemicals (e.g., pesticides, air-fresheners etc.), cigarette smoke, alcohol, and deep fried foods. Also, free radicals can arise from endogenous sources as a result of normal aerobic respiration, metabolism, and inflammation. At low/moderate level, ROS/RNOS (e.g., superoxide radical and nitric oxide) perform important biological functions, for example, in defense against infectious agents[16], and in the function of many cellular signaling pathways.[17] At high level, however, these free radicals from both sources attack various vital cellular components (Fig. 1A).

Cells are protected against these damages by two antioxidant systems. First, antioxidant scavenging enzymes against ROS include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP_X), and glutathione reductase (GRed) whose functions are to convert free radicals to nontoxic and nonradical forms (Fig. 1B). Second, dietary free-radical scavengers (e.g., vitamin E, ascorbic acid, glutathione, carotenoid, and flavonoids) neutralize these free radicals by donating one electron to the radical in non-enzymatic reactions (Fig. 1B). Minerals are also the other dietary antioxidants that are critical to the activity of vital antioxidant enzymes in the body. For example, selenium is required for GP_X activity. Zinc is essential for the activity of CAT and SOD.

When the antioxidant defense system is overwhelmed by an increased oxidant level or reduced antioxidant supply, oxidative stress occurs and then causes oxidative damage to biomolecules and cellular components. Oxidized proteins (e.g., oxidized thiols, protein carbonyls) and oxidized lipids (e.g., lipid peroxides) are formed during these damages. Such oxidized lipids are broken down into aldehydes (e.g. malondialdehyde -MDA), which can crosslink proteins, particularly oxidized forms. Both oxidized and cross-linked proteins are resistant to degradation and thus accumulate over time. Additionally, these oxidative damages to lipids and proteins located on organelle membranes of cells result in the loss of membrane integrity and ion leakage. DNA damage (e.g., 8-hydroxydeoxyguanosine -8-OH-dG) is another form of oxidative damage, which tends to interfere with gene expression. MtDNA is even more vulnerable to oxidative damage than nuclear DNA and its damage causes mitochondria to shut down. Collectively, these impaired cellular components fail to accomplish their native roles and result in accelerated cell aging.

Glycation

Glycation (Maillard reaction) is another cause of aging. It is a reaction in which glucose and other sugars react spontaneously with free amino groups of proteins, resulting in irreversible cross-linked proteins called Advanced Glycation End-products (AGEs). AGEs are slowly formed and accumulate in long-lived structural proteins such as collagen and elastin, thereby leading to increased stiffness of blood vessels and joints, and impaired functions of the lung, kidney, heart, and retina.[18] These are commonly seen as features of aging.

Telomere shortening

The telomeres lie at the tips of the chromosome (Fig. 2A) and protect chromosome them from being recognized as break points by the DNA repair machinery, which could recombine with their homologous sequences at the ends of other chromosomes. As for the cellular senescence theory of

aging, telomere shortening acts as mitotic counter that determines the proliferative lifespan of the cells. Prior to complete erosion of the telomere, such a critical telomere length activates a DNA damage response pathway involving p53 and Rb, mediating the cell to become senescent (Fig. 2B). However, such cells can enter into a state of senescence rapidly and independently of their intrinsic mechanisms when exposed to various extrinsic physiologic factors including oxidative stress, DNA-damaging agents, oncogene overexpression, and other metabolic perturbations.[19] Therefore, apart from being considered as a biological program involved in the replicative senescence, telomere shortening is also induced by physiologic stress. Both aspects of telomere shortening gives rise to senescent cells that are terminally arrested but remain intact and viable with altered phenotypes[20], contributing collectively to the aging of tissues. However, telomere shortening appears not to deleteriously affect post-mitotic tissues such as brain, heart, and skeletal muscle because these cells cannot divide although they may function throughout adult life.

Accumulation of toxic and non-toxic garbage

Examples of this garbage include toxic and inert by-products of cellular metabolism (e.g., cross-linked proteins and lipids, lipid peroxidation debris, and AGEs), as well as other modified proteins formed by other reactions independent of ROS such as racemization, deamination, and alkylation reactions. In addition, lipofuscin (age pigment) is regarded as a product of lysosomes containing hydrolytic enzymes to degrade proteins, lipids and damaged organelles. As lysosomes engulf large amounts of the garbage that are resistant to these hydrolytic enzymes, they are inevitably bloated with indigestible content, thus accumulating in cells as lipofusin granules.[21] High levels of metals, especially lead, aluminum, and iron, also tend to accumulate in cells and cause toxic effects to them. This garbage appears to be a primary cause of aging for non-dividing cells because these cells cannot dilute the garbage away whereas mitotic cells efficiently do during division.

Cellular senescence & cell death: The mechanisms responsible for cellular aging

Cellular senescence and cell death are the cellular responses to damage or stress through distinct molecular mechanisms and are mainly responsible for cellular aging. Their roles in the aging process are described below.

Cellular senescence (Arrested cell growth)

In addition to replicative senescence, the second form of cellular senescence called stress-induced senescence is subsequently given and viewed as a general cellular response program. Several studies have indicated that normal cells can undergo senescence rapidly in response to various physiologic stresses.[22] Levels of the p53 and Rb activity triggered by these stresses through signaling pathways determine whether cells enter senescence. In fact, cells decide whether to undergo a transient growth arrest, cellular senescence, or apoptosis depending on the type of cellular stress and its severity and the cell type. Accordingly, cellular senescence is one of several programs activated in normal cells in response to physiologic stresses.

As for the mechanisms of cellular senescence, it is triggered through activation of the p53 and Rb following the presence of a critically short telomere. Cellular senescence is a major mechanism responsible for aging in mitotically competent cells since as these cells become senescent, they display a drastically altered phenotype. For example, they express genes that encode degradative enzymes and inflammatory cytokines. Thus, the accumulation of these senescent cells can disrupt the tissue structure and gradually decrease

tissue function, resulting in aging and age-related disease (Fig. 3A). Moreover, not only do senescent cells disrupt the tissue architecture but also secrete growth factors, so they might stimulate the proliferation of cells that harbor preneoplastic mutations (Fig. 3B). On the contrary, post-mitotic cells are non-dividing and thus do not enter senescence as a result of telomere shortening.

Cell death (apoptosis)

Apoptosis is an active mode of cell death that allows organisms to eliminate damaged or dysfunctional cells in a controlled fashion without damage to surrounding tissues. The first change in a cell undergoing apoptosis is cell shrinkage. Next, small bubble-like protrusions of cytoplasm ("bleb") start forming at the cell surface as the nucleus and other cellular structures begin to disintegrate. The chromosomal DNA is then degraded into small pieces and the entire cell breaks apart, forming small fragments known as apoptotic bodies. Finally, the apoptotic bodies are swallowed up by phagocytes (Fig. 4A). As for its mechanisms, apoptosis can be triggered in a cell through the extrinsic pathway or the intrinsic pathway (Fig. 4B). In the extrinsic pathway, physiological signals (e.g., tumor necrosis factor, Fas ligand) bind to death receptors on the outer surface and then trigger the caspase cascade. In the intrinsic pathway, damaged DNA stimulates p53 accumulation, leading to alteration in mitochondrial membranes, cytochrome c release, and activation of the caspase cascade

Post-mitotic cells contain toxic and inert garbage that is not extensive enough for the removal, so they progressively have impaired functions and exhibit aging features. As this damage increases, these cells are subsequently removed, thereby resulting in a decrease in overall cell number and tissue functions. Hence, cell death causes

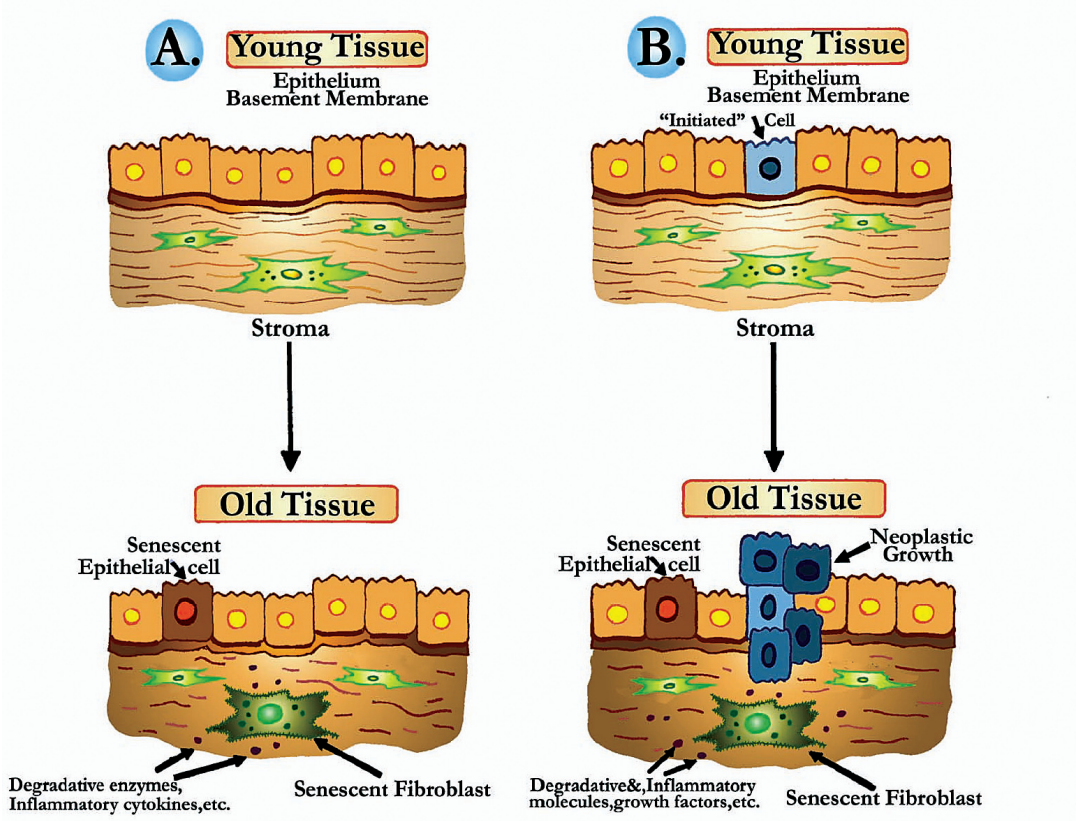

Fig. 3 Senescent cells may contribute to aging (A) and age-related pathology e.g., cancer (B)

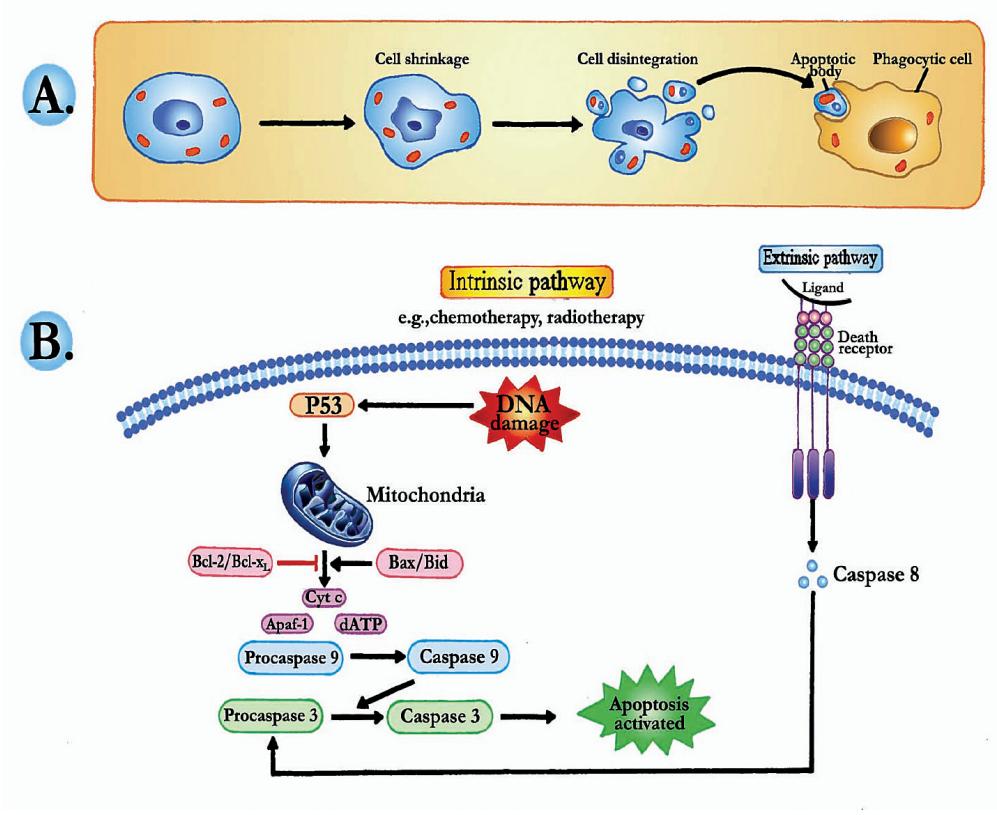

Fig. 4 (A) Membrane and morphological changes in apoptotic cells (B) Intrinsic & extrinsic pathways and series of biochemical steps in apoptotic cell death

detrimental effect leading to aging phenotype in post-mitotic cells that cannot be readily replaced such as neurons, cardiac myocytes, and skeletal muscle cells. In contrast, this apoptotic mechanism is less efficient in causing aging to some mitotically competent cells because they can dilute out the garbage.

Importantly, there is evidence that cellular senescence and apoptosis are powerful tumor-suppressive mechanisms in relatively young organisms. Indeed, dysfunctional or damaged cells that undergo senescent or apoptosis cannot further transform into cancer cells, at least early in life. Nevertheless, as more senescent cells accumulate in tissues and apoptosis depletes more cells from post-mitotic tissues, these two mechanisms eventually contribute to aging phenotype late in life.

Interventions to delay aging

Calorie restriction (CR) is the only intervention to improve health and extend lifespan in a variety of species[23], including primates.[24] The mechanism that could explain the effect of long-term CR on aging is related to the reduction of body fat and insulin signaling as well as ROS produced during breathing. Although the effects of CR on human longevity are not yet available, there is now significant evidence that eating appropriate foods or foods with antioxidants has beneficial effects on increasing the functional lifespan, if not the maximal lifespan.[25]

Conclusion

Aging is a complex process that involves different mechanisms. Theories that explain cellular aging can generally be divided into the structural damage and programmed theories of aging. A

variety of factors induce physiologic stresses to all kinds of cells. Notably, postmitotic and mitotic cells differ in their proliferative capacity and undergo aging in response to these stresses via distinct mechanisms of cell death and cellular senescence, respectively. These aged cells accumulate over time and eventually cause dysfunction of aged tissues.

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บทคัดย่อ

ความชราระดับเซลล์

พินทุสร หาญสกุล
สาขาชีวเคมี สถานวิทยาศาสตร์พรีคลินิก คณะแพทยศาสตร์ มหาวิทยาลัยธรรมศาสตร์

ความชราภาพ (Aging) เกี่ยวข้องกับการเปลี่ยนแปลงทางสรีระแบบไม่ย้อนกลับ และมีการสะสมเพิ่มมากขึ้นตามเวลา ซึ่งจะนำไปสู่การเสียชีวิตในที่สุด ทฤษฎีความชราภาพที่ได้รับการยอมรับมากที่สุดในปัจจุบัน แบ่งได้เป็นทฤษฎีชราภาพที่เกิดจากการสะสมความเสียหายในเซลล์ (structural damage theories) และทฤษฎีชราภาพที่เกิดจากการกำหนดทางชีวภาพ (Programmed theories) สามารถแบ่งสาเหตุต่างๆ ที่ทำให้เกิดความชราได้เป็นสี่กลุ่มหลัก คือ อนุมูลอิสระ (free radicals) การเกิดกระบวนการไกลเคชั่น (glycation) การหดสั้นของเทโลเมียร์ (telomere shortening) และการที่เซลล์มีการสะสม by-product ที่ได้จากกระบวนการต่างๆ ในเซลล์ รวมทั้งสารเคมีและโลหะที่มีการย่อยสลายยาก (accumulation of toxic and non-toxic garbage) ปัจจัยเหล่านี้กระตุ้นให้เกิดภาวะเครียด (stress) ต่อเซลล์ทุกชนิด สำหรับเซลล์ชนิด postmitotic และ mitotic นั้นมีการตอบสนองต่อความเครียดโดยเข้าสู่กระบวนการตาย (cell death) และกระบวนการแก่ชรา (Cellular senescence) ตามลำดับ จำนวนที่ค่อยๆ เพิ่มขึ้นของเซลล์ที่แก่ชราเหล่านี้ ส่งผลให้เกิดการเสื่อมของเนื้อเยื่อที่มีการทำงานลดลงหรือผิดปรกติในที่สุด

คำสำคัญ: ความชราระดับเซลล์, กระบวนการชรา, อะพอพโทซิส