The Biology of Aging and Frailty

Neal S. Fedarko, PhD

KEYWORDS

• Frailty • Aging • Apoptosis • Senescence • Inflammation

In developing and validating the concept of frailty as a geriatric syndrome, it has been necessary to distinguish the clinical expression of frailty from normal age-related changes and other age-related disease pathologies. Fried and colleagues[1–5] have provided a framework for excluding potentially confounding disease and a working clinical tool to diagnose frailty, and they have shown associations between frailty and other pathophysiologies. However, investigating the underlying biologic basis for the geriatric syndrome of frailty by studying basic homeostatic pathways and mechanisms has not proceeded at the same rate. The following article provides an overview of the homeostatic pathways emphasized in research on aging and explains how this science may help to stimulate frailty research.

NORMAL AGING

Aging can be defined as the decline and deterioration of functional properties at the cellular, tissue, and organ level. This loss of functional properties yields a loss of homeostasis and a decreased adaptability to internal and external stress, increasing vulnerability to disease and mortality.[6] Aging is a breakdown in maintenance of specific molecular structures and pathways, a loss of homeostasis, and a failure in homeodynamics.[7] Homeodynamics refers to biologic systems that do not actively mandate stasis; instead, they dynamically reorganize and reset points of balance in response to internal and external change to maintain their functional capacity over time.

Individuals vary a great deal in the onset of the aging process and the rate and extent of its progression. Differences in the manifestations of aging reflect differences in functional capacity. Functional capacity is a direct measure of the ability of cells, tissues, and organ systems to operate properly/optimally and is influenced by genes and environment. Optimal cellular, organ, and organism operation reflects homeodynamic mechanisms and maintenance pathways. Mechanisms of maintenance include DNA repair, synthesis, and fidelity surveillance; detection and clearance of defective proteins

This work was supported in part by Grant No. AG000120 from the National Institutes of Health.
Division of Geriatric Medicine and Gerontology, Department of Medicine, Johns Hopkins University School of Medicine, Johns Hopkins University, 5501 Hopkins Bayview Circle, Baltimore, MD 21224, USA
E-mail address: ndarko@jhmi.edu

Clin Geriatr Med 27 (2011) 27–37
doi:10.1016/j.cger.2010.08.006 **geriatric.theclinics.com**
0749-0690/11/$ – see front matter

and lipids; clearance of defective organelles and cells; and defense against pathogens and injury. Many of the physiologic theories of aging are direct counterparts of these maintenance mechanisms (eg, DNA damage, error catastrophe, free-radical, mitochondrial damage, and immunosenescence theories). These maintenance mechanisms, in turn, affect homeodynamics through the cellular responses of apoptosis, senescence, and repair and the systemic response of immune activation/inflammation. For example, when DNA damage is too great to be repaired, cells undergo apoptosis.[8] Cells can respond to free-radical damage to DNA by inducing senescence[9] or initiating apoptosis.[10] Oxidative damage and apoptosis are correlated negatively with the repair mechanism autophagy.[11] Proteins aberrantly modified with nonenzymatic glycation[12] or free radicals[13] can induce inflammation. Inflammation and immune responses resolve, in part, through targeted immune cell apoptosis.[14] In addition to critical telomere length, senescence can be triggered by oxidative stress[9,15] and protein glycation/cross-links.[16,17] Hormones contribute to homeodynamics, in part, through modulation of apoptosis, senescence, and inflammation.[18–20]

FRAILTY

Frailty is a geriatric syndrome characterized by weakness, weight loss, and low activity and is associated with adverse health outcomes. Frailty manifests as age-related, biologic vulnerability to stressors and decreased physiologic reserves yielding a limited capacity to maintain homeostasis.[3] The validated and widely used 5-item frailty criteria for screening—self-reported exhaustion, slowed performance (by walking speed), weakness (by grip strength), unintentional weight loss (4.5 kg in the past year), and low physical activity[1]—are composite outcomes of multiple organ systems. The surrogate endpoint markers—elevated cytokines and chemokines[21–23]; reduced insulinlike growth factor 1(IGF-I), dehydroepiandrosterone sulfate, and leptin[24]; perturbed neutrophil, monocyte, and white blood cell distribution[25,26]—indicate multiple systems are dysregulated in frailty.

This definition of frailty (multisystem dysregulation yielding decreased physiologic reserves and increased vulnerability to stressors) has commonality to that of aging (loss of molecular/cellular functional properties yielding decreased adaptability to internal/external stress and increased vulnerability to disease and mortality). Both have a basis in loss of homeostasis, although with aging, the failure in homeodynamics is global, whereas with frailty, the failure in homeodynamics cycle around energy metabolism and neuromuscular changes. Because researchers have characterized frail elderly populations, the observed changes in functional performance and biomarker distribution are distinct from the corresponding age-related changes observed in nonfrail (normal) individuals.[1–4]

CELLULAR RESPONSES TO STRESSORS

For aging and frailty, loss of homeostasis results in an increased vulnerability to stressors. An organism's reaction to stressors involves the cellular responses of apoptosis, senescence, and repair. At the cellular level, stressors (eg, free radicals, DNA damage, cell injury/insult) challenge maintenance mechanisms (**Fig. 1**). The evolved cellular response, apoptosis, removes damaged/aberrant cells through controlled cell death; senescence alters their phenotype and blocks further proliferation; and repair removes damaged proteins, lipids, and organelles and recycles constituent parts. Failure in these responses leads to transformed cells/neoplasms that can radically compromise organ function and survival. Dysregulation of these cellular responses can contribute to tissue pathology when, for example, increased

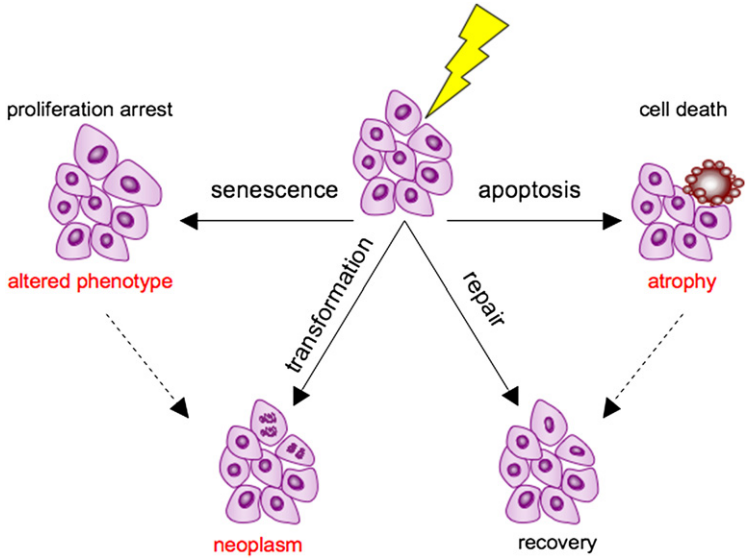

Fig. 1. Cellular responses to stressors. Stressors (free radicals, DNA damage, nutrient or oxygen constriction, cell injury [represented by the *lightening bolt*]) challenge cellular homeostasis. The cellular response can be senescence, apoptosis, repair, or neoplastic transformation of the cell. Senescence, a tumor-suppressive response is associated with an altered secretory phenotype. The controlled cell death of apoptosis can also be tumor-suppressive, although many cells, especially immune cells, normally exit through apoptosis. Apoptosis can, however, yield tissue/organ atrophy. Repair enables recovery of homeostasis. In some cases, apoptosis is a precursor of repair and recovery (*dotted line* with *arrow*). Also, the senescent cell phenotype is sometimes a precursor/contributor to neoplasm formation and cancer progression.

apoptosis leads to tissue/organ atrophy or when expression of the senescent cell phenotype increases proinflammatory cytokine release. All these cellular responses have been implicated in normal aging and are discussed in greater detail in the following sections. The balance between apoptosis and senescence or the acceleration of either may well precipitate changes in multiple systems and, ultimately, in frailty and related late-life vulnerability.

Apoptosis

Apoptosis is an orderly process of cellular self-destruction, a process as crucial for survival of multicellular organisms as cell division. Apoptosis is important in embryogenesis: morphogenesis (eliminating excess cells, such as webbing between digits in embryos), selection (eliminating nonfunctional cells, as in neuronal pruning), immunity (eliminating dangerous cells, such as self-antigen recognizing cells), and organ size (eliminating excess cells).[27] Apoptosis is also important in adults in tissue remodeling (eliminating cells no longer needed, as in mammary gland involution after lactation), maintains organ size and function,[28] and eliminates damaged/dysfunctional cells.[29,30]

Apoptosis can be initiated by external signals that bind to physiologic receptors on cell surfaces or by intrinsic damage that propagates cytosolic signals. Both pathways converge with mitochondrial signals, which lead to a caspase cleavage cascade, which, in turn, results in the orderly proteolysis of proteins and DNA, the cross-linking

of cell corpses, and their subsequent engulfment. Although death by necrosis and oncosis (ischemic cell death) invokes major inflammatory responses and collateral damage, apoptosis is a controlled cellular demolition with no inflammatory response. Key apoptosis steps (chromatin condensation, vesicle formation, and activity of hydrolytic enzymes) have a high energy demand and thus, apoptosis is an adenosine triphosphate (ATP)-dependent process.[31] Thus, at insufficient ATP levels, cells shift from apoptotic to necrotic cell death.[32] Consistent with this, an age-related decline in cellular ATP levels was found to promote necrotic fibroblast cell death over apoptosis in response to oxidative stress.[33] These molecular processes may stimulate chronic inflammation in older adults, which probably facilitates frailty and late-life decline through mechanisms discussed below.

As a sentinel homeodynamic cellular response, apoptosis can have pathophysiologic consequences for aging. For example, too much apoptosis can yield tissue degeneration,[34] whereas too little apoptosis allows dysfunctional cells to accumulate or differentiated immune cells to persist.[35] Evidence suggests that sarcopenia is apoptosis-driven.[36] Conversely, failures in apoptosis can contribute to the senescent cell phenotype as well as rogue cell proliferation.[37] It has been shown that apoptosis is an important cellular defense mechanism in maintaining genetic stability, and centenarians who have aged successfully possess cells that are more prone to apoptosis.[38] The proinflammatory marker interleukin 6 (IL-6) seems to be protective against apoptosis[39] and its serum levels are known to increase with increasing age[40] and have an inverse correlation with apoptosis.[41]

Senescence

Cellular senescence is a response of normal cells to potentially cancer-causing events. The term replicative senescence identifies the subset of senescent cells in which the arrest in proliferation is associated with the high number of cell divisions (the Hayflick and Moorhead[42] limit—typically between 40 and 60 cell divisions). The mechanism for replicative senescence involves each division resulting in a shortening of the telomere region of chromosomes, such that a cumulative critical length is reached that does not support the DNA replication machinery. Senescent cells exhibit an irreversible arrest of cell proliferation, an altered function, and in some cases, a resistance to apoptosis. Besides telomere shortening, inducers of senescence include DNA damage, oncogene expression, supermitogenic signals,[43] and telomere-independent pathways, which include cytoskeletal, interferon-related, IGF-related, mitogen-activated protein kinase, and oxidative stress pathways.[44] The arrest in proliferation is imposed and maintained on cells by the induction of cyclin-dependent kinase inhibitors p16 and p21 that implement cell-cycle arrest.[45] Cellular senescence is associated with typical phenotypic changes, such as enlarged morphology, activation of senescence-associated β-galactosidase, elevated expression of proteases, cell-cycle inhibitors, and proinflammatory cytokines.[43]

The contribution of cellular (replicative) senescence to organismal aging has been controversial, although increasingly; evidence seems to link cellular senescence to aging.[46,47] Senescent cells accumulate with age and at sites of age-related pathology.[47] The senescent phenotype (eg, secretion of IL-6) may contribute to the proinflammatory state observed in normal aging that is exacerbated in frailty.

Repair

The repair cellular response involves removal of damaged proteins/lipids and organelles and recycling of constituent components via the catabolic/degradative machinery of cells—proteasomes, lysosomes, and autophagosomes.[48] Proteasomes

are large cellular protein complexes that degrade unnecessary or damaged proteins tagged with ubiquitin. Lysosomes are cellular organelles that fuse with vacuoles and dispense enzymes that degrade proteins, polysaccharides, nucleic acids, and lipids present in the vacuoles. Autophagosomes are involved in sequestering cytosolic components/organelles through phagophore formation, fusing the formed autophagosome with lysosomes, and degrading material by the lysosomal machinery (ie, autophagy). Normal cycling (flux) of these cellular catabolic vacuoles prevents the accumulation of damaged/aberrant molecular and cellular components.[49]

The removal and recycling phases of repair have gained considerable interest in research on aging. Conditions that modulate lifespan—mutations in the insulin/IGF-I signaling system, treatments that reduce the expression/activity of the transcription factor mammalian target of rapamycin (mTOR), and caloric restriction all increase autophagy.[49] Aging cells exhibit an increase in mitochondrial DNA mutations and a decline in mitochondrial function.[50] Also, free radical generation by damaged mitochondria increases. Autophagy normally maintains homeostasis through mitochondrial turnover. Dysregulation of autophagy,[51] proteasomes,[52] and lysosomes[53] have all been observed with increasing age. Thus, functional capacity to remove oxidized, cross-linked, and/or unfolded proteins, nucleic acids, lipids, and polysaccharides is impaired, and these products increase with age. Lipofuscin ("aging pigment") arises in lysosomes through the oxidation of unsaturated fatty acids through iron-generated free radicals. Lipofuscin is insoluble and refractile to cellular removal and recycling, accumulates with age, and may compromise organelle functional capacity by "overstuffing" organelles or diverting hydrolases and lipases away from their normal substrates.

SYSTEMIC RESPONSES TO STRESSORS: INFLAMMATION

Inflammation is a sentinel systemic response to stressors that plays a central role in the aging process in normal, healthy individuals.[54] The classic definition of inflammation refers to 5 cardinal signs: *calor* (heat), *dolor* (pain), *rubor* (redness), *tumor* (swelling), and *functio laesa* (loss of function). Inflammation modulates the cellular responses to stressors, and cellular responses can regulate components of the inflammatory response. Immunosenescence is the decline in the function of the adaptive immune system that occurs during aging that is associated with thymic involution, alterations in T-cell subsets, and reduction in antibody production. As adaptive immunity declines, innate immunity systems exhibit low-level but chronic activation that, with oxidative stress, leads to a low-level but chronic proinflammatory phenotype. A proinflammatory state may underlie several pathologies, including cancer,[55] cardiovascular disease,[56,57] diabetes mellitus,[58] osteoporosis,[59] rheumatoid arthritis,[60] and cognitive disorders, such as Alzheimer and Parkinson disease.[61] It is thought that these altered cytokines, in the local tissue microenvironment, perturb cellular functional capacity facilitating disease progression. "Inflammaging" is the term used to describe this proinflammatory state associated with aging,[62] which has also been termed molecular inflammation.[63,64]

Several studies have shown significant association of elevated IL-6 levels with frailty in older adults.[21,22,25,65,66] The altered inflammatory state observed in frailty may contribute to several frailty-associated pathologies. For example, proinflammatory cytokines affect the growth hormone/IGF-I axis.[67] "Sickness behavior" (fatigue, malaise, loss of interest in social activities, difficulty concentrating, and changes in sleep patterns) is triggered by the production of proinflammatory cytokines by macrophages and other cells of the innate immune system in response to immune

Table 1
Cellular and systemic response regulation by key transcription factors

	Transcription Factor			
Response	p53	Rb	NF-κB	FOXO
Apoptosis	↑[72]	↓[73]	↓[74]	↑[75]
Senescence	↑[76]	↑[45]	↑[77]	↑[78]
Repair/autophagy	↓[79]	↓[80]	↓[81]	↑[82]
Inflammation	↓[83]	↓[84]	↑[85]	↓[86]

The induction (↑) or repression (↓) of cellular and systemic responses by key transcription factors and the references supporting that assessment are given in parenthesis.

challenge.[68] The *cytokine hypothesis of depression* suggests that proinflammatory cytokines can act as neuromodulators—key factors in the (central) mediation of the behavioral, neuroendocrine, and neurochemical features of depressive disorders.[69,70] The central action of cytokines may also account for the hypothalamic-pituitary-adrenal (HPA) axial hyperactivity that is frequently observed in several age-related disorders, because proinflammatory cytokines disturb the negative feedback inhibition of circulating corticosteroids on the HPA axis.[71–86]

CROSS TALK BETWEEN CELLULAR RESPONSES AND INFLAMMATION

The tumor suppressors retinoblastoma protein (pRb), p53, and forkhead transcription factor (FOXO) are key modulators of apoptosis, senescence, and repair responses. The central transcription factor in inflammatory responses is nuclear factor kappaB (NF-κB). NF-κB also modulates apoptosis, senescence, and repair responses. The proteins p53, pRb, FOXO, and NF-κB are controlled by complex pathways involving upstream regulators, downstream effectors, and cytosolic inhibitors (for NF-κB) that regulate expression of other genes, modulate cell-cycle progression, and are crucial for allowing normal cells to sense and respond to apoptosis and senescence signals. These transcription factors have direct effects on increasing or decreasing cellular responses (**Table 1**).

Interaction between p53 and pRb is well known in their role in determining whether DNA damage can be repaired or whether apoptosis should occur.[87] Cross talk also occurs between p53 and FOXO[88] and pRb and NF-κB.[89] The communication between response regulators can be multileveled. For example, p53 acts in at least 2 stages of inflammation—as a general inhibitor of NF-κB-dependent transcription and, through an unknown mechanism, as a positive regulator of neutrophil clearance by macrophages.[90]

SUMMARY

The cellular responses of apoptosis, senescence, and repair and the systemic response of immune activation/inflammation have evolving roles in contributing to the aging phenotype. Homeostatic mechanisms effect change through these responses; thus, they are likely candidates for pathways that may contribute to the failure in homeodynamics seen in frailty. Dysregulation of apoptosis may contribute to the frailty traits of sarcopenia and weakness. Similarly, the cellular senescent phenotype, secreting proinflammatory cytokines, may contribute to the dysregulated inflammatory state seen in frailty. One can further speculate that deficits in repair in

specific tissues (eg, muscle, nerves, bone) contribute to frailty. Facets of senescence, apoptosis, and repair response have yet to be carefully studied in the setting of frailty. It may be expected that the patterns observed in normal aging between these different cellular and systemic responses would be further perturbed or accelerated in the syndrome of frailty. Future investigations targeting these areas will provide the data necessary to test these hypotheses related to the biology of aging.

REFERENCES

1. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci 2001;56(3):M146–56.
2. Walston J, McBurnie MA, Newman A, et al. Frailty and activation of the inflammation and coagulation systems with and without clinical comorbidities: results from the Cardiovascular Health Study. Arch Intern Med 2002;162(20):2333–41.
3. Fried LP, Ferrucci L, Darer J, et al. Untangling the concepts of disability, frailty, and comorbidity: implications for improved targeting and care. J Gerontol A Biol Sci Med Sci 2004;59(3):255–63.
4. Bandeen-Roche K, Xue QL, Ferrucci L, et al. Phenotype of frailty: characterization in the women's health and aging studies. J Gerontol A Biol Sci Med Sci 2006; 61(3):262–6.
5. Chang SS, Weiss CO, Xue QL, et al. Patterns of comorbid inflammatory diseases in frail older women: the Women's Health and Aging Studies I and II. J Gerontol A Biol Sci Med Sci 2010;65(4):407–13.
6. Holliday R, Understanding ageing. Developmental and cell biology series; 30. Cambridge (UK); New York: Cambridge University Press; 1995. p. xiv, 207.
7. Lloyd D, Aon MA, Cortassa S. Why homeodynamics, not homeostasis? ScientificWorldJournal 2001;1:133–45.
8. Roos WP, Kaina B. DNA damage-induced cell death by apoptosis. Trends Mol Med 2006;12(9):440–50.
9. Chen Q, Fischer A, Reagan JD, et al. Oxidative DNA damage and senescence of human diploid fibroblast cells. Proc Natl Acad Sci U S A 1995;92(10):4337–41.
10. Ozawa T. Oxidative damage and fragmentation of mitochondrial DNA in cellular apoptosis. Biosci Rep 1997;17(3):237–50.
11. Wohlgemuth SE, Seo AY, Marzetti E, et al. Skeletal muscle autophagy and apoptosis during aging: effects of calorie restriction and life-long exercise. Exp Gerontol 2010;45(2):138–48.
12. Ramasamy R, Vannucci SJ, Yan SS, et al. Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. Glycobiology 2005;15(7):16R–28R.
13. Nabeshi H, Oikawa S, Inoue S, et al. Proteomic analysis for protein carbonyl as an indicator of oxidative damage in senescence-accelerated mice. Free Radic Res 2006;40(11):1173–81.
14. Marshall JC, Watson RW. Programmed cell death (apoptosis) and the resolution of systemic inflammation. Can J Surg 1997;40(3):169–74.
15. von Zglinicki T. Role of oxidative stress in telomere length regulation and replicative senescence. Ann N Y Acad Sci 2000;908:99–110.
16. Nagaraj RH, Sell DR, Prabhakaram M, et al. High correlation between pentosidine protein crosslinks and pigmentation implicates ascorbate oxidation in human lens senescence and cataractogenesis. Proc Natl Acad Sci U S A 1991;88(22):10257–61.
17. Ravelojaona V, Robert AM, Robert L. Expression of senescence-associated beta-galactosidase (SA-beta-Gal) by human skin fibroblasts, effect of advanced

glycation end-products and fucose or rhamnose-rich polysaccharides. Arch Gerontol Geriatr 2008;48(2):151–4.
18. Evans-Storms RB, Cidlowski JA. Regulation of apoptosis by steroid hormones. J Steroid Biochem Mol Biol 1995;53(1–6):1–8.
19. Hertoghe T. The "multiple hormone deficiency" theory of aging: is human senescence caused mainly by multiple hormone deficiencies? Ann N Y Acad Sci 2005; 1057:448–65.
20. Roubenoff R. Molecular basis of inflammation: relationships between catabolic cytokines, hormones, energy balance, and muscle. JPEN J Parenter Enteral Nutr 2008;32(6):630–2.
21. Leng S, Chaves P, Koenig K, et al. Serum interleukin-6 and hemoglobin as physiological correlates in the geriatric syndrome of frailty: a pilot study. J Am Geriatr Soc 2002;50(7):1268–71.
22. De Martinis M, Franceschi C, Monti D, et al. Inflammation markers predicting frailty and mortality in the elderly. Exp Mol Pathol 2006;80(3):219–27.
23. Qu T, Yang H, Walston JD, et al. Upregulated monocytic expression of CXC chemokine ligand 10 (CXCL-10) and its relationship with serum interleukin-6 levels in the syndrome of frailty. Cytokine 2009;46(3):319–24.
24. Hubbard RE, O'Mahony MS, Calver BL, et al. Plasma esterases and inflammation in ageing and frailty. Eur J Clin Pharmacol 2008;64(9):895–900.
25. Leng SX, Xue QL, Tian J, et al. Inflammation and frailty in older women. J Am Geriatr Soc 2007;55(6):864–71.
26. Leng SX, Xue QL, Tian J, et al. Associations of neutrophil and monocyte counts with frailty in community-dwelling disabled older women: results from the Women's Health and Aging Studies I. Exp Gerontol 2009;44(8):511–6.
27. Haanen C, Vermes I. Apoptosis: programmed cell death in fetal development. Eur J Obstet Gynecol Reprod Biol 1996;64(1):129–33.
28. Mondello C, Scovassi AI. Apoptosis: a way to maintain healthy individuals. Subcell Biochem 2010;50:307–23.
29. Muradian K, Schachtschabel DO. The role of apoptosis in aging and age-related disease: update. Z Gerontol Geriatr 2001;34(6):441–6.
30. Pollack M, Phaneuf S, Dirks A, et al. The role of apoptosis in the normal aging brain, skeletal muscle, and heart. Ann N Y Acad Sci 2002;959:93–107.
31. Kass GE, Eriksson JE, Weis M, et al. Chromatin condensation during apoptosis requires ATP. Biochem J 1996;318(Pt 3):749–52.
32. Richter C, Schweizer M, Cossarizza A, et al. Control of apoptosis by the cellular ATP level. FEBS Lett 1996;378(2):107–10.
33. Miyoshi N, Oubrahim H, Chock PB, et al. Age-dependent cell death and the role of ATP in hydrogen peroxide-induced apoptosis and necrosis. Proc Natl Acad Sci U S A 2006;103(6):1727–31.
34. Adams JD, Mukherjee SK, Klaidman LK, et al. Apoptosis and oxidative stress in the aging brain. Ann N Y Acad Sci 1996;786:135–51.
35. Gupta S. Molecular mechanisms of apoptosis in the cells of the immune system in human aging. Immunol Rev 2005;205:114–29.
36. Marzetti E, Leeuwenburgh C. Skeletal muscle apoptosis, sarcopenia and frailty at old age. Exp Gerontol 2006;41(12):1234–8.
37. Hsu HC, Scott DK, Mountz JD. Impaired apoptosis and immune senescence - cause or effect? Immunol Rev 2005;205:130–46.
38. Franceschi C, Monti D, Scarfi MR, et al. Genomic instability and aging. Studies in centenarians (successful aging) and in patients with Down's syndrome (accelerated aging). Ann N Y Acad Sci 1992;663:4–16.

39. Biffl WL, Moore EE, Moore FA, et al. Interleukin-6 delays neutrophil apoptosis. Arch Surg 1996;131(1):24–9 [discussion: 9–30].
40. Giuliani N, Sansoni P, Girasole G, et al. Serum interleukin-6, soluble interleukin-6 receptor and soluble gp130 exhibit different patterns of age- and menopause-related changes. Exp Gerontol 2001;36(3):547–57.
41. Kovalovich K, Li W, DeAngelis R, et al. Interleukin-6 protects against Fas-mediated death by establishing a critical level of anti-apoptotic hepatic proteins FLIP, Bcl-2, and Bcl-xL. J Biol Chem 2001;276(28):26605–13.
42. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res 1961;25:585–621.
43. Muller M. Cellular senescence: molecular mechanisms, in vivo significance, and redox considerations. Antioxid Redox Signal 2009;11(1):59–98.
44. Fridman AL, Tainsky MA. Critical pathways in cellular senescence and immortalization revealed by gene expression profiling. Oncogene 2008;27(46): 5975–87.
45. Ohtani N, Yamakoshi K, Takahashi A, et al. The p16INK4a-RB pathway: molecular link between cellular senescence and tumor suppression. J Med Invest 2004; 51(3–4):146–53.
46. Campisi J. Cellular senescence and apoptosis: how cellular responses might influence aging phenotypes. Exp Gerontol 2003;38(1–2):5–11.
47. Jeyapalan JC, Sedivy JM. Cellular senescence and organismal aging. Mech Ageing Dev 2008;129(7–8):467–74.
48. Korolchuk VI, Menzies FM, Rubinsztein DC. Mechanisms of cross-talk between the ubiquitin-proteasome and autophagy-lysosome systems. FEBS Lett 2010; 584(7):1393–8.
49. Vellai T, Takacs-Vellai K, Sass M, et al. The regulation of aging: does autophagy underlie longevity? Trends Cell Biol 2009;19(10):487–94.
50. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet 2005;39: 359–407.
51. Cuervo AM, Bergamini E, Brunk UT, et al. Autophagy and aging: the importance of maintaining "clean" cells. Autophagy 2005;1(3):131–40.
52. Chondrogianni N, Gonos ES. Proteasome dysfunction in mammalian aging: steps and factors involved. Exp Gerontol 2005;40(12):931–8.
53. Chondrogianni N, Fragoulis EG, Gonos ES. Protein degradation during aging: the lysosome-, the calpain- and the proteasome-dependent cellular proteolytic systems. Biogerontology 2002;3(1–2):121–3.
54. Stout RD, Suttles J. Immunosenescence and macrophage functional plasticity: dysregulation of macrophage function by age-associated microenvironmental changes. Immunol Rev 2005;205:60–71.
55. Aggarwal BB, Shishodia S, Sandur SK, et al. Inflammation and cancer: how hot is the link? Biochem Pharmacol 2006;72(11):1605–21.
56. Osiecki H. The role of chronic inflammation in cardiovascular disease and its regulation by nutrients. Altern Med Rev 2004;9(1):32–53.
57. Diomedi M, Leone G, Renna A. The role of chronic infection and inflammation in the pathogenesis of cardiovascular and cerebrovascular disease. Drugs Today (Barc) 2005;41(11):745–53.
58. Duncan BB, Schmidt MI. The epidemiology of low-grade chronic systemic inflammation and type 2 diabetes. Diabetes Technol Ther 2006;8(1):7–17.
59. Ginaldi L, Di Benedetto MC, De Martinis M. Osteoporosis, inflammation and ageing. Immun Ageing 2005;2:14.

60. Wong SH, Lord JM. Factors underlying chronic inflammation in rheumatoid arthritis. Arch Immunol Ther Exp (Warsz) 2004;52(6):379–88.
61. McGeer PL, McGeer EG. Inflammation and the degenerative diseases of aging. Ann N Y Acad Sci 2004;1035:104–16.
62. Giunta S. Is inflammaging an auto[innate]immunity subclinical syndrome? Immun Ageing 2006;3:12.
63. Chung HY, Kim HJ, Kim KW, et al. Molecular inflammation hypothesis of aging based on the anti-aging mechanism of calorie restriction. Microsc Res Tech 2002;59(4):264–72.
64. Chung HY, Cesari M, Anton S, et al. Molecular inflammation: underpinnings of aging and age-related diseases. Ageing Res Rev 2009;8(1):18–30.
65. Leng SX, Yang H, Walston JD. Decreased cell proliferation and altered cytokine production in frail older adults. Aging Clin Exp Res 2004;16(3):249–52.
66. Hubbard RE, O'Mahony MS, Calver BL, et al. Nutrition, inflammation, and leptin levels in aging and frailty. J Am Geriatr Soc 2008;56(2):279–84.
67. Johnson RW, Arkins S, Dantzer R, et al. Hormones, lymphohemopoietic cytokines and the neuroimmune axis. Comp Biochem Physiol A Physiol 1997;116(3): 183–201.
68. Dantzer R, Bluthe RM, Laye S, et al. Cytokines and sickness behavior. Ann N Y Acad Sci 1998;840:586–90.
69. Myint AM, Kim YK. Cytokine-serotonin interaction through IDO: a neurodegeneration hypothesis of depression. Med Hypotheses 2003;61(5–6):519–25.
70. Schiepers OJ, Wichers MC, Maes M. Cytokines and major depression. Prog Neuropsychopharmacol Biol Psychiatry 2005;29(2):201–17.
71. Porter RJ, Gallagher P, O'Brien JT. Effects of rapid tryptophan depletion on salivary cortisol in older people recovered from depression, and the healthy elderly. J Psychopharmacol 2007;21(1):71–5.
72. Harbour JW, Dean DC. Rb function in cell-cycle regulation and apoptosis. Nat Cell Biol 2000;2(4):E65–7.
73. Fridman JS, Lowe SW. Control of apoptosis by p53. Oncogene 2003;22(56): 9030–40.
74. Cardoso SM, Oliveira CR. Inhibition of NF-kB renders cells more vulnerable to apoptosis induced by amyloid beta peptides. Free Radic Res 2003;37(9): 967–73.
75. Jagani Z, Singh A, Khosravi-Far R. FoxO tumor suppressors and BCR-ABL-induced leukemia: a matter of evasion of apoptosis. Biochim Biophys Acta 2008;1785(1):63–84.
76. Wesierska-Gadek J, Ranftler C, Schmid G. Physiological ageing: role of p53 and PARP-1 tumor suppressors in the regulation of terminal senescence. J Physiol Pharmacol 2005;56(Suppl 2):77–88.
77. Bernard D, Gosselin K, Monte D, et al. Involvement of Rel/nuclear factor-kappaB transcription factors in keratinocyte senescence. Cancer Res 2004;64(2):472–81.
78. Kyoung Kim H, Kyoung Kim Y, Song IH, et al. Down-regulation of a forkhead transcription factor, FOXO3a, accelerates cellular senescence in human dermal fibroblasts. J Gerontol A Biol Sci Med Sci 2005;60(1):4–9.
79. Maiuri MC, Galluzzi L, Morselli E, et al. Autophagy regulation by p53. Curr Opin Cell Biol 2010;22(2):181–5.
80. Tracy K, Dibling BC, Spike BT, et al. BNIP3 is an RB/E2F target gene required for hypoxia-induced autophagy. Mol Cell Biol 2007;27(17):6229–42.
81. Copetti T, Bertoli C, Dalla E, et al. p65/RelA modulates BECN1 transcription and autophagy. Mol Cell Biol 2009;29(10):2594–608.

82. Zhao J, Brault JJ, Schild A, et al. Coordinate activation of autophagy and the proteasome pathway by FoxO transcription factor. Autophagy 2008;4(3):378–80.
83. Zheng SJ, Lamhamedi-Cherradi SE, Wang P, et al. Tumor suppressor p53 inhibits autoimmune inflammation and macrophage function. Diabetes 2005;54(5): 1423–8.
84. Ying L, Marino J, Hussain SP, et al. Chronic inflammation promotes retinoblastoma protein hyperphosphorylation and E2F1 activation. Cancer Res 2005;65(20): 9132–6.
85. Tak PP, Firestein GS. NF-kappaB: a key role in inflammatory diseases. J Clin Invest 2001;107(1):7–11.
86. Snoeks L, Weber CR, Wasland K, et al. Tumor suppressor FOXO3 participates in the regulation of intestinal inflammation. Lab Invest 2009;89(9):1053–62.
87. Hooper ML. The role of the p53 and Rb-1 genes in cancer, development and apoptosis. J Cell Sci Suppl 1994;18:13–7.
88. You H, Mak TW. Crosstalk between p53 and FOXO transcription factors. Cell Cycle 2005;4(1):37–8.
89. Kim YA, Lee WH, Choi TH, et al. Involvement of p21WAF1/CIP1, pRB, Bax and NF-kappaB in induction of growth arrest and apoptosis by resveratrol in human lung carcinoma A549 cells. Int J Oncol 2003;23(4):1143–9.
90. Zhou M, Gu L, Zhu N, et al. Transfection of a dominant-negative mutant NF-kB inhibitor (IkBm) represses p53-dependent apoptosis in acute lymphoblastic leukemia cells: interaction of IkBm and p53. Oncogene 2003;22(50):8137–44.