Felipe Sierra · Ronald Kohanski *Editors*

Advances in Geroscience

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Foreword

In an introduction to a 2003 special issue of Science [1], my colleagues and I emphasized that "We have focused on physiological mechanisms underlying processes of aging, rather than on the large array of debilitating and costly disorders that so commonly emerge during the latter half of the life-spans of human beings." That was before the days of Geroscience, however! But what exactly *is* Geroscience? It is a term coined by my colleague and friend, Gordon Lithgow, a professor at the Buck Institute for Research on Aging who, true to his Scottish heritage, is thrifty with the use of all instruments of commerce, including terminology. Gordon used that nomenclature to summarize a new interdisciplinary research enterprise at the Buck Center; although it needs some grammatical editing, it is quite informative: "We consider that the relationship between aging and age-related disease (is) an important problem that can be tackled through an interdisciplinary approach" (http://www.geroscienceonline.org/index.php). This view emphasizes the concept that it is not only difficult to disassociate fundamental processes of aging from the numerous diseases of aging, but that joint investigations of these two domains of scholarship are essential if we are to unravel the pathogenesis of atherosclerosis, myocardial infarctions, strokes, non-ischemic heart failure, benign and malignant neoplasms, dementias of the Alzheimer type, frontal temporal dementias, Parkinson's disease and Lewy body dementias, peripheral neuropathies, cataracts and age-related macular degeneration, presbycusis, chronic obstructive pulmonary disease, type 2 diabetes mellitus, the metabolic syndrome, osteoporosis, osteoarthritis, sarcopenia, glomerulosclerosis, etc.

The editors of this volume, Felipe Sierra and Ronald Kohanski, have taken this concept to an exciting new level of implementation. As the Director of the Division of Aging Biology of the National Institute on Aging, Felipe approached his counterparts at more than twenty sister NIH institutes with narrow interests in specific diseases of aging with the following paraphrased sales pitch: "Let's have a discussion about how basic processes of aging are the major risk factor for your disease X. But first, I want to assure you that I do not want your money for my own institute! I want to start a 'Geroscience Interest Group' so that we can work together to accelerate progress towards the enhancement of healthy human

aging." The response was overwhelmingly enthusiastic and resulted in a series of productive joint conferences, including a 2013 Summit meeting at the NIH [2]. Readers are urged to learn more about this trans-NIH initiative via Felipe's excellent video on the subject: http://www.nia.nih.gov/about/links/2013/07/video-dr-felipe-sierra-discusses-trans-nih-geroscience-interest-group-and-more.

This volume, despite its 19 chapters and its stellar list of authors, can best be viewed as just a beginning step for Geroscience. Given the extent of research funding that it richly deserves – far more than the current NIH pay lines – we can anticipate major basic and translational advances in our healthspans, at which point we can go to the Food and Drug Administration and point out that the side effect of our research – increased longevity – can really be a good thing for humanity. As the late Charlie Chaplin pointed out, "we are all amateurs; we don't live long enough to become anything else" [1].

Seattle, WA, USA
June 5, 2015

George M. Martin, M.D.

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Preface

Geroscience is a new field that aims to bridge two communities: biologists focused on understanding the basic mechanisms that drive aging, and geriatricians attempting to improve the quality of life of elderly patients. Geroscience has been defined (Wikipedia) as "an interdisciplinary field that aims to understand the relationship between aging and age-related diseases." Because aging is the major risk factor for most chronic diseases, the "Geroscience Hypothesis" posits that common biological mechanisms of aging play important roles in the susceptibility of aged individuals to multiple chronic diseases.

The role of aging as a driver of chronic diseases is often downplayed under the assumption that aging is a non-modifiable risk factor. Yet we know that the rate of aging (however that is defined, as decay in function or susceptibility to disease) is modifiable by simple changes in the environment: a healthy diet, moderate exercise, and other elements of a generally healthy lifestyle will increase a person's chances of leading a longer and healthier life. In fact it is a dietary intervention, diet restriction, that provided the first handle to biologists' intent to understand the underpinnings of the aging process. That work, coupled with genetic experiments in short-lived simple organisms ranging from yeast to flies and worms, allowed scientists to unravel some of the major mechanisms involved. Furthermore, driven primarily by the Intervention Testing Program (created and supported by the National Institute on Aging), the field has moved recently to defining pharmacological interventions that expand lifespan in rodents (mainly mice). In addition to these impressive advances in terms of increasing lifespan, recently there has been a shift in focus, towards measuring healthspan as well as lifespan. Indeed, while some interventions (such as resveratrol) only increase health but not lifespan in mice, a handful of other interventions have been shown to increase both, although with the strong caveat that no manipulation has achieved that goal without having some secondary negative effects.

The enormous aging of the population worldwide, with the oldest-old being the age segment with the fastest growth, poses an urgent dilemma: if aging is indeed the largest risk factor for most chronic diseases, this increase in the proportion of elderly will necessarily pose an insurmountable challenge to the world's economic and

health-care systems. Fortunately, at the same time that this demographic change is reaching a critical stage, our understanding of aging biology is allowing scientists to consider the possibility of intervening to delay aging, and hopefully, with it all major chronic diseases. This improved knowledge has allowed the organization of concepts into six to eight hallmarks or pillars, believed to be the main drivers of the process. While some details still need further clarification, there is broad agreement within the research community about these major drivers, and differences only reflect different biases and granularity.

These advances and the emerging opportunity to modify the process of aging by pharmacological means spawned the appearance of the new field of Geroscience. The initial concept came from a group at the Buck Institute for Research on Aging which, under the leadership of Dr. Gordon Lithgow, put forward a successful proposal to the Common Fund of the National Institute of Health. This project, Interdisciplinary Research Consortium on Geroscience, was funded for 5 years between 2007 and 2012. Subsequent to that effort, the editors of this book started a trans-NIH effort following the same line of thinking and resulting in the formation of the trans-NIH GeroScience Interest Group (GSIG). The effort attracted the attention of over 20 different institutes within the NIH, as well as wide support from both the scientific community and, importantly, non-federal advocacy and support groups. With the imprimatur of so many NIH institutes, the subsequent growth of the field was impressive and culminated in the organization of a large Summit, Advances in Geroscience: Impact on Healthspan and Chronic Disease, held in the NIH Campus on October/November 2013. In turn, that effort resulted in the publication of a White Paper in November 2014 in the Journal *Cell*.

For whom is the book written? Geroscience is an interdisciplinary field attempting to address the mechanisms by which aging biology is the main risk factor for chronic diseases. As such, this book examines those mechanisms and it provides an emerging overview of the new discipline of Geroscience. Each chapter aims at connecting the clinical manifestation of specific age-related chronic diseases with the major pillars of aging biology. These pillars are suspected (or in some cases, known) to play a role in the etiology of these diseases, not just singly but in multiple diseases because aging is the major risk factor for their appearance. As such, each chapter combines features of clinical science and basic biology, and it is hoped that this approach will be informative and enlightening to both these communities: physicians will hopefully learn about the basic underpinnings of aging that might affect the outcome of chronic diseases and/or treatments, while basic scientists might profit from learning about clinical aspects of their disease of interest in the context of aging. Altogether, the editors and authors anticipate that this book will raise further awareness of the molecular mechanisms which might become targets for further investigation and, ultimately, new targets to combat multiple comorbidities at once.

This book examines the biological mechanisms and clinical consequences of aging by providing specific coverage on a wide range of chronic diseases, from arthritis and cancer to dementia and stroke among others. This book begins with an introduction of the general principles, including both a description of current

thinking in aging biology and a description of the Geroscience Hypothesis, with some background on the critical role that epidemiology has played in defining our basic understanding of age-related chronic diseases. Each of the following 16 chapters focuses on one particular disease, or group of related diseases, with an emphasis on how aging is a risk factor. This book finishes with a global discussion of pain in the elderly and a commentary on the important topic of translation into the clinic and the necessity of cross-fertilization between clinicians and basic scientists. Unfortunately, not all diseases afflicting the elderly have been covered, and this omission is simply the result of space limitations. It is hoped that this book will entice experts in those areas to think more deeply about the basis on which aging as a risk factor for their specialty disease, and perhaps publish their thoughts on this subject.

Contributors to this volume represent a large range of disciplines. An effort was made, whenever possible and appropriate, to engage in each chapter both basic scientists and clinicians; the editors are enormously thankful to all authors for the effort and sense of shared responsibility. Importantly, the editors thank all members of the GSIG executive committee for their support during the preparation of this book and the help provided on the selection of authors. Similarly, GSIG members are to be commended for the additional editorial work to complete this volume.

The editors believe that this book presents vital information and ideas that can help readers better understand how aging is a critical – but malleable – risk factor in chronic diseases of the elderly. The potential to alter the rate of aging is at the heart of Geroscience, and achieving that goal should improve lifespan and healthspan in the human population.

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Abbreviations

AAV	Adeno-associated virus
Aβ	Amyloid beta
ACEi	Angiotensin converting enzyme inhibitor
ACL	Anterior cruciate ligament
ACOS	Asthma-COPD overlap syndrome
ACT	Asthma control test
AD	Alzheimer's disease
ADCC	Antibody-dependent cellular cytotoxicity
ADE	Adverse drug events
AF	Atrial fibrillation
AGE	Advanced glycation end product
AHR	Airway hyperresponsiveness
AIDS	Acquired immunodeficiency syndrome
AKT	Protein kinase B
AMD	Age-related macular degeneration
AMPK	Adenine monophosphate-activated protein kinase
ANI	Asymptomatic neurocognitive impairment
APP	Amyloid precursor protein
ARB	Angiotensin 2 receptor blocker
ART	Anti-retroviral therapy
ATP	Adenosine-5'-triphosphate
BALF	Bronchoalveolar lavage fluid
BBB	Blood brain barrier
BDNF	Brain-derived neurotrophic factor
BER	Base excision repair
BMD	Bone mineral density
BMI	Body mass index
BMP	Bone morphogenetic protein
BMSC/B-MSC	Bone marrow-derived stem cell
BMU	Basic multicellular unit
Breg	Regulatory B cells

BV/TV	Bone volume fraction
CAC	Coronary artery calcium
CBT	Cognitive behavior therapy
CCI	Chronic constriction injury
CD	Cluster of differentiation
CDC	Center for Disease Control and Prevention
CDK	Cyclin-dependent kinase
CEE	Conjugated equine estrogens
CFA	Complete Freund's adjuvant
CFB	Complement factor B
CGRP	Calcitonin gene-related peptide
CHF	Congestive heart failure
CLBP	Chronic low back pain
CM	Conditioned medium
CMV	Cytomegalovirus
CNS	Central nervous system
CNV	Choroidal neovascularization
COLD	Chronic obstructive lung disease
COMP	Cartilage oligomeric protein
COMT	Catechol-O-methyl transferase
COPD	Chronic obstructive pulmonary disease
COX2	Cyclooxygenase-2
CPM	Conditioned pain modulation
CR	Caloric restriction
CRP	C-reactive protein
CSE	Cigarette smoke extract
CT	Computed tomography
CTGF	Connective tissue growth factor
CVD	Cardiovascular disease
DA	Dopamine
DAergic	Dopaminergic
DBP	Diastolic blood pressure
DBS	Deep brain stimulation
DC	Dendritic cell
DDI	Drug-drug interactions
DG	Dentate gyrus
DHEA	Dehydroepiandrosterone
DLB	Dementia with Lewy bodies
DLCO	Carbon monoxide diffusing capacity
DMM	Destabilized medial meniscus
DPP	Diabetes prevention program
DR	Dietary restriction
DRG	Dorsal root ganglia
DSB	Double strand break
DSR	Differential stress resistance

dsRNA	Double-stranded RNA
DSS	Differential stress sensitization
DXA	Dual energy X-ray absorptiometry
E2	Estradiol
Ea/Aa	Ratio of early to late diastolic mitral annular velocity
EBV	Epstein-Barr virus
ECM	Extracellular matrix
EF	Ejection fraction
EF	EnhanceFitness
EMT	Epithelial-mesenchymal transition
EPC	Endothelial progenitor cell
eQTL	Expression quantitative trait loci
ER	Endoplasmic reticulum
ERK	Extracellular-regulated kinase
ETC	Electron transport chain
EWAS	Epigenome-wide association study
FDA	Food and Drug Administration
FFA	Free fatty acid
FGF	Fibroblast growth factor
FGFR2	Fibroblast growth factor receptor 2
FIRKO	Fat tissue-specific insulin receptor knockout
FOXO	Forkhead box protein O
FRP	Frailty-related phenotype
FS	Fractional shortening
FTIRM	Fourier transformed infrared microepectroscopy
GA	Geographic atrophy
GAD	Glutamic acid decarboxylase
GALT	Gut-associated lymphoid tissue
GDF-11	Growth and differentiation factor 11
GDNF	Glial-derived neurotrophic factor
GERD	Gastroesophageal reflux disease
GFAP	Glial fibrillary acidic protein
GFP	Green fluorescent protein
GH	Growth hormone
GHR	Growth hormone receptor
GTT	Glucose tolerance test
GWAS	Genome-wide association study
GzmB	Granzyme B
HAD	HIV-associated dementia
HANA	HIV-associated non-AIDS
HAND	HIV-associated neurocognitive disorder
HAT	Histone acetyl transferase
HCV	Hepatitis C virus
HDAC	Histone deacetylase
HERV	Human endogenous retroviruses

HFD	High-fat diet
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HPA	Hypothalamo-pituitary axis
HPC	Hematopoietic progenitor cell
HPLC	High pressure liquid chromatography
HPV	Human papillomavirus
HR	Heart rate
HSC	Hematopoietic stem cells
hsCRP	High-sensitivity C-reactive protein
HSP	Heat shock protein
HSV	Herpes simplex virus
HT	Hormone therapy
ICH	Intracerebral hemorrhage
ICS	Inhaled corticosteroids
I-FABP	Intestinal fatty acid binding protein
IFN	Interferon
IGF	Insulin-like growth factor
IGFBP	IGF binding protein
IGT	Insulin glucose tolerance test
IL	Interleukin
IND	Investigational new drug
iNOS	Inducible nitric oxide synthase
IOM	Institute of Medicine
IPF	Idiopathic pulmonary fibrosis
iPS/iPSC	Induced pluripotent stem cell
IR	Ionizing radiation
IRAP	IL-1 receptor antagonist protein
IRS1	Insulin receptor substrate 1
ISH	Isolated systolic hypertension
ITM	Intima-media thickness
ITP	Interventions Testing Program
KMT	Lysine methyl transferase
LBP	Low back pain
LBP	LPS binding protein
LDL	Low density lipoprotein
L-DOPA	Levo-DOPA
lncRNA	Long non-coding RNA
LOA	Late onset asthma
LPS	Lipopolysaccharide
LSA	Long-standing asthma
LSD	Lysine-specific demethylase
LV	Left ventricle
LVH	Left ventricular hypertrophy
MAC	Membrane attack complex

MAO-B	Monoamine oxidase B
MAP	Mean arterial pressure
MCAo	Middle cerebral artery occlusion
MCAT	Mitochondrial-directed catalase
MD2	Myeloid differentiation factor 2
MDP	Mitochondrial-derived peptides
miRNA/miR	MicroRNA
MMP	Matrix metalloproteinase
MMSE	Mini-mental status exam
MND	Mild neurocognitive disorder
mPTP	Mitochondrial permeability transition pore
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MSC	Marrow stromal cell
MSC	Mesenchymal stem cell
mtDNA	Mitochondrial DNA
mTOR	Mechanistic target of rapamycin
mTORC1	mTOR complex 1
mtROS	Mitochondrial ROS
NAD+	Nicotinamide adenine dinucleotide
NADC	Non-AIDS-defining cancers
ncRNA	Non-coding RNA
NER	Nucleotide excision repair
NET	Neutrophil extracellular trap
NHEJ	Non-homologous end joining
NIA	National Institute on Aging
NIH	National Institutes of Health
NK cell	Natural killer cell
NLRP3	Nucleotide-binding domain and leucine-rich repeat pyrin domain containing 3
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NOX	NAP(P)H oxidase
NPC	Neural precursor/progenitor cell
Nrf2	Nuclear factor erythroid-derived 2
NRTI	Nucleos(t)ide reverse transcriptase inhibitor
NSAID	Nonsteroidal anti-inflammatory drug
nvAMD	Neovascular AMD
OA	Osteoarthritis
OPG	Osteoprotegerin
OR	Odds ratio
ORF	Open reading frame
PAMP	Pathogen-associated molecular pattern
PAWH	People aging with HIV
PBMC	Peripheral blood mononuclear cell

PD	Parkinson's disease
PD1	Programmed cell death protein 1
PDGF	Platelet-derived growth factor
PEDF	Pigmented epithelium-derived factor
PET	Positron emission tomography
PGC-1α	PPAR-gamma coactivator 1-alpha
PGE2	Prostaglandin E2
PHB1	Prohibitin 1
PI	Protease inhibitor
PI3K	Phosphatidyl inositol 3 kinase
PKA	Protein kinase A
PKC	Protein kinase C
PMN	Polymorphonuclear cell
Polg	DNA polymerase gamma
PPARγ	Peroxisome proliferator-activated receptor gamma
pQCT	Peripheral quantitative computed tomography
PRR	Pattern recognition receptor
PSNL	Partial sciatic nerve ligation
PSVT	Paroxysmal supraventricular tachycardia
PTEN	Phosphatase and tensin homolog
PTH	Parathyroid hormone
RAAS	Renin-angiotensin-aldosterone system
RAGE	Receptor for advanced glycation end product
RANKL	Receptor activator of nuclear factor kappa B ligand
RAP	Retinal angiomatous proliferation
RAS	Renin-angiotensin system
RB	Retinoblastoma
RCT	Randomized control trial
RI	Recombinant inbred
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RPE	Retinal pigmented epithelium
RSV	Respiratory syncytial virus
RT	Reverse transcriptase
RTL	Recombinant T cell receptor ligand
SABA	Short-acting b adrenergic 2 receptor agonist
SA-βGal	Senescence-associated beta galactosidase
SAHF	Senescence-associated heterochromatic foci
SAM	Senescence-accelerated mouse
SASP	Senescence-associated secretory phenotype
SBP	Systolic blood pressure
sCD	Soluble cluster of differentiation
SD-OCT	Spectral domain optical coherence tomography
SERM	Selected estrogen receptor modulator
SGZ	Subgranular zone

SHR	Spontaneously hypertensive rat
SIRT1	Sirtuin1
SIV	Simian immunodeficiency virus
SNAE	Serious non-AIDS events
SNP	Single nucleotide polymorphism
SNpc	Substantia nigra pars compacta
SNS	Sympathetic nervous system
SOD	Superoxide dismutase
SPF	Specific pathogen free
SPT	Skin prick testing
SRM	Selected reaction monitoring
STAT	Signal transducer and activator of transcription
SVZ	Subventricular zone
T	Testosterone
T2DM	Type 2 diabetes mellitus
TB	Tuberculosis
TC	Tai Chi
TCR	T cell receptor
TERT	Telomerase reverse transcriptase
TGFβ	Transforming growth factor beta
TIA	Transient ischemic attack
TIMP	Tissue inhibitor of matrix metalloproteinase
TLR	Toll-like receptor
TNFα	Tumor necrosis factor alpha
TNFR1	Tumor necrosis factor receptor 1
tPA	Tissue plasminogen activator
Treg	Regulatory T cells
TSA	Trichostatin A
UPR	Unfolded protein response
UPS	Ubiquitin-proteasome system
UPS	Unfolded protein stress
VE	Ventricular ectopy
VEGF	Vascular endothelial growth factor
VEGFR	VEGF receptor
VFA	Vertebral fracture assessment
WHI	Women's Health Initiative
WRN	Werner's

The Geroscience Hypothesis: Is It Possible to Change the Rate of Aging?

Steven N. Austad

Contents

1 Introduction

In the 200,000 year history of anatomically modern humans, we have never lived remotely as long as we do today. The rate of change in our life expectancy has been breathtaking. For the past 175 years, the mean age-at-death has increased steadily by about 2.5 years per decade, or 6 h per day, among the longest-lived countries [1]. While in the early part of the twentieth century, the rise in life expectancy was

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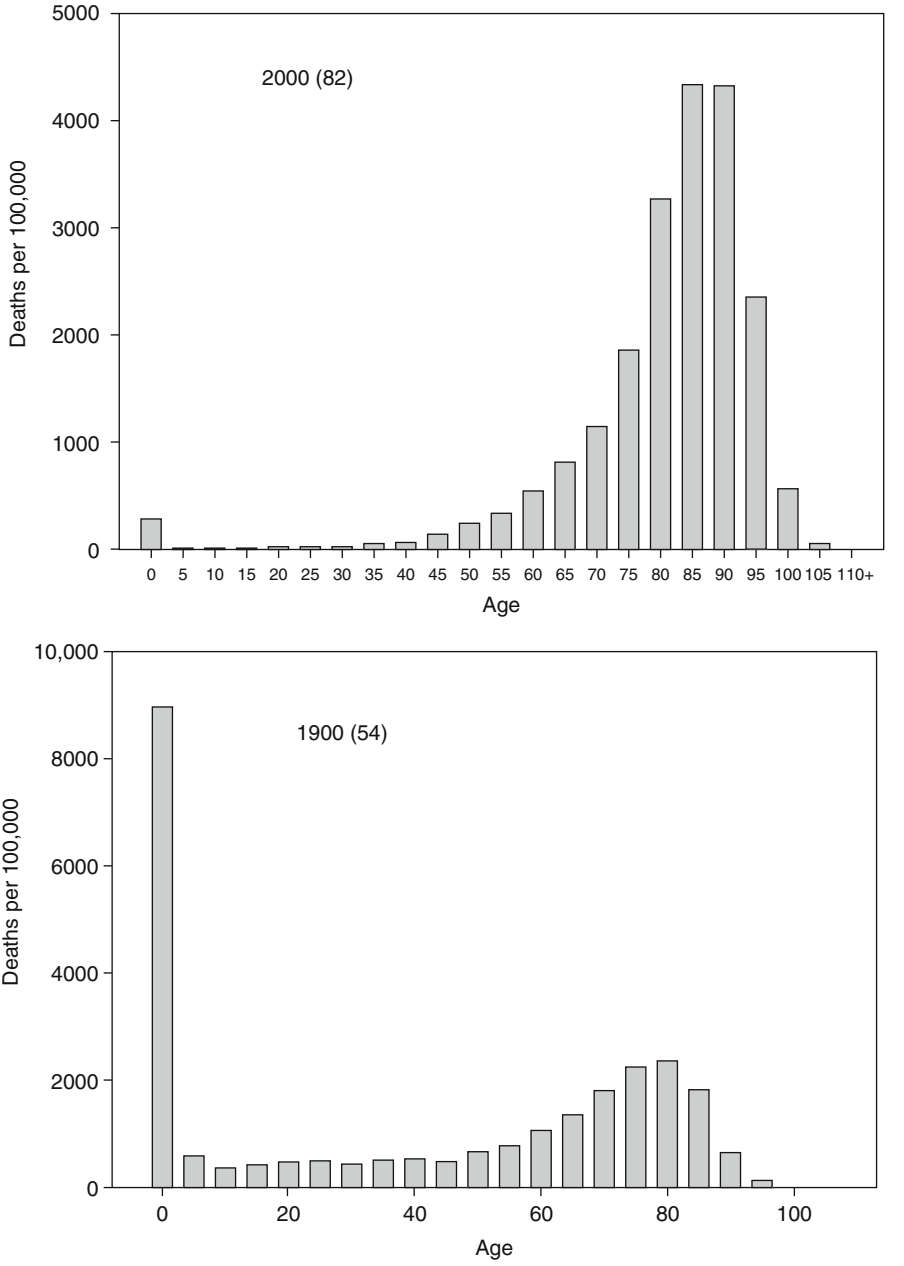

Fig. 1 Distribution of deaths as a function of age among Swedish women from the years 1900 and from 2000. Birth and death data from Swede are among the most reliable in the world. Note that more than 90 % of the population reached age 65 in the year 2000 compared with only 50 % reaching that age in 1900. Numbers in parentheses are life expectancies at birth (Data from the Human Mortality Database (downloaded November 2014))

driven mainly by reduced infant and young adult mortality, more recently bigger advances have been made in combating later life diseases (Fig. 1). As a consequence chronic health problems associated with aging, such as sarcopenia, osteoporosis, and Alzheimer's disease, which were once rare, have become common. As the global population continues to age over the coming decades, maladies of aging threaten to overwhelm our healthcare infrastructure, disrupt our national economies, and potentially poison relations among generations. Fortunately, understanding of the basic biology of aging has also progressed rapidly in the past several decades such that the promise of medical interventions that enhance and lengthen healthy life is no longer an empty promise promulgated only by avaricious quacks and charlatans. The economic impact of generalized health extension could be stunning. According to one analysis, slowing the rate of human aging by 20 % would be worth more than $7 trillion over the next 50 years in the United States alone [2].

The likelihood that we will ultimately be able to slow human aging depends on our understanding of underlying processes. I have claimed that such understanding has progressed rapidly in recent decades. What is the evidence for such a claim? How realistic is the promise of medically extended healthy life? Those are the topics of this chapter.

2 Aging and Its Relation to Disease

No 60 year old – even the healthiest, hardest-training, and most disease-free 60 year old – can sprint as fast or throw as far as she could as a healthy 25 year old. This is *prima facie* evidence that aging, the progressive decline in physical function that accompanies growing older, occurs even in the absence of disease. However aging is so intimately intertwined with numerous diseases and disabling conditions that almost any discussion that begins with aging ends on disease. Although aging occurs

Table 1 Death rates from selected diseases in the United States (2010) * indicates the disease is essentially nonexistent at this age.

	Age group					
	35–44	45–54	55–64	65–74	75–84	85+
Malignant neoplasms	29	112	300	666	1202	1730
Diabetes mellitus	4	13	32	68	144	286
Diseases of the heart	26	82	187	409	117	4285
Alzheimer's disease	*	0.3	2	20	185	987
Parkinson's disease	*	0.2	1	12	75	166
Influenza & Pneumonia	2	4	10	28	102	426
COPD	2	10	39	146	370	691
Stroke	5	13	30	82	288	994

Data from Murphy et al. [3]

Rates are per 100,000 population in the specific age group based on the 2010 U.S. Census

even in the absence of disease, it clearly increases *vulnerability* to multiple diseases. In other words, aging is a risk factor for diseases. In fact, it is by far the biggest risk factor for virtually all of the chronic diseases that strain our health care system today and it increases the chances that a given disease will be fatal (Table 1). For instance in 2010, an American 75–84 years old had a 42-fold greater chance of dying of cancer, and a 45-fold greater chance of dying of heart disease, than a 35–44 year old. The chances of dying from Alzheimer's disease increase more than 600-fold between age 50 and 80 [3]. For comparison, smoking only increases overall mortality rate by threefold compared with nonsmokers (http://www.cdc.gov/tobacco/data_statistics/fact_sheets/health_effects/tobacco_related_mortality/) and having two copies of the ApoE4 allele, the most common genetic risk factor for Alzheimer's disease, increases an individual's chances of contracting that disease by only 12-fold relative to those with two copies of the ApoE3 allele [4]. Looked at from this perspective, aging is by far the biggest threat to human health in the developed world today. Geroscience, the topic of this book, is an interdisciplinary field seeking to understand the basis for the relationship between aging and disease vulnerability.

The Geroscience Hypothesis posits that, because aging underlies so many diseases and disabling conditions, interventions that would retard aging would also simultaneously prevent or delay the onset of multiple chronic diseases. In recent years there has been success at delaying cardiovascular diseases. Over the first decade of the twenty-first century, the age-adjusted death rate from heart diseases fell by more than 30 % and for stroke fell by more than 35 % [5, 3]. One contributing factor is the discovery of treatments that address underlying risk factors such as high blood pressure and high cholesterol. There has also been progress against a major behavioral risk factor – smoking. Importantly though, aging is a bigger risk to health than high blood pressure, cholesterol, and smoking combined. If we could similarly learn to treat the risk factor of aging, the health benefits would be enormous, not only for delaying fatal diseases but in delaying many nonfatal conditions such as hearing and vision loss, osteoporosis, and arthritis that degrade the quality of later life.

3 Experimental Organisms in Aging Research

3.1 Uses and Caveats in the Use of Model Organisms

For most of its history, basic aging research relied on standard laboratory animals such as fruit flies, mice, and rats. The chief advantage of these animals was that their laboratory husbandry was established and that they were short-lived. That is, rats and mice are short-lived among mammals, fruit flies are relatively short-lived among insects. Initially, basic aging research focused on describing physiological changes occurring during aging in the hope that the nature of these changes would reveal underlying aging mechanisms. Short-lived animals were useful because individuals could be monitored throughout their lives and the longevity of different

groups could be compared and contrasted. Until recently, lengthening of life was assumed to be sufficient evidence that aging had been slowed. This view has recently been questioned as will be discussed later, but it has dominated the history of experimental aging research.

After dietary restriction (DR), simply reducing the amount of available food, was discovered to lengthen life in many laboratory rats and mice, attention shifted to searching for mechanism(s) by which DR had these effects and also searching for other methods of life extension. Again, the rate limiting step for such studies was the length of the animals' lives. But even the shortest-lived species commonly used in this research lived months (fruit flies) or years (mice and rats), and because the focus was on increasing lifespan, aging studies were particularly time-consuming compared with other areas of biomedical research.

It is important to understand why the focus so quickly fell on lengthening life rather than shortening it. In principle, understanding basic aging processes could be studied much more quickly by accelerating them rather than retarding them. The practical difficulty with this logical approach is that there are many ways to shorten animals' lives by inducing pathological processes that may have nothing to do with normal aging processes. The problem is how would we know the difference between those aberrant pathologies and normal aging processes? This doesn't mean that so-called accelerated aging models, which do exist, are not informative. It does mean that such models are difficult to evaluate with respect to normal aging and findings from them need to be interpreted with considerable care. For instance, the so-called Senescence Accelerated Mouse (SAM mouse) is a series of exceptionally short-lived mouse strains, created by accidental outbreeding of an AKR/J inbred strain with an unknown other strain. Despite their short lives – most live less than 1 year – they have had virtually no impact on the larger mouse aging research field, because like all so-called accelerated aging models, they replicate at best a few of the features of normal aging and the fidelity of that replication is not clear.

Experiments that lengthen life are much less problematic to interpret. Animals are unlikely to live longer if we haven't retarded at least *some* normal aging process, such as the increasing susceptibility to cancer. We may not have retarded them all (however many that may be), but we must have retarded some. To verify that one had identified a mechanism regulating aging, generally, the mantra for many years was that both mean (or median) and maximum longevity must be extended. Maximum longevity is generally defined as the mean longevity of the oldest $x\%$ of the starting population, where x often equals 10 %. The focus on maximum longevity implies that ameliorating a specific disease process may impact mean longevity, but only by affecting aging itself would both the mean and the length of life of the longest-lived animals be longer. For example, if group A displays longer mean or median survival, but no difference in maximum survival than group B, then group A must have experienced higher mortality rate than group B in the latter part of life. Higher mortality late in life is not a trait that one would associate with slower aging. For this reason exercise, which consistently increases mean longevity in both rats and people [6, 7] and has manifold beneficial health-preserving effects, is not gen-

erally considered to retard aging by researchers in the basic aging research community. By comparison, DR increases both mean and maximum longevity in many (but not all) species and genotypes, therefore has generally been considered the gold standard of aging retardation. As will be noted later, the over-reliance on longevity as the cannonical metric of aging is now being re-thought by many researchers.

3.2 *Worms (*Caenorhabditis elegans*)*

A major breakthrough in the field, which had previously relied on fruit flies and laboratory rodents, was the widespread adoption of the model nematode, *Caenorhabditis elegans*, for aging research in the 1990s [8–10]. A 1 mm long, free-living, soil nematode introduced to the biological research community in the 1960s by Sydney Brenner, *C. elegans* proved to be a wonderful model for the study of aging as well (Table 2). Not only did *C. elegans* develop rapidly and live only a couple of weeks rather than a couple of months like fruit flies, they could be maintained in much larger numbers in much smaller space than flies. They were also naturally inbred, which mitigated the problem of inbreeding depression and unpredictable genetic background effects, and they were more genetically tractable than flies, particularly after the discovery that expression of individual genes could be suppressed with ease by genetically altering their *E. coli* food to produce RNAi against the gene of interest [11]. A final advantage of worms is that because they are transparent, reporters such as green fluorescent protein (GFP) can be employed on living worms to assess gene expression and protein location [12].

A key feature of worm biology that turns out to be highly relevant to its aging biology is that under conditions of overcrowding, food shortage, or high temperature – conditions not conducive to successful reproduction – developing worms enters an alternative 3rd larval stage called dauer. Dauer is a nonfeeding, metabolically and transcriptionally quiescent, highly stress-resistant and long-lived stage of arrested development from which worms emerge only when crowding eases, food

Table 2 Relevant biological traits of traditional animal species used in basic aging research

	Caenorhabditis elegans	*Drosophila melanogaster*	*Mus musculus*
Body size	1 mm (length)	3 mm (length) 1–1.5 mg	30–45 g
Genome size (bp)	1.0×10^8	1.2×10^8	2.8×10^9
Stem cells	No	Yes, limited	Yes
Neuron number	302	135,000	75,000,000
Wild-type longevity[a]	2–3 weeks	2–3 months	2.0–2.5 years
Max. life extension	10×	2×	1.75×

[a]Wild-type longevity equals the longevity of laboratory-adapted animals

becomes abundant once again, or the temperature falls. Adult worm longevity, upon emerging from dauer and completing development, does not appear to be related to the length of time it spent in dauer [13]. In nature, worms are often found in dauer, which appears to be a specialized dispersal phase [14]. Thus dauer appears to be an important part of the worm's natural life cycle and the genetics of dauer entry and exit have been extensively investigated [15]. Longevity, as typically reported in the worm literature, is adult longevity. The 3 day larval period, whether or not worms went through dauer, is ignored.

The reason that dauer is a key life history feature for aging research is that many of the hundreds of known worm longevity genes are part of the dauer regulatory network. As dauer larvae are very long-lived, partial induction of the dauer regulatory network is likely to lengthen adult life. Possibly for this reason, an order of magnitude more longevity-enhancing genes have been found in worms than in any other species and the magnitude of genetically-induced life extension achieved in

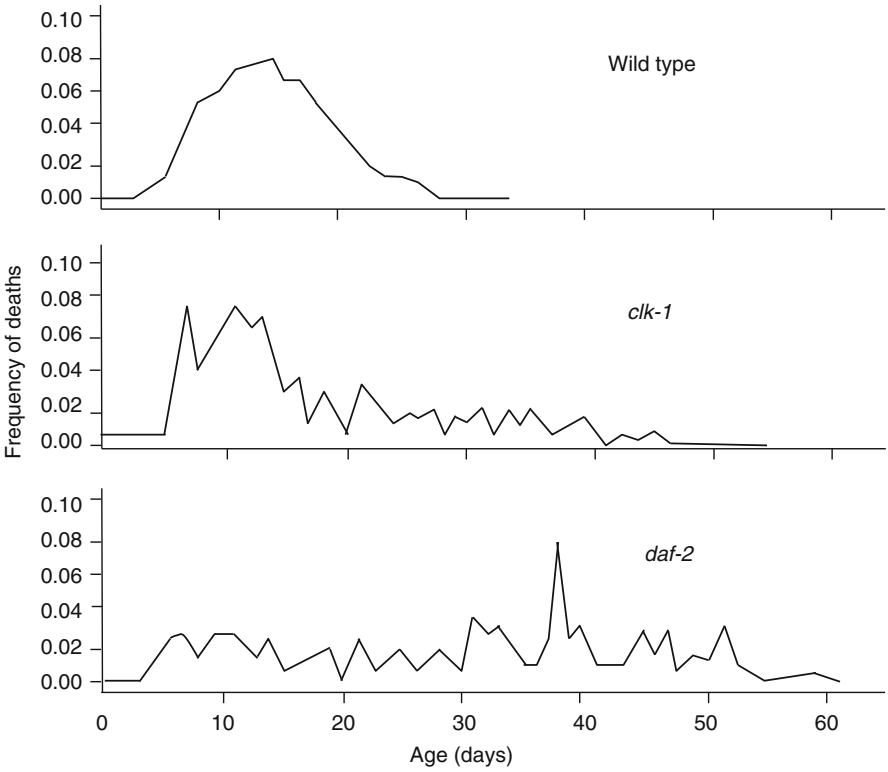

Fig. 2 Distribution of deaths in *wild-type*, long-lived *clk-1* and *daf-2 C. elegans* mutants. Note that only deaths in wild-type worms bear any resemblance to the distribution of deaths in modern humans, as shown in Fig. 1 (Adapted from Chen et al. [17] by permission of the Gerontological Society of America)

worms is proportionally much longer than any other species. For instance, one worm mutant has been reported to increase adult longevity by nearly tenfold [16].

In addition to changes in mean or median longevity among long-lived worm mutants, the *distribution* of deaths varies dramatically among the mutants (Fig. 2) [17]. Deaths in the wild-type strain were concentrated between 5 and 20 days. Considerably less clumped deaths were seen in the *clk-1* mutant, and in the longest-lived *daf-2* mutant, there is virtually no clumping of deaths but a slow steady trickle of them for 60 days. This is not a standard pattern of senescence-related mortality. Only the wild-type strain has a death distribution resembling to any degree that of senescent deaths of humans.

For all their many benefits, worms also have their limitations as aging models. They have a limited behavioral repertoire making assessment of their physical and cognitive health status difficult. The food they are fed in the laboratory, *E. coli*, is not part of their natural diet and in fact is slightly toxic to them. No one yet knows what their natural diet is, but when fed *Bacillus subtilis* rather than *E. coli*, wild-type worms lived about 40 % longer [18]. It is difficult to monitor food consumption in *C. elegans*, so controlled feeding trials are challenging. And all somatic cells in adult worms are postmitotic, so that studying the aging biology of actively replicating cells is not possible with worms.

*3.3 Fruit Flies (*Drosophila melanogaster*)*

The laboratory fruit fly, *Drosophila melanogaster* has lost its pre-eminence as a genetic model for aging studies, but it still has its place in the traditional aging research bestiary. Although not as genetically tractable and short-lived as worms, they are considerably more tractable and shorter-lived than any vertebrate. Moreover, they are behaviorally much more complex than worms, facilitating assessment of cognitive as well as physical aging [19]. Flies also have real organ systems like eyes, heart, and Malpighian tubules that have analogs if not homologs in vertebrates. Their dietary requirements are much clearer and have been extensively investigated [20, 21]. Monitoring and controlling food intake in flies is not routinely done, but techniques are available to do so [22] and if employed would add considerably to the utility of the model. Adult flies, while being mostly composed of postmitotic cells, also have several pools of stem cells, which allow the study of tissue maintenance by cell replacement. In particular, the *Drosophila* genetic toolkit can be deployed to understand stem cell dynamics and functional changes with age [23, 24]. Numerous similarities have been identified between fly and mammalian stem cell behavior [25].

*3.4 Laboratory Mice (*Mus musculus*)*

Since the 1980s when targeted genetic manipulation of laboratory mice became possible, they have eclipsed rats to assume the role of pre-eminent mammalian model for biomedical research of all types [26]. Basic aging research is no

exception. Husbandry practices for mice are particularly well developed, including the maintenance of mice under specific-pathogen-free (SPF) conditions. Mouse physical, sensory, and cognitive phenotypes and the manner in which they change with age have been extensively explored as has the pathophysiology typical of various mouse strains (http://phenome.jax.org/). The longevity and disease profile of mouse strains – a strain is the product of at least 20 generations of brother-sister inbreeding – varies considerably [27]. Some strains should be strenuously avoided for aging research because they are particularly prone to die early of a single specific disease [28], meaning that studies of those strains are useful for investigating the disease process but not informative with respect to generalized aging processes. By far the most common strain used in aging research is C57BL/6 and by far the most common sex is male. In well-maintained SPF colonies, median longevity of C57BL/6 males is 26–30 months [29, 27].

The dominance of aging research by this single sex and strain is unfortunate. The presumed advantage of standardization, facilitating comparison of experimental results among laboratories and between studies, is purchased at the expense of generality. One never knows if a result is an idiosyncrasy of a particular genetic background or is a more general phenomenon. All inbred strains have their idiosyncrasies. For instance, C57BL/6 mice are particularly prone to idiopathic ulcerative dermatitis as they age [30], they are also particularly prone to lymphoma [31]. Like many laboratory strains, they exhibit defective melatonin synthesis due to mutations in both necessary biosynthetic enzymes [32]. Most recently it was discovered that the C57BL/6 J sub-strain – and only that sub-strain – has a single nucleotide change in a brain-specific tRNA that interferes with the protein translation machinery [33]. Although that phenotype was only revealed when a second mutation (in *gtpbp2*) led to a major neurodegenerative phenotype only in the C57BL/6 J sub-strain, it raises concern about whether "wild-type" animals of that commonly used sub-strain, might be affected in unknown ways by aberrant protein translation in the brain. So over-reliance on any single mouse strain or sub-strain limits our ability to spot cryptic aberrancies affecting what is classified as a healthy state.

One approach to the problem of cryptic strain idiosyncrasies that combines some generality with some genetic control is the use of genetically heterogeneous mice generated in a repeatable fashion. An example is the UM-HET3 mouse stock [34]. Originally created for gene mapping studies, this stock is created by interbreeding two F_1 hybrids of inbred strains. The resultant F_2 mice are each genetically unique full siblings representing a broad swathe of genetic diversity within the laboratory mouse. *Populations* with the same genetic diversity can be recreated at any time. One reason that inbred mouse strains became so popular was the belief that they would be phenotypically more uniform that outbred populations. However, at least for longevity, UM-HET3 mice are no more variable than C57BL/6 mice (Miller, R.A. 2015, personal communication). This is the mouse stock that is currently used in the National Institute on Aging's Intervention Testing Program (ITP) [35].

Over-reliance on a single genetic background is not a research phenomenon confined to mice or to aging research. Virtually all *C. elegans* research employs the same N2 genetic background. However both mice and fly researchers have discovered that genetic background makes a dramatic difference in the impact of longevity interventions. For instance, overexpressing human superoxide dismutase 1 (SOD1)

in adult fly motorneurons significantly increased longevity in both males and females, by approximately 30 % and 40 %, respectively, in a particular laboratory strain. However the same mutation introduced into ten inbred, wild-caught strain found that females lived significantly longer in only 6 of the 10 strains and male lived longer in only 1 of the 10 strains compared to controls [36].

3.5 Other Species

Each of the three animal species described above is well-suited for research in the discovery of genetic interventions that modulate laboratory life. Together they represent more than 600 million years of evolutionary divergence from one another. Some phenomena such as reduced insulin/IGF signaling leading to lengthened life have been found in all three models. Does this mean that these species are sufficient for investigating *both* fundamental aging processes *and* age-related disease processes relevant to people? I would argue that these species are not enough for either and that we need to expand the traditional bestiary of aging models for the following reasons. First, our workhorse invertebrate models have undergone extensive gene loss since their divergence from our common ancestor. This can be seen by noting that more than 10 % of genes identified in a more distant human relative, the cnidarian *Acropora millepora*, have clear human orthologs that are missing from worm and fly genomes [37]. Thus there is a genomic universe of unknown size that may be relevant to aging processes not susceptible to investigation in worms or flies. Additionally, worms have no somatic cell division in adulthood and flies have limited cell division or regenerative capacity. Consequently, a key anti-senescence process – regenerative capacity – is difficult to study in these species. Finally with respect to these traditional invertebrate models, both worms and flies employ specialized nonaging life history stages during times of environmental stress (dauer in worms, reproductive diapause in flies) which have no human equivalent. Partial induction of these stages could retard aging via mechanisms not available to humans. In this sense, worm and fly findings could provide false clues to a deeper understanding of human aging biology. Thus new models, both vertebrate and invertebrate, with additional human orthologs, greater regenerative capacity, or lacking some type of diapause would be valuable to develop [38, 39].

Second, the use of the laboratory mouse as the sole representative of our own mammal clade warrants rethinking. Mice are the main species we rely on to model specific human disease processes and develop interventions to mitigate these processes. Yet despite their comparatively close relationship to us and the sophistication of genetic manipulation which we can deploy in mice, therapeutic successes in mice across a number of diseases such as stroke, cancer, and Alzheimer's disease (AD) have not translated well to patients. Alzheimer's disease is the most spectacular example of translational failure, with more than 300 drugs showing significant benefit in mouse models, but to date none have replicated that promise in humans [40]. A largely unexplored possibility that warrants attention is that mouse disease

models may fail because researchers typically induce diseases of aging in young animals. For instance, various AD mouse models have been created using a wide variety of genetic strategies leading to cognitive deficits in the mice by 3–18 months of age [41]. Even the latest onset of these is barely in the comparable range of age as a fraction of lifespan of the most aggressive human familial mutation [42]. It may be that aging is a critical component of the disease etiology, say for instance, requiring some vascular injury as an initiating event. Therefore, aged mouse models might be more representative of the human disease. The problem may lie even deeper than the age of onset in the mouse models. It may be that the mouse brain just cannot be humanized with respect to AD sufficiently to make it a therapeutically useful tool. Provocatively, cage enrichment of current mouse models of AD significantly improve cognitive deficits and reduce neuropathological hallmarks of the disease [43–45]. In this case, other species may serve better. A transgenic rat model has been developed that replicates a fuller spectrum of AD pathology than any mouse model [46], for instance, and the emergence of CRISPR-Cas9 technology [47] may allow the development of a better AD model in a small, short-lived primate such as a mouse lemur or marmoset. However, some aspects of human biology may be unique to humans, and AD is a good candidate for a human-specific condition. Nothing representing the full spectrum of cognitive and pathological signs of AD has been found even in our closest relatives, chimpanzees and gorillas [48].

Third, all model organisms currently used in aging research are distinguished by their lack of success in resisting fundamental aging processes. That is, they deteriorate and die quickly. That is one of their advantages for the type of aging research that requires lifespan studies. However, there is another research paradigm available for basic aging research. Some species are already well-known for their exceptionally long and healthy lifespans in the natural world and for being able to resist aging processes much better than the longest-lived, most robust model species. So a complementary research paradigm is to investigate the cellular mechanisms underlying their resistance [49–51]. Given the phenomenal advances in sequencing power in recent years, insight into the genomes and transcriptomes of some of these exceptionally senescence-resistance organisms as well as tools for their further investigation could follow rapidly. An unresolved issue is whether the aging research community is more likely to gain novel insights about aging from the study of animals that are long-lived for their body size such as naked mole-rats or any of a large number of bat species, but not long-lived relative to humans, versus animals that are absolutely long-lived – substantially longer-lived than humans – as some whales, fishes, or diverse taxa of invertebrates such as sea urchins or bivalve mollusks [52].

4 Is It Really Possible to Change the Rate of Aging?

I stated rather blithely above that the promise of medical interventions that enhance and lengthen healthy life is no longer an empty promise. What evidence do I have for that claim? The most obvious evidence is that nature has achieved this feat many

times over the course of evolution. Lifespan has approximately doubled, for instance, in the 6 million years since humans split from our chimpanzee ancestors. However, this evidence is not completely satisfactory as change over that time period could involve hundreds to thousands of variations in genes or gene expression. By far the most compelling evidence – presented below – is that we now are able to lengthen life and enhance a number of aspects of health in multiple ways by simple interventions in model organisms in the laboratory. Some of those ways are likely not to be relevant to humans, who are after all, many times more successful at resisting aging than any of our model organisms. The hope is that some of our successes with model animals will be relevant and translatable to humans and the more targets we identify in animals, the more likely this will be.

4.1 Dietary Restriction: The First Experimental Paradigm

4.1.1 Rodent Studies Past and Present

In the 1930s while investigating the impact of energy intake on growth rate, Clive McCay discovered that feeding rats substantially less than they would eat if given unlimited food increased their longevity [53]. Follow-up studies by McCay and a host of others confirmed this longevity-enhancing result of DR in both sexes of rats, mice, and a number of invertebrate species. Generally these studies found increases in both mean and maximum longevity (defined in the rest of this chapter as the longest-lived 10 % of the population) in both sexes and gradually over the next several decades information accumulated that many, although not all, maladies of aging laboratory rodents were also delayed [54]. By the 1990s a generalized – albeit premature – consensus emerged among aging researchers that DR universally extended life and health and that reduced caloric intake rather than restriction of specific macronutrients or the timing of food availability was the key to this "DR effect."

It is tempting to speculate on whether, if McCay's research had focused on obesity rather than growth rate, might DR might have been considered merely the absence of obesity or excess fat rather than a special ultra-lean state? This thought also illustrates how difficult it is to translate the circumstances of laboratory animals into human terms. The issue of whether DR is the absence of obesity or something else has not yet been determined, as the differing interpretations of the two existing macaque studies of DR illustrates. Although both studies report health benefits of DR, one study finds no effect on survival [55], the other does [56]. Control animals in the study that finds a survival effect weigh roughly 6–11 % more than the national average for captive monkeys of the same species whereas control animals in the study that finds no effect are roughly 8–16 % below the national average. Captive monkeys, in fact captive mammals of virtually all species including mice, are typically obese compared to animals in the wild [57, 58]. So how to think about the results in terms of which animals should be considered obese, which normal, which

restricted, is anything but obvious. One approach, implemented in fruit flies, could be to observe the reproductive effects of different dietary regimes and designate a regime as DR only if it is accompanied by a reduction in reproductive output due to nutrient limitation [59]. Again though, how to translate this to humans remains problematic.

Because calorie intake per se was assumed to be the key to the DR effect, considerable research effort went into exploring the energetic consequences of DR. Particularly after the damaging effects of reactive oxygen species (ROS) became known [60], a clean and neat mechanistic hypothesis to explain the DR effect was developed. This hypothesis proposed that restricting energy intake reduced daily metabolic rate, which in turn reduced ROS production and its consequent tissue damage. This led *ipso facto* to increased health and longevity. Like many a clean and neat hypothesis before it, the reduced metabolism hypothesis crashed on the rocks of experimentation and biological complexity. Both mice and rats experiencing DR have an initial transitory period where metabolic rate really did fall; however, after a few weeks energy consumption stabilizes at about the same rate in ad lib-fed and DR animals when measured as metabolic rate per gram of lean body mass [61, 62]. While these observations seemingly killed the reduced metabolism hypothesis, the reduced ROS hypothesis was left largely unscathed, as it became clear that there is not a simple one-to-one relationship between metabolic rate and ROS production. DR mice and rats in fact were consistently found to produce lower levels of ROS than controls [63]. The ROS hypothesis was seriously challenged, though, by a series of genetic manipulations of mouse cellular antioxidants, both under- and overexpressing them, which in turn reduced or increased ROS damage to tissues, yet which produced no consistent changes in lifespan [64].

A number of other simple physiological hypotheses were advanced by logic and slain by experimentation, including the glucocorticoid cascade hypothesis [65, 66] and the advanced glycation end-products hypothesis [67, 68]. This simple dietary manipulation, reducing food intake, alters so many cellular and physiological aspects – hormones, growth factors, inflammation, cell and protein turnover, body temperature, etc. – of rodent biology that no single factor could be determined to completely explain the DR effect. One notable discovery has been that chronic DR consistently enhances a range of protective responses from apoptosis of damaged cells to increased expression of DNA repair enzymes to elevated xenobiotic detoxification processes to the activation of the proteostasis network [69–74]. Indeed, DR was found to protect rats and mice from a broad range of carcinogens, cardiotoxins, hepatotoxins, and neurotoxins [75].

By the early 2000s the consensus on the primacy of reduced calories over specific dietary macronutrients and the universality of the DR effect began to come apart. That consensus, particularly as it related to reduced caloric intake per se, had been based on one study on one sex, male, of one inbred rat strain, F344, fed a diet that made that sex/strain particularly prone to age-related nephropathy [76, 77]. Several reports that restriction of sulfhydryl-containing amino acids in otherwise isocaloric diets extended mean and maximum longevity in the F344 rats were published in the 1990s, but in the early 2000s these studies were extended to several rat

genotypes [78] and then to mice [79]. More recently, a thorough "nutritional geometry" approach to mouse diet composition and caloric content – this approach systematically varies macronutrient ratios and caloric density – has called into question several aspects of the standard rodent DR paradigm [80]. The results of this study are complex, but two basic themes emerge. First, reduced caloric intake does not necessarily increase longevity. Unlike previous studies, caloric intake was manipulated by diluting individual diets with indigestible cellulose, which is more like the typical DR paradigm used in invertebrate models. Second, median longevity, though unrelated to total caloric intake, was related to the balance of macronutrients. Specifically, mice lived longer when fed diets that were low in protein and high in carbohydrates. Whether the lengthened lifespan associated with reduced protein consumption was due to reduced consumption of specific amino acids as the earlier studies might suggest, was not investigated. It should be noted that this was a single study with a single mouse genotype with modest sample sizes for each of the 25 dietary/survival groups. Nevertheless when combined with the methionine restriction studies published earlier, and the invertebrate studies discussed below, it certainly calls into question the traditional wisdom that calories – not specific macronutrients – are what modulate lifespan.

Also called into question has been the conventional wisdom that the DR effect is close to universal. Despite the fact that laboratory mouse or rat strains, due to the random fixation of alleles during inbreeding, are expected to have their idiosyncrasies, DR had been reported to extend life in a wide variety of strains and outbred stocks of laboratory rodents [54]. Even exceptionally long-lived Ames dwarf mice were observed to live longer with DR [81]. However, recent work on a panel of 41 recombinant inbred (RI) mouse lines at two independent animal facilities found that slightly more of the strains had their lives shortened by 40 % DR – the standard mouse protocol – as had their lives lengthened [82, 83]. This was true for both sexes at one facility (Fig. 3). Only females were examined at the other. The results were strikingly similar to findings with respect to variation in replicative lifespan in 166 single gene deletion strains in yeast [84]. The mouse results should be considered preliminary, as the number of individual lifespans per treatment and strain was small (N = 5 in one study, N = 10–12 in the other) and the strain results varied somewhat between facilities. Yet the indication of substantial genetic variability in the response to DR offers a promising tool for the genetic dissection of the rodent DR effect and its physiological mechanisms the same way it has proven to be a useful tool in yeast.

4.1.2 Dietary Restriction in Flies

The lack of success in discovering mechanisms of the DR effect in rodents even after decades of intensive study led by the 2000s to exploration of its effects in worms and flies, the hope being that the genetic power available in these model invertebrates would provide insight into DR not easily available in mice. In addition, because fly and worm lifespans are short and large numbers of animals can be

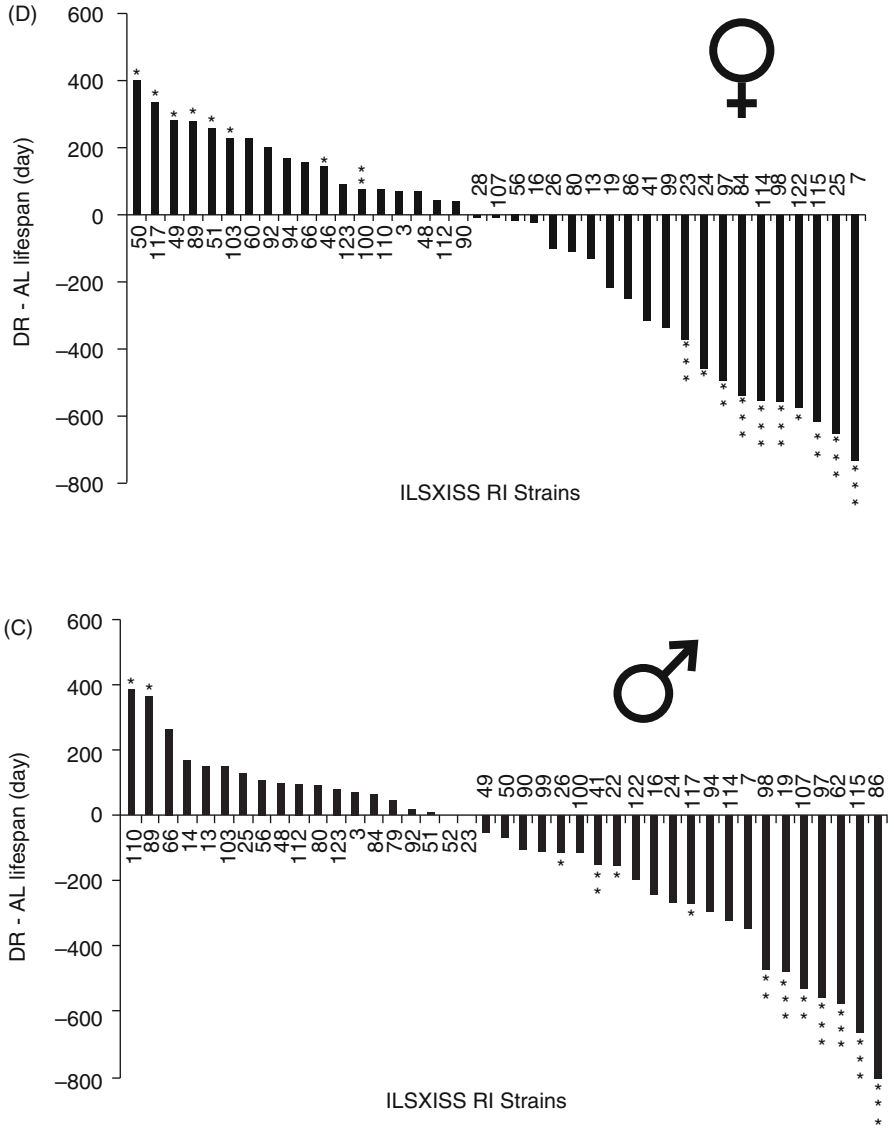

Fig. 3 Variation in the impact of 40 % dietary restriction depending upon mouse genotype. The data are from 41 recombinant inbred lines derived from an original cross of 8 laboratory strains followed by divergent selection for alcohol sensitivity (Reproduced with permission from Liao et al. [82]. *Aging Cell*. ©Blackwell Publishing Ltd/Anatomical Society of Great Britain and Ireland 2010)

easily maintained, experimental refinements such as including many dietary treatments within a single experiment can be routinely done. An implicit assumption of these studies – that DR affected longevity and aging via similar mechanisms in

laboratory rodents and invertebrates – is an assumption that has yet to be validated. Both fruit flies and worms had previously been shown to respond to reduced food availability with longer life [85, 86]. Investigation of DR in these small invertebrates turned out to have its own difficulties, but has also provided its own insight into both genetic and nutritional processes.

The major issues in performing DR experiments in worms and flies is determining the composition of the diet and even more important how much food is actually being eaten. In rodents, it is well-known what their natural diet consists of (seeds plus associated insect larvae) and there had been years of investigation into formulating healthy diets, even specialized breeding versus maintenance diets [87]. More importantly, food consumption can be, and often is, directly measured.

The normal laboratory diet of fruit flies is an agar-base combination of yeast, sugar or molasses, cornmeal, and other carbohydrates [88]. In most laboratory diets, yeast is the primary protein source. There is no true standard laboratory diet and the nutrient concentration of the food can vary as much as tenfold among laboratories. Flies typically eat only 1–2 μg of food daily, so quantifying food consumption is technically challenging, although possible [89]. As a consequence, food consumption in fly studies is rarely assessed. Given the different nutrient concentrations of different laboratories' standard diets, nutrient consumption of standard-fed flies can vary dramatically among laboratories. Until recently, DR experiments in flies were typically performed by simply diluting the lab's standard diet and as long as only a crude relative change in food intake was needed to interpret the experimental results, this procedure was adequate. However as fly nutrition and longevity studies became more sophisticated, experimental procedures also became more sophisticated. It has now been established that flies will compensate for nutrient density by altering overall consumption. One particularly rigorous study found that increasing nutrient density by fivefold from a base diet less than doubled total food intake. Increasing nutrient density from five- to tenfold above the base diet increased consumption only 33 % more, and increasing from 10 to 15-fold above the base diet did not alter food intake at all [89]. To emphasize the diversity of fly diets in use, the 15-fold higher density in this study is the standard diet in other studies. Now it is common for nutritional studies to include an array of food concentrations, both less than and more than, the standard diet for the lab.

Fly research has shown most compellingly that macronutrient composition rather than calories alone has the most dramatic effect on longevity. In particular, several studies have concluded that reduced protein rather than calories is the key determinant of fly longevity [90, 91, 21, 92]. The role of specific amino acids has not yet been completely clarified, although as with laboratory rodents methionine appears to be a particularly important amino acid [91]. Recently Piper formulated a chemically defined diet for flies that should allow further refinement of the relationship between nutrition and aging in this species [93]. One particularly interesting discovery is that even the aroma of extra yeast is enough to shorten fly lifespan [94].

Nutritional research in flies has also illuminated a potentially serious confound in assessing genetic or even the pharmacological influence on aging and longevity in studies that rely on ad lib feeding as virtually all do. The impact of a gene or a drug may be sensitive to dietary factors or may affect the amount of food eaten. For

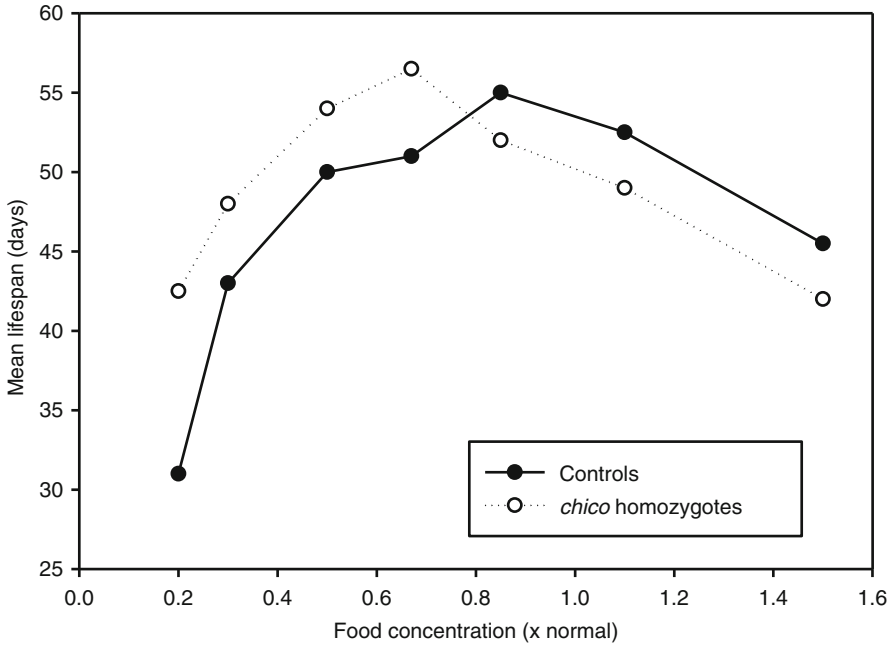

Fig. 4 Impact of nutritional density on lifespan in flies. The *chico* mutation is reported to make flies live longer (Clancy et al. 2001). Note, however, that the nutritional density of a "normal" diet varies dramatically among laboratories, such that what is considered a "normal" diet could affect whether the same mutation is a "short-gevity" or a longevity mutation and that at no food concentration is *chico* significantly longer-lived than control flies on their optimal longevity diet (Redrawn from Clancy et al. [96]. With permission from AAAS)

instance, certain mutations in the *Drosophila* gene *chico* have been reported to extend life [95]. However, this life extension was seen only at some food densities [96]. At other densities *chico* is shorter-lived than controls (Fig. 4) and at no density is *chico* longer-lived than the control flies on their own optimal-longevity diet. As the food density of a "normal" diet in flies is completely arbitrary, the finding that this *chico* mutation extends life is a happenstance of a particular standard diet. Other labs would have observed the same mutation to be life-shortening under their standard conditions. As long as studies are performed over a broad range of food densities, this should not be a problem. However, genetic studies of longevity rarely examine a range of nutrient conditions. In mouse studies, for instance, it is virtually never done.

4.1.3 Dietary Restriction in Worms

Turning to worms, their normal diet is unknown. Furthermore, the standard laboratory diet, live *E. coli* OP50, while having numerous advantages in terms of convenience and standardization, is clearly not a healthy worm diet. In fact, it is toxic. Worms fed killed *E. coli* live 16–40 % longer than worms fed live *E. coli* [97, 98] and worms fed a different bacterial species, *Bacillus subtilis*, live about 40 % longer

than those fed live *E. coli* [18]. Despite these findings, the standard worm diet continues to be live *E. coli*. An obvious interpretive difficulty this presents is whether longevity enhancement resulting from feeding less of a toxic diet is in any way analogous to the rodent DR paradigm. Worms can also be fed a chemically-defined, axenic diet [99], which avoids the problem of frank toxicity – worms typically live much longer on an axenic versus an *E. coli* diet – but for either type of diet it is difficult to determine how much is being eaten. Because it increases longevity and decreases fertility relative to standard worm diet, axenic rearing is considered by some a form of DR [100].

Greer and Brunet (2011) identified 12 methods of life-extending DR that have been used in worms, including two synthetic liquid diets and one mutation that reduces pharyngeal pumping rate. Surprisingly few worm studies use more than two feeding levels (control vs restricted) even though considerably more information can emerge from multiple feeding level studies [101]. Any hope that the powerful genetic tools available in the worm would quickly clarify the molecular pathway or pathways responsible for the DR effect in other species has been dashed however as the genes that are necessary and sufficient for life extension by DR depend on the method of DR employed. Which of the various paradigms is most analogous to rodent DR is not obvious. One of the favored hypotheses among rodent DR specialists, that reduced insulin or IGF signaling is responsible for much of the DR effect, does not appear to be the case for worms.

So, as these examples illustrate, after 80 years of research the key mechanisms by which DR lengthens life remains an unsolved puzzle.

4.2 Genetic Approaches to Retarding Aging

If worm biology has not yet helped solve the mechanistic mystery of the DR effect, it has been instrumental in dissecting the genetics of longevity. Several hundred worm genes significantly extend life when wholly or partially inactivated. Given that the active forms of these genes were selected over millions of years of evolution, this large number is surprising to say the least. It will be interesting if anything like this turns out to be true of other model organisms or whether this is a quirk of worm biology, perhaps due to the centrality of the dauer larval stage in its life history. Still, some of the largest effects on worm longevity are still due to some of the earliest genes discovered to affect aging. Given that a complete review of the numerous genetic influences on aging and longevity is beyond the scope of this chapter, I will focus on just the two that seem at this juncture to be the most robust.

4.2.1 Insulin/IGF Signaling

The first two longevity mutants discovered in worms were both in the insulin/IGF signaling pathway, a phosphatidylinositol-3-kinase (*age-1*) [8] and the insulin/IGF receptor (*daf-2*) [9]. They lengthened worm life by as much as 75–100 %. Insulin

and IGF (insulin-like growth factor) signaling are combined in this section because in worms and flies there is a single receptor that mediates the activity of multiple ligands. Subsequent mutations that reduce signaling through homologous pathways causing both dwarfing and lengthened life were discovered in flies [95, 102] and mice [103–105]. There is even evidence that reduced IGF-1 signaling may play a role in exceptional human longevity [106]. Thus signaling via insulin and/or IGF appears to be a highly conserved modulator of growth, metabolism, reproduction, and longevity – at least under laboratory conditions. Some evidence suggests that in more challenging circumstances the longevity effect may disappear or even be reversed [107, 108].

The evidence in mice deserves some special attention because in some ways it is weaker than in other model systems perhaps because the genetic tools are less robust but also possibly because the effect is less significant in mice or in mammals. It is important to note that too great a reduction in insulin or IGF signaling is life-shortening or lethal. It is also important to note that most of the mouse evidence for involvement of the insulin/IGF networks in longevity is indirect. For instance, possibly the most robust longevity-enhancing genetic treatment in mice is to reduce or eliminate signaling, not through insulin or IGF, but through the growth hormone receptor. The impact on longevity is substantial in both sexes (25–50 % increase) and unlike most mouse genetic longevity enhancements, it has been replicated in multiple genetic backgrounds, in multiple laboratories, and multiple times in the same laboratory [105, 109–111]. Although eliminating growth hormone signaling dramatically reduces circulating (but not locally produced) IGF-1, growth hormone also has effects independent of circulating IGF-1. Directly reducing IGF-1 activity by using mice haploinsufficient for the IGF-1 receptor has a substantially smaller longevity effect (5–11 %) in females and no effect in males [112, 113]. Haploinsufficiency of other members of the IGF signaling cascade such as insulin receptor substrate 1 or 2 also have small or sex-specific effects on longevity [114, 115]. Knocking out insulin – as opposed to IGF – signaling specifically in adipose tissue also modestly extends life (~18 %) in both sexes in mice [116]. Another effect of disruption of the growth hormone receptor is reduced plasma insulin. So potentially the large longevity increase with growth hormone receptor disruption is a combination of alterations to both insulin and IGF. On the other hand, researchers may be overlooking a key component of growth hormone signaling that is independent of insulin or IGF or it may be that haploinsufficiency or complete inactivation of activity in specific tissues are too crude manipulations to understand the context-specific roles of the signaling activity Both worm and fly mutations in insulin/IGF signaling that most effectively extend life are modulated reductions in signaling, rather than its complete ablation.

4.2.2 mTOR

The mechanistic Target Of Rapamycin (mTOR) is a highly conserved serine/threonine kinase that lies at the hub of a complex cellular signaling network that is centrally involved in metabolic processes, many diseases, and even aging itself.

Integrating environmental information on nutrient availability and a variety of stressors, mTOR activation promotes anabolism. Its suppression does the reverse – plus it modulates various stress responses [117]. First described in yeast, mTOR is a component of two distinct complexes, mTOR Complex 1 (mTORC1) which mediates most of the processes mentioned previously, and is directly inhibited by the drug rapamycin, and mTOR Complex 2 (mTORC2), which functions in the modulation of metabolism and various aspects of the cell's cytoskeleton and is not directly responsive to rapamycin. It attracted the attention of aging researchers due to its nutrient responsiveness and the known effects of DR on longevity [118]. Genetic inhibition of mTOR or its downstream mediators increases longevity in worms [119], flies [120], and mice [121]. Some types of DR in worms are ineffective when components of the mTOR network are suppressed. In flies, lifespan extension by reduced essential amino acid availability is also modulated by mTOR and not by insulin/IGF signaling [122]. DR in mice inhibits mTOR activity and pharmacological inhibition of mTOR extends life and health (see below). Therefore, it seems reasonable to speculate that inhibition of mTOR may play a role in DR's effects on health and longevity. Now that mouse genotypes that fail to respond to DR with increased life and health have been identified [82, 83], the role of mTOR and insulin/IGF signaling in the mouse DR response should be accessible.

4.2.3 Healthspan Versus Lifespan

What if an apparently frail and feeble mouse genotype seems to live on and on compared to more robust and vigorous control mice? To a number of researchers, the Ames or Snell dwarf mice appeared to be just such mice. Although they live dramatically longer than littermate controls (24–60 % longer), they are tiny, and because of their small size, they are particularly sensitive to cold. Females are sterile, males sub-fertile, and, when young, animals of neither sex moved around in their cages as much as controls. However, some aspects of their aging process appear to correlate with better health, rather than simply increased longevity. For example, some cognitive abilities appear better preserved with age [123], and neurogenesis continues later in life. Specifically, while early in life their spontaneous neuronal activity rate is lower than controls, Ames dwarf mice maintain this activity rate with age, such that later in life, it surpasses that of old control mice. Nevertheless, their small size and seeming frailty, I believe, led many researchers to raise questions about the quality of life associated with the longer lives Ames or Snell dwarf mice lived – particularly as people begin to consider the possibility of translating these successes from laboratory species to humans. These are legitimate questions with enormous societal implications. The simple assumptions that extended life equals extended health or that extended life will reduce the period of debility near life's end need to be critically evaluated and more difficult questions may follow. For instance, if we medically retard aging, giving most of us an extra 10 years of healthy life, but an additional 10 years of unhealthy life as well, is this worthwhile? Philosophers and economists might well differ in their opinions, but without

evidence, these will be sterile debates. We need information. The Geroscience Hypothesis dictates that basic aging researchers need to define and evaluate healthspan as well as lifespan in their experiments.

Assessing healthspan in laboratory animals turns out to be considerably more difficult – and prone to interpretation – than assessing lifespan. The easiest case to evaluate would seem to be rodent DR, because researchers have been investigating its impact on a spectrum of physiological variables throughout life for decades [54]. There is consensus that many rodent genotypes on DR are still physically fit at ages when their ad lib-fed brethren are all dead and that DR animals are more likely to die without detectable pathological lesions than fully-fed animals. Whether the known downsides of DR – reduced libido, muscle mass, and bone density, slower wound healing and some immunosuppression – would be worth the extended lifespan, assuming that the health benefits in humans were the same, would be a matter of personal psychology. However, as obesity rates are rising world-wide, this is one treatment that even if proven to effectively extend health and reduce morbidity, would not likely be adopted *en masse*.

However, rodent DR is an exception to the rule. Generally, we know relatively little about the health consequences of our various life-extending treatments, particularly with invertebrate species – and what we do know is not necessarily reassuring. For instance, one reasonable metric of health might be resilience in the face of physiological stress, an increase in which often accompanies increased longevity [124, 125]. Yet, genetic variation for reduced mortality in ten inbred lines of *Drosophila* failed to exhibit any correlation with genetic variation for resilience to cold-stress, even though both traits varied [126]. Only recently has substantial effort gone into assessing the health consequences of some of the many longevity-enhancing mutations found in *C. elegans* [127]. A recent examination of four different worm longevity mutations, including the most robust of the known mutations, *daf-2(e1370)*, employing four carefully thought out metrics of worm health (heat and oxidative stress resistance plus activity in liquid or solid media) found that none of the mutations compressed morbidity (defined as a loss of ≥50 % the capacity of a young adult) relative to wild-type by any metric. Moreover, in only 5 of 16 possible cases (4 longevity mutations × 4 health measures) was healthy life extended, and in all of these the unhealthy period of life was also extended. In 7 of 16 possible cases, the period of healthy life was actually shortened compared to wild-type and the unhealthy period extended. While these results should be considered provisional as the investigation of *C. elegans* health is in its infancy, they are clearly provocative.

Research into laboratory rodent health has a much longer history, is considerably easier to assess, and is undoubtedly more relevant to what we might expect in humans. Moreover, what is known about the health consequences of life-extending interventions in mice is considerably more promising than evidence to date from the invertebrates (see below). However, functional metrics are performed (or at least reported) haphazardly and it is never clear whether all investigated metrics have been reported or there was a selection for reporting those that improved. What is needed in rodent aging research is a widely-agreed upon panel of functional indica-

tors that is implemented for any new life-extending interventions. Such a panel is currently under development (Richardson, et al., in press).

4.3 Pharmacological Approaches to Retarding Aging

Arguably the biggest and most exciting advance in basic aging research recently is success in extending mouse life pharmacologically. Although various drugs and supplements have been reported to extend the lives of laboratory rodents at least since the early 1960s [128–132], until recently none had withstood the test of independent replication. A difficult problem with rodent longevity studies is that the cost and time involved in doing them makes replication rare. However, this is essential, so that scientists do not spend years pursuing dead ends arising from anomalous experimental results. A recent example is the 1999 report that a targeted mutation in the mouse $p66^{shc}$ gene increased lifespan by 30 % [133], a finding that was never independently validated until 15 years later when it could not be replicated [134].

For this reason and others, the National Institute of Aging's (NIA) Interventions Testing Program (ITP) represents a major advance in the implementation of drug testing protocols for mouse life extension. A key feature of the ITP is that compounds are tested at three independent research sites (University of Michigan, Jackson Laboratories, University of Texas Health Science Center San Antonio) using identical experimental protocols at the three sites [34]. As a consequence, a positive hit for longevity is immediately confirmed. A second key feature is the use of both sexes of genetically heterogeneous mice – the previously mentioned UM HET3 mouse stock – which prevents a positive hit because of a quirk of an individual inbred strain like C57BL/6 or because of a difference between the sexes.

To date, results from 16 different compounds have been published (Table 3). Some of the compounds have been tested at several doses and/or initiated at several different ages. One major result is that none of the compounds tested to date has significantly shortened mouse lifespan. Another rather astonishing result is that so far 5 of the 16 compounds have significantly extended life in either males alone or in both sexes.

Each of these successful interventions deserves a few words. Aspirin is a familiar nonsteroidal anti-inflammatory drug with anti-thrombotic and antioxidant properties. As chronic, low level inflammation is a hallmark of aging itself and damage from ROS have been implicated in numerous disease processes, aspirin had multiple rationales for being tested. At the single dose tested, there was a small (8 %) but statistically significant lengthening of median lifespan in males but no significant effect in females and no effect on maximum longevity (last surviving 10 %) in either sex [135]. Further investigation indicated that females had less bioactive plasma levels of aspirin and its metabolites. As a large (19,000 participants), multinational randomized clinical trial of low dose aspirin, looking at its impact on variety later life maladies is now underway [136], there is no follow-up to the aspirin study planned for the NIA ITP at this point.

Table 3 Compounds tested and results from the NIA Interventions Testing Program

Compound	Concentration in food	Age at initiation (months)	Effect in males	Effect in females
Aspirin	20 ppm	4	8 %	0
NFP	200 ppm	4	0	0
NDGA	2500 ppm	9	12 %	0
NDGA	800 ppm	6	8 %[a]	–
NDGA	2500 ppm	6	10 %[a]	–
NDGA	5000 ppm	6	9 %[a]	0
4-OH-PBN	315 ppm	4	0	0
CAPE	30 ppm	4	0	0
CAPE	300 ppm	4	0	0
Enalapril maleate	120 ppm	4	0	0
Rapamycin	14 ppm	20	9(28)%[b]	13(38)%[b]
Rapamycin	14 ppm	9	10 %	18 %
Rapamycin	4.9 ppm	9	3 %	16 %
Rapamycin	14 ppm	9	13 %	21 %
Rapamycin	42 ppm	9	23 %	26 %
Simvastatin	12 ppm	10	0	0
Simvastatin	120 ppm	10	0	0
Resveratrol	300 ppm	12	0	0
Resveratrol	1200 ppm	12	0	0
Resveratrol	300 ppm	4	0	0
Oxaloacetic acid	2200 ppm	4	0	0
Green tea extract	2000 ppm	4	0	0
Curcumin	2000 ppm	4	0	0
Medium chain triglyceride oil	60,000 ppm	4	0	0
17α-estradiol	4.8 ppm	10	12 %	0
Acarbose	1000 ppm	4	22 %	5 %

Effect = % median lifespan increase (all statistically significant)

[a]Survival curves not yet complete, results provisional

[b]Numbers are percent life extension from the time (20 months) the mice began receiving rapamycin

NDGA (nordihydroguaiaretic acid) is a natural product produced by the creosote bush with both anti-inflammatory and antioxidant properties, which is used in some traditional medicine pharmacopeias. NDGA had a slightly larger (12 %) effect on median lifespan than aspirin also in males only [137] with no statistical effect on females and no effect on maximum longevity in either sex (Table 3). Males appear to metabolize NDGA considerably slower than females and so maintain its levels in their blood considerably longer [137]. Although complete survival data are not yet available, NDGA follow-up studies begun a little earlier in life (6 months versus 9 months for the original study) using a lower dose, the same dose, and twice as high a dose as the original study appear to have very similar results. At all doses median

male survival of those getting the drug is 8–10 % greater than controls and at no dose do females appear to be living longer [138].

A nonfeminizing estrogen with little or no affinity for the classical estrogen receptors, 17α-estradiol has been repeatedly reported to have neuroprotective and antioxidant properties [139]. It was hypothesized that males in particular might benefit from 17α-estradiol. Males did. Median longevity of males increased by a statistically significant 12 %, but as with aspirin and NDGA there was no significant effect on median longevity of females or on maximum longevity of either sex. An interesting aspect of the 17α-estradiol studies is that there was a dramatically larger effect (28 % increase in median longevity) at one facility than either of the others (a nonsignificant 3 % at each). The larger effect was not due to longer-lived treated mice at that site but to shorter-lived controls [138]. So this general result should be treated somewhat cautiously until more research is done.

The fourth compound found to extend mouse longevity is acarbose, commonly used clinically particularly in Europe in the treatment of type II diabetes. It ameliorates the postprandial spike in plasma glucose. Acarbose is not metabolized, it inhibits α-glucosidases in the intestines and therefore slows the breakdown of dietary carbohydrates into glucose. For that reason, it was hypothesized that it might act as a DR mimetic. Median male longevity was increased at all sites by 22 % for the pooled data. Maximum male longevity was also increased significantly (by 11 %). For females, the data are more complex. The pooled data showed a 5 % increase in median female longevity, which reached statistical significance ($p=0.01$). However, there was essentially no absolute median difference at one site, a nonsignificant 7 % increase at another site, and a marginally significant ($p=0.04$) 7 % difference at the third. As no statistical corrections were done for multiple comparisons, this result, like the female 17α-estradiol results, should be interpreted with caution. Surprisingly, maximum longevity of females was increased at all sites in absolute terms, by 9 % overall, a highly significant ($p=0.001$) result. Recall that these are genetically heterogeneous mice, so the puzzling results where there are large inter-site differences or a marginal result in median longevity but a more significant result in maximum longevity could be a consequence of that genetic heterogeneity. The drugs could be beneficial for some genotypes and not for others.

To summarize the ITP results to this point. Four of 16 drugs tested have shown highly statistically significant effects on median male longevity, only one of those four (acarbose) showed a statistically significant increase in median female longevity and there is reason to interpret that one result cautiously. Only one of the four drugs – again, acarbose – increased maximum longevity significantly, and it did so in both sexes. Follow-up studies on acarbose are currently underway.

By far the biggest success of the NIA ITP has been the discovery that rapamycin treatment increases longevity robustly in both mouse sexes even when initiated relatively late in life [140]. Used clinically as part of an antirejection cocktail primarily for people receiving kidney transplant [141, 142], for some cancer chemotherapeutic regimes [143], and to inhibit overgrowth of coronary artery stents [144, 145], rapamycin and its close chemical relatives (“rapalogs”) are well-known to

directly inhibit mTOR kinase [146]. The biology of mTOR and the effects of its inhibition are too complex to cover here except cursorily as above, but many fine reviews are available [118, 147, 117]. In 2009 the NIA ITP reported that rapamycin when initiated at 20 months of age (the demographic equivalent of about 60 years old in human terms) lengthened life 9 % in male mice and 14 % in female mice as well as significantly extending maximum lifespan in both sexes [140]. Measured from the time at which rapamycin feeding had begun though, males lived 28 % longer than controls and females lived 38 % longer than controls. Follow-up studies with mice begun on the same dose of rapamycin at 9 months of age found a highly significant 10 % increase in median lifespan in males and an 18 % increase in females as well as 16 and 13 % increases in maximum longevity, respectively.

The longevity enhancing impact of rapamycin has now been replicated in both sexes, in both genetically heterogeneous and inbred mice, in a variety of labs, when administered in food or injected, at a variety of doses, and when started in adolescence, adulthood, or later in life [148–152]. In addition, it has now been shown to delay or even reverse cognitive deficits in mouse models of Alzheimer disease [153–155] and Huntington's disease [156], attenuate the progression of atherosclerotic plaques in atherosclerosis models [157, 158], prevent or delay both spontaneous and genetically-induced mouse cancers [149, 159, 160], and delays the onset of symptoms in a progeria model [161].

On top of these improvements in mouse models of various human diseases, rapamycin also improves a number of mouse health indicators. For instance, it enhances and broadens vaccine response [148, 162], delays normal cognitive decline during aging, reduces anxiety and depression [155, 151], increases spontaneous physical activity, improves cardiac function, and enhances sleep consolidation in older mice [163, 164, 151]. In sum, rapamycin administered to mice increases longevity, prevents or delays many diseases, and preserves many aspects of health. It might almost be said to retard aging itself.

All drugs have side-effects, rapamycin is no exception. Are any of these side-effects severe enough to eliminate it from consideration as a potential senescence-retarding intervention in humans? Because it has been in clinical use for years already, we know quite a bit about rapamycin's side-effects in people with various serious diseases. However because it is typically used in combination with other drugs and never given to completely healthy people, we know little about its side-effects in healthy people. From what is known, however, the side effects of most concern are immunosuppression, hyperglycemia, glucose intolerance, and insulin resistance [165–167]. However, in a genetically heterogeneous mouse stock, these effects were seen in young male mice during the first 6 weeks of rapamycin treatment but were substantially diminished and even reversed in some cases by 5 months of treatment [168]. Similar metabolic dysregulation was seen in both genetically heterogeneous and C57BL/6 J male mice for the first 4 months of treatment whether the mice were fed a normal or high-fat diet, but these effects disappeared within 2 weeks after cessation of treatment [167]. So at least in male mice, metabolic changes produced by chronic rapamycin treatment disappear quickly when treatment is halted

and may be transient even with continued treatment. It will be enlightening to see whether these effects also occur in female mice and in both sexes of other species.

The use of rapamycin as a component of anti-rejection therapy following organ transplant suggests that if used chronically it may enhance susceptibility of infectious diseases. Without question rapamycin suppresses aspects of immune function [169–171]. However, it enhances other aspects, and consequently has been termed an immunomodulator rather an immunosuppressant [148, 172]. Chronic enteric rapamycin administration has been found to enhance resistance to pneumococcal pneumonia in elderly mice [173], although no such protection – and possibly reduced protection – was found against West Nile virus [174]. Moreover, a 6 week course of injected rapamycin prior to influenza vaccination has been found to enhance protection again influenza in both mice and humans [148, 172]. Therefore, the impact of chronic rapamycin on disease susceptibility in healthy humans is far from clear and should not by itself discourage trials in species other than mice.

5 Future Directions

As previously shown, dietary, genetic, and pharmacological interventions have been found that robustly extend life in model organisms and some have also been shown to extend multiple features of health. Where do we go from here if we are serious about ultimately discovering new ways to prolong human health? One obvious way forward is to solidify our knowledge base. That means replicating and optimizing successful interventions for both health and longevity in both sexes in other genotypes and other species. A surprising number of successful longevity interventions, both genetic and pharmacological, have had sex-specific effects (Austad & Bartke, *Gerontology*, in press). That also means evaluating interventions that have not already been approved for human use in other mammal species. Mice, particularly laboratory mice, are not an acceptable stand-in for all mammals. They have displayed a notable lack of success in predicting therapeutic efficacy in human diseases such as Alzheimer's disease, stroke, or even cancer. Mice have their obvious quirks such as their extreme susceptibility to cancer and limited cognitive sophistication. Their robust longevity response to constitutively-reduced growth hormone signaling has never been seen in another species and has failed to be observed even in their close relative, the laboratory rat [175]. Fortunately, additional mammal species such as the domestic dog and the small, short-lived primate, the common marmoset, appear to be excellent candidates for such studies.

Geroscience, as I hope this chapter has shown, is advancing more rapidly than almost anyone supposed. Its promise to enhance and extend human health could transform not only human health in the twenty-first century but also all the social institutions that depend on human health. In the year 2100, we may look back at the year 2000 and consider it as medically unsophisticated as we now think of the year 1900.

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Etiological Role of Aging in Chronic Diseases: From Epidemiological Evidence to the New Geroscience

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Contents

1 Premise – Evolution of the Science of Chronic Diseases and Current State of the Field

1.1 Introduction

According to Wikipedia, "*Epidemiology is the science that studies the patterns, causes, and effects of health and disease conditions in defined populations. It is the cornerstone of public health, and informs policy decisions and*

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evidence-based practice by identifying risk factors for disease and targets for preventive healthcare". Consistent with this definition, over the last few decades, epidemiological studies identified a number of genetic and environmental risk factors for the majority of chronic diseases. There is no doubt that epidemiology has contributed tremendously to both the science of understanding of disease and to the science of prevention, both of which are necessary to achieve population health. It is currently believed that the increased longevity in the population and the decline in cardiovascular morbidity and mortality resulted from interventions on risk targets that were first identified in epidemiological studies.

Notably, however, the research on etiology of chronic diseases, which initially was mostly on cardiovascular disease and cancer, has been carried out in ways that, inadvertently, precluded understanding their relationship with aging. Since age and sex were considered unchangeable risk factors, they were generally factored out from all analyses as "potential confounders". "Adjusting for age and sex" was almost a *mantra* for seasoned epidemiologists; this, unfortunately, translated into a lost opportunity. Indeed, age is by far the strongest and most pervasive risk factor for almost all chronic diseases and medical conditions. The effect of aging on the risk of developing cardiovascular disease, cancer, diabetes, osteoarthritis and dementia, just to cite a few diseases, is several magnitudes higher than the risk attributed to all other known risk factors. The idea of "adjusting for age" obscures consideration of the effect of age, and also overlooks the critical nuance that chronological age is a poor approximation of biological aging. There is increasing heterogeneity with age between individuals in the physical and functional consequences of the aging process, which probably results from differentials in exposures across the life course and the intrinsic rate of biological aging. Understanding how the intrinsic biological mechanisms of aging affect most aspects of health in humans is a fascinating scientific challenge that has captured the attention of the greatest scientific minds over the centuries. However, with the current aging of the population, estimating biological aging is now also recognized as important for practical clinical purposes. To some extent, geriatricians and gerontologists have approached this problem through the conceptualization and operational definition of frailty as a diagnosable clinical syndrome that is a hallmark of the aging process and is marked by susceptibility to stress, definable biology, underlying loss of resiliency and diminished functional reserve. However, as research on the biology of aging in animal models progresses, it complements the work on mechanisms of aging-related dysregulation in humans; the two lines of investigation together suggest that a core set of mechanisms may reside at the basis of aging and resulting frailty. These same mechanisms may also contribute to disease and may be modifiable with appropriate interventions. In this chapter, we propose the idea that many chronic diseases in older age and frailty both originate, at least in part, from accelerated aging, and may mutually precipitate or exacerbate one another. We propose that this concept has enormous translational potential and is consistent with the new evidence emerging from the fields of Geroscience and Precision Medicine.

1.2 Traditional Approach to Chronic Diseases

The study of chronic diseases, etiological mechanisms, signs, symptoms and consequences, as well as potential treatments, has been focused primarily on analysis of organ-specific diseases and their clinical manifestations. Over the last 60 years, science has gone through a number of stages of such analysis and evidence. This progression began with population-based epidemiological studies that described the prevalence and incidence of chronic diseases, identified their etiologic risk factors and mechanisms, and led to the development – and evaluation – of clinical and population-based interventions, from Coronary Care Units to behavioral and pharmacologic therapies and primary prevention initiatives. Further, epidemiologic investigation led to evidence that there were independent predictors, namely environmental and behavioral risk factors, for specific chronic diseases that were potentially modifiable. Randomized controlled trials have shown that modification of such risk factors resulted in substantial primary prevention of morbidity and mortality. At the same time, intrinsic risk factors, such as the presence of hypertension, diabetes or elevated LDL cholesterol, have been shown to be predictive of subsequent cardiovascular disease and that screening for early identification of these conditions can lead to behavioral change or treatments that are effective in primary or secondary prevention of subsequent disease. Clinical and community-based guidelines, as well as health policies, have gone on to implement these recommendations on a population scale. These advances in knowledge and delivery of public health and medical science have been followed by a dramatic decline in cardiovascular morbidity and mortality. Age-adjusted death rates per 100,000 persons in the U.S. decreased from a peak of 307.4 in 1950 to 134.6 in 1996, an overall decline of 56 %; and they are continuing to decline today [1].

Overall, much population-based and clinical research has demonstrated that significant portions of chronic disease mortality and even the incidence of morbidity and resulting disability are either preventable, or can be delayed in onset. Following this line of research, geriatricians and gerontologists hypothesized that interventions could be developed to promote healthy and active aging, and that those interventions would include – but not be limited to – the primary and secondary prevention of chronic diseases [2]. The ultimate aim of those interventions is the "compression of morbidity" to the latest years in the human life span, including the delay of chronic disease morbidity and the onset of physical and cognitive disability. There is now a substantial literature to support the effectiveness of prevention of chronic diseases into the oldest ages [3], while the possibility to ultimately prevent physical and cognitive disability is still unanswered.

The many decades of science briefly summarized above have followed two pathways of reasoning. The traditional medical approach to chronic disease is to accomplish a diagnostic classification that is as precise as possible, based on symptoms, signs, clinical tests and other clinical elements. A correct and precise diagnosis allows access to the wealth of experience acquired in clinical medicine, including prognosis, and effective disease-modifying and symptomatic treatment of a specific disease. Physicians tend to work backwards in the etiologic pathway from making a disease diagnosis based on external clinical elements to generating hypotheses about patho-

physiological mechanisms and the risk factors. Treatments aimed at prevention and cure are then administered that work 'forward' in the etiologic pathway, thereby correcting the clinical manifestation of diseases. A corollary of this method is the assumption that each syndromic manifestation has an underlying specific pathophysiology.

The traditional medical approach to human diseases has been quite successful in the care of young and middle-aged patients and prevention in these age groups. However, it has substantial limitations in the care of older patients for several reasons. First, the signs, symptoms, clinical manifestations, prognosis and response to treatment for many chronic diseases vary with age, and the specificity of symptoms decreases with increasing age. For example, in older persons episodes of hypoglycemia are often asymptomatic and signs of a previous acute myocardial infarction are often found in people with no history of symptoms. Second, the high likelihood of geriatric conditions and multimorbidity in older ages blurs diagnostic boundaries between diseases and complicates treatment choices. Third, in many older adults, the manifestations and clinical course of diseases are strongly affected by the underlying status of the "host" as well as by other coexisting diseases. Because of these reasons, considering aging as a 'confounder' in the study of chronic diseases ignores the complexity of the interactions between aging, disease and frailty. We now know that aging plays a central role in the pathogenesis, clinical presentation and response to treatment of many chronic diseases. Therefore, the patient's age (both biological and chronological) should be a primary clinical element that should affect choices of diagnostic, preventing and therapeutic interventions.

Emerging evidence on multi-morbidity and the frailty syndrome lays out the basis for making substantial progress in translating these concepts into improved care of older patients. Promising developments are coming, as well, from the rising interest in Geroscience and Precision Medicine [4]. The convergence of these scientific disciplines can be transformative in our understanding of the interplay between aging, frailty and disease, with the potential of producing dramatic improvements in public health.

In this chapter, we explore the evolution and current state of the science pertaining to possible links between aging and chronic disease(s), with a specific focus on the epidemiological evidence that such association is robust and not exclusively explained or sustained by a stochastic process. We seek to link together the mounting evidence that biological mechanisms that underlie aging lead to dysregulation of multiple physiological systems, loss of homeostatic capabilities and increased susceptibility to stress, and that these changes facilitate the emergence of both multimorbidity and clinically apparent frailty. Then, we consider whether the epidemiological literature is consistent with the stated hypothesis. Finally, we examine our current understanding of the biology of frailty as a basis for generating hypotheses about the biological mechanisms that link aging and chronic diseases.

1.3 From Clinical Presentation to Etiology and Biology

For over a thousand years, chronic diseases have been studied from the starting point of their externally apparent clinical manifestations. This approach led to the development of a number of classification systems, some relatively simple (such as

the now disproven distinction between inflammatory and degenerative diseases) and some extremely precise and sophisticated (such as classification of lymphomas based on histological characteristics). While the ability to recognize specific diseases and to treat them successfully has increased tremendously, the limitations of these approaches have also become apparent. These are evident, in part, from the advent of applications such as high throughput genetic, genomic, proteomic and imaging biomarkers to the study of chronic diseases, in combination with the evidence from prospective/longitudinal epidemiological, population-based studies. Biomarker studies – perhaps better than any other scientific approaches – have offered evidence that, in many cases, diseases that are driven by different mechanisms converge into the same pathological and clinical manifestations. For example, it is now widely accepted that under the label of "Alzheimer's disease" exist a number of conditions with different underlying mechanisms [5]. Conversely, diseases that appear quite different from the perspective of phenotypic and end-organ manifestations are now known to have shared etiologic biomarkers (e.g., inflammation); this suggests that they are driven by the same pathophysiologic mechanisms, and that multiple, seemingly unrelated, chronic diseases share biomarker signatures. Interestingly, such biomarkers are often also related to aging itself and predict the development of frailty, a major adverse health outcome associated with aging. This is consistent with evidence that the biology of aging is associated with chronic disease development through mechanisms beyond the length of time for exposure and cumulative risk from external risk factors; rather, the evidence actually points to aging as playing a powerful causal role in development of chronic diseases.

2 Epidemiologic Interrogation of Chronic Disease

Over the last 60 years, we have learned – in large part through epidemiologic investigations - how the causes of morbidity have dramatically changed in our population. With the increase in longevity due to the demographic transition, chronic diseases have become the dominant causes of morbidity and mortality in the developed world, and are rapidly reaching that dominance globally. According to a recent report, over 85 % of US Medicare beneficiaries have at least one of nine chronic diseases [6].

The frequency of most of the major chronic diseases rises with age. However, we should not assume that the relationship between aging and disease is monotonic; in fact, it is quite complex. Some chronic diseases, such as those due to genetic defects, exposures during gestation, or environmental perturbation may become clinically evident early in life and are unlikely to emerge after a certain age. Some other diseases have a typical age of emergence and only rarely occur outside a certain time window (e.g., rosacea or lupus). However, the majority of chronic diseases such as dementia, cardiovascular diseases, cancer or osteoarthritis, show increased incidence and prevalence with aging, although many individuals never develop those diseases during their lifespan. Finally, some pathological conditions occur in everyone with aging (e.g., decline in renal function, decline in lean body mass) and, because they occur in everyone, we assume they are not a disease but rather part of "normal aging".

Given the extreme variability of human pathology, making a generalization is difficult. However, it is plausible that biological aging plays an important pathophysiologic role in diseases whose incidence and prevalence increase with aging. To offer the counterarguments first, there are basically two objections to this theory. The first is that not everyone develops those diseases as they age, although for some diseases, such as Alzheimer's disease, it has been argued that if they lived long enough, everyone would eventually develop the disease. The second argument is that not everyone develops diseases in the same sequence. In both cases, objections can be easily overcome by hypothesizing that the clinical emergence of disease results from a tradeoff between organ or tissue-specific susceptibility, the rate of progression by which subclinical processes become clinical, and the overall dysregulation induced by the aging process. As an example of the latter, aging can facilitate an imbalance in cholesterol metabolism, but such imbalance may never emerge clinically in individuals who do not have a certain genetic susceptibility and maintain a healthy diet and weight. Further, the clinical presentation of disease may be delayed by behavioral compensations, such as walking more slowly in patients with pulmonary diseases – so as not to experience the symptoms – or increased walking to improve muscle efficiency in utilizing oxygen in patients with peripheral artery disease – and thus decreasing the symptoms.

Unfortunately, modern medicine, public health and much of science has focused almost exclusively on mechanisms that create susceptibility to a single disease, and have substantially ignored the clues as to the potential direct contribution of aging and related biology to health as well as to chronic diseases. In this context, it is understandable why age has been considered merely a 'confounder'. In surveying the literature on chronic disease, the attributable fraction of the burden of chronic diseases to the health burden of aging appears quite high across diseases, even when the effect of powerful risk factors such as hypertension or smoking are factored out. However, such analyses have rarely been conducted comprehensively, because most studies have focused on one disease outcome and ignored the effect of competing risk or aggregate impact. Future studies are needed to estimate in large, representative cohorts the population-attributable fraction to aging and health of multiple chronic diseases after adjusting for known risk factors and using a multivariate approach that addresses competing risk and selective mortality. Such studies would help to estimate the extent to which the burden of morbidity in older persons is attributable to aging per se.

3 Where Does Aging Fit in the Study of the Etiology of Chronic Disease?

An interpretation of the epidemiologic literature strongly suggests that aging contributes independently to the pathogenesis of many chronic diseases, and there are truly very few exceptions, largely in the form of rare diseases. Almost counterintuitively, the only diseases that have been interrogated to reveal aging effects are the

differential mechanisms for congestive heart failure by age [7], and the progeroid syndromes. Whether progeroid syndromes truly recapitulate the effect of aging in their early emergence of the aging phenotypes is unclear [8]. In general, very few of the findings of these studies have been translated into better understanding of the interface between aging and disease in people who have an average lifespan.

To go beyond "age as a confounder" or "aging as a process" independent of – and unrelated to – chronic diseases in the study of human pathology requires a shift in perspective. It is essential to go from the study of the clinical manifestations of a given disease and then its proximal drivers, to an agnostic consideration of the pathophysiologic events that precede the development of single and multiple disease(s) over the aging process, and to relate these outcomes to the processes and outcomes of aging including, but well beyond, disease(s). Theoretically and ideally, such a shift would require:

1. A basic understanding of the biological mechanisms of aging in humans. While we are far from having this knowledge in its entirety, some of the basic theories of aging have been developed and, to some extent, supported experimentally in model organisms (mostly yeast and worms, and in some cases, mice). Technology is currently available or in an advanced stage of development that should allow the testing of some of these theories in humans. Fully developing and testing such technology – and then the theories themselves – is clearly an important priority in aging research.
2. Criteria for disease diagnosis and multimorbidity classification should be developed that are not exclusively based on clinical manifestations and that do not ignore the role of the aging process or novel insights about causal pathways [9].
3. A list of potential biomarkers that change with aging and may be modified by chronic diseases – or that themselves modify disease – should be developed, and their ability to predict the decline in physical and cognitive function that occurs with aging should be evaluated. Ideally, these biomarkers should belong to pathways that have been demonstrated to be altered in animal models of aging and frailty.

3.1 Multi-morbidity and Aging

As outlined above, one of the landmarks of the interface between aging and diseases is multi-morbidity. In the U.S., nearly 80 % of Medicare beneficiaries 65 and older have at least 2 chronic conditions, and more than 60 % have at least 3 chronic conditions [10]. In fact, the condition of multi-morbidity is the most frequent medical condition that affects individuals 65 years and older. In a cross-sectional study that included 1.7 million patients in Scotland, UK, Barnett and colleagues found that 30.4 % of the population aged 45–64 years, 64.9 % of people ages 65–84 years, and 81.5 % of people aged 85 years and over reported at least two chronic conditions. Data from the InCHIANTI study, a longitudinal study of aging performed in Italy in a population-based sample, suggest that the average number of chronic diseases

present in a person increases exponentially with aging, and the longitudinal rise in multi-morbidity is even higher than what can be estimated from a cross-sectional survey because of selective mortality [10]. Of course, the scenario of morbidity is more complex than just the counts of specific chronic diseases or considerations about their individual etiology. For example, in most studies on multimorbidity only a limited number of diseases are considered, typically 10–20, which is only a fraction of the diseases that affect aging humans. Prior reviews have discussed the significance of multimorbidity in the context of aging [11–17].

The development of multiple diseases in the same person occurs, in part, by chance alone, as a result of the length of exposure and cumulative development of the resulting pathophysiologic alterations. However, recent data suggest that the development of multi-morbidity is not merely the result of multiple independent, disease-specific pathophysiological processes. Their development can derive from shared etiologic factors, which predict many different chronic diseases that are age-related. That is, there are many environmental and behavioral risk factors (e.g., smoking) and shared intrinsic mechanisms, such as chronic inflammation, which drive development of many seemingly unrelated end-organ manifestations and diseases, from cardiovascular disease to diabetes to cancers and COPD [11]. Further, a number of studies have recently suggested that this is only part of the story. A landmark paper that tested the hypothesis that certain diseases tend to appear in clusters in some specific individuals was published by van den Akker and colleagues in 1998 [15]. Working in a large general practice, they used data on disease prevalence and co-prevalence to calculate the expected prevalence of multi-morbidity under the assumption of association by pure chance. The authors found that more people than expected did not have any disease or had four or more diseases, while less than expected had one or two diseases. This suggests that some individuals develop a global susceptibility to multiple diseases, while others appear to be unusually resistant, and that the co-occurrence of some specific diseases is not due to chance alone. These authors and others have suggested that "the force of aging" is different in different individuals and makes them more or less susceptible to multi-morbidity. This hypothesis has never been empirically evaluated, due to lack of data. However, since 1998, numerous studies, largely heterogeneous for sample size, age, settings, indices of multi-morbidity and statistical methods (observed-to-expected ratio, cluster analyses, factor analyses and multiple correspondence analyses), have been trying to describe possible patterns of associative multi-morbidity in order to generate helpful evidence for actual clinical practice and management [10, 17]. Compromising this research is the absence of standardization of methods, which has resulted in high variability among studies and inconsistency in results, making it difficult to compare them. Interestingly, chronological aging appears to be a strong predictor of mortality even in analyses that take into account the contributions of multiple diseases and risk factors [18, 19]. While this is not a surprising finding to clinicians, it clearly underlines the fact that that the parameters that are usually observed and considered in medical care do not exhaust all of the changes in health status that occur with aging. In other words, there are biological processes that occur with aging that are not revealed by clinically evident disease. It is quite possible that the excess mortality associated "purely" with old age may be due to

subclinical disease, unobserved confounding and/or measurement error in assessing the full effect of diseases on prognosis. However, an alternative hypothesis is that some fundamental biological processes essential for life across cells, tissue and the entire organism deteriorate with the aging process and facilitate the emergence of both diseases and a general decline in health status that is not classified as "disease" but increase the risk of mortality.

An example based on the mitochondrial theory of aging may clarify this idea. A number of studies have found that aging is associated with loss of mitochondrial function; the mitochondrion being the key organelle responsible for cellular energy production in tissues and organs. Although mitochondrial volume appears to increase with aging, the electrical and chemical trans-membrane potential of the inner mitochondrial membrane is generally reduced and there are also functional defects in the electron transport chain that impair the efficiency of oxidative phosphorylation and limit the production of adenosine-5′-triphosphate (ATP) [20]. In addition, impairment in mitochondria may affect apoptosis as well as autophagy mechanisms.

When mitochondria are not functioning properly, tissues that have high energy demands suffer first. This is manifest in genetic mitochondrial disorders, which mostly affect the nervous system, heart and skeletal muscle [21]. A decline in mitochondrial function, regardless of its origin, has also been associated with central obesity, sarcopenia, fatigue, loss of physical fitness and diabetes [22]. Studies in animal models and in humans have demonstrated that the macroautophagy mechanisms aimed at removing and recycling defective mitochondria (mitophagy) become defective with aging, leading to the persistence of damaged mitochondria that generate excessive Radical Oxygen Species (ROS) and trigger a chronic inflammatory response. It is notable that inadequate energy support, excessive and unopposed oxidative stress as well as chronic inflammation have all been associated with the syndrome of frailty and with almost all chronic diseases whose incidence and prevalence increase with aging as well as with sarcopenia, gait disorders, and brain dysfunction. The translation of these biological events into both multi-morbidity and frailty is direct and intuitive. Further, it is easy to see how age-associated mitochondrial dysfunction can produce a number of vicious cycles that further impair mitochondrial physiology, for example, by causing ischemia or metabolic derangement. There are now many other examples implicating a single biological mechanism that, while driving the development of aging as a phenotype and aging-related frailty, also causes multi-morbidity. What is important here is that the triangle of aging-multimorbidity-and frailty may derive from the same root cause and, plausibly, they may all respond to the same interventions.

3.2 Frailty

We suggest above that the process of aging, and in particular "accelerated aging", may be best approximated by the concept of frailty. We develop further here the concept and the evidence that underlying mechanism(s) of aging – as exemplified

above by the example of mitochondrial changes - may lead to this syndrome of frailty. Frailty is associated with a dysregulation of the complex adaptive mechanisms that support organismal resilience; the dysregulation leading to loss of homeostatic capabilities and increased susceptibility to stress. The net result is the emergence of a distinct clinical syndrome with a characteristic phenotype that is predictive of a range of adverse clinical outcomes.

We will first describe frailty as a prototypical constellation of signs and symptoms that allows a clinical diagnosis and then, working backwards in the causal pathway to etiology, we will consider how what we currently know about frailty informs understanding of aging and "accelerated aging".

There is now strong evidence to support frailty as being a clinical syndrome characterized by a constellation of symptoms and signs: muscle weakness, slowed gait, low physical activity, perceived low energy or "exhaustion", and weight loss that is not explained by a distinct disease process [23]. When a critical number of such signs and symptoms occur in the same individual they identify the frailty syndrome [23]. It is noteworthy that studies have demonstrated that the phenotypic criteria of frailty co-occur in ways that are consistent with the definition of a medical syndrome [24]. That they predict the clinical outcomes associated with being frail provides criterion validity.

The clinical presentation of frailty is consistent with dysregulation in multiple systems and organs [23], although it is, as yet, unknown whether such dysregulation is the primary causal event, a collateral, or a consequence. Operationally, when multiple of these criteria are present (three or more) the individual is considered "frail" and consistently, "frail" individuals have high risks – over 3 years – of mortality, disability, falls, hospitalization, loss of independence, and slower recovery from illness or surgery and greater postoperative adverse outcomes [23, 25]. Further, those who meet these criteria for frailty show decreased responsiveness to treatments, for example for HIV or renal failure [26]. This susceptibility to adverse outcomes occurs frequently in the context of a stressor, such as illness, hospitalization or surgery.

Clinical frailty develops progressively, so that testing positive for one or two criteria predicts the development of the full syndrome, with weakness and slowed gait being the most common earliest predictors [27]. Studies have found that the greater frailty is associated with greater risk for disability and loss of independence, for example, in the absence of an acute precipitant [28].

Clinical frailty is also associated with the presence of specific chronic diseases, particularly those with an inflammatory etiology, and the risk of frailty rises with the number of such diseases present [29]. While frailty incidence rises with increasing age, independently of ongoing chronic diseases, the association with the *subsequent* appearance of chronic diseases, including cardiovascular, kidney and rheumatologic diseases, suggests that there may be both a primary, aging-related phenotype of frailty, and a phenotype of frailty that is secondary to chronic disease. Further, new evidence indicates that obesity and aggregate risk for coronary artery disease in midlife predicts the development of pre-frailty and frailty 26 years later. Together these observations may implicate a shared etiology of aging (the process) and frailty (the clinical syndrome) [29].

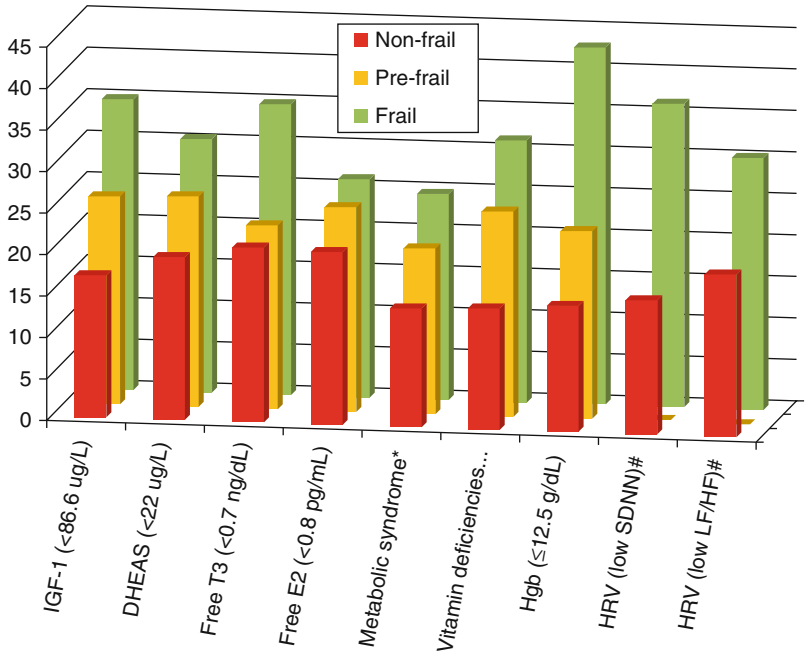

Fig. 1 Abnormal levels of biomarkers of multiple physiologic systems with frailty status (frail v. pre-frail vs. non-frail) in older adults. * Based on a 9-year incidence in the Cardiovascular Health Study. # Data only available for Frail versus non-frail. All other data from the Women's Health and Aging Study I and II, baseline (women 70–79 years old)

Although epidemiological research suggests that frailty is likely to cause an irreversible deterioration of health, there is some initial evidence that frailty can be prevented. For example, as shown by the InCHIANTI study, following a Mediterranean diet was protective against development of frailty as well as the development of cardiovascular diseases over a 6-year follow-up [30].

A large body of evidence indicates that frailty in all its clinical manifestations could be driven by a specific, although complex, pathophysiology that leads to dysregulation of multiple physiologic systems. Since aging is pervasive across the entire body, the more systems that are dysregulated, the greater the likelihood that the clinical manifestation is the result of accelerated aging or frailty rather than to specific disease. Longitudinal studies have found that dysregulation and loss of function tends to occur harmonically across multiple systems (Fig. 1), thus suggesting the existence of an underlying biological alteration, such as mitochondrial dysfunction, that may be altering energy availability and function for many systems. However, many of the systems affected (hormonal, inflammatory, muscle, anemia, autonomic) also mutually regulate each other, suggesting interrelatedness driving mutual dysregulation and consistent with one of the principles of a complex adaptive system. Further, the relationship goes beyond any particular dysregulated system: simply counting the number of systems dysregulated predicts the frailty

phenotype, and the risk increases exponentially with the number of physiological systems involved [31]. The latter is consistent with the fraying of a complex dynamical and adaptive system that is essential for a resilient and robust organism. In other terms, we can hypothesize that some fundamental housekeeping mechanism important for homeostasis and probably related to energetics becomes impaired and diminishes the functionality of important physiological systems at the organismal level. To generate frailty, the level of physiological impairment should be severe enough to impair other downstream compensatory mechanisms– towards a downward spiral that leads to the clinical presentation of frailty [23], an emergent state which tends be irreversible.

The alteration of specific physiologic functions may be involved in the vulnerability to adverse outcomes characteristic of frailty. For example, in the Women's Health and Aging Study II, women 85–95 years old underwent a glucose tolerance test (GTT) to evaluate glucose, insulin and metabolic responses to this physiological stressor [32, 33]. Women who were frail had a significantly greater increase in insulin and glucose during GTT than those not frail or pre-frail. Further, at 120 min after the oral glucose load, the frail old-old women had glucose levels 67 mg/dl higher than non-frail women (adjusting for age and BMI), and normalization of glucose back to baseline was delayed, compared to non-frail women. Further, frail women showed a pattern of elevation in glucose- raising hormones and decrease in glucose-lowering hormones not seen in the non-frail [33]. These findings indicate that the entire physiological network of signals that regulate glucose homeostasis tends to be altered in frailty. Interestingly, most of the genetic mutations that have been associated with longevity in animal models are related to the insulin/IGF-1 pathway, which is involved in carbohydrate metabolism. Importantly, the diminished regulation of physiological responses to a stressor identifies the frail. However, while the frail were highly dysregulated compared to pre-frail and non-frail individuals, dysregulation was a generalized state for these women 85 and older, with the difference being only one of degree between the non-frail, pre-frail and frail. Specifically, only 27 % of the entire group of old-old women evaluated in this study had normal fasting glucose and normal OGTT, while 71 and 78 % of the full group met criteria for prediabetes and diabetes, respectively [34]. This suggests that findings of frailty are at the more severe end of dysregulation associated broadly with aging, and that the dysregulation of aging is interpretable, in this case, as disease [4].

The findings summarized above suggest that some specific cellular alterations might be key to maintaining the robust complex dynamic system of the human organism which is essential for health and resilience. The case of mitochondrial dysfunction described above is just an example of the many potential mechanisms that could be involved. The true underlying mechanisms of biological alteration that lead to frailty, and aging itself, remain unknown. The existence of a common causal pathway between aging and frailty could explain why the prevalence of frailty increases geometrically with aging and why the criteria used to define the frailty syndrome clinically include dimensions, such as sarcopenia and mobility, that are strongly modified by aging in all individuals and across species.

4 Conclusion

The analogies between aging and frailty may also explain why the clinical manifestations, evolution, prognostic implications and response to treatment of many chronic diseases are substantially different according to the age of the affected individuals. The multisystem nature of frailty, and of aging itself, may further offer insights into why many trials of single-agent replacement therapies in older adults have failed to improve targeted health outcomes. Thus, an important area of future research is to determine the physiological links that explain such age-associated differences in the manifestations of chronic morbidity – incorporating appreciation of multisystem phenomena and underlying drivers - so that this knowledge can be inserted into new clinical guidelines for the diagnosis and treatment of a series of pathologies in older, complex patients. Knowledge of the change in disease mechanisms, as well as the development of multimorbidity and frailty in association with age, offer the framing for breakthroughs in these research areas.

In summary, a focus on disease that avoids considering the biological processes of aging can lead to misunderstanding of the full scope of disease etiology in older patients and miss new therapeutic opportunities. Considering aging illuminates the question of how it is that biologic drivers of disease differ in the old compared to the young. Ultimately, it is possible that, as our understanding of the biology of aging grows, a new chapter of geriatric medicine will open. Perhaps the next generation of precision medicine is a disruptive vessel through which to accomplish some of this goal. Precision medicine is an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person. The extension of this method to aging and frailty appears to be the natural evolution of this idea and one that synergizes well with the new impetus of the Geroscience initiative. Placing this information in the context of the full human population experience can lead to understanding of the place of aging itself and the generalizable import of processes observed.

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The Impact of Aging on Cancer Progression and Treatment

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Contents

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1 Introduction

In higher organisms, cancer reflects the cost of the need for long-lived self-renewing somatic stem cells in proliferative tissues functioning throughout the lifespan. Such cells are necessary for the constant production of new cells to replace damaged or shed effector cells, thereby maintaining tissue and organ homeostasis. Somatic stem cells and their replicating progeny exhibit a staggering capacity for proliferation, but they also can undergo malignant transformation. DNA mutations in these cells along with changes of their epigenetic state almost inevitably allow for the emergence of aberrant self-renewing clones on their way to full neoplastic conversion. Mutations occur in these self-renewing compartments as a result of exposure to external carcinogens and genotoxic agents, but also occur solely through bad luck; for example, as an error of DNA replication [1]. These mutations provide the finite number of genetic alterations required for malignant transformation. Given the daily production of immense numbers of new cells, it is actually remarkable that highly replicating tissues only very rarely undergo neoplastic conversion. The finding that oncogenic events that characterize malignancy are very common, even present at birth [2], whereas cancer is an unusual disease mainly affecting the elderly, demonstrates the existence of very effective tumor suppression mechanisms.

In this Chapter, we will discuss the molecular basis of cancer and aging, with particular emphasis on the mechanisms that associate increased incidence of cancer with aging. Specifically, we will discuss how time-dependent accumulation of genetic and epigenetic alterations in self-renewing cells as a result of imperfect homeostatic mechanisms can act as a common molecular basis for aging and cancer; how tumor suppression mechanisms that have evolved to prevent cancer can cause age-related functional attrition of self-renewing cells, which in turn contributes to certain aging-related pathologies; and how aging-associated physiological changes can contribute to cancer initiation and progression. From these discussions, we hope to identify preventive measures that can minimize the risk of cancer while slowing the rate of aging.

2 Molecular Basis of Cancer

Cancer can largely be considered a genetic disease. That is, aberrant expression of proteins that normally regulate cell growth and proliferation cause cancer, either by over- or under-expression of normal versions of cellular proteins, or expression of mutant proteins that acquire de novo oncogenic functions. These changes in protein expression largely arise from fixed DNA mutations that occur as a result of replication error, carcinogen exposure and other genotoxic stresses. Cancer-causing mutations are grouped into two classes: activating events on *oncogenes* and inactivating events on *tumor suppressor genes* (Table 1). Ample evidence suggests that both

Table 1 The role of oncogenes and tumor suppressors in cancer and aging

	Cellular function	Role in cancer	Role in aging
Oncogenes	↑Cell cycle ↑Cell growth ↑Survival ↓Differentiation ↓Apoptosis	Gain-of-function mutation "One-hit" model Commonly initiating event "Oncogene addiction"	Loss-of-function impairs stem cell maintenance (pro-aging) Gain-of-function enhances stem cell self-renewal potential (anti-aging), but can activate tumor-suppressor response to induce senescence or apoptosis, or lead to clonal dominance of stem cells with defective differentiation potential (pro-aging)
Gatekeeper Tumor Suppressor	↓Cell cycle ↑Differentiation ↑Apoptosis	Loss-of-function mutation "Two-hits" Model Inactivation is required for tumor maintenance	Loss-of-function enhances stem cell function (anti-aging) but may induce stem cell exhaustion (pro-aging) Aberrant activation impairs stem cell function (pro-aging), but physiologically regulated increase in gene dose can in some cases extend lifespan by preventing cancer.
Caretaker Tumor Suppressor	DNA repair Telomere maintenance ROS detoxification Epigenetic maintenance	Loss-of-function mutation "Two-hits" Model Inactivation promotes (epi-) genomic instability Inactivation is not required for tumor maintenance	Loss-of-function impairs stem cell maintenance (pro-aging) Gain-of-function effects on aging are currently unclear

classes of mutations are needed for full malignant transformation, and generally multiple (>5) such events are required in adult cancers.

2.1 Oncogenes

Oncogenes are genes whose protein products promote neoplastic transformation of normal cells. They are often mutated or over-expressed forms of normal cellular genes (sometimes termed "proto-oncogenes"), but can also be encoded by certain strains of oncogenic viruses and acquired by normal cells following viral infection (e.g. SV40 large T antigen). Cellular proto-oncogenes encode proteins that play essential roles in regulating cell growth, survival, proliferation and differentiation. As a result, many proto-oncogenes are important regulators of embryonic development [3, 4], while some are specifically required for somatic stem cell maintenance and tissue homeostasis in adult mammals [5]. Given their unique ability to regulate cell growth and survival, proto-oncogenes are the targets of oncogenic mutations.

Oncogenic mutations either increase the gene's normal activity, or confer de novo oncogenic function to the mutated genes. Upon activation, a proto-oncogene becomes an oncogene, gaining the ability to confer growth and survival advantage to normal cells and promote cancer development. Since only one copy of the proto-oncogene needs to be mutated to exert its oncogenic function, activating mutations of proto-oncogenes can follow the "one-hit" model and often occur early during cancer development.

Oncogenes can be activated in a number of ways. For example, the mutant protein product possessing enhanced enzymatic activity or resistance to degradation/inactivation can be generated as a result of DNA mutations in the coding region of the oncogene (e.g. BRAF-V600E). Alternatively, increased expression of the oncogene protein product can occur as a result of gene amplification or promoter mutation (e.g. TERT), or DNA mutations affecting transcription efficiency or transcript stability. Lastly, chromosome rearrangement events involving one or more oncogenes can generate fusion proteins that acquire increased transcript stability or de novo oncogenic function (e.g. BCR-ABL, MLL-AF9).

An important concern is whether a given oncogene contributes only to cancer initiation or is it also required for the continued survival and expansion of cancer cells (termed 'tumor maintenance' or 'oncogene addiction'). Inhibiting oncogenic pathways involved in tumor maintenance from cancer cells causes tumor regression through increased cell death and/or cell cycle arrest [6]. Clearly, oncogenes to which a cancer is 'addicted' make better targets for cancer therapy. For example, the development of inhibitors against mutant BRAF (Vemurafenib) or the BCR-ABL fusion protein kinase (Imatinib) have substantially improved the treatment of BRAF-mutant melanoma and Philadelphia chromosome-positive chronic myelogenous leukemia (CML) patients, respectively. However, as with any disease based on clonal evolution, drug resistance frequently emerges in cancer cells. Thus a better understanding of the molecular function of oncogenes and their normal cellular counterparts may help to identify cooperating pathways which, when inhibited, can cause synthetic lethality of the drug resistant cancer cells.

While oncogenes are best known for their roles in cancer, dysregulation in their activity may also contribute to aging under physiological or pathological conditions. Because many proto-oncogenes are critical regulators of somatic stem cell function and maintenance in adult tissues, insufficient proto-oncogene activity may contribute to age-related functional attrition of somatic stem cells and aging of self-renewing tissues. This is best illustrated by studies of telomerase reverse transcriptase (*TERT*). Telomerase is activated in human cancers through several genetic mechanisms and is critical to transformation in some tissues (e.g. melanocytes). Loss of telomerase activity with attendant telomere shortening and dysfunction causes attrition of certain self-renewing cells and the manifestation of certain aspects of aging (e.g. pulmonary fibrosis, hair greying, cirrhosis, and bone marrow failure) in humans [7]. Another potential example is EZH2, a histone methyltransferase that is activated in a variety of cancers including lymphoma, melanoma and certain forms of lung cancer. EZH2 is a critical mediator of stem cell epigenetic state and self-renewal capacity [8], and EZH2 inhibitors are a promising new class

of anti-cancer agent undergoing active clinical testing. However, a concern is that long-term use of EZH2 inhibitors may promote aging by augmenting somatic stem cell attrition.

A second way proto-oncogenes can contribute to aging is through the activation of tumor suppressor genes. Oncogene activation can trigger tumor suppressor responses in host cells, resulting in oncogene-induced apoptosis, senescence or differentiation. As activating mutations of oncogenes can accumulate in the stem cell pool over time, this can cause functional attrition of stem cells with age, resulting in reduced regenerative potential of aging tissues (discussed in detail in the next section).

A third and related way whereby oncogenic events can contribute to tissue aging is through the induction of advantaged clones that are defective for normal stem cell function. For example, work in human hematopoietic stem cells (HSCs) has shown that clones harboring a small number of oncogenic events may exhibit a modest increase in replicative capacity, in the absence of frank transformation [9–11]. In this case, these pre-neoplastic HSCs can out-compete normal HSCs, thereby "taking over" the bone marrow compartment. Although these pre-neoplastic HSCs may have an advantage in terms of proliferation and self-renewal compared to normal HSCs, they may also be defective in the production of differentiated blood elements, and thereby contribute to anemia and leukopenia, which are not uncommon features of hematopoietic aging in humans. It is likely that this type of stem cell competition resulting from oncogenic events that cause tissue dysplasia contributes to aging phenotypes in tissues beyond the bone marrow.

2.2 Tumor Suppressor Genes

Given the danger posed by stochastic oncogene activation or acquisition of viral oncogenes, mammalian cells are equipped with a variety of tumor suppression mechanisms to prevent and counter oncogene activation. These tasks are performed by so-called tumor suppressor genes. Tumor suppressors function to either prevent the emergence of neoplastic cells by maintaining genomic stability or to restrict the growth and proliferation of already damaged cells. For malignant transformation to succeed, tumor suppressor genes must be inactivated either by DNA mutations or epigenetic silencing. As both copies of tumor suppressor genes usually need to be inactivated to abolish their function, mutations of tumor suppressor genes in cancer cells often follow the "two-hit" model. There are some exceptions, as tumor suppressor genes can be 'haploinsufficient', meaning the loss of a single copy of the gene is sufficient to confer growth advantage to mutant cells; in other cases, mutation of the tumor suppressor proteins can create dominant negative or a gain-of-function form of the protein that is sufficient to drive neoplastic transformation. In these cases, mutations of tumor suppressor genes can act as an initiating event in malignant transformation.

Tumor suppressor genes have been functionally divided into at least two major categories (Table 1). One group of tumor suppressor genes are constitutive

housekeeping genes that act as "caretakers" in the cells to maintain genomic and epigenetic stability thereby minimizing the risk of oncogene activation. Examples of this are genes such as *BRCA1* or *ATM* whose protein products prevent cancer by promoting genomic stability. In contrast, a second group of tumor suppressor genes are thought to be activated by characteristic features of early neoplasms, such as DNA damage or uncontrolled mitogenic signaling. Such tumor suppressor genes have been termed "gatekeepers" whose function is to restrict aberrant cell growth and proliferation [12]. While this classic distinction has intellectual appeal, limitations of this conceptual framework have emerged with a modern understanding of the cellular mechanisms of tumor suppression. For example, certain tumor suppressor genes such as *TP53* perform both caretaker and gatekeeper types of functions. Moreover, several recently appreciated tumor suppressor genes (e.g. *LKB1* or *PTEN*) are potent regulators of intracellular signaling, and their loss augments metabolism and growth in a way that does not obviously fit into either category. These limitations notwithstanding, we will discuss these tumor suppressor mechanisms and their relation to aging as grouped through this framework.

2.2.1 Caretaker Tumor Suppressor Genes

Caretaker tumor suppressors act to maintain genomic integrity or epigenetic stability, and do not directly regulate cell growth or proliferation [13]. The best characterized examples of caretaker tumor suppressors are genes involved in DNA damage response and repair.

DNA lesions occur constantly in all cells as a result of the intrinsic chemical instability of DNA, errors in DNA replication, or exposure to cell intrinsic (such as reactive oxygen species (ROS) generated from cellular metabolism) or extrinsic genotoxic agents (such as UV, radiation or chemical carcinogens). To deal with the many types of DNA lesions, mammalian cells have evolved diverse DNA repair mechanisms. When the lesion only occurs on one strand of the DNA double helix, it can be faithfully repaired using information from the complementary strand through mechanisms such as base excision repair (BER) or nucleotide excision repair (NER). In the case of DNA double strand breaks (DSBs), the lesions can either be repaired by the homologous recombination (HR) mechanism that uses the information from the homologous sister chromatid to faithfully repair the damage, or by the more efficient but error-prone non-homologous end joining (NHEJ) mechanism. In each case, the cell detects DNA lesions through sensor proteins, which recruit the appropriate DNA repair machineries; if the lesions occur during the cell cycle, additional checkpoint proteins are activated to arrest the cell cycle, allowing enough time to repair the lesion before resuming division. If the repair is not successful, more familiar gatekeeper tumor suppressors such as $p16^{INK4a}$ and p53 are activated to induce programmed cell death (apoptosis) or permanent cell cycle arrest (e.g. cellular senescence or terminal differentiation) to prevent the damaged cells from further propagation.

Given their critical role in maintaining genomic integrity, mutations in DNA repair genes are frequently associated with an increased incidence of cancer [14].

For example, women with a single copy of a germline mutation in *BRCA1* or *BRCA2*, which contribute to HR-mediated DSB repair, are at significantly increased risk of developing cancers of the breast, ovary and other tissues; while patients with *ATM* inactivation are most predisposed to leukemia and lymphoma. Interestingly, many congenital DNA repair deficiency diseases show a varying degree of accelerated aging in addition to their cancer-prone phenotype [13, 14]. Likewise, with the prominent exception of Hutchinson-Gilford progeria, nearly all the genetic segmental progerias result from mutations in genes associated with DNA metabolism (e.g. WRN), and generally lead to a cancer-prone phenotype. This relationship between DNA metabolism and aging is not entirely due to the secondary effects of cancer as patients of DNA repair deficiency diseases such as Cockayne Syndrome and Trichothiodystrophy do not have increased risk of cancer while still exhibiting some features of premature aging [13]. Likewise, external exposures that cause DNA damage also induce aspects of premature aging, providing an additional link between DNA metabolism and life-long somatic stem cell function. These observations, together with the findings that DNA repair genes are essential for maintaining long-term self-renewal potential of somatic stem cells throughout life at least in mouse [15], suggest DNA repair genes are not only essential for preventing neoplastic transformation, but may also play a role in regulating the rate of physiological aging. It is however unclear whether enhancing the activities of caretaker genes could delay the onset of stem cell aging.

While mutations of DNA repair genes can promote cancer initiation and clonal evolution, their continued inactivation is generally not required for cancer cell growth and proliferation. In fact, due to frequent defects in various DNA repair mechanisms, many types of cancer cells are critically dependent on specific DNA repair pathways for survival. This cancer cell specific dependency can be exploited therapeutically to target cancer cells without harming normal cells with a wholly intact repertoire of DNA repair activities [16, 17]. For example, cancer cells with *BRCA1/2* gene mutations are defective in the repair of double strand breaks via homologous recombination, and treating these cells with PARP1 inhibitors that cause accumulation of single strand breaks, which are later converted into DSBs, results in synthetic lethality in the mutant cells but not wild type cells [18, 19]. These findings of in vitro synthetic lethality in turn translate into significant clinical antitumor activity of PARP inhibitors in *BRCA1/2*-deficient malignancies. Several other defects in DNA repair and/or DNA damage-induced checkpoints are exploited therapeutically to allow for enhanced killing of cancer cells versus non-malignant tissues.

DNA repair genes are not the only caretaker tumor suppressors; genes involved in telomere maintenance, reactive oxygen species (ROS) detoxification and epigenetic modification are often also characterized as caretaker genes due to their ability to maintain genomic and epigenetic stability of the cell. However, their roles in cancer are more context-dependent. For example, telomerase is essential for maintaining genomic integrity by protecting chromosomes from fusing to each other, therefore acting as a caretaker against neoplastic transformation. However, several lines of data now show that telomerase activity is also oncogenic, presumably by enabling the immortalization of neoplastic cells. Similarly, while DNA and

histone modification enzymes are important for maintaining the integrity of epigenetic information in the cell, aberrant activation of these genes is an emerging theme now noted in many types of cancer. A final example is the surprising role of NRF2 in squamous cancers of the aerodigestive tract. NRF2 regulates many genes involved in the detoxification of ROS. Excess ROS has traditionally been considered oncogenic, through effects on intra-cellular signaling as well as by inducing macromolecular damage. Large tumor sequencing studies such as the Cancer Genome Atlas, however, have firmly established that excess NRF2 activity is potently oncogenic in squamous cancers of the lung, oropharynx and esophagus, challenging the long held view of ROS as being oncogenic [20]. It is perhaps not a coincidence that the notion that ROS promotes physiological aging, as in the "free radical theory of aging", is also being seriously questioned in modern gerontology [21, 22]. Therefore, unlike gatekeeper tumor suppressors (discussed next), the activity of caretaker tumor suppressors does not directly inhibit cancer cell growth, and depending on the cellular context, can actually promote cancer progression.

2.2.2 Gatekeeper Tumor Suppressor Genes

Gatekeeper tumor suppressor genes are negative regulators of cell proliferation. Unlike the caretaker genes, gatekeeper genes are generally not essential for routine cellular function; instead they are only activated upon sensing persistent cellular stress such as chronic DNA damage or oncogene activation.

Perhaps the best known gatekeeper tumor suppressor gene is *TP53*, the so-called "guardian of the genome". *TP53* is the most highly mutated gene across all human cancers, and mice with heterozygous or homozygous loss of *TP53* are highly prone to sporadic cancers. *TP53* encodes a multifunctional transcription factor (p53) that, in response to a variety of stresses, controls the expression of genes involved in a wide range of cellular activities including DNA damage repair, cell cycle control, apoptosis and autophagy. Critically shortened telomeres and double strand DNA breaks can activate p53 through the checkpoint kinases ATM and CHK2, which then upregulate the expression of the cyclin-dependent kinase (CDK) inhibitor $p21^{CIP1}$ to induce cell cycle arrest to facilitate DNA repair. Moreover, p53 can also respond, in a poorly understood manner, to excess mitogenic signaling (e.g. "oncogenic stress") mainly through activation of the ARF (a.k.a. $p19^{ARF}$ in mouse or $p14^{ARF}$ in human) tumor suppressor protein (Fig. 2). ARF is encoded by the *CDKN2A* locus, which also encodes another important gatekeeper tumor suppressor, $p16^{INK4a}$, and *CDKN2A* expression is induced by a wide-variety of oncogenic signals [23, 24]. ARF binds to and inhibits the activity of MDM2, a potent negative regulator of p53, thereby leading to p53 protein stabilization and p53-mediated cell cycle arrest or apoptosis. Similar to p53, mice lacking one or both alleles of *Arf* are cancer-prone, and deletion of *ARF* is common in human malignancies. The cellular response to p53 activation depends on the nature and duration of the cellular stress, as well as the cellular context [25]. While strong, persistent signals are likely to induce apoptosis or senescence; low intensity, transient signals tend to induce reversible cell cycle arrest [26].

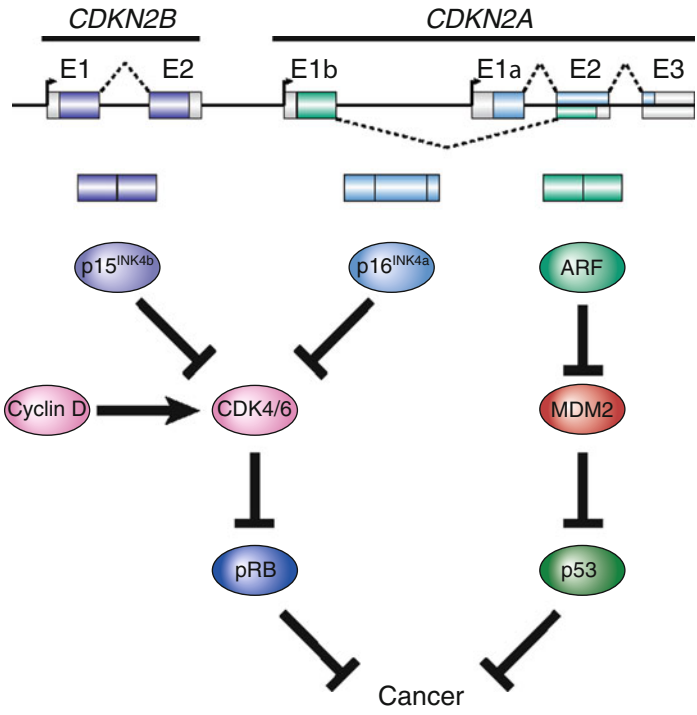

Fig. 1 The $p16^{INK4a}$-RB and ARF-p53 tumor suppressor pathways. The *CDKN2A/B* locus on human chromosome 9p21 encodes three archetypical tumor suppressor proteins: $p16^{INK4a}$, ARF and $p15^{INK4b}$. $p16^{INK4a}$ and ARF share common second and third exons, but are transcribed from separate promoters and first exons in alternative reading frames, thus yielding different proteins with distinct cellular functions. $p16^{INK4a}$ and $p15^{INK4b}$ belong to the INK4 family of cyclin dependent kinase inhibitors, which bind to CDK4/6 and inhibit the formation of cyclin D-CDK4/6 holoenzyme, leading to the stabilization of the RB family transcription factors and blocking cell cycle entry. ARF binds to and inhibits the activity of MDM2, a potent negative regulator of p53, leading to p53 protein stabilization. Activation of the $p16^{INK4a}$-RB and ARF-p53 pathways upon oncogenic stress or persistent DNA damage can lead to cellular senescence or apoptosis, which serve as important tumor suppression mechanisms to prevent malignant transformation

A second group of important gatekeeper tumor suppressors involves the control of mammalian cell cycle progression, including the Retinoblastoma (RB) family proteins and several types of cyclin-dependent kinase inhibitors (Fig. 1). RB family proteins (RB, p107, and p130) are important regulators of the G1-S cell cycle transition, and they play a redundant role in binding and inhibiting the activity of E2F family transcription factors, which control the expression of genes important for DNA synthesis and cell division. Among the RB-family proteins, RB in particular is important for the induction of cellular senescence and for tumor suppression [27, 28]. RB protein activity is regulated by various cyclin/CDK protein complexes. Upon sensing mitogenic signals, activated cyclin-CDK complexes promote cell cycle entry by phosphorylating and inactivating RB proteins. The activity of the

cyclin D-CDK4/6 complex is regulated by the INK4 family proteins (including $p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$ and $p19^{INK4d}$), which bind to CDK4/6 and inhibit the formation of cyclin D-CDK4/6 holoenzyme [29]. While the INK4 family proteins appear to be structurally similar, mouse genetic models and mutational studies in human cancers suggest $p16^{INK4a}$, and to a lesser extent $p15^{INK4b}$, carry out the most important tumor suppression functions. This may be due to the different expression pattern and activation mechanism of each INK4 protein.

As mentioned, $p16^{INK4a}$ and ARF are encoded by the *CDKN2A* locus located on human chromosome 9p21 (Fig. 1). Their open reading frames share two exons translated in alternate reading frames, as well as different first exons and promoters. The *CDKN2A* locus is the most frequent site of cytogenetic deletion in human cancers, and point mutation or epigenetic silencing of the locus is also well-described. For the most part, $p16^{INK4a}$ and ARF are not expressed by normal proliferating cells in vivo, and are largely dispensable for normal development (with the exception of a role for Arf in eye development). Mice lacking both $p16^{INK4a}$ and *Arf* demonstrate normal development, but are highly tumor prone, even more so than animals lacking only $p16^{INK4a}$ or only *Arf*. Although these genes do not participate in normal cell cycle control, they are highly activated, albeit with delayed kinetics (1+ weeks), by a variety of oncogenic stimuli including in vitro culture, excess mitogenic signaling, increased cell density, disordered chromatin structure and many noxious stimuli (e.g. UV light and ionizing irradiation). The precise origin and nature of the signals that induce $p16^{INK4a}$ expression in vivo are poorly understood, and can be both intra-cellular and extra-cellular. For example, we recently showed that the presence of a small number of neoplastic cells within a given tissue will rapidly induce $p16^{INK4a}$ expression in nearby non-malignant cells [30]. Unlike $p21^{CIP1}$, $p16^{INK4a}$ is not acutely induced by DNA damage and does not play a role in the initial DNA damage-induced cell cycle arrest. Instead, the main function of $p16^{INK4a}$ appears to be to initiate and/or maintain a highly durable if not permanent cell cycle arrest (called 'cellular senescence') to prevent malignant transformation. In addition to the INK4 family proteins, another group of CDK inhibitors, the CIP/KIP family proteins (including $p21^{CIP1}$, $p27^{KIP1}$ and $p57^{KIP2}$), also represses the cell cycle by inhibiting the activity of the cyclin E-CDK2 complex. Their role in tumor suppression appears to be more limited. With the exception of bladder cancer where $p21^{CIP1}$ mutation is seen in 15 % of tumors, mutations of these proteins are unusual in most human malignancies, and mice deficient for $p21^{CIP1}$ or $p27^{KIP1}$ only exhibit a mildly increased risk of cancer.

Some evidence suggests the ARF-p53 and $p16^{INK4a}$-Rb tumor suppressor pathways are inactivated, through a wide variety of mechanisms, in almost all human cancers. Unlike caretaker tumor suppressors, the continued inactivation of gatekeeper tumor suppressors is required for cancer cell survival and proliferation, as re-expression of key gatekeeper genes such as p53 is sufficient to cause cancer regression [31, 32]. Activation of these tumor suppressor pathways may contribute to the therapeutic response to certain classes of anti-cancer agents; e.g. p53-mediated apoptosis may be important in the response to certain DNA damaging chemotherapy drugs. To date, however, re-activation or re-introduction of tumor suppressor

genes in established cancers has proven technically difficult, and is currently not achievable in most cancers.

In addition to its critical role in cancer prevention, there is a large literature linking p53 with physiological aging (reviewed in [33]), but this relationship is complex. For example, as a critical regulator of genomic stability, p53 has been suggested to play a critical anti-aging role by minimizing the accumulation of macromolecular damage. Alternatively, excess p53 activation has been suggested to be age-promoting, perhaps through the induction of decreased function in somatic stem cell compartments. In support of this notion, mice expressing constitutively activated p53 exhibit a premature aging phenotype and have reduced life spans [34, 35], and hematopoietic stem cells isolated from these mice are functionally deficient in competitive transplantation settings [36]. However, mice engineered to carry an extra copy of the entire *Cdkn2a* locus and/or *p53*, whose activities are under physiological regulation, exhibit enhanced cancer resistance without detectable negative effects on aging and lifespan [37–39]. Therefore, excess, unbridled p53 may promote aging, whereas physiological p53 activation is likely anti-aging.

There are even stronger data linking $p16^{INK4a}$, and to a lesser degree ARF, to mammalian aging. The expression of both products of the *CDKN2a* locus increases exponentially with aging in all mammalian species studied [40–42], and this is thought to represent the accumulation of senescent cells with aging (covered below). The deletion of $p16^{INK4a}$ in mice appears to partially rescue the functional defects of old somatic stem cells in the nervous and hematopoietic system [43, 44], as well as improve the regenerative potential of aged lymphocytes and pancreatic beta cells [45–48]. Therefore, activation of $p16^{INK4a}$ appears to lead to a reduced replicative capacity in several somatic, self-renewing compartments, with an attendant decrease in physiological reserve. Alternatively, $p16^{INK4a}$ expression also appears to restrain forms of excess proliferation, malignant and non-malignant, that are pathogenic. For example, aberrant macrophage proliferation in atherogenic plaques appears to be restrained by $p16^{INK4a}$ and Arf expression, with reduced atherogenesis [49]. Beyond a wide range of murine studies supporting these relationships, there are also compelling genome-wide unbiased evidence for this model in humans. Genome wide association studies (GWAS) have linked regulation of the *CDKN2A* locus to many age-associated conditions such as cancer susceptibility, stroke, myocardial infarction, aortic aneurysm, glaucoma, and type 2 diabetes [50]. For cancer and atherosclerotic disease, it appears increased expression of *CDKN2A/B* transcripts is protective, but it is believed that excess expression of these transcripts may alternatively underlie the susceptibility to some of the other linked phenotypes.

2.2.3 Other Important Tumor Suppressors

As mentioned, there are additional genes that perform important tumor suppressor functions, but which do not fit neatly into either the caretaker or gatekeeper category. The Phosphatase and Tensin homolog (PTEN) protein converts phosphatidylinositol (3,4,5)-trisphosphate to phosphatidylinositol (4,5)-bisphosphate to

inhibit downstream AKT signaling, thereby negatively regulating cell growth and proliferation [51]. PTEN mutations are frequently found in human brain and epithelial cancers, and mice engineered to delete *Pten* in somatic cells are cancer-prone. The human *STK11/LKB1* gene encodes a serine/threonine kinase that regulates energy metabolism and cell polarity through activation of a wide range of downstream kinases including the adenine monophosphate-activated protein kinase (AMPK) family of kinases. Somatic mutations of *LKB1* are found in a large fraction of human lung adenocarcinomas and cutaneous melanomas, and murine studies suggest loss of *LKB1* promotes cancer development, with a particularly strong association with distant metastases [52, 53]. The *NF1* gene encodes neurofibromin, a RAS GTPase-activating protein that negatively regulates RAS signaling. Loss of *NF1* causes hyperactive RAS signaling, leading to enhanced growth and decreased apoptosis of the mutant cell. Germ line mutation of *NF1* causes the tumor predisposition syndrome neurofibromatosis type I in humans, and somatic mutations of *NF1* are found in a wide range of human cancers. In all cases, these genes function as negative regulators of growth factor signaling and clearly act as tumor suppressors. Certainly the pathways regulated by these proteins, e.g. cellular growth and metabolism, have been linked to physiological aging through a variety of approaches, but the role of these specific proteins in human aging is unclear.

3 Cellular Mechanisms for Tumor Suppression

Upon detecting oncogenic stress, a proliferating cell can activate three major cellular tumor suppression mechanisms to prevent malignant transformation: permanent cell cycle arrest (cellular senescence), programmed cell death (e.g. through apoptosis, necroptosis or autophagy), or terminal differentiation (in the case of somatic stem and progenitor cells) (Fig. 2). Although much is known about the respective molecular biology of these processes, under which circumstances a damaged cell chooses each of these potential fates is not well-understood. Failure to activate the tumor suppression mechanisms, however, is a cardinal feature of all cancers.

3.1 Cellular Senescence

Among cellular tumor suppression mechanisms, the most important appears to be "cellular senescence", an irreversible form of cell cycle arrest, which is also intimately associated with aging. Senescence occurs in response to cellular stresses and displays features that distinguish it from other forms of growth arrest such as "quiescence" or "terminal differentiation" (reviewed in [54]). Several lines of evidence suggest that the senescence mechanism has beneficial features such as preventing cancer as well as non-neoplastic diseases characterized by pathogenic proliferation, and may also play an important role in wound healing. Importantly, however,

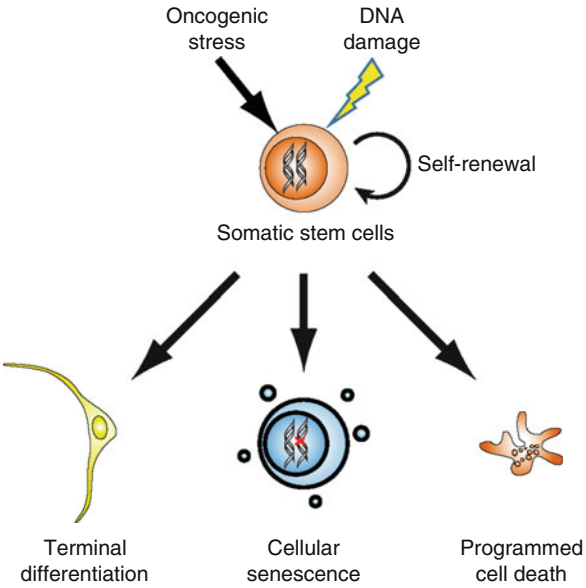

Fig. 2 Cellular tumor suppression mechanisms. Upon sensing oncogenic stress or persistent DNA damage, somatic stem cells and proliferating progenitors can activate three major cellular tumor suppression mechanisms to prevent malignant transformation: terminal differentiation, permanent cell cycle arrest (cellular senescence), or programmed cell death (e.g. through apoptosis, necroptosis, or autophagy). The choice to activate which specific mechanism is cell type and developmental stage dependent, and can lead to distinct physiological consequences

significant human and murine data suggest that senescence plays a causal role in some aspects of aging.

With regard to tumor suppression, senescence appears to be more critical than apoptosis or other forms of regulated cell death such as Autophagy. Mice with even mild defects in senescence activation are highly tumor-prone. In contrast, animals with near-complete deficiency in apoptosis (e.g. lacking Bax and Bak) display several developmental phenotypes and excess numbers of certain classes of hematopoietic cells, but are not generally cancer-prone [55]. In humans, unbiased genome-wide screens have demonstrated that inactivation of the senescence-promoting *CDKN2A* and *TP53* loci are common in familial cancer syndromes, and the Cancer Genome Atlas has established that loss of $p16^{INK4a}$ and p53 functions are the most common somatic genetic events in human cancers, with possibly all tumors harboring one or multiple events targeting senescence (reviewed in [56, 57]). Even for a gene like *TP53*, which modulates both apoptosis and senescence, the available data suggest that its ability to promote cell cycle arrest is considerably more important to its ability to repress tumors than its effects on cell death [58, 59]. Therefore, not only is p53- and $p16^{INK4a}$–mediated cell cycle arrest (e.g. senescence) important for preventing tumorigenesis, it appears to have evolved in mammals to be the *most*

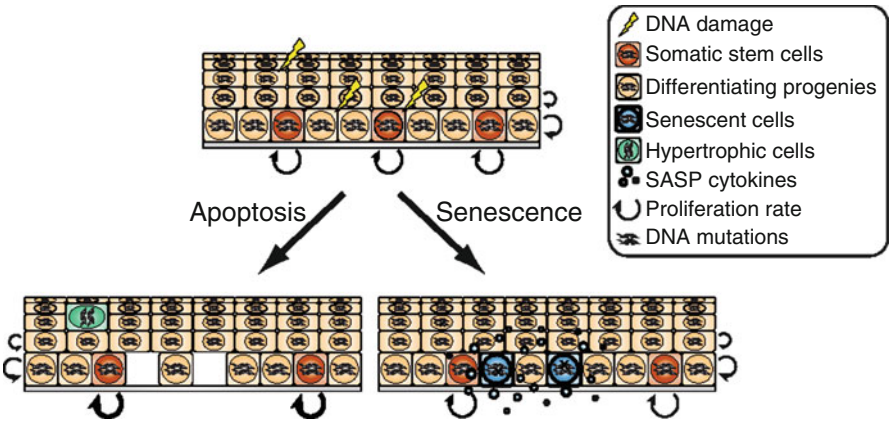

Fig. 3 Problems of Apoptosis vs. Senescence. While both are effective at preventing damaged cells from further propagation, programmed cell death (apoptosis) and cellular senescence have distinct advantages and disadvantages as tumor suppression mechanisms. The major advantage of apoptosis is that it irrevocably removes the damaged cells in question, however, this can leave "holes" in the tissue that need to be filled either by hypertrophy of remaining cells or increased proliferation of somatic stem and/or progenitor cells, which can compromise stem cell or tissue function. In contrast, while cellular senescence may have the advantage of preserving semi-functional cells after damage thus requiring less compensatory proliferation of the stem and progenitor cells, senescent cells can secrete harmful cytokines that interfere with normal tissue function, and senescent stem cells may occupy limited "niche" space leading to decreased overall function of the stem cell pool

critical anti-cancer mechanism. This fact suggests that senescence possesses some evolutionary advantage over apoptosis as a tumor suppressor mechanism.

One potential explanation for this observation is teleological: senescence did not evolve to suppress tumors, but rather was perfected through evolution to enhance species fitness by playing a role in physiologic processes such as the stress response or wound healing. By this argument, tumor suppression is merely a collateral benefit of the senescence mechanism, in the same way that decreased cancer is a benefit of more faithful DNA repair. This argument may be correct, but it has been hard to demonstrate what other function certain components of the senescence machinery (e.g. $p16^{INK4a}$) perform beyond limiting aberrant proliferation. For example, mice (or even in some rare cases, humans [60]) lacking $p16^{INK4a}$ appear phenotypically normal until the development of a wide variety of malignancies at an early age. So, even if the senescence response developed for additional reasons, the main phenotypic readout in mammals appears to be tumor predisposition.

Another possible reason for an evolutionary preference of senescence over apoptosis to censor tumors might be that apoptosis can produce new problems (Fig. 3). Since organ size and function of many tissues is regulated throughout the mammalian lifespan, a damaged cell censored by apoptosis would leave a niche "hole" that would then be filled either by hypertrophy of the remaining cells, or through increased proliferation of somatic stem cells. Hypertrophy is associated with tissue dysfunction in

many organs (e.g. the heart), and would not solve the problem where diversity through cell number is important (e.g. the cellular immune system). Additional replication, however, may be undesirable as S-phase and mitosis provide opportunities for further errors of replication. Furthermore, the loss of certain cells to apoptosis in response to cellular stresses might, in particular, be functionally non-advantageous. For example, senescence, not apoptosis, is clearly the principal barrier to melanocyte transformation. As the primary function of melanocytes is to provide protection from damaging UV light through the synthesis of melanin, it could be evolutionarily undesirable for melanocytes, damaged as a result of UV exposure, to undergo apoptosis. Melanocyte senescence leaves a semi-functional, possibly protective melanocyte while diminishing the risk of malignant transformation. Therefore, senescence may be evolutionarily preferable to apoptosis as it may lead to a reduced lifetime proliferative burden on stem cells by leaving resident cells able to perform some desirable functions of related non-senescent cells (e.g. melanin synthesis). If correct, this model predicts that the clearance of senescence cells, for example through the use of 'senolytic' small molecules selectively toxic to senescent cells, might have unwanted toxicities. In contrast to this line of reasoning, Van Deursen and colleagues have reported that a genetic depletion of senescent cells is beneficial with regard to some aspects of aging in a strain of progeroid mice [61], although these experiments did not extend longevity or address the role of senescent cells in physiological aging. Ongoing experiments will address the consequences of senescence ablation in an aged individual.

3.2 Programmed Cell Death

As a tumor suppression mechanism, the major advantage of programmed cell death (such as apoptosis) over cellular senescence, is that it irrevocably removes damaged cells, thereby eliminating the potential for detrimental mutations to further propagate. This may be desirable under certain cellular contexts; for example, during early embryonic development it may be advantageous to eliminate cells with DNA damage instead of attempting repair, to prevent mutations from being transmitted into a large population of the cells. A similar situation may also apply to germ cells where the maintenance of genomic integrity is of utmost importance. Consistent with this notion, embryonic stem cells, early developing embryos and germ cells are all exquisitely sensitive to ionizing radiation-induced DNA damage, and they rapidly induce apoptosis without undergoing cell cycle arrest and DNA repair [62, 63]. These observations support the hypothesis that apoptosis may have evolved primarily as a mechanism to safeguard the genomic integrity against genotoxic stress, and the modest tumor suppression afforded by apoptosis in some tissues is only a collateral benefit. Nonetheless, apoptosis does play important roles in tumor suppression. Somatic mutations that affect genes regulating cell survival and apoptosis have been found in human cancers, particularly in tissues that have high intrinsic sensitivity to apoptosis such as lymphocytes; and pharmacological or biological means to induce cancer cell apoptosis are important therapeutic strategies to treat cancer.

3.3 Terminal Differentiation

Terminal differentiation is another form of permanent cell cycle arrest distinct from cellular senescence in that it is a developmentally programmed process for stem cells to generate normal effector cells, whereas senescence is stochastically induced by cellular stresses that distort homeostasis. The notion that terminal differentiation prevents oncogenic transformation is not new, as it has been demonstrated that the tumor suppressor protein APC prevents colon cancer by balancing the self-renewal and differentiation of intestinal stem cells through the regulation of cellular beta-catenin levels. However, the activity of APC is constitutive and not influenced by oncogenic stimuli. The first evidence suggesting terminal differentiation may act as a checkpoint against oncogenic stress comes from the finding that oncogene activation can induce differentiation. Over-expression of the *MYC* oncogene in the epidermis results in a long-term depletion of stem cells while accumulating differentiated keratinocytes [64]. Later, it was shown that DNA damage by ionizing radiation can directly trigger the differentiation of melanocyte stem cells and hematopoietic stem cells [65, 66]. These observations support the hypothesis that induced differentiation may be a conserved checkpoint mechanism to limit the self-renewal potential of somatic stem cells upon DNA damage or oncogene activation to prevent neoplastic transformation. However, it remains to be determined whether this mechanism applies to additional stem cell populations, and whether any cancer develops as a result of escaping the differentiation checkpoint mechanism.

4 Oncogenic Mutations Require a Proper Cellular Context for Malignant Transformation

While activating mutations of oncogenes and inactivating mutations of tumor suppressors are required by nearly every cancer, the particular genes that are preferentially mutated in each type of cancer are highly cancer-type specific. This suggests the non-genetic cellular context, that is the cellular epigenetic state, also plays a critical role in the impact of oncogenic mutations on cell biology. For example, activating mutations of the *H-RAS* oncogene cannot promote cancer in cells that do not express the *H-RAS* mRNA. Likewise, mutations that occur in replication incompetent cells (e.g. that are terminally differentiated) also do not drive oncogenesis. More subtly however, there are many examples in which the oncogenic potential of a mutant protein is restricted to a specific cell type or a particular developmental stage. For example, the loss of the VHL tumor suppressor is largely restricted to one type of advanced human cancer: renal carcinoma. VHL plays an important role in oxygen sensing, and VHL-inactivated cells act as if they are hypoxic. This observation can be interpreted as a demonstration that hypoxia does not promote cellular growth in most contexts, but is a stimulus to grow and divide in specific renal cells. Therefore, while cancer is a genetic disease, the specific cellular context determines the ability of oncogenic events to drive particular types of cancers.

5 How Does Cancer Relate to Aging?

Broadly defined, aging is the process of age-dependent decline in body function and increase in mortality that affect most living organisms. Aging is not a disease, rather, it is a collection of symptoms that reflect progressively decreased cellular function and disrupted tissue homeostasis over time. On the cellular level, aging is characterized by the time-dependent functional attrition of non-dividing (terminally differentiated) cells and impairment in somatic stem cell self-renewal and differentiation as a result of cell intrinsic and extrinsic stresses. These lead to impaired tissue homeostasis, disrupted tissue architecture, reduced organ function and altered energy metabolism, all of which contribute to the reduced fitness of aged organisms. Since aging is not caused by a single factor, no single intervention can be expected to treat all aging symptoms. The process of aging is not restricted to the aged. Mammalian cells experience DNA damage and other forms of age-promoting stresses on a daily basis. We are equipped with sophisticated repair mechanisms to deal with these challenges. Only when the extent of cellular and tissue damage exceeds the ability of our body to cope with these forms of damage do the symptoms of aging start to appear. Therefore, while completely preventing or reversing aging may be unlikely, minimizing these forms of daily damage may slow the rate of aging, while also reducing the incidence of cancer.

5.1 Aging as a Result of Time-Dependent Accumulation of Cellular Damage

One prominent feature of aging is the age-dependent accumulation of damage in various cellular components. Terminally differentiated cells such as neurons are sensitive to the accumulation of damaged lipids and proteins over time, largely due to their inability to dilute such molecules through successive cell divisions. In contrast, accumulation of genetic damage, including unrepaired DNA damage, is particularly detrimental to the function of long-term self-renewing cells including somatic stem cells. It is unclear if increases in macromolecular damage that are characteristic of aging solely reflect a lifetime's accumulation of toxic events, or also result from a reduced capacity to clear or repair damaged proteins and DNA with aging.

DNA damage impairs somatic stem cell function in several ways: it may lead to the generation of defective proteins that directly interfere with cellular function, or it may induce tumor suppression mechanisms as a result of persistent DNA damage or oncogene activation, which then limit the self-renewal potential of somatic stem cells through mechanisms such as senescence, cell death or differentiation. Therefore, the efficiency of somatic stem cells to successfully repair various types of DNA damage is a critical determinant of the rate of aging. As mentioned, several human progeroid syndromes (e.g. Bloom, Werner, Cockayne, Fanconi Anemia, etc.) are associated with mutations in DNA repair

genes [67]. Telomere dysfunction is a specialized form of DNA damage, and mice deficient in telomerase have reduced somatic stem cells function and exhibit features of premature aging [15].

Therefore, despite their striking differences in presentation, aging and cancer seem to share a common origin: that is, the time-dependent accumulation of cellular macromolecular damage. When oncogenic mutations occur in a dividing cell as the result of DNA damage, the outcomes between cancer and aging depend on whether tumor suppression mechanisms can be successfully activated to prevent the damaged cell from further propagation: cancer arises when tumor suppression mechanisms fail, while aging results, at least partially, from the progressive functional attrition of self-renewing cells due to tumor suppression.

5.2 How Is Cellular Senescence Related to Aging?

Cellular senescence is thought to promote aging by reducing the regenerative potential of self-renewing cells and/or by leading to the production of detrimental cytokines and other biomolecules; however, some components of the senescence machinery (e.g. $p16^{INK4a}$) also appears to play a beneficial anti-aging role in some tissues by preventing the development of certain aging-associated pathologies (such as cancer and atherosclerosis). Therefore, while it may seem attractive to attempt to delay aging by attenuating tumor suppressor function or clearing senescent cells, more research is required on this topic to prevent unintended consequences.

5.2.1 Adverse Effects of Senescence with Regards to Aging: Loss of Self-Renewal

Although the expression of senescence markers is associated with aging, this observation does not establish a causal relationship between senescence and the loss of tissue replicative capacity associated with aging. To address this issue, a number of studies have been performed examining the effect of genetic ablation or overexpression of $p16^{INK4a}$ in self-renewing tissues and cell types (e.g. beta-cells, neural stem cells, HSCs, lymphocytes) on aging and age-related stresses. In mice, these studies have consistently shown that loss of $p16^{INK4a}$ can partially ameliorate the loss of tissue-specific replicative capacity with aging [43–48, 68], indicating that increasing levels of $p16^{INK4a}$ are not only associated with aging, but in part play a causal role in these tissues. In each of these compartments, $p16^{INK4a}$ deficiency attenuated the decline in proliferation and function as a function of advancing age, and the effects of $p16^{INK4a}$ loss are consistent across disparate self-renewing tissues. In no organ or tissue studied, however, does $p16^{INK4a}$ loss completely abrogate the effects of aging, indicating that $p16^{INK4a}$-independent aging also occurs in these compartments. Moreover, tissue specific

ablation of $p16^{INK4a}$ in B-lymphocytes, as opposed to T-lymphocytes, was strongly associated with the development of cancer [46], making explicit the organismal costs of diminished $p16^{INK4a}$ function in some tissues.

Related observations have likewise suggested that activation of p53 or its effectors may compromise self-renewal to promote aging in mice [34, 35, 69] and humans [70]. The case for p53, however, is more complicated because it and its downstream effectors such as $p21^{CIP1}$ play important roles in regulating the DNA damage response, explaining why moderately increased gene dosage of *Cdkn2a* and *p53* in mice is associated with reduced aging [35, 37]. Additionally, HSCs from $p21^{CIP1}$–deficient mice demonstrate premature exhaustion [71], consistent with the notion that a p53- and $p21^{CIP1}$-dependent cell cycle pause in response to DNA damage or other stressors may be important for enhanced self-renewal and stem cell longevity in vivo. These results suggest that p53 activation can be both pro-aging and anti-aging depending on the nature and duration of the stress behind its activation.

Several lines of evidence have suggested that telomere dysfunction may contribute to mammalian aging by attenuating self-renewal and replicative capacity. Telomerase-deficient mice that have been serially backcrossed to harbor human length telomeres demonstrate regenerative failure in multiple organs due to a decline in stem cell proliferative capacity and tissue repair ability [72, 73]. In addition, human CD28 negative T cells, which have lost optimal telomerase activity, accumulate in vivo with age and display impaired function [74], and patients with congenital defects in telomerase activity display lymphopenia and lymphocyte hypo-proliferation [75, 76]. Together, these results support the notion that age-dependent activation of the cellular senescence program in proliferating cells impairs their self-renewal potential and promote aging.

5.2.2 Adverse Effects of Senescence with Regards to Aging: Gain of the Senescence Associated Secretory Phenotype (SASP)

In addition to a loss of replicative capacity, the accumulation of senescent cells with aging appears detrimental because of newly acquired functions of senescent cells. In particular, senescent cells display certain cell surface molecules and produce a raft of secreted SASP molecules associated with the ‘sterile inflammation’ characteristic of aging. While the production of SASP factors may be beneficial during transient episodes such as wound healing [77–79], the exponential accumulation of senescent cells with aging produces a monotonic increase in levels of SASP cytokines. Although numerous inflammatory markers increase with aging, the best studied with aging is IL-6, which is a reasonable aging biomarker whose expression is associated with worsening of age-associated phenotypes (reviewed in [80]).

Although perhaps beneficial in the short-term (e.g. in wound repair), elaboration of SASP cytokines appears detrimental in the long-term for several reasons. SASP factors may impair cellular differentiation and disrupt tissue homeostasis in otherwise undamaged tissues. Additionally, SASP factors appear to promote tumor

growth in a paracrine manner [81–83]. Several cytokines associated with increased tumor progression are produced by senescent cells including IL-6, IL-8, GRO-alpha and VEGF; each of which could potentially promote tumor progression in nearby cells harboring oncogenic events. Finally, the chronic activation of immunity and associated immune dysfunction resulting from the potent pro-inflammatory cytokines associated with the SASP is associated with many aspects of aging (reviewed in [84]).

5.2.3 Benefits of Senescence with Regard to Aging

Although the field has perhaps focused more recently on the age-promoting roles of senescence, it is now clear that the senescence machinery also contributes important anti-aging roles. As mentioned, the clearest benefit of *p53* and *CDKN2A* activation is tumor suppression, and since neoplastic disease is strongly associated with aging, the anti-cancer functions of senescence attenuate this important age-associated phenotype. Animals lacking these pathways rapidly succumb to cancer, whereas mice engineered to exhibit a physiologically regulated increase in *Cdkn2a* and *p53* function exhibit a reduction in tumorigenesis with attendant longevity extension [37]. More recently, it has also become clear that the senescence machinery may limit non-malignant, but pathogenic proliferation in other age-associated settings. For example, work on humans and mice has suggested that *CDKN2A* expression plays an important role in preventing atherosclerosis by limiting disease-causing proliferation of monocyte/macrophage or smooth muscle progenitors [49, 85, 86]. It is also likely that activation of proteins associated with senescence may play beneficial roles in the prevention of other non-malignant diseases associated with aging (e.g. autoimmune conditions). While the notion that cancer and some aspects of aging are opposite outcomes based on the failure or success of senescence may be generally correct, these observations suggest that one should be careful as to how a given age-related phenotype is classified (i.e. as excess, aberrant proliferation, or replicative failure). Moreover, while activation of senescence may be “antagonistically pleiotropic” (that is, beneficial in young age but adverse in old age), the data seem clear with regard to senescence that some of the beneficial functions in youth actually provide a lifelong benefit, manifesting as a decreased incidence of certain classic age-associated conditions such as cancer and atherosclerosis.

6 Why Does the Incidence of Cancer Increase with Age?

The incidence of cancer increases sharply with aging (Fig. 4). For example, common epithelial malignancies of the breast and colon are highly unusual in individuals below the age of 40, with an exponential increase in incidence with aging such that such tumors are highly common in adults over the age of 65. Several reasons have been suggested for this intimate relationship between aging and cancer (Fig. 5):

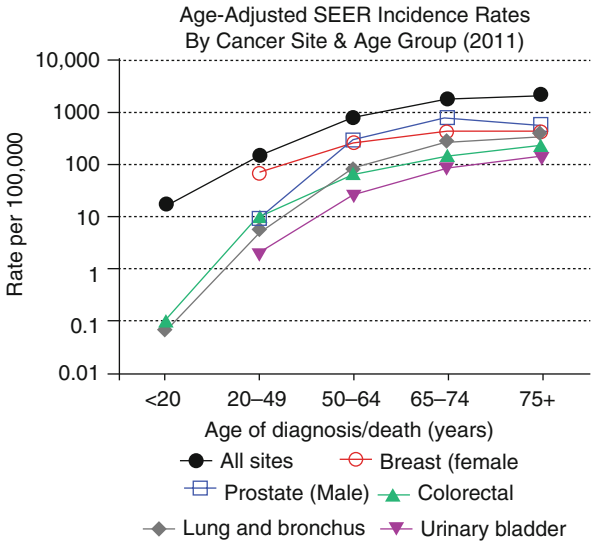

Fig. 4 Cancer incidence increases with aging. The incidence of most common types of human cancers, including lung, colorectal, urinary bladder, breast (female) and prostate (male), increases exponentially with age. While most types of cancers are extremely rare in young people, they are highly common in adults over the age of 65. Cancer incidence data are from the 2011 SEER (Surveillance, Epidemiology, and End Results Program) 18 areas. Rates are per 100,000 and are age-adjusted to the 2000 US Std Population (19 age groups – Census P25-1130). Cancer incidence data include all races and both sexes unless otherwise noted

6.1 Accumulation of DNA Mutations

Cancer requires the serial accumulation of a requisite number of DNA mutations in a long-lived self-renewing cell. One major source of time-dependent accumulation of DNA mutations likely comes from the intrinsic chemical instability of the DNA molecules. This is supported by the recent findings that most types of human cancer exhibit age-dependent enrichment of C>T substitutions at NpCpG trinucleotides, which is likely the result of the relatively elevated rate of spontaneous deamination of 5-methyl-cytocine at these locations [87]. The fact that this particular mutational signature is the only type that is strongly correlated with age across many cancer types suggests its accumulation is predominantly time dependent.

Another major source of DNA mutations likely occurs as accidents during DNA replication as cells divide throughout life. Since many of the self-renewing cells divide infrequently, the requisite number of mutations needed for malignant conversion may necessarily accumulate over years or decades. For example, HSCs represent the best understood system of human stem cell transformation. An adult human has fewer than 10^5 such cells that divide very rarely, approximately once every 40 weeks [88]. It is thought that these cells make roughly one mistake per replicative event, with the vast majority of errors in DNA replication being of no significance. As this small number of cells divides over decades, rare DNA mutations

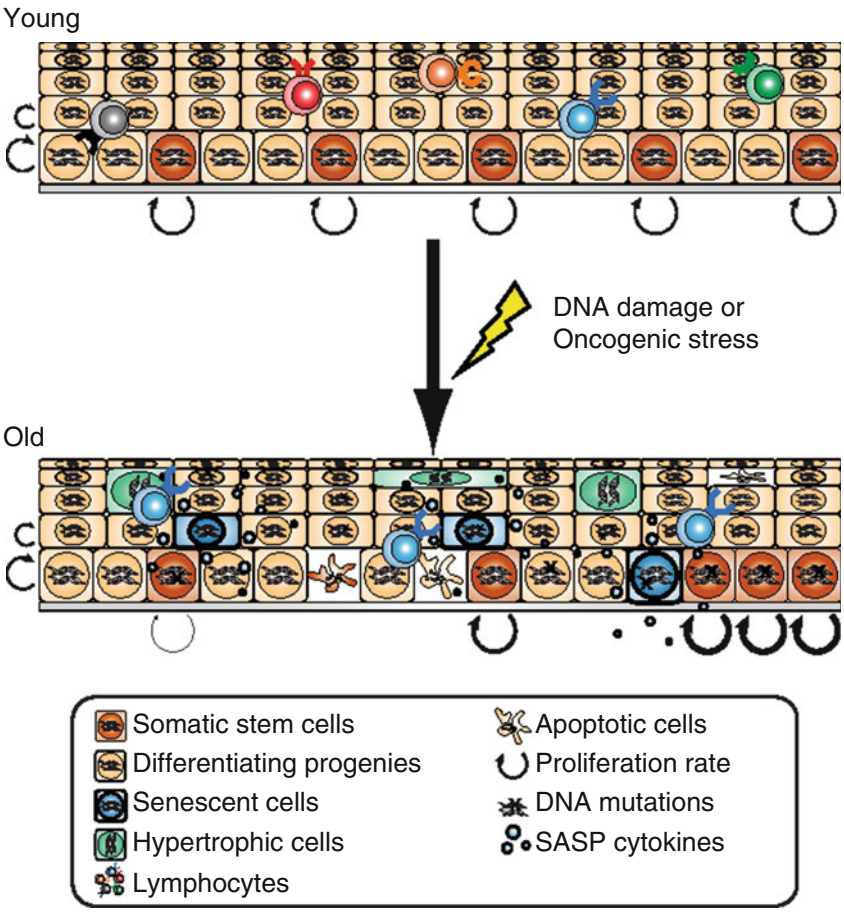

Fig. 5 Aging promotes cancer initiation and progression. Aging promotes neoplastic transformation and cancer development in several ways: (1) The time-dependent accumulation of DNA mutations in somatic stem cells can lead to the emergence of hyper-proliferative clones that may progress to full-blown cancer. (2) The functional attrition of somatic stem cells during aging as a result of genetic mutations and/or activation of cellular tumor suppression mechanisms can induce compensatory proliferation of remaining stem cells, which may lead to accumulation of new oncogenic mutations. (3) The age-dependent decline in adaptive immune system function as a result of reduced number, diversity, and functional competence of lymphocytes can lead to impaired immune surveillance and clearance of damaged or senescent cells from the body, which may contribute to increased cancer incidence in the elderly. (4) Accumulation of damaged and senescent cells in old tissue can disrupt tissue architecture and secrete pleotropic pro-inflammatory cytokines that contribute to cancer initiation and/or progression

occur stochastically which provide a selective advantage to a specific HSC, which then begins to overgrow the bone marrow. The increased replicative rate of this clone allows for further DNA mutations, and the serial accumulation of oncogenic driver events with inactivation of tumor suppressor mechanisms eventually leading to HSC cancer; that is, leukemia [89]. Presumably, cancer arises through similar

mechanisms in other tissues, and in all cases, significant evidence suggests the period from the earliest stem oncogenic events to full malignant conversion can in some tissues last several decades. The more rapid induction of the same cancer type (leukemia) in mice over a much compressed period (~2–3 years) likely reflects several species differences including murine HSC dynamics (e.g. more rapid HSC proliferation) and reduced tumor suppressor barriers (e.g. differences in DNA repair, telomere biology and p53/p16^{INK4a} function).

Besides stochastic DNA mutations, genomic instability can also occur as a result of telomere shortening. In cells with intact DNA damage response machineries, critically shortened telomeres can trigger p53–mediated cellular senescence or apoptosis. In the absence of p53 however, telomere shortening instead causes chromosome end-to-end fusions, leading to chromosome breakage during the subsequent round of cell division, which can result in chromosomal abnormality and aneuploidy [90]. Human somatic cells start with approximately 15 kb of telomere sequence during early embryonic development [91]. In the absence of telomere maintenance mechanisms, each cell division is accompanied by a net loss of 100–200 bp of telomeric sequence. Even in stem cells such as HSCs that possess telomerase activity, telomere length decreases with age in vivo. Therefore, age-associated telomere shortening can contribute to increased cancer incidence in the elderly by inducing genomic instability and mutagenesis. Indeed, short telomere length has been associated with significantly increased risk of several types of human cancers [92, 93], and people with genetic deficiencies in telomere maintenance are predisposed to the development of certain cancers such as leukemia and keratinocytic cancers in addition to exhibiting features of premature aging [88]. Moreover, the human *TERT* locus on chromosome 5p has been associated through genome-wide association studies with a phenomenal range of human cancers, but in a complex manner. For example, common alleles with reduced telomerase activity (and presumably shorter telomeres) appear to increase human risk for the same cancers noted in the syndromes of congenital telomerase deficiency (e.g. leukemia), whereas the same alleles appear to decrease the risk for other cancer types such as melanoma, where telomerase clearly appears to be oncogenic [94].

6.2 Loss of Stem Cell Function with Compensatory Hyper-Proliferation

Given this model of how serial mutations occur in self renewing cells to yield malignant clones, it becomes obvious that anything which increases the chances of DNA mutation or which increases the rate of proliferation of tissue-specific stem cells will also increase the risk of malignant conversion. Carcinogens such as UV light and tobacco smoke are well known mutagens, which promote cancer by damaging DNA. Likewise, however, it is becoming clear that somatic stem cell function is compromised with aging in many tissues. This may occur as a result of tumor suppressor mechanisms such as senescence, which in turn occurs as a result of telomere

shortening or other intrinsic and extrinsic cellular stresses. Importantly, however, the loss of even a fraction of a tissue's somatic stem cell compartment imposes a significant replicative burden on the remaining stem cells of that tissue. This compensatory increase in replication in turn translates into a greater risk of cancer, as well as additional possible stem cell attrition. Familiar examples of this process in humans are bone marrow failure syndromes such as aplastic anemia and myelodysplasia, where HSC numbers are sharply compromised, and malignant transformation to acute leukemia is highly enhanced.

6.3 Waning Immunity

Indubitably, the cellular immune system plays an important role in the surveillance and prevention of neoplastic clones. In fact, it appears that a substantial fraction of human cancers develop specific mechanisms to evade the immune system, thereby allowing for progression into later stages [95]. The ability of the immune system to repress malignancy may be particularly important in certain kinds of cancers that are very immunogenic; for example, those resulting from viral infections or which have tumor neo-antigens. Since the immune system plays an active role in cancer repression throughout life, the waning of immunity with aging, and in particular cellular immunity as mediated by T cells, likely plays an important role in tumorigenesis. Loss of immune function has been suggested to explain an observation in human melanoma: tumors driven by mutations of the *NRAS* oncogene (~25 % of melanoma) occur in patients significantly older than in those with tumors driven by mutations of the *BRAF* oncogene (~50 % of human melanoma). NRAS tumors are more strongly associated with chronic sun damage, and exhibit a larger number of antigenic mutations than *BRAF* tumors. Therefore, one hypothesis is that *NRAS* tumors are more efficiently cleared by younger individuals, but become more common with aging as cellular immunity decreases.

6.4 Changes in the Cellular Milieu

Beyond cell intrinsic events, it is also likely that extracellular changes that occur with aging also promote malignancy. For example, an increase in the number of senescent cells in aging tissues is thought to cause a significant increase in the local concentrations of pro-inflammatory cytokines secreted by senescent cells. The molecules elaborated by senescent cells are highly pleiotropic, causing many effects such as disruption of cellular differentiation, increasing proliferation, degradation of tissue matrix and increased cellular survival, which may in turn promote cancer initiation and/or progression. It should be noted that this model is somewhat controversial, with many authors suggesting senescence-related cytokines can also deter tumorigenesis, and therefore the effect of senescent cells with regard to cancer are likely tissue- and context-specific.

7 Preventing Cancer and Aging

Clearly, cancer and aging are intimately linked, such that cancer to some extent may be an almost inevitable consequence of advanced age in the setting of the accumulation of macromolecular damage, loss of somatic stem cell function, waning immunity, etc. While our understanding of the molecular basis of cancer and aging continues to grow, it is possible to design strategies to both decrease the incidence of cancer as well as slow the rate of aging.

7.1 Minimizing Cellular Damage

As it becomes increasingly clear that a time-dependent accumulation of cellular damage (particularly DNA damage and mutations) serves as a common molecular basis for both cancer and aging, reducing the total amount of damage our cells experience throughout life can be expected to protect against both cancer and aging. One way to achieve this is to minimize the exposure to environmental mutagens such as smoking, UV radiation and chemical carcinogens that can cause direct DNA damage and mutations. Indeed, it has been shown that quitting smoking has a myriad of health benefits in both target tissues such as the lung (e.g. reduced risk of lung cancer, emphysema and pulmonary fibrosis), as well as tissues less directly affected by tobacco inhalation (e.g. a reduction of cancer in many tissues, enhanced immunity, less vascular disease). Evidence suggests that tobacco exposure widely promotes aging-like phenotypes in many tissues, and therefore has been argued to represent the prototypical human 'gerontogen' [96, 97]; that is, an environmental exposure that promotes physiological aging. Similarly, avoiding UV exposure can dramatically decrease the incidence or cutaneous melanoma and slow skin aging. In addition to naturally occurring mutagens, cytotoxic agents that directly damage DNA are commonly used for treating a wide range of human malignancies. As a result, patients, particularly young children, who received cytotoxic cancer therapy, are at increased risk of developing secondary malignancies [35], and often display features of accelerated aging affecting a wide range of tissues [98]. The latter is believed to be partly due to reduced somatic stem cell function, either as a direct result of therapy-induced stem cell attrition (through cell death, senescence, or differentiation), or as the result of compensatory proliferation-induced exhaustion. Therefore, finding ways to protect stem cell function in cancer patients may help to reduce the aging-promoting effects of cytotoxic therapy in this special scenario. This issue is addressed in more detail in Chap. 4. The Impact of Cancer Treatments on Aging. Another potential way to reduce cellular damage is through modulating energy metabolism. It has long been observed that interventions such as dietary restrict (DR) and pharmacological inhibition of the mTOR pathway, which slow cell growth and metabolism, reduce the incidence of cancer and extend life span. One possible explanation for this is that decreased cellular energy metabolism may reduce the generation of harmful

byproducts that cause cellular damage including DNA mutations. Alternatively, lowered cellular metabolism may slow cell proliferation, resulting in reduced replication-associated DNA damage and mutations.

7.2 Enhancing Immune System Function

The immune system plays an important role during both aging and tumorigenesis. As humans age, the function of the immune system declines sharply, resulting in increased susceptibility of the elderly to external infection. This aging-associated decline in adaptive immune response may also contribute to impaired immune surveillance and clearance of damaged or senescent cells from the body, which can contribute to aging and cancer development. On the other hand, an aberrant immune response such as autoimmune diseases also increases with age. Therefore, finding ways to augment the normal immune system function without over-activation in old individuals may ameliorate certain aspects of aging while providing an important anti-cancer defense. One suggested approach has been to augment thymic function, therefore delaying T cell aging, through supplementation with cytokines such as IL-7 [99].

7.3 Maintaining a Functional Stem Cell Pool

A key feature of aging is the loss of regenerative potential in self-renewing tissues as a result of functional attrition of somatic stem cells. Therefore, an important goal of aging research is to find ways to maintain functional stem cells throughout life. Functional attrition of stem cells with age occurs in part as the result of DNA damage accumulation that subsequently activates tumor suppression mechanisms. As DNA damage primarily occurs during DNA replication, the number of cell divisions a given stem cell goes through is expected to be negatively associated with their function. In support of this notion, many types of somatic stem cells including HSCs are found to be highly quiescent, and loss of quiescence is associated with premature stem cell exhaustion. Therefore, reducing the rate of stem cell proliferation may provide a way to slow stem cell aging. Alternatively, providing an exogenous source of healthy somatic stem cells, for example through a regenerative medicine approach, could provide a means to reduce both aging and cancer.

8 Summary

Serial accumulation of mutations in long-lived somatic stem cells as a result of imperfect DNA repair mechanism contributes to both cancer and aging. The choice between these two seemingly distinct outcomes depends on the effectiveness of

cellular tumor suppression mechanisms against oncogenic mutation – failed tumor suppression results in cancer, while successful tumor suppression causes functional attrition of self-renewing cells, contributing to physiological aging. The increased incidence of cancer with aging reflects the time-dependent accumulation of mutations in somatic self-renewing cells, as well as waning immune surveillance and possibly pro-oncogenic changes to the tissue milieu with aging. While preventing aging or curing cancer is unlikely, minimizing cellular damage, enhancing immune system function, and maintaining a functional stem cell pool may slow the rate of aging while simultaneously reducing the risk of cancer.

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The Impact of Cancer Treatments on Aging

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1 Introduction

Owing to early detection and improved treatment, cancer survival rates in the US have recently been increasing [59]. This opens a new era in cancer care that is challenged with not only enhancing treatment efficacy but also improving the quality of the clinical outcome. There is an urgent need to address the highly complex and multifaceted problems that arise as a result of the therapy itself.

Modern cancer therapy evolved from a cytotoxic approach, using chemotherapeutics and ionizing radiation that are limited in their ability to distinguish healthy and cancerous cells, to drugs with specific molecular targets, such as tyrosine kinase inhibitors or antibodies recognizing specific receptors. Despite the leap in sophistication achieved through research and development, toxicity still remains the major limiting factor for many types of therapy, including the more targeted ones. This is especially relevant considering that many of the patients receiving these toxic therapies are older and are likely to have co-morbidities. More importantly, undesired side effects pose serious clinical challenges, including a negative impact on the quality of life and healthspan of cancer survivors.

We are now faced with an unprecedented range of dysfunctions and diseases resulting from the very interventions that have significantly improved the chance and length of survival after cancer diagnosis. This is an issue with multifaceted roots which requires a multilevel approach. There is much to be considered when addressing adverse health issues secondary to cancer treatment: (1) age-dependent co-morbidities affecting the great majority of patients who are older adults, (2) the potential compromise to health- span and quality of life, especially among childhood cancer survivors, (3) the incomplete understanding of the complex molecular pathways that can sensitize cancer cells while protecting healthy cells, (4) the multimodality nature of cancer treatments that involve a mix of molecular targets, and (5) a relatively short time frame to eliminate side effects compared to other chronic diseases.

The emerging interdisciplinary field of geroscience may hold the key to understanding the mechanisms involved in preventing and treating many of these side effects. This is especially important as the side effects involve damage to a wide-range of tissues, cells, organelles, and molecules that manifest over time and which can resemble phenotypes observed during aging. For example, cancer treatments such as chemotherapy or radiation can cause DNA alterations and mutations overlapping with those caused by oxidative damage and/or replication errors observed during aging.

2 Cancer Therapies

Traditionally, surgery has been a key treatment for solid tumors (e.g. breast, prostate, colorectal, gynecologic, and lung). The modern cancer therapy arsenal has greatly benefitted by the addition of therapies including chemotherapy and

radiotherapy which have provided a broader control of the disease locally and systemically. Unfortunately, some of these therapies themselves can be toxic to the patient and also promote secondary tumors. Here we discuss the adverse effects that are known to affect patients during and long-after cancer therapy, ranging from mild side-effects, to long-term adverse secondary health problems and secondary cancers.

2.1 Chemotherapy

The beginning of modern chemotherapy began with the 1942 toxic mustard nitrogen gas studies by Goodman and Gillman, commissioned by the US Department of Defense in search for therapeutic properties of chemicals developed for chemical warfare during World War II [259]. After that, it took over 30 years for legislation by the US Congress to create the first federal program for anticancer drug discovery, leading to major improvements in the efficacy and reduction of the toxicity of chemo drugs. However, chemotherapy remains largely based on semi-targeted cytotoxity to rapidly proliferating cells [50, 143, 200]. Although chemotherapy was first thought to be quite selective, we now know normal cells including non-dividing cells also experience severe toxicity, leading to dose-limiting side-effects such as cardiotoxicity, myelosuppression, gastrointestinal damage, and fatigue. Although all chemotherapy agents produce reactive oxygen species (ROS) during apoptosis, some generate particularly high levels of ROS, such as the anthracyclines (doxorubicin, daunorubicin, and epirubicin), alkylating agents (cyclophosphamide and busulfan), platinum-based drugs (cisplatin, carboplatin, and oxaliplatin), podophyllotoxins (etoposide and teniposide), and camptothecins (topotecan and irinotecan) [50]. The US Food and Drug Administration (FDA) has approved 132 cancer chemotherapy drugs, 56 of which have been reported to cause oxidative stress, which leads to collateral damage to normal cells which is likely to overlap with that caused by ROS endogenously generated during mitochondrial respiration, and believed to contribute to aging phenotypes [41].

Anthracyclines produce the highest level of ROS by redox cycling and iron chelation, leading to mtDNA lesions and respiratory chain dysfunction, as well as disruption of antioxidant defense and repair systems [114, 115, 130, 219, 244]. Doxorubicin (a.k.a. Adriamycin) is an anthracycline widely used for the treatment of many cancers which exerts its antineoplastic effects by DNA intercalation, topoisomerase II inhibition, and oxidative stress. Its clinical use is limited due to common side-effects including myelosuppression, nausea, fatigue, but above all, cardiotoxicity [13, 137]. Cardiotoxicity is exponentially dose-dependent, and secondary to doxorubicin-induced oxidative stress with an estimated 7 % of patients developing congestive heart failure (CHF) after a cumulative dose of 550 mg/m^2 [218]. Although rare and generally transient, acute toxicity can be evident within a few minutes following administration [78]. Chronic cardiomyopathy usually occurs during the first year following treatment, but can manifest up to 20 years later and is

irreversible. Doxorubicin-related cardiotoxicity is probably multifactorial, but most of the damage can be attributed to oxidative stress and lipid peroxidation that leads to mitochondrial DNA damage and dysfunction, mirroring what is observed during cardiac aging [62, 89, 165, 168, 214].

Cisplatin (cis-diamminedichloroplatinum(II)) is another chemotherapy drug widely used in many cancers, which suffers from oxidative stress-induced side effects, prominently in the kidneys and ear [53, 58, 194, 195, 202, 220].

2.2 Radiotherapy

Radiation-based treatment is a major arm of cancer therapy. Irradiating biological material leads to a rapid burst of ROS generated primarily because of the ionization of water molecules and direct ionization of target molecules [72, 189]. Ionizing radiation (IR) such as X-rays and γ-rays can cause direct macromolecular damage by energy deposition, but 60 % of damage is caused by ROS which is produced through the interaction of energy with water [11]. Although radiation-dependent ROS are effective in killing cancer cells, they also damage normal cells leading to dysfunction [18, 173, 256, 270]. It has been reported that even localized small field radiotherapy of the head and neck in patients can cause oxidative damage at the organismal level, and may lead to secondary mutations [193]. The use of the free-radical scavenger amifostine significantly reduced the side effects caused by radiotherapy [21, 28, 30], but additional studies are needed to understand how or if it affected the toxicity to the cancer cells.

Other studies also indicate that administering antioxidant enzymes with radiotherapy reduces the toxic side effects. For example, SOD was found to protect against radiation induced cystitis in bladder carcinoma without reduction in antitumor efficacy [92]. However, the use of antioxidants such as vitamin C is not expected to provide differential protection effects to normal and cancer cells, although it may do so with specific types of tumors.

2.3 Target-Based Therapeutics

Beyond the more traditional cytotoxic therapies, a new generation of target-based drugs has greatly enhanced the anti-cancer arsenal. There are various approaches to interfere with specific molecular targets that have been largely executed by antibodies and small molecules [121]. Although these inflict much less damage compared to the more traditional interventions, they can also promote damage in multiple systems, which could affect either the patient's aging process or cause significant damage resulting in loss of redundancy and frailty. For example, a kinase inhibitor such as rapamycin, well established to extend longevity in model organisms [19, 97, 190], is also effective in treating kidney cancer, but can contribute to either

immunosuppression or immunostimulation, depending on the dose and mode of administration, which can have profound implications for the functionality of the immune system during aging [144, 145].

The concept of using immunity in cancer therapy was proposed in the late nineteenth century [47, 65] but it only progressed thanks to the introduction of hybridoma technology that enabled targeted antibody design and engineering [121, 204]. Targeted monoclonal antibodies can attach to cancer cells, based on the presence of an antigen on certain tumor cells, and can act as immunomodulatory agents [152, 175, 251], inhibit specific receptors or ligands [33, 135, 204, 261], or deliver cytotoxic agents such as radioisotopes, genes, and toxic drugs [25, 259]. Small molecules designed to target specific molecular pathways have also been actively developed recently, including various kinase inhibitors [102, 138, 271], cyclin-dependent kinase (CDK) inhibitors [163], drugs acting on hypoxia-related pathways [151, 257], and those targeting the PI3K/AKT/mTOR pathway [60, 79, 107, 198, 241, 242].

With the increasing appreciation of the role of metabolism in cancer, the concept of metabolic targets for cancer therapy has gained traction [81]. Metabolic dysfunction and mitochondrial alterations are common underlying characteristics of various age-dependent diseases, and several of the drugs have been proposed/tested off-label as cancer treatments or have multiple indications including those currently approved/developed for diabetes [66, 182], autoimmunity [29, 234], mitochondrial function [69, 80, 227], and other scopes [225, 238]. Because aging is a leading risk factor for most major diseases, older individuals often suffer from multiple conditions. Each disease can affect the other and accelerate the process. For instance, obesity and diabetes often develop with age, and a recent study with 5.24 million participants showed a strong association between being overweight/obese (BMI $\geq$25 kg/m^2) and 17 of 22 frequent cancers. Each 5 kg/m^2 increase in BMI was associated with higher risks of cancers of the uterus, gallbladder, kidney, liver, etc. [16]. However, obesity is not significantly linked to all types of cancer and the correlation shows clear sex-specific differences [229]. Also, diabetes is associated with an increased risk of some cancers, including liver, pancreas, endometrium, colorectal, breast, and bladder. This could be directly affected by hyperinsulinemia, hyperglycemia, and inflammation [84].

Although targeted therapeutics cause significantly lower toxicity than the traditional chemotherapeutics or ionizing radiation, they are not free of side effects [9, 85, 147, 187, 205, 224, 238]. Careful long-term follow-up studies on these newer therapeutics should be encouraged to better understand their immediate and long-term impact on cancer survivors.

3 The Clinical Impact of Cancer Therapy on Aging

Despite the fact that cancer therapeutics enter the clinic after tremendous scrutiny on safety, the side effects are still the major limiting factor. It is estimated that at least 50 % of cancer survivors will suffer from treatment-related side effects at the

physical and psychosocial level [231]. Furthermore, subtler and less visible alterations secondary to cancer therapy may have profound effects later in life and affect the way we age. Importantly, these late-life effects are not scrutinized within the FDA process for approval of the drugs. This section discusses the potential impact these therapies have on lifespan/healthspan (including secondary tumors arising from therapy) and quality of life. The following section will discuss the molecular aspects of these events in detail.

Clinical and epidemiological studies have shown that long-term cancer survivors are at risk for late effects of their cancer treatment including secondary malignancies, cardiac and vascular abnormalities, pulmonary complications, infertility, endocrinopathies and other chronic conditions. Understanding the mechanistic details of damage, protection and death in both normal and malignant cells will be critical to develop adjuvant therapeutics to selectively protect the patient, but not the tumor. Because of its interdisciplinary and comprehensive nature, Geroscience is well-positioned to address these issues.

The treatment of pediatric cancers has witnessed remarkable improvements leading to a 5-year survival rate nearing 80 % [191]. Because most pediatric cancers are now curable, the adverse effects of treatment as patients age, including growth/development, fertility, secondary cancer, organ failure, and psychosocial challenges, are emerging issues for long-term childhood cancer survivors [191]. However, the more subtle but wider effects of a variety of treatments on cellular and organismal aging are likely to be underestimated. Based on a recent study of 10,397 adult survivors of childhood cancer, it is estimated that by 30 years from their cancer diagnosis, 73 % of them develop at least one chronic health condition, and 42 % exhibit a severe, life-threatening, or disabling condition or die from a chronic condition [170]. This is considerably higher than chronologically comparable controls [170]. Also, treatment-related secondary malignancies are a serious concern [17, 169, 255]. Ionizing radiation has been favored for the treatment of childhood cancers, leaving the survivors at increased risk of secondary malignancies, including those arising from the skin, breast, thyroid, and brain [8, 24, 110, 166, 176, 213]. Selected chemotherapeutics have also been successful for childhood cancers, but they can also cause adverse consequences. These include alkylating agents [117, 154], anthracyclines [125, 232], antimetabolites [7, 35, 185], corticosteroids [51, 70], platinum-based [253], and vinca alkaloids [82, 112]. Notably, long-term childhood cancer survivors also experience increased risk for metabolic dysfunctions, including obesity, diabetes, and hypertension, that are known to bring about additional major age-related diseases.

4 Geroscience and Cancer Treatment: Current Status

Current interventions to treat cancer affect several pathways that regulate the aging process. The undesired off-target events and their consequences may cause collateral damage to normal cells, alter the course of aging and also cause secondary

damage to the patient as mentioned in the previous section. Here, the latest research on aging will be reconciled with some of the major molecular pathways affected by cancer therapies. Aging involves complex and interdependent biological processes many of which are affected by chemotherapy and radiotherapy and could contribute to accelerated aging.

4.1 Genomic Instability

Both chemo- and radiation therapies are aimed at destroying rapidly dividing cells, primarily by inducing damage to their replicating DNA. The lack of specificity in their actions, as discussed above, means that adjacent, non-cancerous cells, are also damaged. Thus, therapy-induced DNA damage may impede our ability to maintain genomic integrity and hasten the aging process. Chemotherapeutics and therapeutic irradiation are well known to cause DNA damage to the somatic nuclear and mitochondrial genomes, leading to functional decline of multiple organs and various diseases [26, 239]. Accumulation and incorporation of these DNA lesions can cause genomic instability, a hallmark of aging. Therefore, these therapies can be considered unintentional accelerators of aging, resulting in several phenotypes observed in older individuals; e.g. cardiomyopathies, and secondary malignancies.

4.1.1 Nuclear DNA

DNA is subject to frequent chemical alterations and breaks on a daily basis that requires sophisticated surveillance and repair to prevent genomic instability. The first line of defense is the chromatin structure where DNA is wrapped around a protein core (histones) in units known as nucleosomes. Then, there are highly specialized repair mechanisms that mend damaged DNA lesions ranging from error-free base excision repair, to error-prone translesion repair and non-homologous end-joining. Nevertheless, none of these systems are able to prevent the accumulation of age-dependent modifications and errors, and in fact, the error-prone systems can even promote point mutations and small DNA insertions and deletions, in an attempt to avoid the more problematic chromosome breaks and rearrangements. Genomic instability can arise from direct damage to the DNA, including single- and double-strand breaks, inter- and intra-strand base cross-linking, and depurination or depyrimidination, leading to transition or transversion mutations, or by progressive mutations, such as additions, deletions, frame-shifts, or substitutions. Chemotherapeutics and ionizing radiation can promote virtually all of the lesions listed above, leading to an accelerated genomic aging [5]. Therefore, further studies are necessary to: (1) assess the role of DNA damaging cancer therapy on the level and type of DNA damage and mutations, (2) determine how/if these change/increase during aging, (3) determine whether in addition to promoting secondary tumors they may play a role in accelerating multisystem aging and/or specific lesions/diseases. In addition to providing important clues

that can help us redesign cancer treatment and improve the information given to patients in the informed consent, these studies can help test the genomic instability hypothesis of aging. Because each treatment causes a different profile of DNA mutations/lesions, these analyses could link a type of DNA damage (for example, point mutations) to specific age-dependent phenotypes (ex. secondary tumors and their type). The collaborative involvement of basic scientists, computational biologists, and clinicians will be necessary to achieve these goals.

4.1.2 Mitochondrial DNA

Chemotherapeutics and radiation can also damage mitochondrial DNA and cause dysfunction. The less sophisticated DNA repair mechanisms present in mitochondria may also contribute to enhancing this toxicity. There have been many reports of increased mtDNA mutations during aging, but the exact degree of causal contribution of mtDNA damage to human aging is still not entirely clear. Oxidative damage to the mtDNA has long been proposed to be involved in the aging process, but recent studies show evidence that replicative infidelity and spontaneous mutations may be more pertinent [210]. Comparison of mitochondria from the pre-frontal cortex of young (<1 year) or old (>75 years) brains showed a non-significant increase of oxidative DNA damage (8-oxodG), but a significant increase in DNA scars resulting from replication infidelity and spontaneous base hydrolysis, indicating that age-related mtDNA damage by ROS may be less than previously thought [116]. In fact, the mtDNA mutator mouse, which expresses a proofreading-deficient DNA polymerase γ ($PolgA^{mut}$), has increased levels of mtDNA mutations including deletions, and shows a premature aging phenotype, although the contribution of small mutations to aging phenotypes in these mice is an area of debate [3, 64, 226, 236, 237, 254]. Aging mitochondria have also been reported to carry large deletions in their DNA with a concurrent decline of mitochondrial energy production [15, 106, 124, 139]. One of the biggest hurdles of understanding mtDNA mutations is the lack of technology that is sensitive and accurate enough to detect single mutations with low background error frequencies.

Although currently less understood than nuclear DNA, mtDNA is also subject to damage by chemotherapy and radiation. Doxorubicin-induced mtDNA damage is proposed to occur via oxidative stress, as 8-oxoG adducts were increasingly detected preferentially in mtDNA over nuclear DNA following treatment in rodents and humans [129, 208]. Also, large mtDNA deletions in cardiomyocytes of mice chronically treated with doxorubicin have been reported [1]. Further, doxorubicin-induced cardiotoxicity is thought to result from the combined oxidative damage to mitochondrial DNA and lipids [129]. Additionally, platinum-based drugs [161, 180] and other chemotherapeutics [266] can damage mtDNA. Furthermore, ionizing radiation causes significant mtDNA damage, including a common deletion (~5 kb) that is more prominent in normal cells compared to cancer cells, and also found in aging mitochondria [181, 274]. Also, γ-radiation causes point mutations in the D-loop region of the mtDNA in an inconsistent manner [162]. As for nuclear DNA damage,

the role of various chemotherapy drugs in causing different types of mtDNA damage can serve as an invaluable tool to determine the role of different categories of mtDNA damage on aging and age-related diseases. Again, it will be first essential to determine in mice and possibly human samples, what are the short-term and long-term effects of each chemotherapy drug on the mtDNA of different cell types.

4.1.3 Telomere Attrition

Telomeres are protective sequences that define and protect the ends of linear chromosomes, consisting of long double-stranded TTAGGG repeats that can reach 9–15 kb in humans and 100 kb in rodents [168]. Telomere length shortens in primary fibroblasts from older humans and also with serial passaging in vitro [89, 95]. Critical telomere shortening can signal cells to enter an irreversible proliferative arrest [20, 235], a condition known as replicative senescence [100]. Notably, ectopic expression of telomerase led to the immortalization of human fibroblasts [20]. Thus, it is not too surprising that telomeres have a critical role in cancer biology, Telomere-shortening and cellular senescence are thought to be important to protect against cancer [233]. However, telomere attrition can also promote oncogenesis by causing unregulated chromosomal rearrangements and genomic instability, as discussed above [260, 263].

There are numerous reports of telomere shortening after chemotherapy and ionizing radiation treatment, with older patients being at a greater risk. An accelerated rate of telomere attrition was found after various cancer chemotherapy treatments, including cisplatin and irradiation (>100-fold) [230], two doses of high-dose cyclophosphamide and Ara-C [186], six to eight cycles of CHOP (cyclophosphamide, Adriamycin, vincristine, and prednisone) [136], in a dose-dependent manner after CHOP, 5-FU, and fludarabine treatments [61]. Furthermore, in long-term survivors of childhood cancers, lower telomerase content was significantly related to treatment-related secondary thyroid cancer, resulting from intensive chemotherapy and ionizing radiation [87]. Also, therapy-related damage to hematopoietic stem cells (HSC) can cause myelodysplasia or acute myelogenous leukemia in recipients of autologous bone marrow and HSC transplant, posing a lethal complication [39]. Further studies should investigate both the potentially negative and positive effects of telomere shortening on aging and diseases, and particularly cancer. In fact, telomere shortening may both play an anti-cancer role by preventing the division of old and damaged cells but could also contribute to cell senescence and the pro-inflammatory effects of senescent cells [32, 223].

4.2 Epigenetic Alterations

In addition to mutations, cancer therapy can also cause epigenetic changes in normal and malignant cells. Epigenetics refers to genetic regulation without alterations of the DNA sequence. The term was first coined to connect genotype to phenotype

and describe the deeply complex processes between them [243]. The currently understood molecular mediators of epigenetics are DNA methylation on CpG sites, histone modification (methylation and acetylation), chromatin remodeling, and non-coding RNAs (such as microRNAs and long non-coding RNAs), all processes involved in regulating gene expression. Unlike genetic information, epigenetic changes are plastic, dynamic, and diverse, making these changes a moving target during aging [73, 104]. There have been recent reports suggesting an age-specific CpG methylation pattern. Horvath reported 353 CpG sites pertinent to cell death/survival, cellular growth/proliferation, organismal/tissue development, and cancer, that can accurately predict chronological age across tissue and cell types to within a few years, starting from newborn and including induced pluripotent stem cells [103]. Hannum et al. built a quantitative model of aging based on >450,000 CpG markers from the blood of 656 individuals (19–101 years old) and showed that one's aging rate and chronological age can be predicted from this methylome. Also, the methylome may help explain the age-dependent epigenetic drift that may impact transcriptional patterns over time [94]. Also, a novel integrative epigenome-interactome approach identified tissue independent age-associated methylation interactome hotspots targeting stem-cell differentiation pathways with validation with independent DNA methylation data sets, encompassing over 1000 samples from different tissue types [252]. Another recent report examined more than 480,000 CpG sites from 965 individual samples and found 162 CpGs significantly associated with age, of which 65 were novel sites [39, 73].

Cytotoxic drugs and radiation can affect the DNA methylation profile and lead to epigenetic side effects that can persist much after treatment, including secondary cancer [52, 240]. Chemotherapy can affect DNA methylation and ionizing radiation can cause stable DNA hypomethylation in both target and bystander tissues. Breast cancer patients who received chemotherapy showed hypomethylation in 8 CpG sites in their blood cells that persisted 6-months after treatment [215]. DNA hypermethylation has also been shown in at least 1 gene in more than half of a set of leukemia patients that was associated with shortened average time to develop secondary therapy-related leukemia (49.3 vs. 133.2 months) [228]. Unlike chemotherapy that is usually delivered systemically, radiotherapy is generally focused on the tumor with precision. However, the bystander effect, which refers to indirect exposure and damage to neighboring tissues, in part by causing persistent epigenetic changes, still poses a serious clinical problem for cancer survivors [109, 123, 247]. In summary, epigenetic changes could contribute to the long-term effects of cancer therapy although they are not expected to promote the severe aging and disease phenotypes that mutations and gross chromosomal rearrangements are likely to promote.

4.3 Mitochondrial Function

Mitochondria are a major target for many cancer therapeutics, and are also strongly implicated in aging and age-related diseases. As mentioned above, mtDNA damage has been long thought to cause mitochondrial dysfunction.

Although the exact mechanisms by which mitochondria contribute to aging are still largely unclear, there are several promising theories. Because many oxygen species are so reactive, the mitochondrial free radical theory of aging has been one of the most cited theories for how mitochondrial and extra-mitochondrial damage occurs during aging. Briefly, the theory states that the superoxide and other free radicals that arise as by-products of mitochondrial respiration can cause oxidative damage to macromolecules including nucleic acids, proteins, and lipids. Indeed, ROS production increases with age and the cellular defense mechanisms against it declines. Recently, additional mechanisms of mitochondrial contribution to aging have been proposed including mitochondria as a signaling organelle [26, 40]. Notably, in worms, inducing a mitochondrial unfolded protein response (mtUPR) in neurons has been shown to transmit a mitochondrial signal to the gut that in turn leads to extended lifespan [63, 258]. The exact identity of the signal is currently under investigation. The emerging biology of mitochondrial-derived peptides (MDP) has also been shown to be involved in the regulation of aging and stress responses [134]. Humanin is a MDP encoded within the mitochondrial DNA that was first discovered through a search for neuroprotective factors from a cDNA library constructed from an unaffected brain fraction of an Alzheimer's patient [98]. Humanin levels decline with age and its expression is regulated by the growth hormone and insulin-like growth factor 1 axis (GH/IGF-I) [61]. Long-lived GH-deficient mice and the GH-deficient Ecuadorian cohort who are immune to cancer and diabetes have higher levels of humanin compared to their normal counterparts [61]. Notably, as discussed in the next section, humanin has recently been shown to protect against cancer treatment-related toxicity. Because cancer survivors experience delayed treatment-induced mitochondrial dysfunction that manifests as skeletal muscle dysfunction [203] and cardiac failure [129, 141], it would be of great benefit to be able to identify early markers of mitochondrial damage at the genetic, functional and signaling levels, and also interventions that can selectively protect mitochondria in normal cells during cancer treatment, such as humanin.

4.4 Cellular Senescence

Cellular senescence can result from different types of cancer treatments. As discussed above, senescence is defined by as an irreversible growth arrest that can be induced by telomere shortening. However, stress-induced premature senescence in the absence of reduced telomere length can occur in response to DNA damage (especially double strand breaks) [126], strong mitogenic stimuli (e.g. oncogenes), and mitochondrial dysfunction, including reactive oxygen species [233, 276]. The molecular mechanisms behind cellular senescence involve a network of cell cycle regulators, including cyclin-dependent kinase inhibitors (CDKis), notably p21 that is activated by the ATM/p53 pathway, and $p16^{INK4a}$, both of which converge on the tumor suppressor RB to arrest cell cycle [233]. Currently, the "senescent" state of a cell is assessed based on several characteristics including (i) enlarged morphology

(e.g. twice the size of non-senescent cells) [99]; (ii) senescence associated β-galactosidase activity that reflects increased lysosomal biogenesis [128, 131]; (iii) senescence associated secretory phenotype (SASP), (iv) DNA-SCARS (DNA segments with chromatin alterations reinforcing senescence) [192], and (v) chromatin remodeling, including senescence-associated heterochromatin foci (SAHF) [4, 272]. More recently, the concept of 'deep' or 'late' senescence is emerging where senescent cells continue to evolve with time [233], including the senescence-associated opening of gene-poor heterochromatic regions where major retrotransposon families, L1, ALU and SVA reside, leading to increased transposition in senescent cells [54]. There are several ways senescence is thought to contribute to organismal aging and age-related diseases including cancer, inflammation, development and tissue/organ deterioration [32, 223, 233].

Traditional genotoxic cancer treatments (i.e. chemotherapy and ionizing radiation) can cause off-target cellular senescence of normal cells. Breast cancer patients who have received anthracycline-based chemotherapy showed elevated levels of $p16^{INK4a}$ in their blood cells immediately and 12 months after treatment [201]. Premature senescence by ionizing radiation and/or chemotherapy in hematopoietic stem cells has been reported in mice [153, 245]. Notably, the number of studies focusing on the role of cancer therapies on cell senescence and its consequent role on organismal aging and diseases is very limited, pointing to the need of additional studies which will also be useful to understand the role of senescence in aging and diseases. Studies could be designed to lead to demonstrations of the link between therapy, the presence of senescent cells and diseases. For example, fibroblasts or lymphocytes could be treated *in vitro* with chemotherapy drugs, taken through a sufficient number of population doublings to induce senescence and then injected into middle aged mice to determine whether the chemotherapy-treated, but also the untreated, senescent cells have effects on aging and age-related diseases.

4.5 Stem Cell Aging

The negative side effects of cancer therapy are often associated with toxicity to stem cells and, in some cases, their exhaustion. Aging is partially attributed to the loss of regenerative capacity with time, resulting in suboptimal tissue maintenance and repair. There are several proposed mechanisms that lead to stem cell aging and deficiency or dysfunction. Age-dependent accumulation of DNA damage and telomere shortening are thought to play a major role in stem cell damage, manifesting in replicative senescence [212]. Epigenetic changes representing proliferation history, independent of telomere length, have also been shown to cause hematopoietic stem cell aging [14]. Stem cells favor a specific microenvironment supported by certain types of cells, called niches, that provide factors such as TGF-β [246], bone morphogenetic protein (BMP) [55], Wnt [44], Notch [2], and cyclic ADP ribose [267]. The age-dependent decline of these cells is also thought to contribute to stem cell exhaustion with time and loss of activity in those remaining [171]. More recently,

metabolism has emerged as an important factor in maintaining stem cell pluripotency. Proliferating cells, e.g. active stem cells and cancer cells but particularly cancer stem cells, require biosynthetic building blocks to synthesize DNA, protein, and lipid membranes to replicate. Rather than relying on increased consumption of extracellular nutrients, highly proliferating cells rely partially on de novo synthesis from glucose and glutamine. This high anabolic demand makes these dividing cells rely on aerobic glycolysis [74, 111]. Mitochondria, being the single most important metabolic organelle, have also risen as a key regulator of cell stemness [74, 111].

Traditional cytotoxic chemotherapy and ionizing radiation target rapidly proliferating cells with little distinction between normal and malignant cells. Therefore, quickly dividing normal stem cells are also targeted by these interventions, leading to undesired side effects that limit the efficacy of chemotherapy. For example, both cyclophosphamide and 5-FU, but also many other chemotherapy drugs targeting DNA, can cause severe damage to bone marrow stem cells leading to immunosuppression which is often severe enough to require bone marrow transplants. Furthermore, stem cells may be subject to therapy-induced DNA damage leading to senescence, and alterations in mitochondrial health that can also reduce their stemness. While the damage to the rapidly dividing hematopoietic stem cells is well documented, even non-dividing cells including muscle cells and neurons can also be severely damaged by several chemotherapy drugs. Therefore it will be important to understand how these drugs affect quiescent stem cells or those that are dividing very slowly such as the resident satellite cells in muscles.

5 Geroscience and Cancer Treatment: Filling in the Gaps

Geroscience is an emerging field that embraces and encompasses a wide range of disciplines, reflecting the complexity of aging. This also provides an entirely new opportunity to approach diseases with a wider net. For example, the toxic side-effects of cancer treatment arise because of the inability of treatments to fully distinguish normal and malignant cells. Therefore, the ability to separate the two cell types, to a degree similar to that attained by antibiotics, which can readily distinguish bacterial cells from ours, would undoubtedly improve our aim at cancer cells and dramatically increase the therapeutic window. Because geroscience is focused on the protection of normal cells from any type of damage, the field is well positioned to identify ways to protect normal and not cancer cells from toxins, not by simply screening for drugs with those properties but by understanding the fundamental mechanisms of protection in healthy and malignant cells. Ideally, normal cells (and the patient) could be protected while malignant cells could be sensitized to treatment. Chemoprotectants such as amifostine, glutathione, mesna, and dexrazoxane have been investigated and shown to provide drug-dependent protection to specific tissues, but the use of these compounds has not been shown to increase disease-free or overall survival [140]. One of the hallmarks of longevity interventions is increased stress resistance of the whole organism (and normal cells).

However, because of acquired mutations that render cells self-sufficient in growth and insensitive to anti-growth signals, cancer cells act autonomously and independently, providing an opportunity for geroscience to exploit the difference by selectively protecting the normal tissue without interfering, or even enhancing, treatment efficacy [93] (Fig. 1). Another opportunity that geroscience may provide is the identification of novel drug targets for cancer and/or the scientific basis for an oncocentric use of already FDA-approved pharmaceuticals.

The following are some geroscience-based interventions that deserve further investigation with the potential to provide a different approach to cancer treatment, whereby the focus not only continues to be on improving the killing of cancer cells, but also on the preservation of the patient's healthspan post-treatment.

5.1 Metabolic Interventions

5.1.1 Dietary Interventions

Dietary interventions have contributed greatly to our understanding of lifespan and stress resistance regulation. Dietary restriction (DR) is the most effective and reproducible intervention to decelerate the rate of aging and increase healthspan in various model organisms ranging from the simple yeast to worms, flies, rodents, and possibly non-human primates [48, 90, 118, 142]. In 1934, Crowell and McCay

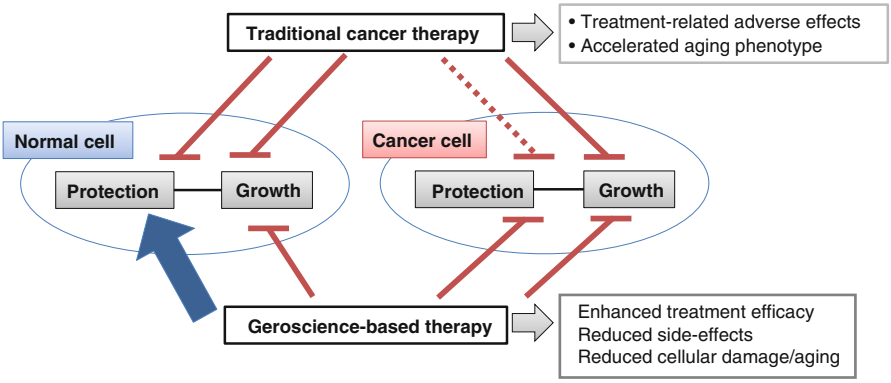

Fig. 1 Geroscience-based approaches have resulted in interventions aimed at protecting normal cells while instead sensitizing cancer cells to therapy. The Differential Stress Resistance and Sensitization strategies to treat cancer have resulted from basic and translation geroscience research that first described how starvation can protect normal cells from a wide variety of toxins and then identified proto-oncogenes as the negative regulators of the resistance. Basic geroscience research also described how cells expressing oncogene analogs have an adaptive disadvantage when expose to extreme and complex environments such as starvation in addition to toxins. These findings were exploited to protect mice and possibly humans from chemotherapy while rendering cancer cells more sensitive to the treatment

reported that rats fed a calorie restricted diet with sufficient nutrients starting after weaning extended lifespan nearly twice [150]. Following this seminal discovery, Walford and Weindruch reported that in mice, adult-initiated DR (undernutrition without malnutrition) which began at 12-months of age [248, 249], and DR initiated at weaning both increased lifespan and reduced tumor incidence [250], confirming the DR studies in rats. A >30-year longitudinal adult-onset DR study in rhesus monkeys performed at the Wisconsin National Primate Research Center (WNPRC) shows that DR (30 %) delayed disease onset and mortality, with a 50 % decrease in cancer incidence [48, 49]. However, a comparable >20-year study performed at the National Institute on Aging (NIA) shows a slightly different result where DR did not extend lifespan, but successfully improved health span, including reduced incidents of cancer and diabetes [149]. The disparities between the WNPRC and NIA studies were largely attributed to differences in diet composition and the genetic origin of the monkeys, suggesting that DR may need to be carefully considered if it is to be practiced in humans.

The mechanisms underlying DR are still unclear, but energy allocation appears to be fundamental. Because a cell can only retain a limited amount of energy at any given time, the cellular energetic network must economically balance the finite energy reserve between reproduction/growth and repair/maintenance [119]. However, under starvation or DR, the favored survival strategy is to discourage reproduction/growth and invest the remaining energy in repair/maintenance [119]. In a way, this switch of energy encourages the entrance into a maintenance mode, which could explain why DR or fasting reduces size and fertility, and increases lifespan and stress resistance [211].

Notably, short-term fasting selectively protects normal cells and mice while sensitizing malignant cells to chemotherapeutics and ionizing radiation, providing a means to induce differential stress resistance (DSR) [133, 183, 196]. Furthermore, short-term fasting may reduce chemotherapy-induced side effects such as nausea and vomiting in cancer patients [197]. Multiple fasting cycles protect hematopoietic cells from chemotoxicity and also promote their self-renewal in mice [42], and has the potential to prevent long-term treatment-related stem cell exhaustion. Short-term DR also enhances skeletal muscle stem cell function [38].

In addition to DR, simply restricting a single essential amino acid increases lifespan and stress resistance [57, 174, 188, 206, 277]. In flies, restoring essential amino acids to DR was sufficient to reverse lifespan extension [88]. Rodents fed a methionine or tryptophan restricted diet (MR and TR, respectively) lived longer and showed significantly less age-dependent diseases and enhanced resistance to oxidative stress [188], including tumor growth retardation in animals fed a MR diet [27, 222], and shows much potential in humans [67]. MR has been shown to have marked negative effects on cancer cells [36], whereas normal cells are relatively resistant to methionine restriction [37]. However, the use of specific amino acid restrictions for cancer treatment in vivo has limited applications since cancer cells can obtain methionine from other cells and tissues, therefore causing potentially more harm to normal cells than cancer cells. As has been shown for fasting, MR can also sensitize cancer cells to chemotherapeutics [86, 105, 221, 122, 268], suggesting its potential

to induce differential stress sensitization (DSS). On the other hand, TR also provides longevity and reduced age-dependent deterioration [57, 174, 207], but has mainly been explored for neurological benefits due to its role in serotonin synthesis. Interventions that can induce DSR may increase the therapeutic window of available cancer treatments, while at the same time protecting normal cells from treatment and preventing long-term secondary adverse health issues in cancer survivors. Fasting and DR are candidate interventions with similar and different characteristics, but short-term fasting may be more clinically feasible because of its brevity, wide range, and efficacy [132]. Nonetheless, fasting is not a trivial intervention, especially for cancer cachectic patients, and fasting-mimicking diets providing high nourishment and a relatively high calorie content are likely to prove more beneficial.

5.1.2 Mitochondria Interventions

The recent identification of short open reading frames (sORFs) in the mtDNA that yields bioactive peptides such as humanin and MOTS-c, represent an entirely novel layer of signals that are inherently mitochondrial [87, 134]. The levels of these mitochondrial hormones decline with age both in the circulation and in relevant tissues in laboratory rodents [87, 136]. Humanin expression is regulated by the GH/IGF-I axis and its levels are positively correlated with longevity in long-lived mouse models [61]. Humanin is a protective factor against various types of stress, chiefly those that are related to oxidative stress. Many labs have reported on the protective effects of humanin in various disease models including Alzheimer's disease [98, 156, 167, 273], atherosclerosis [172, 269], and ischemic injury [164, 264, 265]. A recent report on bortezomib, a drug in clinical trial for childhood cancers, shows that humanin treatment successfully prevented bortezomib-induced toxicity to growth plate chondrocytes that lead to growth arrest without interfering with its anti-cancer effects [46, 68].

5.1.3 Glycolysis Blockade

Glucose is a major source of energy and carbon for mammalian cells. Inhibitors of glucose catabolism or generation, also considered calorie restriction mimetics, have been shown to increase lifespan in mice. Acarbose reduces the breakdown of starches and disaccharides to glucose by inhibiting α-glucosidases in the intestine, and thus limits glucose supply to cells. Acarbose treatment increased the median lifespan of male and female mice by 22 % and 5 %, respectively [96]. Acarbose is currently used to treat type 2 diabetes, but in addition it has been shown to also exhibit cardio-protective benefits [43]. In flies, feeding acarbose reduced tumor growth and improved survival [101].

2-deoxyglucose (2-DG) is a glucose analog that is phosphorylated by hexokinase to 2-DG-phosphate, which cannot be further metabolized and therefore blocks gly-

colysis. Rats fed 2-DG showed similarity to those under CR with improved glucose and insulin regulation [263], and increased recovery from stress [170]. 2-DG is actively being investigated for cancer treatment and although its use as a single modality drug in still debated [230], combination treatments with chemotherapy and radiotherapy are very promising [148, 186].

5.1.4 Intervening in the GH/IGF-I Axis

Dietary interventions alter a wide range of processes. However, there are certain key pathways that have been elucidated to mediate and/or mimic their effects. DR and fasting both reduce circulating levels of growth hormone (GH) and its downstream effector insulin-like growth factor 1 (IGF-1) [132]. In fact, it is suggested that the GH/IGF-I axis is a major mediator of the beneficial effects of DR [22], and a major regulator of lifespan and stress resistance [90, 118, 142]. GH deficient mice are resistant to stress, are smaller in size (dwarf), have reduced fertility and reduced levels of circulating GH/IGF-I, insulin, and glucose [113, 142, 160]. Conversely, mice overexpressing GH have a shortened lifespan [12]. Ecuadorian individuals with GH receptor (GHR) mutations coupled with severe GHR and IGF-1 deficiencies (Laron syndrome) are immune to diabetes and dramatically reduced cancer incidence [91]. Serum from Laron subjects protected human mammary epithelial cells from oxidative stress, concurrently reducing the expression of downstream signaling elements including RAS, protein kinase A (PKA), and mTOR. In addition, cells of long-lived mice with GH/IGF-I axis deficiencies are more resistant to oxidative stress (H_2O_2, paraquat), UV, genotoxins (methylmethanesulfonate, MMS), heat, and cadmium [160, 199], suggesting that enhanced stress resistance is at least partially responsible for longevity, and the possibility to enhance protection by interventions such as DR or down-regulation of the GH/IGF-I axis.

All things considered, the GH, IGF-I and insulin pathways appear to be major mediators/regulators of aging and stress resistance [22]. Therefore, interventions targeting the GH/IGF-1 axis may provide normal cells with increased stress resistance against cancer treatment and thus prevent secondary adverse health problems in cancer survivors. For instance, octreotide, a somatostatin analog that antagonizes GH production, has been shown to ameliorate chemoradiotherapy-induced diarrhea [216, 275]. Pegvisomant is a dominant negative GH mimetic that is FDA-approved for the treatment of acromegaly. It would be of interest to investigate how octreotide and Pegvisomant affect both the short-term and long-term effects of chemotherapy and other cancer treatments.

5.1.5 Intervening in the Nutrient Sensing Pathways

Nutrient sensing pathways are involved in longevity regulation and thought to partially mediate DR [75]. Many of these pathways are downstream of the GH/IGF-1 axis, including mTOR/S6K, PI3K/AKT, RAS, and AC/PKA and are highly

conserved from single-celled yeast to humans [75]. In yeast, deleting human homologs of RAS (*RAS2*) and/or AKT (*SCH9/S6K*) increased lifespan to more than twofold while providing increased stress resistance against oxidants, genotoxins, and heat-shock [142]. Similarly, in *C. elegans*, mutations in the human homologs of insulin/IGF-1 receptor (*daf-2*) and PI3K (*age-1*) extended lifespan by twofold and increased resistance to thermal and oxidative stress [120]. In *D. melanogaster*, mutations in the insulin receptor substrate (*chico*) led to a 50 % lifespan extension [83].

Pharmacological manipulations can also increase lifespan, as shown in mice fed rapamycin [23], metformin [146], or SRT1720 [157, 158]. Rapamycin inhibits the mammalian target of rapamycin (mTOR), which can function downstream of IGF-I but can also be activated independently of IGF-1, and acts as a major regulator of cellular proliferation, metabolism, and stress [184, 262]. Rapamycin, the most experimentally successful longevity agent tested in model organisms, has recently been shown to extend both mean and maximum life span of both male and female mice, and in several genetic backgrounds [23]. Although rapamycin was initially used in the clinic as an immunosuppressant for organ transplants, its potential for cancer treatment was recognized more recently [34]. Analogs of rapamycin (rapalogs) with improved pharmacokinetics and solubility, including temsirolimus and everolimus, are being developed. Temsirolimus has been approved by the FDA for treating renal cell carcinoma, and clinical trials to test its efficacy in other cancers are underway [45, 209]. Everolimus is an oral rapalog that is also FDA-approved for various cancer treatments, including advanced renal carcinoma [155]. In addition to its anticancer effects, rapamycin has been shown to prevent stem cell senescence, protect mice from ionizing radiation-induced loss of proliferative basal epithelial stem cells [108], and enhance stem cell niche support [267].

Metformin is a front-line drug of choice for the treatment of type 2 diabetes, with several proposed mechanisms of action [76], that has recently gained much attention in cancer therapy [177]. An early report suggested that diabetes patients that received metformin as part of their treatment had a 23 % reduction in the risk for cancer [71]. A meta-analysis on 25 studies recruiting 579,621 patients reported that metformin use was associated with an overall 27 % reduction in the risk of developing any malignancy [77]. In particular, breast cancer has received much attention with promising results supporting the efficacy of metformin use in cancer [179]. Much of these studies involve diabetes patients who are at a higher risk for cancer [84], thus further randomized controlled clinical trials are needed to evaluate the efficacy of metformin in non-diabetic cancer patients. Furthermore, the use of metformin as a preventive measure of cancer should be considered [159]. In worms, metformin has been shown to extend lifespan through mitohormesis via the peroxiredoxin PRDX-2 [56], and by targeting the folate cycle in their bacterial feed, causing a methionine-restricted diet [31]. In mice, metformin (0.1 % w/w in diet) starting at middle age extended healthspan and lifespan in male mice, while a higher dose (1 % w/w) was toxic [127]. Notably, the combination or rapamycin and metformin may successfully antagonize cancer cells while protecting normal fibroblasts or epithelial cells, and thus prevent secondary health problems in cancer survivors [6].

Resveratrol activates Sirt1, an NAD^+-dependent deacetylase that has been shown to increase lifespan in lower organisms [251, 259]. In mammals, resveratrol treatment improved lifespan and healthspan in mice on a high-fat diet [65], and transgenic mice with moderate over-expression of Sirt1 showed an improved metabolic profile in multiple models of insulin resistance and diabetes [10, 178]. In addition to Sirt1, resveratrol is thought to act through multiple additional targets. In contrast, SRT1720 is a specific activator of SIRT1 that improves lifespan and healthspan in mice on both standard and high-fat diets [121, 157]. The effect of SRT1720 on cancer is mixed, with one study showing promotion of breast cancer cell migration and metastasis [217], and another showing increased apoptosis of breast cancer [135] and multiple myeloma cells [47].

6 Future Prospects for Geroscience and Cancer Treatments

Interventions aimed at affecting the vastly complex nature of the aging process require the understanding of its effect on a wide range of biological processes. However, the rate of aging is also affected by environmental factors such as chemotherapy which can profoundly alter its course. The topics discussed above have been categorized to help us understand this vast biology, but in fact they cover overlapping components of a biological network, each one influencing the other. For instance, telomere attrition can cause genomic instability that can lead to senescence, which in turn can affect inflammation (SASP) and stem cell exhaustion. Similarly, mitochondrial damage and increased ROS generation can damage DNA and cause genomic instability and senescence. The cytotoxic ripples inflicted by cancer chemotherapy and radiotherapy are wide and are among the most impactful interventions affecting aging and age-related diseases. Because many types of cellular damage caused by cancer therapy seem to accelerate those that occur naturally with age, a gero-centric approach may provide a more comprehensive solution both at the level of prevention and treatment. Geroscience can largely contribute to cancer therapy in at least three ways: (1) provide novel interventions and/or targets; (2) provide a method or intervention to selectively protect the patient, based on the stress-resistant phenotype of many long-lived model organisms, and (3) both enhance the killing of cancer cells while protecting the patient. On this line, geroscience-based intervention(s) should be promoted and urgently investigated to grasp the broader landscape of healthspan and quality of life of cancer-survivors. Lastly, there is a need to educate the patients of long-term consequences of cancer therapy and how geroscience can contribute to their decision-making and their post-treatment choices aimed at optimizing healthspan and quality of life.

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Cardiovascular Disease and Aging

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1 Introduction

Aging impairs the cardiovascular system and is the dominant risk factor for cardiovascular disease (CVD). The incidence and prevalence of most CVD increase with advancing age, and CVD are the leading cause of death for populations over 65 years of age. According to the heart disease and stroke statistics 2014 update published by the American Heart Association, Americans 60–79 years of age have over 70 % prevalence rate and Americans over 80 years of age have over 80 % prevalence rate of cardiovascular diseases [1]. However, most of the research efforts on prevention of these diseases have ignored age and have focused instead on development of interventions that target "traditional" CV risk factors such as hypertension, high blood cholesterol and triglycerides. In addition to the increased prevalence of cardiovascular diseases, aging is also associated with impaired responses to cardiovascular diseases. Aging also leads to deterioration of the structure and function of the heart and vasculature in individuals without overt cardiovascular disease, as reflected in cardiovascular measurements that are made in healthy individuals at rest and during exercise, both of which reveal aging-related changes. Therefore, it is important to understand the molecular mechanism of cardiovascular aging and how the age-related changes in the cardiovascular system interact with the pathophysiological mechanisms that lead to cardiovascular disease. In this chapter, we will describe the characteristics of cardiovascular aging in humans and in mammalian models, and review the roles of different hallmarks of aging in cardiovascular aging. Valvular degeneration in the aging heart is not addressed in this review, and the reader is referred to other sources [2, 3].

2 Cardiovascular Aging in Humans

2.1 Cardiac Aging

2.1.1 Cardiac Structure/Function at Rest

A continuum of expression of cardiac structural and functional alterations occurs with age in healthy humans (Fig. 1). On the one hand, a unified interpretation of identified cardiac aging changes at rest in otherwise healthy persons (Fig. 1) suggests that these changes are at least in part adaptive, occurring to some extent in response to changes that occur within the arterial tree that result in increased load on left ventricular ejection [4]. On the other hand, these age-associated cardiac changes also have direct relevance to the steep age-associated increases in left ventricular hypertrophy (LVH), chronic heart failure, and atrial fibrillation (AF).

2.1.2 Cardiac Structure

Cross-sectional studies of subjects without hypertension or clinically apparent CVD indicate that with advancing age the walls of the left ventricle increase in thickness, largely because of an increase in ventricular myocyte size and an increase in vascular

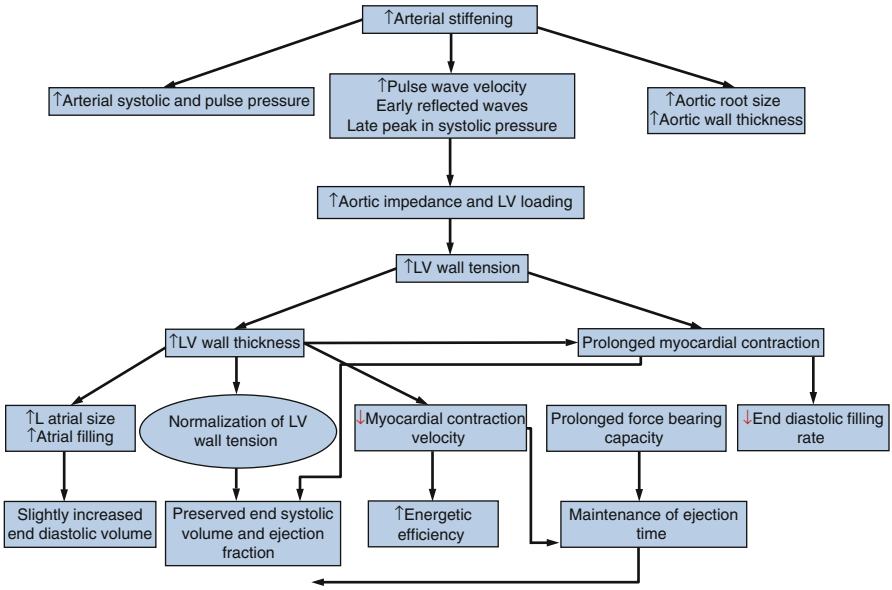

Fig. 1 Arterial and cardiac changes that occur with aging in healthy humans (Adapted from Lakatta [252])

impedance, and this helps moderate the increase in LV wall tension (Fig. 1). Modest increases in collagen levels and non-enzymatic cross linking, rendering collagen stiffer, also occur with aging. In older hospitalized patients without apparent cardiovascular disease, however, the cardiac myocyte-to-collagen mass ratio in the older heart either remains constant or increases because of an increase in myocyte size.

2.1.3 Left Ventricular Function at Rest

Increased central arterial stiffness is a factor that leads to an increase in the afterload on the left ventricle, leading to increased left ventricular wall thickness (Fig. 1). The left ventricle and the central arteries have bidirectional constant interactions. One useful index of this interaction is termed arterial-ventricular coupling. This tight heart-arterial coupling is thought to allow the cardiovascular system to optimize energetic efficiency. The LV ejection fraction (EF) at rest, the most commonly used clinical measure of heart-arterial crosstalk, is preserved during aging (Fig. 2). The average value of resting EF is approximately 65 %, and very few healthy, sedentary, community-dwelling older individuals have EF <50 % (which would indicate impaired LV-arterial crosstalk [5].)

Prolonged contraction of the thickened LV wall maintains a normal ejection time in older persons in the presence of the increased resistance to pulsatile blood flow from the heart. This preserves the systolic cardiac pumping function at rest. One disadvantage of prolonged contraction is that at the time of the mitral valve opening, myocardial relaxation is relatively more incomplete in older than in younger individuals. The LV early diastolic filling rate progressively slows after the age of 20

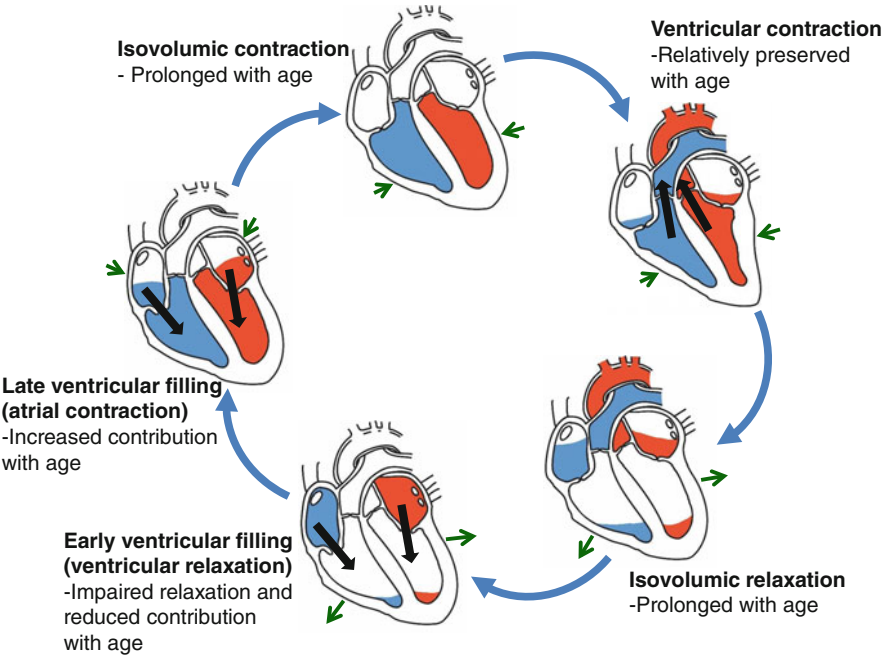

Fig. 2 The age-related changes in different phases of the cardiac cycle. With age, ventricular systole remains relative preserved while isovolumic relaxation and contraction are prolonged. Ventricular relaxation is impaired and the relative contribution of early ventricular filling is reduced, with an increased contribution of atrial contraction (late filling)

years [6–8], so that by 80 years the rate is reduced, on average, up to 50 %. Structural (fibrous) changes within the LV myocardium or residual myofilament Ca^{2+} activation from the preceding systole are putative mechanisms for a reduced early diastolic LV filling rate. Structural changes and functional heterogeneity occurring within the left ventricle with aging may also contribute to this reduction in peak LV filling rate. However, concomitant adaptations – left atrial enlargement and an enhanced atrial contribution to ventricular filling (Fig. 1) – compensate for the reduced early filling and prevent a reduction of the end diastolic volume. Thus, despite the age-associated changes in the diastolic filling pattern in older, healthy persons, their left ventricular end-diastolic volume in the supine position is not compromised and does not substantially differ from that of their younger counterparts [9, 10]. Thus, neither EF nor cardiac output at rest declines with advancing age.

2.1.4 Cardiovascular Reserve

During exercise, impaired heart rate acceleration and impaired augmentation of blood ejection from the left ventricle, accompanied by a modest increase in LV end-diastolic volume are the most dramatic changes in cardiac reserve capacity that

occur with aging in healthy, community-dwelling persons [11]. Mechanisms that underlie the age-associated reduction in maximum ejection fraction are multifactorial and include a reduction in intrinsic myocardial contractility, an increase in vascular afterload, and arterial-ventricular load mismatching. Although these age-associated changes in cardiovascular reserve, per se, are usually insufficient to cause clinical heart failure, they do affect its clinical presentation, the threshold for symptoms and signs, and the severity and prognosis of heart failure secondary to any level of disease burden. Notably, this loss of reserve limits exercise capacity and contributes to frailty in the elderly.

Sympathetic neural impulses to the heart via beta-adrenergic receptor stimulation of heart and vascular cells elicit the recruitment of cardiovascular reserve capacity during stress. The essence of sympathetic modulation of the cardiovascular system is to ensure that the heart beats faster, to ensure that it retains a small size by reducing the diastolic filling period, reducing LV afterload, to augment myocardial contractility and relaxation, and to redistribute blood to working muscles and to skin to dissipate heat. A sizeable component of the age-associated deficit in cardiovascular reserve is composed of diminished effectiveness of the autonomic modulation of heart rate, LV contractility, and arterial afterload.

Multiple lines of evidence support the idea that the efficiency of postsynaptic beta-adrenergic signaling declines with aging in numerous species [4]. One line of evidence stems from the observation that cardiovascular responses to beta-adrenergic agonist infusions at rest decrease with age in humans and mammalian models. A second type of evidence is that acute beta-adrenergic receptor blockade changes the exercise hemodynamic profile of younger persons to make it resemble that of older individuals. Significant beta blockade–induced LV dilation occurs only in younger subjects. The heart rate reduction during exercise in the presence of acute beta-adrenergic blockade is greater in younger versus older human subjects, as are the age-associated deficits in LV early diastolic filling rate, both at rest and during exercise. In older dogs it has also been observed that an age-associated increase in aortic impedance during exercise is abolished by acute beta-adrenergic blockade [12].

Apparent deficits in sympathetic modulation of cardiac and arterial functions with aging occur in the presence of exaggerated neurotransmitter levels. Plasma levels of norepinephrine and epinephrine, during any perturbation from the supine basal state, increase to a greater extent in older compared with younger healthy humans. The age-associated increase in plasma levels of norepinephrine results from an increased spillover into the circulation and, to a lesser extent, reduced plasma clearance. Deficient norepinephrine reuptake at nerve endings is a primary mechanism for increased spillover into the circulation of older persons during acute, graded exercise (exercise intensity is progressively increased until the subject reaches a self-imposed fatigue level). During prolonged exercise, however, diminished neurotransmitter reuptake might also be associated with depletion and reduced release and spillover in older persons.

2.1.5 Heart Rhythm

Beat-to-beat fluctuation of heart rate (HR) at rest is known as HR variability. It is a physiological phenomenon that reflects different inputs to the cardiac conduction system and variation results from normal fluctuation in these inputs (not to be confused with arrhythmia, described below). However, HR variability declines steadily with age and this reduction is an indicator of cardiac autonomic dysregulation commonly found in older people; it has been linked to increased risk for morbid and fatal outcomes [13].

An increase in the prevalence and complexity of both supraventricular and ventricular premature beats and arrhythmias, whether detected by resting ECG, ambulatory monitoring, or exercise testing, occurs in otherwise healthy older patients and are generally not associated with heart disease, even in older persons. Over a 10-year mean follow-up period, isolated atrial premature beats, even if frequent, were not predictive of increased cardiac risk in these individuals [14].

Short bursts of paroxysmal supraventricular tachycardia (PSVT) are observed in 1–2 % of apparently healthy individuals older than 65 years who were rigorously screened to exclude disease. PSVT at rest or induced by exercise is an early clue that some healthy individuals are at an increased risk for future atrial fibrillation (AF). Another risk factor for AF may be the increase in left atrial size (Fig. 1) that accompanies advancing age in otherwise healthy persons [4].

Limited data available in older persons without apparent heart disease support a marked age-associated increase in the prevalence and complexity of ventricular ectopy (VE); i.e., premature heart beats originating from the ventricular myocardium, both at rest and during exercise, at least in men. A steep increase in the prevalence of VE with advancing age occurs in both clinically free of heart disease individuals and in unselected populations [15]. Neither the prevalence nor the complexity of VE at rest was a determinant of future coronary events over a 10-year mean follow-up period [14].

2.2 Arterial Aging

Intima-media thickening increases with advancing age in animal models that are devoid of atherosclerosis [16], thus indicating that these age-associated alterations are due to the aging process and not to superimposed atherosclerosis. Aging is also associated with thickening of the walls of the central arteries. Intima-media thickness (IMT) rises nearly threefold between the third and ninth decades of life, which is mainly attributable to an increase in intimal thickness in the context of inflammation [17]. A growing body of literature has shown that traditional cardiovascular risk factors and prevalent CVD are associated with increased IMT, and that IMT is itself a potent and independent predictor of adverse CV events. Diffuse intima-media thickening, however, should not be construed as synonymous with

"subclinical atherosclerosis," particularly in the absence of plaques. This thickening is due to myriad age-associated biochemical, cellular, and morphologic changes in the arterial wall, which are modulated by the same factors that have been implicated in the genesis of various CVD [18]. Thus, IMT remains a useful marker of an arterial risk factor associated with aging [19]. In contrast to *central* arteries, IMT is only modestly correlated with *coronary* artery disease [20].

One of the hallmarks of central arterial aging is an age-associated increase in arterial wall stiffness. The age-associated increase in stiffness has frequently been attributed to the fraying and breakdown of elastin due to the lifelong repeated cycles of distention and recoil of the central aorta as well as the increased deposition and covalent cross-linking of collagen molecules. It is now recognized that arterial stiffening can be modulated by several factors besides aging, including lifestyle (e.g., salt intake, exercise, or weight loss) [21], signaling molecules (e.g., nitric oxide) [22], inflammation, and genetics [23]. Manifestations of arterial aging vary among the different vascular beds, reflecting differences in the structural compositions of the arteries and perhaps differences in the age-associated signaling cascades that modulate the arterial properties, or differences in the response to these signals across the arterial tree. For example, in contrast to the central elastic arteries the stiffness of the muscular arteries does not increase with age (e.g., brachial and femoral arteries).

Aortic pulse wave velocity has been anointed the "gold standard" for the noninvasive assessment of central arterial stiffness [24]. Pulse wave velocity has been shown to be an independent predictor of morbidity and mortality in healthy subjects and in individuals with various levels of cardiovascular risk. It is likely that arterial stiffness is not only a risk marker but also a risk factor for cardiovascular diseases.

Increased central arterial stiffening is a likely explanation of the age-associated changes in blood pressures, whereby systolic blood pressure continues to increase with advancing age, and diastolic blood pressure increases until the fifth decade, then levels off and starts to decrease after the age of 60 years [25]. The decrease in diastolic blood pressure may compromise coronary blood flow, which occurs predominantly in diastole, and a further increase in pulse pressure, which can be twice as high in older vs. younger persons. Numerous clinical and epidemiologic studies in several different populations with varying prevalence of cardiovascular diseases have demonstrated that central pulse pressure is an important predictor of adverse outcomes, often more potent than systolic or diastolic blood pressures. Increased central arterial pulse pressure is transmitted to small arteries of the kidney and heart, damaging these vessels and organs, often resulting in stroke, myocardial infarction and chronic renal disease which increase exponentially with advancing age. Both animal and clinical studies have recently demonstrated that arterial stiffness precedes the development of hypertension [26–28]. Interventions to prevent or to delay arterial stiffening have predominantly focused on pharmacologic antihypertensive therapies. However, these strategies are aimed at lowering blood pressure, whereby the reduction in stiffness is a secondary effect due to reverse remodeling of the arte-

rial wall in response to the lower pressures. Because central arterial stiffness is a potent predictor of mortality and morbidity independent of blood pressure, a more direct approach that would target the stiffening process is desirable.

2.3 Clinical Implications of Cardiovascular Aging

In summary, the most dramatic changes in cardiac function that occur with aging in healthy, adult, community-dwelling subjects, ranging in age from 20 to 85 years, are increased LV wall thickness, alterations in the diastolic filling pattern, impaired LV ejection and HR reserve capacity, and altered heart rhythm. Although these age-associated changes do not usually result in clinical heart disease per se, they do compromise the cardiac reserve capacity and affect the threshold for symptoms and signs, as well as the severity and prognosis of heart failure secondary to any given disease-related challenge. This is true for both systolic and diastolic heart failure. Thus, age-associated changes in the heart structure and function that occur in the absence of a clinical diagnosis of heart disease explain the increased risk for left ventricular hypertrophy, atrial fibrillation, congestive heart failure and diastolic heart failure, all of which occur at markedly higher rates in older persons than in younger persons. These three cardiac diagnoses become interrelated in older persons, in part because of this link with age-associated cardiac changes. An age-dependent increase in left ventricular mass increases the stiffness of the left ventricle and promotes an increase in end diastolic filling pressure, which is an important contributor to diastolic heart failure in older persons. In addition, increased diastolic filling pressure results in left atrial dilation, predisposes the heart to AF. When associated with tachycardia and loss of atrioventricular coupling, AF reduces diastolic filling time and eliminates atrial systolic contribution to left ventricular filling, thereby compounding the predisposition to diastolic heart failure.

Age-associated changes are increasingly recognized as risk factors for CVD. Because many of the age-associated alterations in CV structure and function, at both the cellular and molecular levels, are specific risk factors for cardiovascular diseases, there is an urgency to incorporate cardiovascular aging into clinical medicine to preserve the healthspan of older persons. In spite of the fact that CV aging is a major risk factor for CV disease, it has remained for the most part outside of mainstream clinical medicine because the pathophysiologic implications of these changes are largely underappreciated and are not well disseminated in the medical community. However, an understanding of the age-associated alterations in cardiac and arterial structure and function at both the cellular and molecular levels [29, 30] provides valuable clues that may assist in the development of effective therapies to prevent, to delay, or to attenuate the CV changes that accompany aging.

3 Cardiovascular Aging in Mammalian Models

3.1 The Cardiac Aging Phenotype in Mammalian Models

Most of what we know about the molecular basis of cardiovascular aging comes from animal models of human aging and heart function. Cardiac aging responses have been characterized in multiple animal models, including nonhuman primates, dogs, rats and mice. However, due to the different species, strains and definitions of age groups used by different studies, the results should be interpreted cautiously.

Rodents, particularly mice, are widely used in cardiac aging studies. While the rodent heart is different from those of primates and other larger mammals (particularly the electrical conduction system), in general, cardiac aging in rodents closely recapitulates the cardiac aging phenotypes seen in humans without overt cardiovascular diseases [31]. Dai and colleagues showed in a mouse longevity cohort that there were significant age-dependent linear trends for several cardiac parameters [32]. They showed by echocardiography that left ventricular mass index and left atrial dimension significantly increased with age. Diastolic function measured by tissue Doppler echocardiography revealed an age-dependent decline in the ratio of early to late diastolic mitral annular velocity (Ea/Aa) and the frequency of diastolic dysfunction [33], defined as Ea/Aa <1, was increased in C57B6 mice over 24 months of age. The proportion of mice with atrial dilation also significantly increased with age [32]. Systolic function, measured by ejection fraction (EF) and fractional shortening (FS) remain relatively unchanged in middle-age and old mice but decreased in 32 month-old senescent mice [34, 35]. The myocardial performance index was significantly worsened with age [36], indicating that a greater fraction of systole is spent to overcome the pressure changes during isovolumic phases; this has been shown to reflect both LV systolic and/or diastolic dysfunction [37]. The aging-associated changes in different phases of the cardiac cycle are summarized in Fig. 2.

At the cellular level, reduced myocyte number and hypertrophy of remaining myocytes have been demonstrated in aging rats and mice [34, 38]. Structurally, LV wall thickness increases in middle-age and old mice, but decreases in senescent mice [34, 35, 39]. This suggests that the hypertrophic growth of the myocytes in middle-age and old mice becomes decompensated in senescent mice, consistent with myocyte loss [34]. At a histopathological level, the aged hearts display interstitial fibrosis, cytoplasmic vacuolization and hyalinization, increased variation in myocyte fiber size, collapse of sarcomeres, mineralization, arteriosclerosis and arterio*lo*sclerosis [40].

The relatively short lifespan and the availability of genetically modified mice also make mouse models useful tools for study of the molecular mechanisms of cardiac aging. In addition, the general absence of common cardiovascular risk factors such as diabetes and hypertension [32, 41], allows intrinsic cardiac aging changes to be distinguished from disease-induced changes.

3.2 The Vascular Aging Phenotype in Mammalian Models

Aged laboratory rodents exhibit a range of vascular changes also observed in humans. These include endothelial dysfunction [42], structural remodeling, arterial stiffening, vascular oxidative stress and inflammation [43], vascular calcification, microvascular rarefaction [44], autoregulatory dysfunction and impaired functional adaptation to hypertension [45, 46], blood brain barrier disruption [45], neurovascular uncoupling [47], impaired cellular stress resistance [48], increased susceptibility for vascular injury, and mitochondrial dysregulation [49, 50]. Thus, laboratory rodents are well-suited models for studying these aspects of aging-induced vascular pathologies. In contrast, laboratory rodents are not ideal models for age-related increases in blood pressure [51]. Aged wild type mice and rats also do not develop atherosclerotic plaques spontaneously (in the absence of genetic depletion of *Ldlr* or *Apoe*) similar to those observed in aged primates.

The age-related damage of the arterial tree in non-human primates is of particular interest, as these species are physiologically and phylogenetically closer to humans than the more commonly studied rodent models. With age, non-human primates exhibit increased arterial stiffness [52], increased central arterial pulse pressure, increased carotid intima-media thickness, hypertension [53], activation of processes involved in atherogenesis [54], development of aneurysms [55], vascular oxidative stress and inflammation [56, 57], endothelial dysfunction and apoptosis [16], alterations of the blood brain barrier [58] and cellular mitochondrial content [59] and impaired cellular stress resistance [57], mimicking the human vascular aging phenotype.

4 Molecular Mechanisms of Cardiovascular Aging

4.1 Mechanisms of Cardiac Aging

While the phenotypes of cardiac aging have been well-characterized for decades, the molecular mechanisms of cardiac aging are just beginning to be revealed. Using genetic models and interventions targeting different pillars of geroscience, researchers have revealed several critical molecular mechanisms of cardiac aging in the past decade that have the potential to translate into cardiovascular healthspan interventions (summarized in Fig. 3).

4.1.1 Macromolecular Damage and Mitochondrial ROS

There are multiple sources of reactive oxygen species (ROS) in the cells but mitochondria, which generate ROS during oxidative phosphorylation, are the origin of most ROS. The heart has a high metabolic demand and is rich in mitochondria and

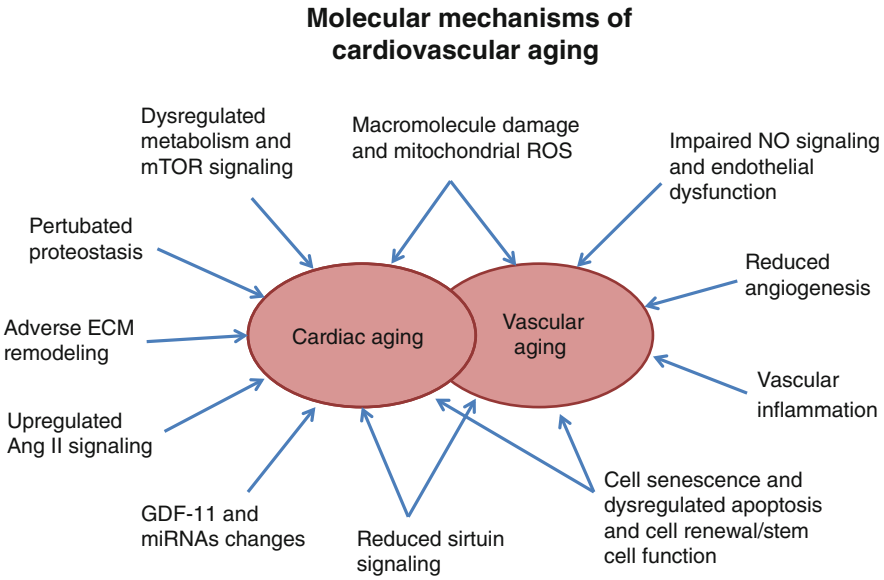

Fig. 3 A diagram summarizing the molecular mechanisms that mediate cardiovascular aging. Multiple pathways and molecules have been demonstrated to play critical roles in cardiac and vascular aging. The details are discussed in Sect. 4

therefore may be especially prone to oxidative damage. Increasing evidence suggests the role of abnormal mitochondrial ROS (mtROS) production and detoxification in mitochondrial dysfunction and cardiomyopathy in old age (reviewed in [60–62]). It has been shown that mitochondrial production of ROS significantly increases in the aging heart [63]. With advanced age, oxidative phosphorylation and mitochondrial state 3 respiration are significantly reduced due to diminished complex I and complex IV activities (see review [64]). The impairment in electron transport chain function may lead to elevated electron leakage and increased mitochondrial ROS production.

Direct evidence of the role of mitochondrial ROS in aging was provided by mice overexpressing catalase targeted to the mitochondria (mCAT). The mCAT mice, but not mice overexpressing wild-type human catalase (naturally delivered to peroxisomes, pCAT), showed an 18 % extension of mean and maximal lifespan [65]. Although cardiac failure was not the major cause of death in these mice, they exhibited great attenuation of many cardiac aging phenotypes, including left ventricular hypertrophy, diastolic dysfunction and impaired myocardial performance, as well as significant amelioration of age-dependent cardiomyocyte hypertrophy, interstitial fibrosis and mitochondrial ultrastructural changes [32, 65]. These cardiac aging benefits in mCAT mice were accompanied by significantly reduced mitochondrial protein oxidative damage and mitochondrial DNA mutation and deletion frequencies [32, 65].

In contrast, mice with homozygous mutation of mitochondrial polymerase gamma ($Polg^{m/m}$) have substantial increases in mtDNA mutations and deletions with age [66, 67], shortened lifespan and exhibit accelerated cardiac aging and developed cardiomyopathy in middle age (13–14 months) [66, 68]. Middle age $Polg^{m/m}$ mice display cardiac hypertrophy, impaired systolic and diastolic function to an extent that is even more severe than wild type mice 24–30 months old. Interestingly, mCAT partially rescues the mitochondrial damage and cardiomyopathy in $Polg^{m/m}$ mice, supporting the role of mitochondrial ROS and mtDNA damage in cardiac aging [68].

In addition to the decline in cardiac function with age, increased susceptibility of the aged heart to stress is also likely related to mitochondrial dysfunction. The aged myocardium has reduced tolerance to ischemia and hemodynamic stress [69], and aged cardiomyocytes have a lower threshold for ROS-induced ROS release and increased susceptibility to mitochondrial permeability transition pore (mPTP) opening [70]. Ischemic preconditioning is also impaired in the aged myocardium (see review [70]), which might be due to a decrease in mitochondrial heat shock protein-70 [71], reduced nitric oxide bioavailability [72], damaged mitochondria that are vulnerable to stress, and diminished PKC translocation into mitochondria [73, 74].

4.1.2 Metabolism and mTOR Signaling

Mechanistic target of rapamycin (mTOR) integrates nutrient and hormonal cues to regulate growth and longevity and is an important modulator of aging and age-related disease [75]. The nutrient sensitive TORC1 branch of the mTOR pathway is a complex that includes mTOR and Raptor (regulatory associated protein of mTOR) and activity of this complex is inhibited by rapamycin. Active TORC1 phosphorylates p70S6K, which accelerates ribosome biogenesis. TORC1 also phosphorylates 4E binding protein 1 (4EBP1) which results in its release from the inactive 4EBP1/eukaryotic initiation factor 4E (eIF4E) complex to allow cap-dependent translation initiation (see review [76]). Studies have shown that increased mTOR signaling impairs – and reduced mTOR signaling improves – resistance to cardiac aging.

In a *Drosophila* model of aging related attenuation of cardiac function, Bodmer's laboratory initially showed that inhibition of the mTOR pathway could attenuate the aging-related decline [77]. Subsequently, they determined that overexpression of 4EBP prevented the aging cardiac decline to the same extent as overexpression of the TOR antagonist TSC, and that, conversely, overexpression of eIF4E leads to a more rapid decline of myocardial function with age [78]. These findings suggest that mTOR signaling through eIF4E plays a primary role in cardiac aging in Drosophila.

In the mouse model, cardiac-specific gene ablation of TSC1, which is an engineered model for increased mTOR signaling, develop cardiomyopathy with the occurrence of scattered foci of enlarged ventricular myocytes and have a median survival of only 6 months [79]. Direct genetic evidence is not yet available in mice to document that decreased mTOR activity is associated with improved cardiac

aging; however data from caloric restriction (CR) and rapamycin support this conclusion.

CR has been shown to extend longevity in numerous animal models and a large body of evidence supports a major role of inhibition of mTOR signaling in mediating the CR benefit [80]. Literature from both rhesus monkey studies and short-duration CR in humans provides strong evidence that CR reduces aging- and/or diet-dependent risk factors associated with heart disease: resting heart rate and blood pressure are decreased, insulin sensitivity is enhanced, lipid profiles are improved, and inflammatory processes that likely contribute to atherosclerosis are reduced [81]. Taffett et al. found that CR of mice had a large positive effect on age-related impaired diastolic function [82]. A later study suggested that in humans that had undertaken CR for a mean of 6.5 years, there was lower blood pressure, lower systemic oxidative stress, and improved diastolic function [83]. In the Dahl salt-sensitive rat (with high-salt induced hypertension), moderate calorie restriction markedly attenuated changes in heart weight, left ventricular mass; wall thickness and echocardiography demonstrated that CR reduced cardiac diastolic dysfunction in this model [84].

The role of mTOR is further supported by the beneficial effects of rapamycin on cardiac aging. Rapamycin has been demonstrated to extend lifespan in model organisms including yeast, fly, and mouse [85–87]. Long-term rapamycin treatment for 1 year initiated at mid-life reduced hypertrophy but failed to restore systolic function in aged male mice [88]. Flynn et al. showed that short-term treatment for 12 weeks initiated at late life can attenuate age-related cardiac hypertrophy and marginally improve systolic function in female mice, and was accompanied by a reduction in age-related inflammation [89]. Our recent study demonstrated that short-term rapamycin (10 weeks) recapitulated the effect of CR to improve diastolic function and LV hypertrophy in old female mice [90]. This was accompanied by restoration of proteomic and metabolic profiles to more youthful phenotypes.

4.1.3 Proteostasis

Protein homeostasis (proteostasis) is a steady state between protein synthesis and degradation. It functions to maintain protein abundance and quality and supports normal physiological function. Failure to maintain protein homeostasis, leading to accumulation of defective proteins, has been observed in several age-related diseases [91], including neurodegenerative diseases [92], cardiac dysfunction [93, 94], cataracts [95], and sarcopenia [96, 97]. Several protein degradation pathways have been implicated in these failures, including autophagy and the ubiquitin-proteosome system.

Perturbation of proteostasis has also been observed in normal aging [98]. One of the hallmarks of aging hearts is the accumulation of myocardial lipofuscin. This “wear and tear” pigment is membrane-bound cellular waste that can neither be degraded nor ejected from the cell and is composed of incomplete lysosomal degradation products, predominantly from damaged mitochondria [99]. This

accumulation is attributable to increased protein oxidation and damage and age-dependent declines in autophagy and ubiquitin-mediated degradation [76, 100]. In normal physiology, removal of damaged mitochondria occurs primarily through fusion and fission, autophagy and lysosomal degradation. When mitochondrial turnover is perturbed by changes in the rates of mitochondrial fission or fusion or alterations in autophagy, the result is an accumulation of damaged and dysfunctional mitochondria. Dysfunctional mitochondria produce high levels of ROS, have impaired ATP production capacity, and likely participate in aberrant signaling [101, 102]. Tissues with accumulated damaged mitochondria may become senescent and cells may undergo apoptosis when a critical threshold of dysfunctional mitochondria have accumulated or when the affected tissue is challenged with an external stress exceeding the cellular functional reserve. Accumulation of damaged, high ROS-producing mitochondria have been demonstrated in aged hearts [101, 102] and are shown to play a role in cardiac hypertrophy [103].

Age dependent cardiac hypertrophy and diastolic dysfunction are also accompanied by cardiac proteomic changes [90]. Briefly, the levels of several mitochondrial proteins, including those involved in tricyclic acid cycle and electron transport chains as well as major metabolic pathways, such as fatty acid beta oxidation, amino acid metabolism, and ketogenesis are significantly reduced in the aged heart, with concurrent increase of proteins involved in glycolysis and gluconeogenesis [90]. Normal adult hearts preferentially utilize fatty acids as the main energy source, while diseased and failing heart use glucose as the main energy source. In addition, consistent with age-dependent cardiac hypertrophy, there are increased levels of extracellular structural proteins (and their associated signaling pathways) with age [90]. These changes may be the result of an underlying decline in protein quality control systems, which in turn lead to the accumulation of damaged proteins that are unable to efficiently perform their biological roles.

As the efficiency of protein degradation decreases in old age, it is generally expected that the overall protein turnover rate should be slower in tissues from older individuals. This was supported by early studies performed using the classical method of measuring the bulk rate of incorporation or wash-out of radioactive label in total protein [104, 105]. However, using a novel non-radioisotope deuterated leucine labeling method followed by proteomics analysis, recent studies demonstrate that the average proteome turnover rate is not significantly different in the aged mouse heart [90]. Similar observations have been made in other tissues, including liver [106] and aged skeletal muscle (Kruse and Marcinek, ms. submitted). These studies examine the individual protein turnover rates simultaneously for hundreds of leucine containing proteins and are independent of differences in amino acid precursor pool sizes [107]. The discrepancy between these recent data and earlier findings might be explained by the greater influence of abundant proteins in the old "bulk" protein measurements, or may be due to precursor pool differences. Other recent studies utilizing a similar metabolic labeling-based mass spectrometry approach to assess in vivo protein turnover have observed turnover rates consistent with our observations in aging mice [108, 109]. Thus, while the overall turnover

rates of proteins are not significantly different or only slightly slower in the aged heart, the increased prevalence of damaged proteins and decreased efficiency of proteostatic maintenance in old age may have a balanced effect on turnover that mediates cardiac aging.

4.1.4 Stem Cells

Despite extensive investigation since the initial reports in 2003, the existence, identities and possible roles of adult cardiac stem cells in cardiac physiology and disease remains under debate. Earlier studies suggest the existence of multipotent populations of cells in the heart, such as c-kit + and sca1+/c-kit- cells, that are capable of differentiating into cardiomyocytes following isolation and culture [110, 111]. In contrast, a recent study demonstrates that c-kit + cells only minimally contribute to cardiomyocytes regeneration during development, aging or in response to injury [112]. Regardless of their role during normal physiology, these cells are apparently insufficient to prevent the progression of cardiovascular aging or to spontaneously regenerate the heart following acute ischemic events. Possible explanations include limited capacity of these cells to regenerate myocardium in the presence of continuous stress (such as pressure overload and ischemia), and the intrinsic aging of cardiac stem cells. Evidence of the latter was provided by experiments in rodents, which revealed that cardiac c-kit + stem cells in older animals had a higher rate of apoptosis and shorter telomeres [113]. In rodent model of diabetic cardiomyopathy, c-kit + cells demonstrate telomere shortening, increased expression of senescence markers p53 and p16INK4a, and an increase in apoptosis. Interestingly, in diabetic cardiomyopathy all of the above changes were attenuated by the ablation of the p66Shc gene [114]. p66Shc is activated by oxidative stress and translocated to the mitochondrial intermembrane space where it binds and oxidizes cytochrome c, producing H_2O_2 [115, 116], suggesting a central role of mitochondrial ROS in aging of cardiac stem (c-kit+) cells. Studies using cardiosphere-derived cells, another type of cardiac stem cell, also demonstrate a significant age-dependent decline in the number and function of stem cells derived from mouse atrial explant [117]. Bergmann et al. used data from environmental ^{14}C in human tissues, and mathematical modeling, to estimate that cardiomyocyte turnover decreased from 1 % per year at the age of 25 to 0.45 % per year by the age of 75 in adult human hearts [118]. Using multi-isotope imaging mass spectrometry to detect ^{15}N thymidine, Senyo et al. reported that the estimated annual rate of cardiomyocytes DNA synthesis is 5.5 % in the young adult and 2.6 % in the old mice [119]. Taking into account the multinucleation and polyploidization of adult cardiomyocytes, the estimated turnover rate of cardiomyocytes is 0.76 % per year in the young adult mice, and the rate further declines with age [119]. At this time, the prevailing view is that cardiac stem cells are present and function during heart development, that few new cardiomyocytes are formed in the adult heart, but these arise from existing cardiomyocytes, whether during normal aging or in response to injury [120].

4.1.5 Extracellular Matrix Remodeling

The extracellular matrix (ECM) provides structural support to the heart, and its composition is a major determinant of the stiffness of the myocardium, which is a major regulator of diastolic function [121]. ECM remodeling is a dynamic process and ECM composition is tightly regulated by the balance of the synthesis and degradation of ECM by matrix metalloproteinases (MMPs) and other proteases. Myocardial fibrosis is a hallmark of cardiac aging and deregulations of ECM synthesis and degradation have both been implicated in cardiac aging and pathology. Cardiac fibroblasts are the primary sources of cardiac ECM proteins, and it has been shown that properties of cardiac fibroblasts and their progenitors are altered with aging [122]. Studies have shown that fibroblasts isolated from old hearts have a lower proliferative capacity and have impaired differentiation into myofibroblasts in response to injury [35, 123]. Cardiac fibroblasts can be derived from several lineages including mesenchymal and myeloid origins, and the Entman group has demonstrated increased differentiation of these progenitors into mesenchymal fibroblasts and myeloid fibroblasts which contributes in increased cardiac fibrosis in aged hearts [122, 124, 125].

Transforming growth factor-β (TGF-β), a family of profibrotic cytokines, induce the expression of ECM proteins and suppress matrix degradation by MMPs [126]. TGF-β1 heterozygous mice showed reduced myocardial fibrosis and stiffness and increased LV compliance at 24 months of age [127]. Connective tissue growth factor (CTGF) is a downstream mediator of TGF-β and its expression increases with aging [128]. Cardiomyocyte-specific CTGF overexpressing mice display accelerated cardiac aging and develop age-related cardiac dysfunction as early as 7 months of age [129]. Consistent with this, Reed and colleagues demonstrated diastolic dysfunction in senescence-accelerated mice at 6 months of age, accompanied by increased LV fibrosis and increased TGF-β and CTGF expression [130]. In another study, Bradshaw and colleagues showed that deletion of secreted protein acidic and rich in cysteine (SPARC) resulted in reduced fibrillar collagen content in the LV and decreased LV diastolic stiffness [131]. This evidence suggests that increased ECM synthesis is an important mediator in diastolic dysfunction with age and reduced ECM synthesis can improve cardiac aging.

MMPs are a family of 25 zinc-dependent enzymes that regulate the degradation of ECM proteins. MMP proteolytic activity is inhibited specifically in the tissue by the tissue inhibitors of matrix metalloproteinase (TIMPs), a family composed TIMP-1, -2, -3, and -4 [132]. The expression levels of MMPs and TIMPs change differentially with age but their roles in cardiac aging have not been well established. In human plasma, the levels of MMP-2, MMP-7, TIMP-1, TIMP-2 and TIMP-4 increase but levels of MMP-9 decrease with age [133]. In CB6F1 mice, the levels of MMPs -3, -9, -14 and TIMP-4 increase from middle-age to old-age [35]. Spinale et al. showed that cardiac-specific MT1-MMP overexpressing mice display increased myocardial collagen deposition and LV dysfunction in middle-age, suggesting accelerated cardiac aging [134]. In a recent study, Chiao et al. showed that MMP-9 levels increase in the LV and plasma of aged C57Bl6 mice [135]. They later

demonstrated that aged MMP-9 null mice exhibit reduced collagen deposition and preserved diastolic function and these attenuated cardiac aging phenotypes are accompanied by reduced expression of pro-fibrotic proteins, periostin and CTGF, and a compensatory increase in MMP-8 levels in the left ventricle [136]. These findings suggest a role of ECM remodeling, under complex regulation by MMPs, in cardiac aging.

4.1.6 Angiotensin Signaling

The renin-angiotensin aldosterone system (RAAS) is a major regulatory system of cardiovascular function. RAAS blockade demonstrates tremendous positive effects on the cardiovascular system, including antihypertensive, anti-inflammatory, reduction of oxidative stress and antiproliferative effects on vascular smooth muscle cells. These beneficial effects clinically translate into protection from hypertensive target organ damage, improvement of chronic heart failure, reduction of atherosclerosis as well as decreased frequency of atrial fibrillation and stroke. The reduction of these aging-related cardiovascular diseases is greater than expected by the effect of blood pressure lowering alone, and hence suggests that RAAS blockade may have a direct role in cardiovascular aging. In the heart, this is supported by the findings that intracardiac Angiotensin II concentrations are significantly increased with age, and many structural, functional and molecular changes found in aged hearts are consistent with the effects of Angiotensin II [32, 137].

Direct evidence for the role of angiotensin II in cardiac aging was provided by the studies showing that inhibition of RAAS by either angiotensin converting enzyme inhibitor enalapril, angiotensin receptor blocker losartan or genetic disruption of Angiotensin receptor type I extended the lifespan of normal rodents and slowed the onset of age related cardiovascular pathologies [138, 139], including reduction of age-related myocardial fibrosis and fibrosis-related arrhythmias [140]. Protection by RAAS blockade is not specific to cardiovascular system, but is also evident in other angiotensin responsive tissues, such as the aging kidney [141].

The mechanism of RAAS protection involves a reduction of ROS. Angiotensin II binds to ATR1, a Gαq protein coupled-receptor (GPCR), which activates NADPH oxidase (NOX2) on the cell membrane and/or NOX4 on the mitochondrial membrane [142]. ROS from NOX2 and/or NOX4 lead to increase mitochondrial ROS production [143, 144]. The fact that mCAT (mitochondrial) but not pCAT (peroxisomal) catalase attenuates Angiotensin II- and Gαq-induced cardiac hypertrophy and failure emphasize the central role of ROS amplification within mitochondria [145]. Mechanisms of ROS amplification may include ROS-induced ROS release: ROS within mitochondria could lead to electron leakage from the electron transport chain, which might further stimulate ROS production. In addition, a ROS-mtDNA damage "vicious cycle" has been postulated in which ROS cause mtDNA mutation, which produce defective respiratory proteins resulting in more ROS production. The involvement of mitochondrial DNA mutations/deletions in this vicious cycle is supported by the observation that primary damage to mtDNA in $Polg^{m/m}$ mice is

sufficient to increase mitochondrial ROS, induce cardiac hypertrophy and systolic dysfunction [68, 145]. Breaking the ROS vicious cycle within mitochondria by transgenic expression of mCAT or mitochondrial targeted antioxidants effectively attenuates both cardiac hypertrophy and failure [146, 147].

4.1.7 Other Factors: Sirtuins, GDF-11 and miRNA

Sirtuin 1 (SIRT1) is a NAD+−dependent protein deacetylase that affects aging and lifespan in model organisms. The Sadoshima lab showed that expression of SIRT1 increases in aged heart, and that low to moderate overexpression (2.5–7.5 fold) of SIRT1 can attenuate cardiac aging responses, while high level overexpression (12.5 fold) resulted in cardiac hypertrophy and myocardial fibrosis at young age and death within a year [148]. In a recent study, mice deficient in the mitochondrial deacetylase SIRT3 displayed accelerated cardiac aging changes, including early age onset of hypertrophy associated with fibrosis, age-dependent increased mitochondrial swelling due to increased mPTP opening, and increased mortality after transverse aortic constriction [149].

Using heterochronic parabiosis, Loffredo et al. recently demonstrated that factors present in the circulation of young mice can reverse cardiac hypertrophy in aged mouse hearts [150]. Using an aptomer-based proteomic approach they identified growth differentiation factor 11(GDF-11) as a circulating factor that declines with age and can reverse age-related cardiac hypertrophy. Recapitulating the effect of parabiosis, treatment with GDF-11 also reduced expression of hypertrophic markers (ANP and BNP) and increased SERCA-2 expression in old hearts [150]. However, a later study has shown that the reagents for GDF-11 detection used in Loffredo's study cross-react with myostatin, and suggested that GDF-11 levels actually tend to increase with age in rat and human sera [151]. Further study will be required to determine the precise role of GDF-11 in cardiac aging.

Increasing evidence suggest that microRNAs (miRNAs) are important regulators of cardiovascular aging and diseases [152, 153]. In heart failure-prone C57Bl6×129Sv mice, van Almen et al. demonstrated that the expression of the miR-17-92 cluster (consisting of miR-18a, miR19a and miR-19b) decreases while the expression of their targets, CTGF and ECM protein thrombospondin-1 (TSP-1) increases. They showed that miR-18a and miR-19b regulated expression of CTGF, TSP-1 and collagen in *in vitro* aged cardiomyocyte cultures, and suggested that these miRNAs mediate age-related ECM remodeling in the heart [154]. In C57Bl6 mice, Jazbutyte et al. demonstrated an age-related increase in miR-22 in hearts and that miR-22 regulates cardiac fibroblast senescence [155]. In a recent study, Boon et al. demonstrated that expression of miR-34a increased in aged mouse hearts and *in vivo* silencing of miR-34a for 1 week can rescue the increase in cardiomyocyte cell death in aged mice [156]. They also showed that aged miR-34a knockout mice have improved contractile function and reduced cardiac hypertrophy compared to wild-type littermates. In the same study, they demonstrated that inhibition of miR-34a can also improve contractile function in Ku80 knockout mice (a mouse model

of accelerated aging). These observations suggest increased miR-34a expression in the aged heart contributes to cardiac aging. However, whether the impact of miR-34a on cardiac aging is mediated by the newly identified target phosphatase 1 nuclear targeting subunit (PNUTS) and/ or other miR-34a targets (e.g. SIRT1) remains to be elucidated.

4.2 Mechanisms of Vascular Aging

The vasculature is a pervasive system whose age-related alterations fundamentally impact the function of every organ. As atherosclerotic diseases are a leading cause for mortality and morbidity, the mechanisms of vascular aging that have direct relevance for atherogenesis are considered, focusing on the role of oxidative stress and chronic low-grade inflammation. Importantly, the microcirculation, with a total length of ~100,000 km, interacts with virtually every cell in the human body. In the past decade a growing number of publications have revised our understanding of the important role of age-related functional and phenotypic alterations of microvascular endothelial cells, both in the aging process and the development of multiple diseases of aging. Thus, we also review recent insights into the mechanisms of microvascular dysfunction in aging and how these might contribute to age-related functional decline of multiple organ systems.

4.2.1 Oxidative-Nitrative Stress and Endothelial Dysfunction in Vascular Aging

It is well-established that nitric oxide (NO) is a crucial factor for the health and function of endothelial cells, regulating vascular tone, structural remodeling, cell proliferation, angiogenic processes, inflammation, hemostasis and barrier function [43]. Considerable evidence has been published that endothelial function is impaired in aging due to an increased production of ROS [42, 50, 157–163], which contributes to the development of a wide range of age-related pathologies, including coronary artery disease and stroke (recently reviewed [43]). Impaired bioavailability of NO due to the functional inactivation of NO by increased O_2^- was shown to result in a severe impairment of flow/shear stress-induced vasodilation in the coronary circulation [42] and other vascular beds [157], compromising adjustment of blood flow to tissue oxygen demand. Impaired flow-induced vasodilation likely contributes to decreased exercise capacity and myocardial ischemia in the elderly. Impaired endothelial release of NO is also responsible for erectile dysfunction in older men. In addition, endothelial cell-derived NO confers significant vasoprotective and cardioprotective effects, including inhibition of inflammatory cell adhesion to endothelial cells and thrombocyte aggregation, disruption of inflammatory processes, inhibition of apoptosis and preservation of endothelial progenitor cell function [43]. Endothelium-derived NO was also shown to regulate mitochondrial biogenesis and

tissue energy metabolism [164, 165]. Thus, the significant age-related impairment of NO bioavailability [166], exacerbated by an age-related decline in eNOS expression [42, 167–170] and availability of tetrahydrobiopterin [171], is likely to promote both vascular inflammation and atherogenesis and lead to cellular energetic imbalance. The key role of endothelium-derived NO in protecting the cardiovascular system during aging is underscored by the finding that eNOS knockout mice exhibit a premature cardiac aging phenotype associated with early mortality [172]. Decreased endothelial NO production has been linked to increased apoptosis of endothelial cells in aging [170, 173], which likely represents an important mechanism involved in age-related microvascular rarefaction (see below). In addition to inactivating NO and eliciting oxidative macromolecular damage, increased production of ROS in the aged vasculature has important signaling roles both in vascular endothelial and smooth muscle cells. Importantly, increased levels of ROS were shown to activate redox-sensitive cellular signaling pathways implicated in inflammatory processes in the aged vasculature [174]. Increased oxidative stress also activates MMPs and promotes pathological vascular remodeling and vascular injury (reviewed in [31, 174, 175]). Caloric restriction was shown to up-regulate eNOS and increase NO bioavailability [165, 176], which contribute both to its vasoprotective and metabolic effects.

One of the major molecular mechanisms underlying vascular oxidative stress in aging is an increased expression and activity of NAD(P)H oxidases (NOX) [42, 159, 163, 177–179]. Previous studies show that inhibition of NOX exerts vasoprotective effects in aging, improving microvascular function [179], restoring penile erection [180] and inhibiting the progression of atherosclerosis [181]. There are multiple pathways which are likely involved in age-related activation and up-regulation of NOX, including hypertension [178] and up-regulation of the local renin-angiotensin II system and TNFα in the vascular wall [182, 183]. Hypertension also elicits increased mitochondrial production of ROS in the vasculature [184] and this effect is exacerbated in aging [185]. Mitochondrial-located Nox4 is a major source of pressure overload-induced oxidative stress in the heart [186] and its expression is up-regulated in the vasculature of hypertensive aged mice [46]. Moreover, angiotensin II induces expression of Nox4 both in smooth muscle cells [187] and in cardiac myocytes [146]. Thus, the effects of oxidative and nitrative stresses in aging are observed primarily in the vascular endothelium, but also have effects in the vascular smooth muscle cells.

4.2.2 Sirtuins in Vascular Aging

There is evidence that SIRT1 is also dysregulated in the aged vasculature [188] and previous studies demonstrated that SIRT1 is a key mediator of the anti-aging effects of caloric restriction [189], including its anti-oxidant and anti-inflammatory vascular effects [176]. The first pharmacological activator of SIRT1 to be widely studied was resveratrol [189]. Resveratrol was shown to confer multifaceted vasoprotective effects in aging, including attenuation of oxidative stress, increases in NO

bioavailability, anti-apoptotic and anti-inflammatory effects [47, 56, 190–192], mimicking many of the effects of caloric restriction. Resveratrol was shown to improve endothelial function in hypertensive patients [193] and to prevent arterial wall inflammation and stiffening in nonhuman primates [190]. Recent data demonstrate that SIRT1 overexpression in vascular smooth muscle cells decreases blood pressure and inhibits vascular remodeling in angiotensin II-treated mice [194] thereby supporting antihypertensive effects. However, resveratrol is not specific to SIRT1 and it was shown to have many other targets in the vasculature, including Nrf2 [195]. More recently, treatment with SRT1720, a synthetic sirtuin activating compound with improved bioavailability and specificity for SIRT1 activation, was also reported to normalize aortic superoxide production, decrease NF-κB activation and down-regulate TNFα in old mice [196]. Several lines of evidence support the concept that activation of SIRT1 confers anti-atherogenic effects. First, there are studies demonstrating an association of genetic variations at the SIRT1 locus with carotid atherosclerosis [197]. Second, $Apoe^{-/-}$ mice expressing inactive truncated SIRT1 (Δex4) in smooth muscle cells demonstrate increased DNA damage-response and apoptosis, increased atherosclerosis and medial degeneration [198]. Third, treatment with the pharmacological SIRT1 activator SRT3025 inhibits atherogenesis in $Apoe^{-/-}$ mice fed a high-cholesterol diet (although it seems to be ineffective in $Ldlr^{-/-}$ mice) [199]. Recent studies suggest that the mitochondrial sirtuins (including SIRT3), which are in part responsible for the regulation of ATP production, metabolism, apoptosis and cell signaling, are also dysregulated in the aged vasculature [188]. However, the role of SIRT3 in atheroprotection is controversial [200].

4.2.3 Vascular Inflammation in Aging

There are substantial experimental and clinical data demonstrating that aging is associated with chronic low-grade inflammation [201], which promotes the development of age-related vascular diseases, including atherosclerosis [174]. Even in normal, healthy aging there is a pro-inflammatory shift in the gene expression profile of vascular endothelial and smooth muscle cells, including an up-regulation of inflammatory cytokines, chemokines, adhesion molecules and iNOS both in laboratory rodents and primates [42, 50, 173, 192, 202–204]. Vascular inflammation in aging contributes to the development of vascular dysfunction [182, 205] and promotes endothelial apoptosis in aging [173, 182]. Secretion of inflammatory mediators from microvascular endothelial cells is also likely to affect the function of cells in the parenchyma of the supplied organs. For example, neural stem cells were shown to lie close to blood vessels, and their function is likely directly affected by pro-inflammatory changes in the specialized microenvironment of this vascular niche [206]. In this regard it should be noted that age-related functional and phenotypic alterations of the microcirculation also promote chronic inflammation indirectly in the brain and other organs. Accordingly, aging is associated with significant blood brain barrier disruption in the hippocampus and other brain regions, which is exacerbated by hypertension [46]. Passing through the damaged blood brain barrier,

plasma constituents, including IgG and fibrinogen, can enter the brain promoting neuroinflammation (e.g. activation of microglia by IgG via the IgG Fc receptors) [207, 208]. Thus, microvascular aging (via endothelial activation and extravasation of leukocytes, secretion of inflammatory mediators and disruption of barrier function) likely contributes to a wide range of age-related chronic diseases.

There is ample evidence that increased NF-κB activation in the aging vascular cells contributes to endothelial activation and pro-inflammatory gene expression [50, 163, 209, 210]. Age-related mechanisms that contribute to NF-κB activation include increased production of mitochondria-derived H_2O_2 [50], Nrf2 dysfunction [56], up-regulation of tissue renin-angiotensin system (RAS) [204, 211–213] and paracrine TNFα signaling [182]. There is strong evidence that age-related vascular inflammation is reversible both by dietary interventions (caloric restriction [176, 214]) and pharmacological treatments that inhibit NF-κB activation [192].

4.2.4 Vascular Cell Senescence, Endothelial Cell Renewal and Apoptosis in Vascular Aging

Role of Cellular Senescence in Vascular Aging

Mitotically competent vascular cells, including endothelial cells, smooth muscle cells, adventitial fibroblasts and pericytes, can react to diverse endogenous and exogenous stressors (e.g. paracrine signals, DNA damage, dysfunctional telomeres) by permanently withdrawing from the cell cycle, a response termed "cellular senescence" [215]. Experiments on endothelial cells *in vitro* suggest that oxidative and nitrative stress are an important stimuli for the induction of senescence [215]. There is increasing evidence that senescent cells accumulate with age in the cardiovascular system. Yet, a controversy exists regarding the exact biological role of senescent cells and the relationship between cellular senescence and vascular aging. Apart from the alterations related to permanent cell-cycle exit, senescent cells acquire distinct phenotypic changes, including the "senescence-associated secretory phenotype" (SASP) [216], which likely contribute to the development of age-related vascular diseases by altering the tissue microenvironment, impairing the function of neighboring cells via the secretion of paracrine mediators and changing the composition of the extracellular matrix. Some of these phenotypic changes are potentially important in altering the regenerative and angiogenic capacity of the vascular endothelium and promoting inflammatory processes and atherogenesis during aging [215].

Increased Endothelial Apoptosis in Aging

Programed cell death might account for some aging phenotypes in various organs [217], as well as the genesis of age-related cardiovascular pathologies. While an attractive hypothesis, the relationship between vascular aging and apoptosis remains

unclear. Aging is associated with increased apoptosis of endothelial cells in the vasculature of non-human primates [16]. The percentage of apoptotic endothelial cells also increases with age in the vasculature of laboratory rodents [173, 182, 192]. The available data suggest that multiple age-related mechanisms, including impaired bioavailability of NO, circulating IGF-1 deficiency, impaired cellular oxidative stress resistance, up-regulation of TNFα and/or mitochondrial oxidative stress are likely to contribute to this increase in apoptotic endothelial cells [173, 182, 218]. Factors present in the circulation of patients with peripheral artery disease were shown to confer significant pro-apoptotic effects in cultured endothelial cells derived from aged rodents [219]. Yet, in human patients no significant correlation was found between patient's age and the number of apoptotic cells in the coronary circulation [220]. In addition to large vessel pathologies, increased apoptotic cell death at the level of the capillaries is also likely to contribute to microvascular rarefaction (see below) and, concomitantly, to the declines in muscle mass [221] and organ function during aging.

Impaired Angiogenesis and Microvascular Rarefaction in Aging

The process of angiogenesis is critical for maintenance of the microvasculature and cardiovascular homeostasis. Previous studies demonstrate that aging is associated with a progressive deterioration of microvascular homeostasis due to age-related impairment of angiogenic processes [43, 222–226]. It is assumed that these changes have a key role in the age-related decline in microvascular density (microvascular rarefaction) [227] that has been observed in multiple organ systems with age, including the heart [228], kidney [229] and skin [230]. Microvascular rarefaction is thought to decrease tissue blood supply, contribute to the development of hypertension and impair adaptation to hypoxia [231–233]. There is also an age-related rarefaction of the cerebral microvasculature, which likely contributes to a decline in cerebral blood flow that reduces metabolic support for neural signaling, promoting cognitive dysfunction in the absence of or preceding neurodegeneration in the elderly [44, 234, 235]. However, the age-related loss of microvascular plasticity has significance beyond metabolic support for neuronal signaling, since neurogenesis in the adult brain is regulated coordinately with capillary growth [44].

The mechanisms by which aging impairs angiogenesis and promotes microvascular rarefaction includes an age-related impairment of the growth hormone/IGF-1 axis (reviewed recently [236]). Previous studies demonstrate that growth hormone supplementation substantially increases cortical vascular density in older rats [234], which was accompanied by a significant improvement of cognitive function. Other factors appear to be involved: pituitary adenylate cyclase-activating polypeptide (PACAP) is an evolutionarily conserved neuropeptide secreted by endothelial cells and neurons that confers important anti-aging effects. Recent studies show that secretion of PACAP by cerebral microvessels also significantly declines with age [237, 238]. In vitro evidence suggests that age-related decline in autocrine PACAP signaling contributes to impairment of endothelial angiogenic capacity with age

[238]. There is also evidence that Dicer1 (ribonuclease III, a key enzyme of the miRNA processing machinery) is essential for normal endothelial angiogenic processes, and that age-related dysregulation of Dicer1-dependent miRNA expression is a potential mechanism underlying impaired angiogenesis and cerebromicrovascular rarefaction in aging [226]. Interestingly, lifelong caloric restriction restores the angiogenic capacity of aged endothelial cells, promoting endothelial cell proliferation and capillary morphogenesis and increasing capillary density in the brain of aged laboratory rodents [214, 239, 240]. SIRT1 also seems to regulate angiogenesis, as suggested by studies on mice with endothelium-specific knockout of SIRT1 [241]. Importantly, Nrf2 has also been implicated in regulation of endothelial angiogenic capacity [242]. In that regard it is significant that cerebral capillary density can be increased in aged mice by resveratrol treatment [191].

Impaired Endothelial Progenitor Cell Function in Aging

The link between vascular aging and a decline in the replicative function of endothelial progenitor cells (EPCs) is controversial. It is still not well established whether aging affects total EPC number [243–245], however, previous studies provided evidence that the function of circulating EPCs is impaired with age [244, 246]. Aging was shown to impair neovascularization, which depends on the intact function of highly proliferative EPCs. A role for age-related alterations of circulating factors in EPC dysfunction is suggested by the findings that the presence of sera from young rats in the culture medium improves the function of EPCs isolated from aged rats [247]. Importantly, IGF-1 was shown to exert beneficial effects on the function of progenitor cells in the cardiovascular system, including antioxidant effects, upregulation of telomerase activity, delaying replicative senescence, and increasing the pool of functionally competent progenitor cells [248]. Both circulating IGF-1 levels and paracrine IGF-1 decrease in aging, which likely exert deleterious effects in EPCs [249]. Indeed, there are studies demonstrating that in human patients age-dependent impairment of EPCs is corrected by GH-mediated increase of IGF-1 [250]. Interestingly, regular aerobic exercise was also shown to increase both the number and migratory activity of EPCs in previously sedentary older men [251].

5 Future Prospects and Interventions

Age-associated alterations in arterial, microvascular and cardiac structure and function represent links that explain, at least in part, the reason why aging is by far the greatest risk factor for cardiovascular disease. It is critical to understand the molecular mechanisms of cardiovascular aging, their interactions with both cardiovascular disease pathogenesis and systemic aging processes, and identify novel pathways that could be targeted for interventions aiming at retardation or attenuation of these

age-associated alterations. The recent studies on the roles of different hallmarks of aging have advanced our understanding of cardiovascular aging and shed light on potential therapeutic strategies. Several examples of such potential therapies are indicated in the sections above, including mitochondrial protective agents, rapamycin and GDF-11. Further understanding of the mechanisms of cardiovascular aging will guide the future translational studies on novel therapeutics to treat age-related cardiovascular disease and to improve healthy cardiovascular aging. Cardiovascular aging is a promising frontier that is ripe for, and in dire need of, attention to prevent age-associated deterioration of healthspan.

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The Impact of Aging on Ischemic Stroke

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Contents

1 Introduction

The high incidence of stroke worldwide is considered a global epidemic. There are two broad types of stroke: hemorrhagic stroke and ischemic stroke. Hemorrhagic stroke is due to weakening of the vessel wall and eventual rupture and spillage of blood in the brain parenchyma. Hemorrhagic strokes are not as common (about 80,000 cases per year in the US) but are more likely to be fatal as compared to ischemic strokes. Ischemic strokes result from blockage or constriction of a cerebral vessel

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resulting in rapid neuronal death due to loss of oxygen and glucose. Ischemic stroke is more common and can run the gamut from mild symptoms to chronic disability and death. Stroke symptoms can vary dramatically from person to person, based in part on the specific blood vessel, the brain region, the duration of ischemia and, crucially, on the general health of the individual. Not surprisingly, stroke is the leading non-martial cause of serious long-term disability and a stroke-related death occurs every 47 s in the US, underscoring the enormous medical, financial and societal burden of this disease. Few therapies are available for stroke patients outside of rehabilitative therapy.

2 Stroke and Aging

In the US, ischemic stroke is considered a disease of the elderly. Thus, while stroke incidence is low among younger demographics, the prevalence of stroke in the sixth–seventh decade of life (60–79) is 6.2 % for men and 6.9 % for women, which then doubles to 13.9 % for males and 13.8 % for females in the 80+ years age group [102]. The increased risk for stroke with age coupled with a growing aging population will lead to an additional 3.4 million people affected by stroke in the next 15 years [209].

Besides elevating the risk for stroke, age also adversely affects stroke outcomes [128]. Stroke outcomes can be assessed by several measures including survival, functional recovery, and length of hospitalization. An early study of 415 patients with transient ischemic attack (TIAs) reported that 1 and 5 years survival was negatively correlated with advanced age [121]. A prospective analysis of 515 stroke patients (Copenhagen Stroke Study) showed that outcome measures such as activities of daily living (measured by the Barthel Index [BI]) were significantly worse in older patients, although neurological outcomes as measured by the Scandinavian Stroke Score were not affected by age [192]. Since ADL status reflects not only recovery from the stroke but also compensation from the non-affected side, it suggests a poorer ability to compensate among this older population. Furthermore, hospitalization length was significantly increased in older patients (>65 years) with stroke [225]. Observational studies in university hospital settings reported that age was a highly significant predictor of poor functional outcome [1, 66, 139]. Moreover, a small study of centenarians also confirmed that strokes were much more severe in this population than in other age groups [207]. Morphologically, aging is associated with decreased salvage of penumbral tissue, and leptomeningeal collateral circulation is reduced in aging stroke patients, which associated with a poorer outcome [15].

2.1 Is Age a Non-modifiable Risk Factor?

Based on the evidence above, age is often referred to as a ‘non-modifiable’ risk factor for stroke. In evaluating this statement, it should be recognized that the aging demographic is a highly variable one. Sex/gender differences, life style factors, and

ethnicity all impact stroke incidence. Additionally, the incidence of hypertension, metabolic disease, hyper-cholesteronemia, that are co-morbid for various neurologic diseases including stroke, all contribute to the variability in the elderly population. Accordingly, while the prevalence of stroke is one-sixth of the 85+ age group, Alzheimer's disease, another neurologic illness, occurs in one-third of the 80+ age group. Thus while age is a contributing factor for many illness, the relatively lower incidence of stroke in the aging population suggest that other factors may also modulate age to elevate the risk for this disease. Some of these factors are considered below.

2.1.1 Sex Differences in Stroke

A principal variable affecting stroke incidence in aging is the sex of the patient. Most ischemic strokes occur in the elderly and among this elderly demographic, women are more likely to get a stroke [217]. In addition to a higher incidence of ischemic stroke at older ages, females display more non-classical stroke symptoms and tend to have worse outcomes from stroke. Thus while stroke is the 4th leading cause of death overall, it is the 3rd leading cause of death in women, and the 5th leading cause of death in men [193]. In fact, the rates of stroke-related death have declined over the last 25 years for men but not women [232]. Furthermore, since women live longer than men, it is projected that stroke-related disability and institutionalization is likely to affect women more than men [145]. Women account for 60 % of stroke-related deaths [169], even after normalization for age. The 5 y stroke recurrence is also disproportionately higher in females (20 %) as compared to males (10 %) in the 45–64 years age range [232]. A Canadian stroke registry study reported that 10 % of women stroke patients were discharged to long term care as compared to 5 % of men [133], despite the observation that stroke size tends not to be different in males and females [248]. In the Danish National Registry analysis, women were reported to have more severe strokes than men although they exhibited a survival advantage compared to men, especially at advanced ages [206].

The increased incidence of stroke among older women, especially when compared to the relative low risk among younger demographics has led to the hypothesis that the loss of ovarian hormones, principally estrogen, at menopause may be a contributory factor. However, analysis of hormone use and stroke incidence in postmenopausal women does not support this conclusion. An early case–control study reported no increased risk for stroke in postmenopausal women who took hormone therapy, relative to those not taking hormones [216]. In a different multicenter case-controlled study, increased lifetime exposure to estrogen was associated with a lower risk of stroke, but interestingly, a lower age at menarche increased the odds of stroke [63]. The influential Women's Health Initiative (WHI) study indicated that hormone use actually increased stroke risk. The WHI study was a randomized, double blind, placebo-controlled multicenter trial, which compared the risk of myocardial infarction, stroke and dementia in women who consumed daily conjugated equine estrogens (CEE) [115]. CEE + progestins [279] or placebo. Hormone therapy

groups showed an increased risk for stroke; however, subgroup analyses indicated that most of this risk was seen in the older age groups. In the CEE trial, increased risk for stroke was statistically significant for the 60–69 years old group but not the 50–59 years old group [115]. In an observational analysis of postmenopausal women in the Nurse's Health Study, estrogen and estrogen + progestin use increased the risk of stroke irrespective of the age of the user or time since menopause [107]. However, the observational arm of the WHI study showed no increased risk for stroke in the CEE or CEE + progestin arm [222, 223]. A possible factor in the discrepancy between the WHI trial and the WHI observational study was that the initiation of hormones was much earlier in the latter study. However other health characteristics among this group can also impact stroke risk in conjunction with hormone therapy (HT). In the observational trial (SHOW study) there were no differences in stroke risk due to HT use; however, HT users were more likely to be normotensive and lean as compared to non-users in this study [42] which was not the case in the WHI study, where hypertension incidence was similar in CEE users and non-users [115]. A similar interaction between HT and hypertension was seen in the Danish Nurses study, where normotensive women who used hormone therapy were not different from controls, while the risk for stroke was elevated among hypertensive women who used hormone therapy [170].

Another modifier of the effectiveness of HT for stroke is the concept of timing. The timing hypothesis postulates that hormone treatment is likely to be cardio- and stroke-protective only if taken during the perimenopause or early after menopause. Subgroup analyses of the WHI study (described above) support this idea, as well as data from a prospective study of Swedish women, where stroke risk was significantly decreased in women who initiated hormone treatment prior to menopause [157]. In a population-based nested case–control study of 50–69 year old women, HT did not significantly elevate ischemic stroke risk [8], further supporting the idea that HT at ages closer to the menopause may be harmless for stroke. Coronary artery calcification, a surrogate marker of cardiac disease, was reduced by estrogen in the youngest cohort of WHI (50–59 years) [177], also signifying that estrogen's effects can be modulated by the age of the user. Finally, a study of non-users found that stroke-related mortality in women 65 and older was higher in women with higher levels of endogenous estrogen [176], implying that elevated levels of hormones in late life, whether exogenous or endogenous, may exert a deleterious effect on stroke.

The accumulated evidence of sex differences in the incidence, mortality and outcome for stroke prompted the recent American Heart Association/American Stroke Association guidelines for sex-specific recommendations for the female stroke patient [41]. This recommendation underscored stroke risk factors that were stronger or more prevalent in females than males, such as migraine, atrial fibrillation, Type 2 diabetes, and hypertension, as well as risk factors specific to women such as pregnancy, gestational diabetes, preeclampsia, oral contraceptives and estrogen therapy. Thus the aging woman may represent a specifically vulnerable population for stroke.

2.1.2 Hypertension and Aging in the Context of Stroke

Hypertension is a significant risk factor for global diseases such as cardiovascular disease, congestive heart failure, stroke and end-stage renal disease. It is a leading risk factor of stroke among the elderly. Hypertension is highly prevalent in the aging population, estimated at 69.6 % of all women aged 65–74, and 64.7 % of all men aged 65–74 [102]. One report estimates that non-hypertensive individuals aged 55–65 are virtually all (90 %) likely to develop stage 1 hypertension and are at a 40 % risk of developing stage 2 hypertension [271], underscoring the close link between aging and hypertension. Systolic blood pressure rises gradually between 30 and 80 years of age, and systolic hypertension is very common after age 50 [219]. Hypertension is seen in 77 % of all stroke patients [169], and the Framingham study reported that hypertension was the factor most strongly associated with stroke in elderly males and females, increasing the odds ratio of stroke 1.9 in aging men and 2.3 in aging women [132]. A similar finding has been reported for both men and women aged 80+ years [13].

While hypertension increases with age, an increase in systolic blood pressure (SBP) was frequently thought to be a normal, and therefore inconsequential, part of the aging process, while increases in diastolic BP (DBP) were seen as the greater threat to cardiovascular and stroke incidence. However, in the US, more than 3/4 of all untreated hypertensive patients 50 years and older are of the "isolated systolic hypertension" (ISH) subtype [90], suggesting that hypertension among the elderly is of a different type [91]. Aging affects hemodynamics, with increases in systolic BP, diastolic BP and mean arterial pressure (MAP) starting at 50–55 years of age. Thereafter, with age there is a leveling off of DBP and MAP, with a steady increase in SBP. This pattern is thought to reflect a transition from age-related changes in peripheral vascular resistance to large artery stiffness [89]. Additionally, the difference between SBP and DBP, or pulse pressure, is currently considered to be a more predictive risk factor for cardiovascular disease. Both aging and hypertension can impair endothelial, and therefore, microvessel function. With age, vascular tone is affected as well as arterial remodeling, resulting in increased pulse wave velocity, a phenomenon referred to as vascular aging [196], a determining factor for cardiovascular disease.

A series of studies examining the effect of anti-hypertensive therapy in elderly patients showed that such interventions reduced all strokes 30–57 % compared to placebo, and reduced fatal strokes by 39–76 % (reviewed in [14]). Thus aggressive management of blood pressure in the elderly may dilute the impact of age on stroke. This may underlie the steady decline in stroke mortality and stroke-associated disability among the elderly that has been reported between 1967 and 1985, although the incidence of stroke remains high in this group [24]. While control of risk factors including hypertension, hyperlipidemia, diabetes and cigarette smoking are necessary at all ages, they are most likely to be beneficial in the elderly [25]. For every 5 mmHg reduction in BP there is a 14 % decline in stroke mortality [51] and a 35–40 % reduction of stroke occurrence [53]. Taken together, the evidence indicates that

there appears to be no age threshold where treatment for hypertension is not likely to be beneficial for stroke [16].

2.1.3 Other Comorbidities and Stroke

Aging-related changes in blood pressure also intersect with dyslipidemia in this group, possibly due to lipid action/accumulation on the arterial wall. Elevated cholesterol and lower levels of high-density lipoproteins are associated with stroke in aging and lipid-lowering drugs reduce the incidence of myocardial infarction [6, 237]. While major trials have indicated that statins are well tolerated in the elderly, the association between hyperlipidemia and stroke is not strong in this group. In a study of high-risk elderly patients (< or = 82 years of age), HMG-CoA reductase inhibitors (statins) appear to lower stroke risk [6]. However, this may also result from other protective actions of statins on the endothelium, including anti-oxidant, anti-inflammatory effects and stabilization of plaques [179]. After age 65, blood lipid levels are less prominent risk factors for cardiovascular diseases and by age 75, blood lipids have little predictive value [29]. In fact, specific lipids may be associated with longevity in the elderly population, for example sphingomyelin in women [103, 238]. Thus among the elderly the risk imposed by hypertension is likely more severe than hyperlipidemia. By contrast, cigarette smoking, whether past or current, also impacts vascular disease [56], and is a risk factor for heart failure even in older adults. In current smokers this risk is elevated irrespective of the 'pack-years' of smoking exposure [104]. Remarkably, prior to 75 years of age, hypertension and diabetes are much less important risk factors as compared to heavy (>2 drinks/day) alcohol consumption at midlife [129].

2.1.4 Diabetes

Cardiovascular complications are the most common non-fatal complication of diabetes among older adults [122]. The prevalence of type 2 diabetes is 16.5 % in men and 12.7 % in women in the 75–84 age range where strokes are common, and glucose intolerance was found in more than 1/3 of all participants in the Framingham study that were 65 or older [281]. Altered glucose metabolism is not necessarily a component of aging, and may represent a sub population that is generally at higher risk for other adverse geriatric processes [131]. Diabetes is frequently associated with cognitive impairment, dementia, depression, and stroke [28]; however, in some cases, it is the co-occurrence of hypertension in diabetic populations that may increase the risk for stroke. Some support for this idea comes from the fact that vascular disease increases before the elevation of glucose levels and more than 25 % of newly diagnosed diabetic patients already have cardiovascular disease [281].

The convergence of comorbid disease and sociocultural stressors during aging as risk factors for stroke fits well with the concept of an 'allostatic load' [182]. Allostatic load refers to the cumulative lifespan exposure to adverse circumstances,

and integrates with the 3-hit hypothesis where disease susceptibility is thought to result from genetic predisposition, early life events and later-life events [60]. Saban and colleagues propose that early life events may predispose an inflammatory epigenetic signature, which is made worse with chronic stressors such as social disadvantage [48] and psychological stress, culminating in increased risk for CVD and stroke [235].

3 Impact of Aging on Conventional Stroke Therapy

Tissue plasminogen activator (tPA; Alteplase) is the only FDA-approved therapy for stroke, and its mode of action consists of proteolytic degradation of the clot, with the goal of re-establishing circulation. tPA has also been shown to increase the risk for hemorrhagic transformation, which occurs subsequent to ischemic stroke and cerebral infarction. Although hemorrhagic transformation (intracerebral hemorrhage) may occur spontaneously after ischemic stroke, thrombolytic therapy occasionally leads to this complication, possibly due to the actions of tPA on matrix metalloproteinases [148, 268]. In animal models, tPA increases permeability of the blood brain barrier in aged (18–20 month old) male Wistar rats as compared to young (3–4 months old) males, and this is related to disassembly of tight junction proteins such as claudin and occludin [134].

The actions of tPA are poorly studied in the elderly, despite the fact that people aged 85 and over are the fastest growing stroke demographic in the US. In the landmark tPA study at NINDS, tPA was noted to cause intracerebral hemorrhage (ICH) in a small proportion of patients and this was embodied in the recommendation that tPA after 3 h was not advised. In this study, the conversion to ICH was 2.87 times greater in patients older than 80 as compared to patients younger than 80 [264], although age was not an independent predictor of ICH. In contrast, age was an independent predictor of hemorrhage in the European Acute Stroke Study [149]. In a small retrospective study of 22 stroke patients who were 90 years or older, most patients had poor outcomes at 30 days post stroke and many died [180]. Similarly, a report of patients from a German registry indicated that the rate of ICH was much higher in older patients (10.3 %; 75 or older) [116]. Although the explanation for this age response is not clear, the greater incidence of underlying vascular pathology including cerebral amyloid angiopathy [105] as well as deficient clearance of tPA in the elderly have been implicated as factors. In fact, in carefully selected older populations, with treatment by stroke specialists and careful adherence to the NINDS guidelines, there does not appear to be an increased risk of ICH in elderly patients treated with tPA [260].

Sex differences in treatment among patients that receive tPA may also factor into the sex differences in stroke outcomes. In a study spanning stroke patients over a decade (1997–2006), men were more likely than women to receive IV tPA, angioplasty/stents, carotid endarterectomy, or cardiac reperfusion. However towards the end of the study period, sex differences in the use of IV tPA were eliminated [267],

which suggests that greater overall tPA use and emphasis on early time-to-treatment may decrease sex differences in acute stroke care. More recently, a comparison of white and black male and female stroke patients found no differences in the outcome of tPA administration in men, but reported that black women were less likely to get tPA than white women [36]. In a regional study, women were more likely to be excluded from tPA for hypertension as compared to men, suggesting that undertreatment of stroke risk factors in women may further impact stroke therapies as well [175]. Increased assignment of tPA therapy to women should be encouraged as treatment outcomes do not differ between tPA treated men and women, while in non-tPA administered groups, males were more likely to have a better neurologic score as compared to women [247].

3.1 Failed Stroke Trials

Although several drugs have been identified in preclinical studies, only a few of these have made it to clinical trials and none have succeeded [44]. These include the SAINT trials that tested the free radical scavenger NXY-059; the RANTTAS trials for Tirilazad mesylate, a lipid peroxidation inhibitor; the INWEST trials using nimodipine, a calcium channel blocker and the Selfotel investigations using an NMDA blocker. While several reasons may explain why the preclinical promise of these drugs was not borne out in clinical trials, an important consideration is the lack of aging animals used in the preclinical studies [166]. Most preclinical studies used healthy young animals as test subjects, which clearly does not approximate the human population [185]. In fact, irrespective of the treatment, stroke outcome was significantly affected by the age of the patient in the combined SAINT trial analyses [70]. Preclinical studies with these drugs routinely failed to use clinically relevant animal models, such as the aged and those with comorbid diseases. A comprehensive review of preclinical studies that lay the groundwork for these failed drugs found that virtually all studies (43/45) used only younger animals [270]. These and other studies provided the impetus for the STAIR recommendations, which specifically included recommendations for clinically relevant animal models [87].

3.2 Preclinical Therapies

Emerging therapies are focusing on interventions that lead to long-term brain repair and plasticity, using cell based therapies and pharmacological therapies.

The use of cell therapies and grafts in stroke has focused on adult stem cells or induced pluripotent stem cells [23, 110]. Both human and animal stroke brains show signs of proliferation, including the aging human [174]. Intra-parenchymal [158] and intra-arterial [159] delivery of bone marrow derived mesenchymal cells is reported to improve neurological outcomes and functional performance when

delivered post stroke. Interestingly, grafts of human umbilical tissue-derived cells have also been shown effective for neural recovery in aged animals [294]. Environmental enrichment appears to improve neurologic function in both young and old animals [38], and further enhances functional recovery when combined with stem cell therapy [117]. Although the mechanism of action is not well understood, several end points are improved including release of trophic factors, anti-inflammatory effects, angiogenesis and cell survival (reviewed in [47]).

Angiogenesis is considered critical to long term stroke recovery [11], and the low rate of vessel formation in the elderly is thought to be associated with low rates of functional recovery. Formation of new blood vessels is a desired therapeutic outcome, and ischemic events provide important signals for new vessel formation, such as secretion of angiogenic and matrix remodeling factors. Angiogenesis is also critical for providing a niche for neurogenesis [210]. Studies have shown that VEGF is angiogenic in the post stroke brain but may also cause blood brain barrier 'leakiness' and hemorrhagic transformation in the early acute phase of stroke [296]. Similarly, stem cell transplant-derived VEGF is also neuroprotective [118]. However, rAAV-mediated VEGF therapy showed that the angiogenic response to this growth factor is attenuated in aged animals [94], while sildenafil treatment post stroke enhanced recovery and angiogenesis in both young and aged animals [293], and adenoviral transfer of adiponectin was more effective in old than young stroke animals [184]. These studies, while promising, underscore the need for preclinical studies to mimic clinically valid aspects of the patient population, including old age and comorbidities.

4 Animal Models to Gauge the Impact of Aging on Stroke

While preclinical models do not measure risk, they can be useful in assessing stroke severity (outcomes) in terms of infarct volume, neurological scores, the impact of comorbid disease and response to therapies. Age differences in stroke outcomes in preclinical models tend to mirror the findings of the clinical studies. With increasing age, there is a greater conversion of ischemic tissue to infarcted tissue in stroke patients as detected by MRI [17]. Similarly, aged animals show more infarcted damage than young animals, and young animals are likely to show spontaneous recovery soon after the ischemic event, while in aged animals, such recovery is usually longer and never reaches the same recovery levels as young animals [38].

Young and aged male and female mice subject to a middle cerebral artery occlusion (MCAo) show that young females sustain a smaller infarct as compared to young males or aged female mice [164] although by 2 weeks post stroke, both young and aged groups were comparable [178]. In a study of neonates (10 day), and adult animals at 2 and 6 months, functional recovery was best in the neonate and was impaired in the older age groups, suggesting that the plastic environment of the immature brain is better suited for stroke recovery [284]. Similarly, post stroke epilepsy, a common complication in this disease, was more common in older animals

as compared to younger ones [135]. In studies comparing young and middle aged animals, the latter group showed worse infarct volume in the spontaneously hypertensive rat strain (SHR) as compared to the normotensive Kyoto Wistar strain [152]. In fact, middle-aged SHR rats show spontaneous white matter disease, cognitive decline and heightened inflammation as compared to normotensive controls [130], accentuating their value as preclinical models for stroke.

Sex differences also modulate stroke outcome in the context of aging in animal models. Adult females have a smaller infarct and better cerebral blood flow than age-matched males both in normoglycemic [4] and diabetic [266] animals. However, although female mice sustain a much smaller infarct [178], they showed significantly more mortality and poorer stroke outcomes as compared to older males. These sex differences prompted several studies addressing the contribution of hormones to stroke outcomes, specifically estrogen. Using natural variations in circulating estrogen levels, Liao and colleagues [160] showed that the extent of ischemic damage was inversely related to circulating levels of estrogen [160]. In fact, replacement with 17β estradiol [77, 234, 243] and its inactive stereoisomer 17α estradiol [249] as well as the conjugate equine estrogen preparation [181] all reduce infarct volume in female animals. Exogenous estrogen replacement is neuroprotective when given prior [77] or subsequent to the injury [167, 286]. However, it should be noted that all these studies were done in young female animals that were ovariectomized to mimic a surgical menopause. In contrast, as mentioned earlier, elevated levels of sex hormones may have a negative effect on stroke in the aged.

Hormone treatment in studies using older female animals does not reliably result in stroke neuroprotection and may in fact exacerbate stroke recovery. While some studies show that estrogen treatment to middle-aged or older female animals is neuroprotective [76], a growing number of studies show that estrogen treatment to ovariectomized middle-aged female animals either has no protective effect [62, 154] or paradoxically, increases infarct volume [243, 244]. Besides gonadal steroids, other endocrine systems are also affected by aging and disease, and it has been proposed that these changes may impact the overall effectiveness of estrogen in an aging model [254]. Support for this idea comes from a study where post stroke IGF-1 treatment to estrogen-exposed middle aged female rats reversed the detrimental effects of estrogen. Conversely, IGF-1 receptor antagonist treatment of young females abrogated the protective effects of estrogen in this group, suggesting that cross talk between hormone systems (in this case estrogen and IGF-1) may be critical for neuroprotection [244]. Thus the loss of both IGF-1 and estrogen in aging females may be responsible for the more severe stroke outcomes seen in this group. Interestingly, IGF-1 levels are higher in young males, while their stroke outcomes are worse than young females, suggesting that the correlation between IGF-1 and neuroprotection may be more complex.

Overall, when key variables that affect stroke outcomes in patients, such as advanced age, sex and hypertension are included in preclinical studies in animal models, they appropriately reflect the outcomes seen in human disease.

4.1 Pathophysiology of Stroke

4.1.1 Ischemia Induced Cascade

Loss of glucose and oxygen to brain cells causes a series of events resulting in neuronal death either through apoptosis or necrosis. Cell death occurs not only in those areas directly affected by the ischemia, but also in neighboring cells as a result of an ischemic cascade initiated in proximal cells. Many of these processes occur simultaneously, beginning with a failure of ATP-dependent systems that result in unregulated calcium entry into the cell. A feed forward process then ensues, whereby calcium-induced release of the excitatory amino acid glutamate, further increases Ca^{+2} accumulation. Consequently, stimulation of calcium dependent enzymes initiate a wide variety of cellular reactions resulting in free radical formation and oxidative stress. Death of ischemic neurons causes toxicity in the local microenvironment, and activates local immune and inflammatory cells, thus amplifying the possibility of cell death (reviewed in [212]).

4.2 Is the Cellular Response to Stroke Different in Aging Animals?

At the cellular level, aged animals are able to mount a cytoprotective response to stroke but the timing of proliferation and activation of key support cells such as glia and endothelial cells is accelerated, resulting in rapid infarct development and poor prognosis in aged animals [221]. Endothelial cells, astrocytes and microglia are the major support cells of the brain and play a critical role in preserving neurons following ischemic injury. A critical way in which these cells interact is the neurovascular unit, where blood brain barrier components (endothelial cells, astrocytes and pericytes) form a functional unit with neighboring neurons. The blood brain barrier itself consists of endothelial cells and their intercellular tight junctions, supported by astrocytic end feet and pericytes [2, 113, 144, 230]. Paracellular transport between adjacent endothelial cells is restricted by the presence of tight junctions, composed of large transmembrane proteins such as claudins and occludins. Collectively, this structure maintains the homeostatic environment of the brain and excludes peripheral cells, proteins, and many molecules, including cytotoxic compounds. Functional changes in the blood brain barrier occur as a result of ischemia, including loss of endothelial tight junctions, the internalization of plasma proteins, and trafficking of peripheral immune cells into the brain parenchyma. Coupled with distress signals from local brain cells, this promotes the intercellular transfer of peripheral immune cells and transcytosis of plasma proteins, thus amplifying the inflammatory response in the ischemic brain. The aging blood brain barrier, and its cellular components, may well underlie the greater stroke severity seen in this group.

4.2.1 Aging and the Blood Brain Barrier

Among the many age-related changes in the brain, alterations in the blood brain barrier are most likely to elevate the risk and severity of stroke. Age-related changes in the microvasculature increase blood brain barrier permeability which is further increased in patients with vascular dementia or Alzheimer's disease [83]. There is growing evidence that hemorrhagic transformation after tPA treatment is due to its action on proteolysis in the neurovascular unit (reviewed in [278]). One possibility is that tPA may act via matrix metalloproteinases (MMP) which are known to promote barrier leakiness. Stroke patients who receive tPA have elevated MMP-9 [119] and MMP9 levels are highest in tPA patients with hemorrhagic conversion [187]. Furthermore, MMP inhibitors significantly reduce the severity of tPA induced cerebral hemorrhage [258]. Increased blood brain barrier permeability with age has been reported in both animals and humans (reviewed in [200]). Following stroke, blood brain permeability is enhanced in older animals and tPA action on the barrier is accompanied by activation of occludin and claudin-5 [134].

Sex differences and alterations in barrier function due to menopause or reproductive senescence are relatively understudied. Experimental studies evaluating the influence of estrogen on blood brain barrier permeability generally indicate a protective function [253]. 17β-estradiol also reduces edema in an experimental stroke model by reducing the activity [199] and abundance [46] of the Na-K-Cl cotransporter. However, the synthetic estrogen ethinyl estradiol has been shown to increase endothelial permeability to albumin [93].

In middle-aged female rats, there is increased permeability of the blood brain barrier in the hippocampus and olfactory bulb as compared to younger females [21]. At the molecular/cellular level, this is accompanied by increased perivascular IgG expression in the hippocampus, a marker commonly used to assess barrier integrity in aging and disease. Furthermore, constitutive expression of claudin-5 and occludin were not altered by age, however junctional localization of these proteins, which is critical for their barrier function was reduced in cerebral microvessels from middle aged reproductively senescent females [19]. Disrupted tight junctions are also seen in aging female hamsters [101]. In fact, cerebral microvessels from a small sample of pre and post-menopausal women also confirmed this reproductive age-related loss of junctional localization [19].

Consistent with the high demand for active transport in these cells, barrier-forming endothelial cells contain significantly greater mitochondrial content than non-barrier forming endothelial cells [205]. In both rats and monkeys, mitochondrial content of endothelial cells is reduced with age [40], while mitochondrial DNA mutations increase during aging and in age-related neurologic conditions such as Alzheimer's disease [190]. Mitochondrial oxidative stress is a leading cause of vascular/endothelial dysfunction in the aging population. With advancing age, the accumulation of reactive oxygen species increases in endothelial cells (reviewed in

[58, 269], resulting in inactivation of the vasodilator nitric oxide (NO), and consequently, reduced vasodilator capacity and perfusion of tissues [57]. Compensatory responses such as increased iNOS in microvessels are also seen in hypertensive subjects [250] and aging male rats [43]. Age-related elevation of oxidative stress is accompanied by a chronic low-grade inflammatory phenotype marked by NF-kB activation [269]. Furthermore, age-associated mitochondrial oxidative stress promotes mitochondrial protein oxidation and mitochondrial DNA mutations (reviewed in [59]), and it is speculated to cause endothelial apoptosis [171, 285]. Decreased density of cerebral arterioles in aging [256] is consistent with the idea of vascular deterioration.

4.2.2 Aging and Angiogenesis

In addition to their central role in the blood brain barrier, cerebrovascular endothelial cells play a critical role in stroke-associated angiogenesis and regulation of blood flow. Angiogenesis or formation of new vessels is an adaptive response to ischemic injury [64, 255]. Angiogenesis is stimulated by hypoxia, which upregulates angiogenic factors such as HIF-1 and VEGF [106, 224]. Post-stroke angiogenesis is closely associated with neurogenesis [7, 52] such that the angiogenic niche promotes neurogenesis [202]. Neurons and astrocytes within the neurovascular unit also secrete angiogenic factors, which in turn enhance proliferation and differentiation of neuronal precursor cells to promote neurogenesis [259]. Thus reduced functional capacity of endothelial cells with age will not only affect vascular repair but also neurogenesis.

The impact of aging on angiogenesis in the stroke brain is poorly studied and the results equivocal. Aged male F344 rats show a significant decrease in capillary angiogenesis compared to young animals following hypoxia [125], while in mice, both young and aged animals display similar microvessel densities 2–3 weeks after hypoxia, although hypoxia-induced upregulation of HIF-1α and Ang-2 was significantly reduced in the aged mice [30]. Aging also impairs the angiogenic potential of senescent human umbilical vein endothelial cells [97], which is associated with reduced VEGF levels [231]. Exogenous VEGF treatment of aging mice, however, did not improve the angiogenic response, suggesting that VEGF-activated downstream signaling pathways may be permanently changed with age [94]. Although HIF-1 activation is a primary step in angiogenesis, PPAR-gamma coactivator 1-alpha (PGC 1α) is also capable of inducing angiogenesis in aged F344 rats [194]. Using gene expression at the neurovascular unit as a marker for angiogenic capacity, resting gene expression of PGC 1α was lower in aging animals, and the angiogenic response to hypoxia was also weaker [191]. Estrogen promotes angiogenesis, decreases free radical production, increases cell survival, and stimulates angiogenesis in cerebral endothelial cells [141]. It also increased microvessel density prior to [10] and 10 days post stroke [9], indicating that the loss of estrogen in aging females may impair repair processes.

4.2.3 Aging and Cerebral Blood Flow

Reduced cerebral blood flow during aging appears to be universal, and has been documented in rats [33, 203], monkeys [197] and humans [240]. Interestingly, cerebral blood flow reduction may occur as early as midlife in humans (50 years) [240]), consistent with elevated stroke risk during this time frame. Impaired endothelial vasodilation, which is an early marker for arterial aging, has been attributed to the twin perils of oxidative stress and inflammation. Accordingly, the vasodilatory compound nitric oxide (NO) is reduced by an imbalance in pro and anti-oxidant systems in the aging vasculature, resulting in high levels of reactive oxygen species and low levels of antioxidant response due to decreases in critical proteins including manganese superoxide dismutase (mnSOD2) and nuclear factor-erythroid 2 p45-related factor 2factor-2 (Nrf2) (reviewed in [80]). This is believed to act as a stimulus for a low -grade inflammation [282] typically seen in aging vessels [290], resulting in a deleterious feed-forward amplification of oxidative stress.

One of the most prominent reasons for NO insufficiency is age, due in part to the age-related reduction of nitric oxide synthase [218] and an age-related increase in arginase, that degrades the natural substrate for NOS [32]. In fact age is the most significant predictor of endothelial-dependent vasodilation [100]. In coronary circulation, endothelial-derived NO decreases dramatically in 70–80 year old patients as compared to 20 year old patients [79], consistent with the observation that cerebral blood flow is negatively correlated with age in a study of 20–100 year old individuals [261]. In contrast, the renin angiotensin system is permissive for hypertension [183] and age-related imbalances in the renin-angiotensin system (RAS) is also a risk factor for cardiovascular disease [12]. In animal models, inhibition of RAS increases lifespan and reduces age-related hypertension (reviewed in [71]). Through production of vasodilatory and vasoconstrictive molecules, the cerebrovascular endothelium plays an important role in regulating blood flow, which is also critical during reperfusion following stroke [109]. Some evidence links the age response to an imbalance between vasoconstrictive and vasodilatory factors, with an elevation in the former [72]. Vascular reactivity is also altered with age, such that adenosine administration induced greater vasodilation in young animals as compared to older animals [127], while intravascular serotonin exacerbates vasoconstriction in older animals [111]. Thus, the aging brain is more likely to be subject to hypoperfusion and potentially, greater neuronal damage in response to ischemic stroke conditions.

4.2.4 The Aging Astrocyte

Astrocytes play an important role in the normal and pathologic brain. Specifically, astrocytes regulate synaptic activity, extracellular matrix secretion, blood–brain barrier integrity and the inflammatory response (reviewed in [252]). Following brain injury, astrocytes become reactive with increased expression of glial fibrillary acidic protein (GFAP) [215, 262]. Astrocytes offer trophic support to neurons

through secretion and/or expression of several soluble factors including neurotrophins [172, 227] growth factors [226, 297] and by regulating local glutamate concentrations [239]. Astrocytes are also a source of inflammatory cytokines/chemokines [137, 150, 226] that can potentially ameliorate or exacerbate the injury response. However, astrocytic response to injury can be modulated by several factors including age and hormonal status.

The aging brain shows distinct changes in astrocyte morphology [272], GFAP expression [195, 233] and astrocyte numbers [112, 189]. Aging accelerates injury-induced astrocyte reactivity [18, 221], and after ischemic injury, this enhanced glial response accelerates glial scar formation [18]. Furthermore, reduced expression of astrocyte-derived SC1, an extracellular matrix-associated glycoprotein, indicates that matrix remodeling may also be impaired in aged rats following focal ischemia [168].

Reduced astrocyte function with age may also impact neurogenesis, a potential endogenous repair mechanism following brain injury. Decreased Wnt3 secretion (a neural stem cell differentiation factor) in astrocytes from middle aged (9 month old) mice resulted in impaired hippocampal neurogenesis in this age group as compared to younger (3 months.) mice [186, 204]. Reductions in astrocyte-derived growth factors and their receptors such as insulin-like growth factor 1 (IGF-1) [78, 155], fibroblast growth factor receptor 2 (FGFR-2) [45] and vascular endothelial growth factor (VEGF) [34] may also contribute to reduced adult neurogenesis.

Both constitutive and injury-induced functions of astrocytes are affected by age. Ex vivo cultures of astrocytes from the olfactory bulb of middle-aged female rats show several phenotypic changes such as increased stress fiber formation, reduced laminin deposition, increased BDNF expression and reduced TrkB expression compared to young astrocytes [156]. Furthermore, aging astrocytes show an impairment in their ability to promote neuronal differentiation of neural progenitor cells [156]. Moreover, ex vivo cultures of ischemia-activated astrocytes from aging females show reduced glutamate uptake as compared to astrocytes harvested from young adult females [155]. The impaired glutamate clearance and metabolic dysregulation observed in the aging astrocyte promotes a more toxic microenvironment in the older brain, thus probably contributing to the increased infarct size observed in old rats [221, 243].

4.2.5 Effects of Sex Hormones on Astrocyte Function

The sex (or gonadal) hormones androgen, estrogen and progesterone and their metabolites play a significant role in regulating astrocyte activity (reviewed in [251]), specifically by increasing expression of astrocyte-derived growth factors [92, 220] and regulating the glial glutamate transporters [213]. In the context of injury, reactive astrogliosis is attenuated following replacement with estrogen, testosterone [26, 61], dihyrotestosterone [61] progesterone as well as the neurosteroids dehydroepiandrosterone, pregnenolone and pregnenolone sulfate [96]. In addition, progesterone, estradiol [95] and the selective estrogen receptor modulators (SERMS)

raloxifene and tamoxifen [27] reduced reactive gliosis in females. Ischemia-activated cortical astrocytes from acyclic middle aged females showed impairments in glutamate clearance and lower growth factor synthesis as compared to astrocyte cultures from young females [155], indicating that a constitutive loss of ovarian estrogen may affect astrocyte function.

Interestingly, neuroprotective interventions by sex hormones may originate from the astrocyte itself or may result from paracrine signaling. Aromatase expression, a key enzyme in estrogen biosynthesis, is enhanced following a sub-lethal pressure increase in astrocyte cultures [98] while estrogen treatment of astrocytes inhibits the steroid hydroxylase CYP7B1 which is responsible for metabolizing dehydroepiandrosterone (DHEA), an important precursor for both estrogen and testosterone [86]. Taken together these data suggest that sex hormones play a supportive role in astrocyte function, and further support the hypothesis that the loss of ovarian hormones at menopause may accelerate stroke severity in females.

4.2.6 Aging Microglia

Microglia are the main effectors of the innate response in the ischemic brain. Microglia are thought to contain multimolecular complexes called inflammasomes, which act as intracellular sensors for host-derived signals in cases of brain injury and stroke [276]. Activated microglia are responsible for phagocytosis of non-functioning cells and synthesis and release of cytokines that can result in cell death. Accordingly, minocycline, an anti-inflammatory compound that targets microglia, reduces microglial activation and improves function and survival rate [114, 165]. In a small clinical trial, minocycline use was reported to improve neurologic function in stroke patients [147]. On the other hand, microglia also promote a neurogenic environment after stroke, since they also produce growth factors that are neuroprotective, and can elevate growth factor synthesis in neighboring cells [287]. Mesenchymal stem cells cultured in microglial media exhibit elevated level of several growth factors including VEGF and IGF-1 [298]. Furthermore, selective ablation of proliferating microglia exacerbates ischemic injury [146] while exogenous application of microglia reduces ischemic injury [138].

Similar to macrophages, at least two activation states have been proposed for microglia. The classical M1 response is induced by microbial agents or T helper cell type 1 secretions and results in the production of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, and IL-12), which are cytotoxic. The M2 activation is induced by Th2 cytokines (IL-4, IL-13), and is characterized by anti-inflammatory responses and tissue repair. Both age [151] and mitochondrial dysfunction [85] impair IL-4 mediated alternate activation and produce a failure to elevate growth factor levels in microglia. With aging, the brain environment develops a more pro-inflammatory profile and aging microglia may be a crucial component of this process. Microglial priming, which occurs with age, is associated with increased expression of inflammatory cytokines and an enhanced activated morphological profile (reviewed in [198]). Oxidative stress and free radical accumulation with age is thought to increase

inflammasome activity, and deletion of NLRP3 inflammasome in cells of myeloid origin reduces the inflammatory profile of microglia in a region specific manner [290].

4.2.7 Peripheral Immune Cells

One of the consequences of blood brain barrier dysregulation after stroke, coupled with elevated expression of specific receptors and adhesion factors by endothelial cells and astrocytes, is the influx of peripheral immune cells into the brain. Consequently, leukocytes including T cells, B cells, neutrophils and monocytes (macrophages) are mobilized to the ischemic region [81, 99], contributing to secondary inflammation and accelerating cell death [173]. The significant loss of splenic weight after transient [201] and permanent [274] ischemia suggests that immune cells stored in the spleen cells are mobilized into circulation after injury and recruited to the brain [241]. Accordingly, splenectomy [3] or treatments that maintain spleen mass such as infusion with human umbilical cord blood cells [274] reduced infarct volume. The latter treatment also reduced ischemia induced elevation of inflammatory cytokines in the spleen [273], suggesting that the spleen may be a good target for stroke therapy.

More recent work has shown that the type of immune cells recruited to the brain may also affect stroke outcomes. Macrophages are immunologically difficult to distinguish from microglia and the reduction of this cell type in the brain after splenectomy indicates that macrophages are mobilized to the ischemic brain [3]. Growing evidence indicates that T cell recruitment contributes to increased stroke pathology [108] and consistent with this evidence, T cell knock out mice have lower infarct volumes [288]. Additionally, specific cohorts of T cells have been shown to have cell protective or cell toxic effects. Thus, the gammadelta IL-17 producing cells (Th17) have been implicated in increased cell death [246], while regulatory T cells (Treg) and IL-10-producing Breg cells are thought to provide cell protection [35, 161]. Irrespective of age, young, middle-aged and aging mice subject to ischemia improved after treatment with recombinant T cell receptor ligand (RTL), which causes T cells to become non-pathogenic [75, 299]. This study supports the idea that different neuroprotective mechanisms may be activated with age. In this context, it is worth noting that while splenectomy is neuroprotective in males, it does not improve infarct volume or neuroinflammation in females, so it remains to be determined whether this surgical process would be equally effective in aging females [74].

The use of older animals in stroke research is particularly critical in light of the evidence that immune and other somatic responses will shape the ischemic response. Franceschi and colleagues have proposed the term inflamm-aging in recognition of the impact of aging on the immune system. Specifically, this denotes the upregulation of the innate immune response in the elderly, as well as the persistent low grade chronic inflammatory state seen in this group [88]. Not only does this condition underlie many neurologic diseases including Alzheimer's and stroke, but also

contributes to a paradoxical condition of immunodeficiency, whereby hyperexcitability of the immune system contributes to immune-suppression and lymphopenia that is often seen in the elderly.

4.3 Global Gene Regulatory Mechanisms in Aging

The large number of gene families and disparate cell types that are affected during aging and ischemia suggest the involvement of global regulatory mechanisms. Aged animals not only exhibit altered levels of specific genes such as inflammatory genes following ischemia as compared to young animals [221], but also display unique patterns of gene expression, with adult (3–4 months old) male rats upregulating genes involved in oxidative stress and aged (19–20 months old) rats displaying increased expression of pro-apoptotic and phagocytosis-promoting genes [39]. During the last decade, significant research has focused on molecular processes capable of exerting widespread effects on the genome. These include both small (microRNA) non-coding RNA species that act as translational repressors, long non-coding RNA (lnc) that can act as transcriptional enhancers or repressors, and chemical modification of the genome.

4.3.1 Non-coding RNA

MicroRNAs (miRNAs) are 18–25 nucleotide-long, non-coding RNA molecules that are important regulators of mRNA transcript stability [65] and mRNA translation [5]. MicroRNAs can occur within exons, introns and polycistronic clusters in the genome [153].

Though relatively few in number, miRNAs are predicted to control a large proportion of the tissue- and cell-specific transcriptome [142, 162] regulating important biological processes including mitosis, tissue-specific cellular differentiation, and cell death [55], maintaining the pluripotent state of embryonic stem cells [120], delaying neuronal maturation [143], or promoting neuronal differentiation [54]. Montano and Long (2010) [188] have proposed that RNA surveillance by regulatory molecules such as miRNA influence life span and longevity. Clusters of miRNA increase or decrease with aging, although these alterations are not always predictive of either benign or maladaptive aging. For example, miR-1 declines with age (day 15) in *C. elegans* and its disappearance has been correlated with muscle aging [124]. However, only 7 % of *C. elegans* survive to day 15, which suggests alternatively that declining miR-1 may be a compensatory process that promotes survival.

Specific pathogenic processes that contribute to stroke are associated with miRNA species, such as miR33 with hyperlipidemia, miR155 with hypertension, miR21 and −126 with atherosclerosis and miR222 with plaque rupture (reviewed in [228]). MiRNA profiles are altered with stroke both in humans [163] and in experimental [67, 126] stroke models. MiR17 is reported to be significantly elevated in

acute stroke patients as compared to controls (patients with vascular risk but no stroke) [136], while plasma levels of miR-210 are lowered in ischemic stroke patients as compared to controls, although stroke outcomes are better in patients where miR-210 levels are higher [292].

A recent study evaluated the association of microRNA polymorphisms with the risk of ischemic stroke in a Chinese population [123]. miR146a/rs2910164 C/G genotypes were significantly associated with increased risk of ischemic stroke, and the association was more pronounced in subjects over 60 years old, females, non-drinkers, and subjects without hypertension or diabetes mellitus. In animal models, miR15a has been specifically associated with endothelial cell loss and blood brain barrier breakdown in stroke [289]. MicroRNA manipulation has also been shown to mediate neuroprotection in stroke models. Specifically, antagomirs to Let7f [242] miR200c [257] mir29c [211] and mir181 [283] have been shown to reduce ischemic injury and improve behavioral function. High levels of miR1 are associated with oxidative stress [277] and anti-miR1 antagomirs reduce cortical infarction in experimental stroke [242]. IGF-1, which is neuroprotective for stroke in middle age females, regulates several microRNA that are implicated in barrier function [20].

Long Non Coding (lnc) RNA on the other hand are >200 nucleotides and can extend to several kilobases in length. LncRNA can enhance or repress transcription by epigenetic silencing or by serving as scaffolds for large protein complexes [229]. At the present time, very little is known about the involvement of lncRNA in stroke as compared to microRNA. In two recent studies, an experimental stroke model identified a large cohort of stroke-responsive lncRNA [68] and a subset of these stroke-responsive lncRNA were found to associate with chromatin modifying proteins including Sin3A and coREST, suggesting a mechanism by which non coding RNA can regulate the genome post-ischemia [69].

Little is known about non-coding RNA regulation following ischemia in aging populations, and this represents an important future direction for understanding stroke pathophysiology. A recent study comparing adult and middle-aged male and female rats showed that a small cohort of circulating miRNAs discriminate groups with small infarct volumes (adult females) versus groups with large infarcts (middle-aged females, young males) [245]. Such analyses may be useful in uncovering new mechanistic targets for understanding the impact of age in stroke outcomes.

4.3.2 Epigenenomic Modifications

Heritable, but potentially reversible, modifications to the genome represent the most fundamental aspect of gene regulation aside from the DNA sequence [140]. Epigenetic modifications can be classified into two categories: those that create an active chromatin state and positively regulate gene expression, or those that create a repressive chromatin state and negatively regulate gene expression [84]. Active/inactive states of the genome are regulated by chemical modifications of nucleotides and/or post-translational modification of histones in conjunction with chromatin compaction (euchromatin or heterochromatin) [49, 214]. Although as many as 60

covalent modifications of DNA and histones have been identified to date [140] most studies have focused on the methylation of cytosine located 5′ to guanine (CpG methylation), and the methylation of lysine residues within the N-terminal tails of histone H3. DNA methylation is typically associated with repressed chromatin at promoters and regulatory elements, while histone H3 methylation of lysines 4, 36 and 79 (H3K4, H3K36, and H3K79) are implicated in activation of transcription, while methylation of lysines 9 and 27 of histone H3 (H3K9 and H3K27) are implicated in transcriptional repression [140]. Similarly, histone acetylation is also associated with unwinding of chromatin.

4.3.3 Histone Acetylation and Aging

Histone acetylation typically results in the unwinding of chromatin, which facilitates gene expression, while histone deacetylation causes chromatin compaction. With age, changes in the activity and cellular localization of histone deacetylases (HDACs) can alter the careful balance of histone acetyltransferases (HATs) and HDACs required for maintaining histone acetylation [22, 73]. Decreased acetylation levels have been reported for animal models of neurodegenerative diseases [236]. Age-dependent changes in synaptic plasticity [291] and memory impairment [214] were also associated with lowered histone acetylation.

4.3.4 DNA Methylation in Aging

Both hypo- and hypermethylation of DNA have been reported in aging. One of the earliest studies linking epigenomic changes and aging reported that spawning fish have global decreases in DNA methylation with age [31]. Global hypomethylation was reported in human embryonic lung fibroblasts resulting from peroxide-induced senescence [295], and conversely, experimentally-induced hypomethylation reduced the lifespan of MRC-5 cells by 25 % [82]. A pervasive loss of methylation at Alu repetitive elements was also seen in a large scale study with humans ranging in age from 55 to 92 years [37]. In age-related dementias such as AD, overexpression of the amyloid precursor protein is associated with hypomethylation of the gene as well as APP promoter demethylation in the cortex [265, 280]. The mechanism underlying DNA hypomethylation is not well understood. One obvious mechanism is via altered expression of demethylases, but methylation can also be reduced due to insufficient methyl donors, or essential nutrients involved in metabolism of methyl groups such as folic acid, Vitamin B12 and choline, suggesting that nutritional deficiencies in aging may impact the epigenome. In the brain, increased DNA methylation but decreased histone methylation has been reported with aging [263].

In view of the crucial role that astrocytes play in the ischemic brain, age-related epigenomic modification in this cell type may critically affect stroke outcomes. Astrocytes isolated from adult mice showed higher expression of genes involved in hemoglobin synthesis and neuronal differentiation than aging astrocytes, while

aged astrocytes showed higher expression of genes implicated in zinc ion binding and an increased inflammatory phenotype indicating that normal aging alters gene expression profiles in astrocytes [208]. Astrocytes harvested from the ischemic cortex of young adult female rats had greater H3K4 specific methyltransferase activity as compared to middle-aged females and consistent with this finding, astrocytes from young adult females displayed more H3K4me3 enriched peaks than middle-aged females [50]. H3K4me3 enriched peaks at the mir17-20 cluster and the VEGF gene were further confirmed by measuring elevated mir20 RNA expression and VEGF protein in astrocytes from young adult females relative to middle aged females [50]. The use of these new tools to address cell specific changes in aging and ischemia will be critical for the development of next generation drug therapies, where the focus will be on global regulatory mechanisms.

5 Conclusions

Stroke occurs more often in the elderly and the outcomes are more severe in this group, but it would be incorrect to call age a 'non-modifiable' risk factor. With greater recognition of stroke risk factors during aging, such as hypertension and diabetes, and aggressive management of these diseases and better access to health care among the elderly, the impact of age as a risk factor should be mitigated. Healthier life style choices have extended life span and the concern is that longevity may bring about greater disability. However, a study that measured cumulative risk (exercise, body mass and smoking) found that cumulative disability was reduced in the elderly with healthy habits and disability was postponed and compressed to the last few years of life [275].

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The Role of Aging in Alzheimer's Disease

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1 Introduction

Aging changes the adult brain both structurally and functionally, facilitating and accelerating cognitive impairments and susceptibility to degenerative disorders even in healthy individuals [1, 2]. Indeed, aging remains the single most predominant risk factor for dementia and related neurodegenerative diseases, including Alzheimer's disease (AD). Considering the rate at which the human population is aging, it becomes imperative to identify means by which to maintain cognitive integrity by protecting against, or even counteracting, the effects of aging. Indeed, according to the Department of Health and Human Services, forty million Americans are older than 65 years of age and by 2030 this number will have doubled, accounting for one in five people in the United States. Hence, halting or reversing brain aging by only a small fraction could delay neurodegeneration and dementia, which would have a significant impact on the quality of life in old persons. Here, we will review the clinical distinctions between age-related cognitive decline and AD and explore the role of established hallmarks of aging in the aged brain and in AD.

2 Alzheimer's Disease as a Clinical Entity

AD may be suspected in a patient who exhibits gradual, progressive cognitive decline [3]. The most common symptom associated with AD is loss of recent memory—that is, difficulty forming new memories. People who know the patient well, like a spouse or other family member, may notice that the patient asks the same question more than once, repeats stories or conversations, or has trouble remembering recent events. By contrast, memory for events in the more distant past may be normal. At first, recent memory loss is typically mild, subtle, and in some cases evident only to the patient; as the amnesia gradually worsens over months to a year or so, it eventually catches the attention of family, friends, and clinicians. A clinician may test memory by providing a list of words or other new information, then distract the patient for a few minutes with other tasks or conversation, and finally ask the patient to recall.

Other cognitive symptoms may occur in addition to or instead of recent memory loss. Common symptoms that may emerge either early or late in the course of AD include word finding deficits, difficulty with navigation or decoding a complex visual scene, and trouble with executive function tasks that include planning, organizing, judgment, or problem solving. Motor deficits typically do not occur until late in the disease, except for apraxia, or loss of the ability to carry out complex motor tasks, which may occur earlier. In some cases of AD, language, visuospatial, or executive deficits may dominate in the relative absence of amnesia.

When the clinician discovers cognitive dysfunction that is objectively evident yet not severe enough to interfere with the patient's ability to perform typical, everyday activities, a diagnosis of mild cognitive impairment may be appropriate [4]. When cognitive dysfunction associates with difficulty performing everyday tasks (e.g., driving, balancing a checkbook, keeping track of appointments, etc.), then a diagnosis of dementia may be given. Dementia may be subcategorized as early- versus late-onset, based arbitrarily on whether symptoms emerge before or after age sixty-

five. Importantly, mild cognitive impairment and dementia are umbrella terms that are used to describe how much a patient's cognitive dysfunction has impacted everyday life; however, because these terms do not convey the suspected cause of the cognitive dysfunction, they are not intended as stand-alone diagnoses. Patients with amnestic mild cognitive impairment (i.e., in whom episodic memory impairment is present) have a 10–15 % annual risk of progressing to dementia, often due to AD [5].

While there are many possible causes of mild cognitive impairment and dementia, AD accounts for approximately two thirds of cases among older adults. Other common causes, each with a slightly different but nevertheless overlapping spectrum of typical symptoms, include stroke (vascular dementia), Lewy body disease (closely associated with Parkinson's disease), and frontotemporal dementia. Many other possible causes, while less frequent, may be worthy of consideration because some are reversible or at least treatable; these include vitamin deficiencies (e.g., Vitamin B12), hormone imbalance (e.g., hypothyroidism), infections (e.g., HIV, syphilis, or Lyme disease), tumors or other cancer-related conditions, psychiatric disease (e.g., depression), medication side effect or toxic exposure, or inflammatory or autoimmune diseases (e.g., multiple sclerosis). A standard medical evaluation includes a detailed history, physical examination, neurological examination, objective cognitive testing (either a brief bedside test or a comprehensive neuropsychological evaluation that includes tests of memory, language, visuospatial function, and executive function), neuroimaging with either computed tomography (CT) or magnetic resonance imaging (MRI), and blood tests for thyroid hormone function and Vitamin B12 levels, among other tests as indicated by the patient's specific situation. While such tests cannot provide direct evidence for AD, they can help to raise or lower suspicion for other diagnoses.

AD is diagnosed when a patient exhibits typical symptoms of the illness, and no other cause is uncovered during the medical evaluation. Importantly, AD is defined by the presence of two distinct protein accumulations in the brain of a patient with progressive cognitive decline: beta-amyloid, which self-aggregates into extracellular plaques between cortical neurons, and the microtubule-associated protein tau, which self-aggregates into tangles within the neurites and cell bodies of cortical neurons [6]. Classically, because these protein aggregates may be observed directly only during microscopic evaluation of the post-mortem brain, a diagnosis of AD in life may only be suspected. However, newly emerging biomarker tests are changing this situation, by allowing clinicians to test for the presence of beta-amyloid or tau pathology in a living patient. These tests, which do not substitute for the certainty of an autopsy but nevertheless may increase or decrease suspicion for AD, include direct measurements of beta-amyloid and tau concentrations in the cerebrospinal fluid obtained by lumbar puncture [7], as well as positron emission tomography (PET) scan after intravenous administration of a radiolabeled tracer that selectively binds either to beta-amyloid [8] or tau deposits [9] in the brain.

Treatment options for AD are limited. An active lifestyle may reduce the rate of future cognitive decline and reduce dependence upon caregivers; regular aerobic exercise, social and cognitive stimulation, and a healthy diet are important lifestyle components. Four medications are currently approved for the treatment of AD: donepezil, rivastigmine, and galantamine are centrally-acting cholinesterase inhibitors that increase the half-life of acetylcholine at synapses in the brain, modestly boosting alertness and cognitive function for many patients with mild-to-moderate

AD dementia. Memantine is a *N*-methyl-D-aspartate-type (NMDA) glutamate receptor antagonist that exhibits similar modest benefits among patients with moderate-to-severe AD dementia. None of these medications exhibits any robust influence over the pace of disease progression, and none has any direct impact on the underlying molecular pathogenesis. Clinical trials of many other potential therapies are underway.

3 Spectrum of Normal and Abnormal Aging

Cognitive change occurs with aging in the absence of any disease [10]. Beginning gradually in middle age, cognitive processing speed slows, fluid intelligence declines, and episodic memory skills wane. Older adults exhibit changes in sustained attention, working memory, and distractibility compared to their younger counterparts [11]. Cognitive change attributed to age alone, while robust enough to register as slight changes over time on neuropsychological assessments, is never sufficiently severe to impact daily function or to merit a clinical designation of mild cognitive impairment or dementia. Moreover, isolated age-associated cognitive decline is very slow, with changes that are perceptible perhaps decade-to-decade, but not year-to-year [12].

It is not the case that cognitive aging is simply a slow-motion version of AD. Rather, there are distinct processes at play in the two conditions. Indeed, fundamental changes occur naturally throughout the brain over the human life span, from macroscopic loss of brain volume to microscopic reductions in neuron and synapse numbers. Altered gene expression affects neural signaling with age; for instance, NMDA receptors, the calcium-permeable ion channels that play a central role in the induction of synaptic plasticity, exhibit a robust age-dependent change in subunit composition that affects how new experiences sculpt synaptic strength [13, 14].

However, the distinction between pathological and non-pathological cognitive decline is generally evident only in hindsight, based on the retrospectively-observed pace and pattern of cognitive change, and the end result in terms of functional status. Prospectively, it is very difficult to know, among a group of similar individuals experiencing the sorts of typical, age-associated cognitive changes described here, who will go on to experience a benign course, versus those who are actually experiencing the first, subtle symptoms of a cognitive illness like AD. A major challenge for the field is the fact that the molecular changes associated with AD and other neurodegenerative illnesses begin years—likely decades—before symptoms become clinically apparent. When symptoms do appear, they are at first subtle and not easily differentiated from the typical effects of aging. Given the likelihood that newly-emerging drug therapies will be effective only at an early stage of the disease, this symptomatic overlap between typical aging and prodromal AD is problematic, as there is a need to identify patients before the damage is indelible.

Even at a molecular level, distinguishing typical aging from disease is not straightforward. Roughly a third of asymptomatic older adults exhibit signs of beta-amyloid plaque deposition on amyloid PET imaging [15]. Similarly, as many as 60 % of adults around age 60 years exhibit at least the first stages of tau protein deposits in the medial temporal lobe of the brain at autopsy [16], far exceeding the proportion who exhibit actual cognitive decline; newer data suggest that tau pathology may begin in the locus coeruleus, a brainstem nucleus containing noradrenergic neurons, as early as middle age [17]. The detection of molecular pathology in the brain, either at autopsy or with newly emerging molecular diagnostic tests, is not synonymous with the presence of a clinical disease. Certainly the presence of abnormal protein accumulations raises the risk of future cognitive decline, but the magnitude of that risk is not yet definitively established by available research. In other words, a cognitively healthy patient who tests positive on an amyloid PET scan has an unclear fate; she may be on the precipice of imminent cognitive decline or may survive many years with normal cognitive health. Even if we could peer into her brain with a high-powered microscope years before her death, we may still lack confidence about her cognitive fate.

It is worth mentioning that beta-amyloid and tau are not unique in their capacity to self-aggregate in the brains of older adults. Lewy bodies, the intracytoplasmic neuronal accumulations of alpha-synuclein protein associated with Parkinson disease and Lewy body dementia, frequently appear in the brains of cognitively healthy older adults. The same is true for TDP-43, a protein that self-aggregates in some forms of frontotemporal dementia. When are plaques, tangles, Lewy bodies, and TDP-43 deposits simply benign findings in an aging brain, and at what point do they become significant enough to be blamed for cognitive changes? To confuse the picture even more, it is rare to find, at autopsy, isolated evidence of a single neurodegenerative disease; rather, Lewy bodies and TDP-43 deposits frequently appear in the brains of patients with AD, and patients with Lewy body dementia often exhibit plaques and tangles. How do we know where one disease stops and the other starts?

The answers to these questions are not yet clear. Indeed, the very notion of what constitutes normality is open to debate. What is clear is that cognitive changes due to the inherent biology of aging versus superimposed neurodegenerative disease—while distinct processes—are not mutually exclusive, and probably exist on a spectrum in every aging adult.

4 Risk Factors for Alzheimer's Disease

Despite the uncertainties outlined above, there are some known risk factors for AD. These may be categorized by whether or not they are modifiable: Modifiable risk factors for AD, while less important than non-modifiable factors in the overall risk profile, deserve close attention because they may be mitigated through lifestyle choices. They include the presence of vascular risk factors and insulin-resistant diabetes mellitus [18]. Repeated concussions or other forms of head trauma associate

with an increased chance of developing AD or other neurodegenerative illnesses later in life [19]. A sedentary lifestyle may increase one's chances of developing AD, whereas regular aerobic exercise is protective [20].

Less exposure to education is an additional modifiable risk factor for AD. Individuals with higher education and perhaps greater cognitive stimulation in midlife appear to be protected from developing symptoms of cognitive decline, even though, at autopsy, they may nevertheless exhibit the neuropathological signs of AD [21]. Such findings have given rise to the concept of cognitive reserve, or the buildup of resistance against the cognitively detrimental effects of damage to the brain. Although the mechanism of cognitive reserve is not known, it is hypothesized that education and ongoing learning in life lead to a greater number of synapses and perhaps even neurons in the brain that can act in a compensatory capacity in the face of a degenerative illness.

Non-modifiable risk factors dominate the overall risk for developing AD. Chronological age stands out as conferring by far the most risk. About a third of individuals aged 85 years and older suffer from AD dementia; many more have the pathological signs of the disease, in the absence of overt symptoms. The vast majority of patients are 75 years or older.

Genetics and family history are also non-modifiable risks. Of the many genes that influence overall risk for late-onset, sporadic AD, the *APOE* genotype has a greater effect than all of the others. The *APOE* gene encodes the apolipoprotein E protein, which plays an important role in cholesterol trafficking; it is expressed in astroglial cells and, to a small extent, in neurons in the brain. There are three *APOE* allelic isoforms: ε3, ε4, and ε2, in decreasing order of population prevalence. Carriers of the ε4 allele exhibit an increased risk of developing AD, such that heterozygotes and homozygotes have a 2–3-fold and 12-fold increased lifetime risk relative to noncarriers [22]. Carriers of the least common ε2 allele have a reduced risk relative to ε3 homozygotes, the most common genotype. Gender also plays a role; female *APOE* ε4 heterozygotes exhibit a greater chance of developing AD than their male counterparts [23]. A history of AD in multiple family members may increase suspicion for an *APOE* ε4 allele or other risk-conferring genetic polymorphisms.

It is important to point out that genetic *polymorphisms* that influence the risk of acquiring late-onset, sporadic AD are very different from genetic *mutations* associated with familial, early-onset AD. Mutations in the genes for amyloid precursor protein (APP), presenilin 1, and presenilin 2 lead to cognitive decline in the third or fourth decade of life, causing AD in an autosomal dominant, completely penetrant fashion. Down syndrome (trisomy 21) associates with early-onset AD, due to overexpression of the APP gene, which happens to localize to chromosome 21. Such genetic cases account for only a tiny fraction of overall AD burden worldwide and are not directly relevant to the vast majority of patients who experience sporadic disease.

While much is known about risk factors for AD, less is known about the predictors of better or worse phenotypes of normal aging in the absence of neurodegenerative disease. As described in the previous section, part of the difficulty with such

research is a lack of clarity about the presence or absence of preclinical neurodegenerative disease in a living individual. For instance, whereas the *APOE* ε4 allele is not associated with any difference in baseline cognition among older adults, it does predict a faster rate of decline [24, 25]; however, given the tight association of *APOE* ε4 carrier status with beta-amyloid accumulation, this observation may be explained by the emergence of AD symptoms among carriers rather than an acceleration of AD-independent aspects of cognitive aging. Most likely, many lifestyle factors that associate with a reduced risk for AD, like exercise and education, also predict a more benign course of cognitive aging. Genetics influences the pace of cognitive aging, although the identity of specific genes is only beginning to emerge [25, 26]. As one example, a polymorphism in the gene encoding klotho, a transmembrane β-glucuronidase enzyme, promotes longevity and associates with improved cognition in older adults [27]. The gene for another protein, forkhead box O3, exhibits a polymorphism associated with longevity [28], and its possible association with cognitive aging should be examined.

5 Structural and Functional Changes in the Brain

A proliferation of advanced neuroimaging technologies and analytical techniques has broadened the perspective on how the brain changes in aging and AD. MRI reveals fine structural details of the brain and permits volumetric analysis and detection of atrophy in subjects who undergo repeated, longitudinal imaging. Indeed, perhaps not surprisingly, atrophy is a robust finding in aging adults. Not only do older adults exhibit smaller brains than younger adults, but also year-to-year volumetric decline occurs among cognitively healthy older adults who do not exhibit any concerning signs for preclinical AD [29–31]. Atrophy accelerates in AD, but this is observed only with serial imaging in the same patient and is not captured with a single scan at one point in time.

The hippocampus is a special area of focus for structural imaging, because postmortem pathology points to this structure as an early victim of neurodegeneration and atrophy in AD [32]. Indeed, hippocampal atrophy is a robust finding that differentiates patients with AD from age-matched, cognitively-normal controls [33]. However, the finding emerges only after averaging images from groups of patients and controls; there is so much variability in hippocampal size from individual to individual that hippocampal volume, whether in raw, unadjusted terms or normalized to overall brain volume, is not strongly predictive of diagnosis until rather late into the course of dementia. In other words, a single MRI in one individual is less sensitive to AD than traditional clinical data. The overlap between healthy aging and AD is evident even when using ultra-high field MRI to resolve a very tiny subregion of the hippocampus known to degenerate first in AD [34].

Functional imaging offers another set of perspectives on how the brain changes with age and with the onset of neurodegenerative disease. Whereas structural imaging yields a finding only after enough wholesale loss of neurons, neurites, glial

cells, etc. occurs to result in macroscopic shrinkage of a brain region, functional imaging offers the ability to detect changes in patterns of neural activation that could predate involution. When subjects perform episodic memory tasks during functional MRI scanning, complex results emerge out of older adults who are cognitively normal or have mild cognitive impairment or AD dementia: compared to young adults, healthy older adults exhibit very similar patterns of hippocampal activation during memory tasks, although they show differential activation of frontal and parietal lobe areas [35]. In patients with mild cognitive impairment, hippocampal activity during episodic memory tasks actually increases relative to age-matched, cognitively healthy controls; as the clinical severity worsens towards dementia, hippocampal activation during memory tasks decreases below that seen in controls [36]. The mechanism and implications of this apparent hippocampal hyperactivity during prodromal AD are not known, including whether it represents a compensatory strategy or is instead an inherent part of the pathophysiology. Interestingly, network dysfunction, resulting in hippocampal overactivation and subclinical seizures, has been observed in mouse models of AD [37]. Antiepileptic therapy, specifically with levetiracetam, improves network dysfunction and cognitive deficits in the mouse models [38] and is now being explored in humans [39, 40].

High-resolution MRI, sufficient to resolve individual hippocampal subfields, yields additional insights. It is clear from post-mortem tissue examination that the hippocampal CA1 subfield is selectively vulnerable to degeneration in AD, and disproportionate CA1 atrophy is observed using a variety of advanced structural MRI techniques in vivo [34, 41, 42]. Functional imaging reveals a slightly different story: In healthy older adults and patients with mild cognitive impairment, it is the dentate gyrus and CA3 areas (which cannot be differentiated with in vivo imaging because of their close proximity) that appear abnormal, exhibiting hyperactivity in association with impairment of pattern separation, or the ability to discriminate novel stimuli from slightly different, previously-encountered stimuli [43, 44]. This finding of dentate gyrus/CA3 hyperactivity – which may possibly underlie the observation of global hippocampal hyperactivity observed with lower resolution functional MRI – differentiated patients with mild cognitive impairment from normal controls, and among healthy controls, the amount of activity in this region correlated negatively with cognitive performance. Together, these data suggest that dentate gyrus/CA3 hyperactivity, while more apparent in prodromal AD than in healthy aging, is nevertheless a feature in both. This contrasts with some other data, derived in part from animal models, suggesting that normal aging and AD are cleanly distinguished by dentate gyrus versus CA1 dysfunction [45]; however, these findings have not been readily reproduced in humans.

Functional MRI, in addition to showing patterns of regional brain activation while performing a task, also reveals areas of the brain that exhibit coordinated activity when the subject is at rest, in so-called resting-state networks. One resting-state network, the default mode network, includes the medial and dorsolateral parietal association cortex, medial prefrontal cortex, and the medial temporal lobe, and is more active than other networks at rest. The integrity of the default mode network

deteriorates in AD [46], and it also deteriorates in healthy older adults at risk of AD because of *APOE* ε4 positivity or the presence of amyloid deposition [47].

In summary, and echoing the theme of earlier sections, brain structure and function clearly changes with aging and with AD, but there is a high degree of overlap between these two states. This overlap may reflect a limitation of available technology or may instead reflect a true overlap in underlying biology.

6 Biological Hallmarks in Brain Aging and Alzheimer's Disease

Given these astonishingly intricate connections between brain aging and AD, how can we possibly untangle and comprehend the extent to which aging is responsible for the development of the disease? In their landmark review, "the hallmarks of aging", López-Otín and colleagues describe nine cellular and molecular, age-dependent changes which have been observed across multiple species, tissues, and cells and can be considered common hallmarks of the aging process [48]. We will use these hallmarks as guides to compare normal brain aging with AD in humans and discuss animal and tissue model studies to support findings and arguments, if appropriate. A few hallmarks for which there is substantial evidence for involvement in AD are discussed in more detail: telomere attrition, epigenetic alterations, loss of proteostasis, stem cell dysfunction, altered intercellular communication and neuroinflammation. To what extent these processes underlie or promote AD is unclear but they provide possible therapeutic targets that might interfere with AD pathogenesis by impinging on the aging process directly. As well, we hope that future studies of these well-defined hallmarks in the aging and AD brain will provide additional insight into the mechanisms underlying AD.

6.1 Genomic Instability and Telomere Attrition

Aging results in the accumulation of damage in nuclear and mitochondrial DNA and interference with DNA repair processes can accelerate aging. Telomeres shorten with cell divisions and restoring telomere length can delay aging. While glial and endothelial cells in the brain have the capacity to divide the vast majority of neurons don't renew and some may live longer than a century. As such, they need to withstand stressors and employ repair mechanisms to maintain proper function. Of particular importance is the preservation of DNA, which can be damaged by reactive oxygen and nitrogen species, replication errors, and other mechanisms. Among other defects this leads to single base damage and single or double strand DNA breaks in neurons [49]. Pathways which increase damage or decrease repair could conceivably contribute to brain aging and AD and there is some evidence in support

of this hypothesis [50, 51]. In a landmark study, Yankner and colleagues showed that with age DNA damage accumulates in the brain, specifically in promoter regions of genes involved in synaptic plasticity, vesicular transport, and mitochondrial function, and that levels of these genes were reduced with age [52]. Remarkably, neurons may regulate DNA errors to control plasticity and cellular diversity and to facilitate chromatin remodeling in these highly transcriptionally active cells. Thus, exploratory behavior and neuronal activity in mice led to the formation of transient double stranded DNA breaks. In APP transgenic mice more breaks were observed and these were maybe more permanent and damaging [53].

Telomere attrition contributes to the age-dependent loss of proliferative capacity in some cell types and provides a strong link to cellular senescence and organismal aging. The activity of telomerase, the enzyme that adds telomeric repeats to terminal DNA, decreases with age in mice [54, 55], and mice lacking telomerase show reduced neural stem cell activity in adults but not during development [54, 56]. This decrease in telomerase activity may in part be dependent on p53 as removal of p53 is sufficient to rescue defects in proliferation, self-renewal, and differentiation of neural stem cells in telomerase-deficient mice. Also, physical exercise, which is known to increase adult neurogenesis and cognitive function, increased telomerase activity in neural stem cells [55]. The role of telomeres in AD and AD mouse models is not well understood and controversial [57]; while there appears to be no significant telomere abnormality in AD brains, several studies reported accelerated telomere shortening in leukocytes from patients compared with age-matched controls [58]. However, among 10 studies investigating telomere length in leukocytes from AD patients, half observed a shortening of telomeres while the others found no change [57]. In a recent study of 274 individuals, leukocytes with either shorter or longer telomeres compared with those found in normal subjects were both associated with mild cognitive impairment, a major risk factor for development of AD [59]. Possibly explaining some of these discrepancies between studies or even within studies are observations that leukocyte telomere length, or better, the ratio between telomerase activity and telomere length, are inversely related to hippocampal volume in early aging [60]. In experimental models of AD, APP mice lacking telomerase (G3tert deficient) show reduced amyloidosis accompanied by microglial activation and impaired neurogenesis [61] suggesting independent effects of telomeres on different AD relevant pathways. Consistent with this interpretation, and this may relate to neuroinflammation and changes in intercellular communication, telomeres in microglia may be altered in the aging brain [62]. Microglia are phagocytic cells and the key representative of the immune system in the brain (see section 6.6). They maintain proliferative capacity in adulthood but seem to show signs of senescence in normal and AD brains [63]. Accordingly, mice lacking telomerase showed reduced dendrites and dendrite branching and increased expression of activation markers in microglia [61]. Adding complexity, telomerase (TERT) has recently been found to exert extranuclear functions beyond telomere maintenance, including protection of mitochondria from oxidative stress and prosurvival effects in mature neurons. Human TERT was detected in mature human neurons and while its total level was unchanged in AD brains, the enzyme colocalized with mitochondria

in advanced stages of the disease [64]. Intriguingly, in these AD brains, TERT was not detected in neurons with tau pathology and in cultured neurons, TERT protected against the accumulation of tau lesions, supporting the idea that the protein confers a protective effect to neurons [64]. Together, these reports suggest that DNA damage and telomerase activity may have a role in both normal brain aging and AD but the exact mechanisms remain to be defined.

6.2 Epigenetic Alterations

Posttranslational modifications of histones and changes in DNA methylation patterns constitute important aspects of gene regulation and they have increasingly been implicated in aging. Epigenetic regulation of gene expression also has a critical role in memory and learning and may thus assume a particularly important role in the aging of this tissue [65]. Consequently, it is likely that age-related changes in epigenetic processes would contribute to cognitive dysfunction and AD. What's more, epigenetic mechanisms are key to neuronal fate determination and function with repressor element 1-silencing transcription factor (REST) playing a dominant role [66]. Together with HDAC, MECP2 and CoREST, this factor regulates developmental and adult neurogenesis as well as gene activity in mature neurons. Interestingly, as cellular stress increases with age, REST appears to be important in promoting expression of anti-apoptotic, anti-oxidant, and other protective genes including BCL2, SOD1, and FOXO [67]. In AD, REST is depleted from the nucleus and appears in autophagosomes instead; moreover, genetic ablation of REST in mice or the REST homologue SPR-4 in *C. elegans* increases neuronal susceptibility to oxidative stress and results in neurodegeneration and shortened lifespan, respectively. Epigenetic regulation of memory and hippocampal plasticity in particular is altered with age in mice undergoing specific learning tasks. Thus, while baseline hippocampal histone H3 and H4 acetylation as well as levels of HDACs and histone acetyltransferases (HATs) were unchanged with age, old mice failed to acetylate histone H4K12 in response to a learning task [68]. Consistent with this lack of H4K12 acetylation, only few genes were differentially expressed based on genome wide transcript analysis or ChIP sequencing in the aged hippocampus in response to the learning paradigm [68]. To identify genes involved in age-related memory loss, Pavlopoulos and colleagues analyzed gene expression in the dentate gyrus, a region of the hippocampus thought to show age- rather than AD-specific deterioration, in pathology-free subjects from 33 to 88 years of age. The histone binding protein RbAp48, which functions in histone acetylation and transcriptional regulation, showed the most prominent decline with age, and mice expressing an inhibitor of RbAp48 showed hippocampus dependent memory deficits associated with a regional decrease in histone acetylation. Upregulation of RbAp48 reversed age-related memory loss and normalized histone acetylation [69].

DNA methylation controls the expression of genes and, with age, these patterns change, although it has not been demonstrated that reversing age-related DNA

methylation can extend lifespan. A study of close to 400 human brains aged 1–102 years showed a strong positive correlation between methylation and age across three brain regions [70]. Two independent, epigenome wide association studies (EWAS) of AD analyzed together multiple brain regions in close to 1,000 autopsied brains and correlated DNA methylation changes with pathology [71–73]. Remarkably, four methylated sites, close to *ANK1*, *RHBDF2*, *RPL13* and *CDH23*, were linked to AD pathology in both studies and all except CDH23 are biologically linked to *PTK2B*, a known AD-associated gene. In a more focused approach, analysis of 28 genetic loci associated with AD in 740 autopsied brains from AD cases and controls showed significant DNA methylation changes in SORL1, ABCA7, HLA-DRB5, SLC24A4 and BIN1, some of which correlated with brain pathology [74]. Using a genome wide approach, Gjoneska et al. recently compared the transcriptome and epigenome of a mouse model of AD-related neurodegeneration and brain tissue dissected from AD brains [75]. They observed a downregulation of synaptic plasticity genes and regulatory regions and a concomitant increase in immune response genes and regulatory regions such as the ETS transcription factor PU.1, a critical factor for the development of the myeloid lineage including microglia [76].

Remarkably, apart from DNA methylation, few studies assessed the role of epigenetic changes in the human brain in normal aging or AD thus far and there is little consensus at this point. Studies investigating miRNA changes in the disease focused frequently only on one or a few specific species and only one study carried out an unbiased screen [77]. Clearly, larger and broader screens will be necessary to gain an understanding of the role of these epigenetic regulators. Using selected reaction monitoring (SRM) proteomics and confirmation by immunoblot, Zhang et al. reported strongly reduced acetylation of histone H3 K18/23 in temporal lobes of AD brains compared with controls in a study of 15 brains in total [78]. Histone modifications have been implicated in AD pathogenesis in mouse models. For example, the deacetylase SIRT1 shows disease-dependent upregulation in neurons in a mouse model of AD and overexpression of SIRT1 reduced neurodegeneration dependent on its deacetylase activity [79]. Subsequent studies by the same group showed that DNA double strand breaks led to a recruitment of SIRT1 in injured neurons where it deaceylated HDAC1 and thus stimulated enzymatic activity, which was necessary for DNA repair [80]. While overall SIRT1 mRNA levels were not changed with age in the cortex of wild type mice [81], SIRT1 activity decreased with normal brain aging in microglia and resulted in increased production of IL1-beta and tau-dependent memory deficits [82]. It is thus possible that, in this fashion, loss of deacetylase activity promotes neuroinflammation and AD pathogenesis.

Overall, it is well known that epigenetic changes are key to learning and memory and some studies suggest that these pathways become dysfunctional during brain aging [65, 83, 84]. It seems clear from recent studies that the AD brain shows methylation changes which are linked to the genetic and biological understanding of the disease. As ChIP sequencing techniques become more reliable and amenable to high throughput studies, it is likely that additional epigenetic modification will be discovered in AD and aid in the understanding of the disease. In general, our knowledge of epigenetic changes with normal brain aging remains scant.

6.3 Mitochondrial Dysfunction

Mitochondria are the cell's purveyors of life and death by regulating energy metabolism and cell death pathways. They also regulate cellular redox potential, calcium levels, cell cycle and influence many other key pathways. It is thus not surprising that these organelles have a major role in aging in general, and in brain aging and AD in particular. For excellent general overviews about this large field of research we refer to [85–87]. Neurons have very high numbers of mitochondria and their dysfunction would be expected to have significant consequences but, at the same time, it could be expected that nature has built in extra protective mechanisms to avoid premature failure of the nervous system. One hypothesis proposes that mitochondrial DNA damage accumulates with age, leading to reduced mitochondrial enzymatic activity, a loss of mitochondrial bioenergetic function, and subsequent cellular degeneration [88]. There is indeed support for an accumulation of mitochondrial damage in the aging normal human brain and in AD [89–91]. Additionally, mitochondrial enzyme levels including the nuclear encoded F1 ATP synthase [52] or activity of complex I and complex IV [92] were reduced in the aging human or rodent brain, respectively. Likewise, it has been reported that pyruvate and ketoglutarate dehydrogenase as well as COX activities are reduced in mitochondria from AD brains compared with normal controls, and that the number of normal-appearing mitochondria is lower in AD [86]. Mitochondrial impairments have also been extensively documented in APP or tau transgenic mouse models for AD [85]. In summary, while it is clear that mitochondria are altered and fulfill a key role in both brain aging and AD, it is debated whether dysfunctional mitochondria are principal drivers of brain aging and AD or primarily a consequence of accumulating beta-amyloid and other brain lesions.

6.4 Loss of Proteostasis

The equilibrium between synthesis and degradation determines the levels of a given protein but many other regulatory pathways contribute to this process as well as the qualitative aspects of the product, including protein folding, polymerization/aggregation, tagging for degradation, etc. Changes in any of these have been implicated in aging and age-related loss of protein homeostasis, and would be expected to have particularly drastic consequences in a tissue with largely post-mitotic cells. Indeed, neurodegenerative diseases are largely characterized by accumulation of protein deposits and strong evidence points to a causal role for protein dyshomeostasis in many such diseases. AD is the archetype neurodegenerative disease where to this day, only the combined presence and abundance of amyloid plaques and neurofibrillary tangles allows for the unequivocal diagnosis of AD by the neuropathologist upon autopsy. We have learned that amyloid plaques are extracellular assemblies of highly ordered fibrils consisting predominantly of 40–42 amino acid fragments

derived from APP, and that autosomal dominant mutations in APP result in amyloid accumulation and disease [93]. Likewise, we know that neurofibrillary tangles form inside neurons, are made of fibrils of the microtubule associated protein tau, and that autosomal dominant mutations in tau result in tangle formation and development of a related neurological disease called frontotemporal dementia [6]. Thus, AD is clearly a disease of proteostasis failure and much of the last two decades of research in the field has tried to understand the basis of this failure in both autosomal dominant and sporadic forms of the disease [93]. Ground-breaking measurements of beta-amyloid steady state levels in brains of living humans by Bateman and colleagues demonstrated that production of beta-amyloid is not increased in sporadic AD, but that instead, impaired clearance is chiefly responsible for its accumulation [94]. This clearance defect may include a combination of problems including impairments in phagocytic capacity of microglia [95] and astrocytes [96], transport of beta-amyloid across the blood–brain-barrier [97], or extracellular degradation of beta-amyloid [98]. Significant evidence exists that beta-amyloid may also accumulate inside neurons and contribute to their dysfunction [99].

Posttranslationally modified tau protein is a sensitive indicator of neuronal injury and forms increasingly more complex and insoluble aggregates in neurons in AD [6]. It has long been maintained that this pathology begins in the entorhinal cortex from which it spreads to hippocampal and cortical areas [100] but, in a herculean effort characterizing tau pathology in more than 2,300 postmortem brains aged 1–100 years old, the same authors reported that subtle tau abnormalities occur already in the youngest brains and that they are first observed in the locus caeruleus [17]. Braak concludes from these findings "that the pathologic process underlying AD is not age-dependent but an uncommonly slowly progressive one that frequently extends into old age". While this interpretation is possible, the lack of fibrillar tau in younger brains could also indicate that age-dependent processes turn relatively harmless tau lesions into neurotoxic ones. Furthermore, the fact that tangle pathology can be replicated in mouse models in a matter of months – albeit with mutant forms of tau – argues for biological processes, rather than time alone, playing a key role in tauopathies.

Both beta-amyloid and tau accumulate in the brain decades before clinical symptoms manifest and changes in extracellular beta-amyloid and tau levels in the CSF appear to be reliable predictors of clinical disease onset [7]. As discussed above, new PET imaging probes, which allow for the detection of abnormal beta-amyloid [8] and tau [9] deposits in living people, seem to support these findings made in CSF. Because many brains from cognitively healthy people show abnormal beta-amyloid and tau deposits, the distinction between normal brain aging and slow disease progression becomes extremely difficult. Studies in mouse models expressing human APP or tau containing familial mutations form pathological protein deposits but, thus far, to our knowledge, no data exist whether such deposits develop with the same kinetics in a young or old mouse brain, within the same mouse model.

Interestingly, part of the machinery that controls protein folding and maintenance and elimination of abnormally folded proteins shows prominent changes in aging neurons and other brain cells. Proteomic studies of AD brains and mouse

models of the disease showed significant oxidation and inactivation of various proteins and enzymes including UCHL1 [101], which is necessary for the degradation of misfolded or damaged proteins by the proteasome, the ubiquitin E3 ligase CHIP, hsp70, ubiquitin, etc. [102, 103]. In support for a central role of the ubiquitin-proteasome degradation system (UPS) in AD, unbiased iTRAQ mass spectrometry of tissue from AD and age-matched controls showed prominent changes of multiple proteins which interact with ubiquitin in the hippocampus and cortex [104]. Genetic approaches in mouse models of AD support the notion that beta-amyloid and phosphorylated tau impair the UPS and that changes in UPS function directly modulate the accumulation of these proteins in vivo [102, 105, 106].

The kinase mechanistic target of rapamycin (mTOR), which is a key regulator of protein homeostasis and inhibitor of autophagy (see below), is highly expressed together with PI3K and Akt in the developing brain, and tightly regulated in the adult brain during learning and memory processes. Sustained activation of this pathway together with an insulin and IGF-1 resistant state has been observed in AD and linked to abnormal phosphorylation of tau and, ultimately, desensitization to insulin receptor and IGF-1 receptor signaling [107]. Beta-amyloid is a strong candidate for activating this PI3K/Akt/mTOR pathway in a feed-forward loop [107]. Inhibition of mTOR by pharmacological or genetic means reduces neurodegeneration, tauopathy, and amyloidosis in mouse models of AD [108]. Inhibition of mTOR by rapamycin, which increased levels of autophagy, ameliorated cognitive deficits and lowered beta-amyloid brain concentrations in APP transgenic mice [109]; likewise, rapamycin mediated an increase in GSK-beta phosphorylation and autophagy induction was associated with fewer cognitive deficits and reduced tau pathology in tau transgenic mice [108]. While some of these effects of rapamycin target autophagy, other more general metabolic and anti-aging pathways will be activated as well.

Interestingly, human brains display a diversity of granular structures containing cross-linked proteins, lipids, and carbohydrates which accumulate prominently with age and occur alongside the classical beta-amyloid and tau lesions in AD. Some of these structures are extracellular, such as corporea amylacea. Others, including Hirano bodies, Marinesco bodies, granulovacuolar inclusions, stress granules, and lipofuscin are found inside neurons or glial cells; with the exception of lipofuscin granules, these structures remain poorly characterized and their origin and significance to neurodegeneration are still unclear [110]. Of particular interest to neurodegeneration are lipofuscin deposits which were first described in neurons by Hannover in 1842 and are now a well-established aging marker for post-mitotic cells [111]. Lipofuscin forms as undegradable material within lysosomes and can accumulate independent of age, e.g. in lysosomal storage diseases, as a result of abnormal autophagy or impaired lysosomal degradation. As proposed by Brunk and Terman [111], during normal aging, oxidative modifications to macromolecules make them undegradable in lysosomes, leading to the recruitment of large amounts of newly synthesized lysosomal enzymes which, however, still fail to degrade the material. Consequently, there will be an insufficient supply of lysosomal enzymes available for autophagy, leading to accumulation of aged mitochondria and other cellular organelles and material. The levels of many lysosomal proteins and enzymes are

increased in the aging brain and more dramatically in AD and lysosomal dysfunction may have a major role in AD pathogenesis [112]. Along with lysosomal dysfunction, or possibly as a consequence, the AD brain is characterized by extensive endosomal abnormalities and the accumulation of autophagosomes [113]. Genetic manipulation of pathways that regulate autophagosomal pathways in APP or tau transgenic mice showed prominent effects on disease progression and accumulation of these proteins [108, 114, 115]. Stress granules, which contain proteins and RNAs that accumulate in response to stress, are gaining interest due to their possible role in neurodegenerative diseases [116]. Relevant to AD, granulovacuolar degeneration bodies were found to contain markers of stress granules, including pS6 and p54/Rck, and to be highly abundant in neurons from AD compared with control brains. Such neurons did not contain tau aggregates and displayed reduced oxidative damage, suggesting a potentially protective function of these granules [117]. In line with these findings, stress granules containing different types of proteins were associated with tau pathology in AD brains and tau transgenic mice and, based on experiments overexpressing the stress granule protein TIA-1, the authors proposed that some stress granules promote tauopathy [118].

In aggregate, these observations strongly support a major role for protein dyshomeostasis in both normal brain aging and AD. The relative importance of various aspects of protein maintenance and turnover in AD pathogenesis are unclear as they are unclear for aging in general. It is likely, however, that pathways that stabilize protein homeostasis will be beneficial for AD.

6.5 Stem Cell Exhaustion

Stem cells are critical to the regeneration of many tissues and with age, the capacity of these cells to self-renew and produce progeny declines. The adult brain was considered in the past to be a post-mitotic tissue without stem cell activity, and it took several decades from the first reports of adult neurogenesis in rats by Altman and colleagues [119] until it became accepted that several mammalian species, including primates, have the capability to generate new neurons in select brain regions [120]. Following a pioneering study by Gage and colleagues demonstrating the uptake of BrdU into dividing hippocampal neurons in cancer patients treated with this drug [121], Frisen and his team produced the most convincing evidence for neurogenesis in the human brain, thus far, by taking advantage of radioactive ^{14}C isotope released into the biosphere following atomic bomb tests to birth date neurons [122]. Their studies showed that a large portion of the human hippocampus is subject to neuronal turnover, that 700 new cells are added per day, on average, and that neurogenesis declines with age – intriguingly, that decline seems less pronounced than the one observed in rodents [122, 123]. Based on extensive studies of the functional relevance of adult neurogenesis in rodents (see below) it is likely that human neurogenesis contributes to cognitive function and, consequently, it is possible that the age-related decline in neurogenesis results in reduced function [120].

Adult neurogenesis in rodents occurs mainly in two distinct brain regions, the subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus, a subregion of the hippocampus. Adult neural stem cells are a relatively quiescent population that can both self-renew and give rise to more rapidly dividing progenitors which in turn produce neurons (neurogenesis), as well as astrocytes and oligodendrocytes (gliogenesis) [124]. Ultimately, newly born neurons in the SVZ migrate and incorporate into the olfactory bulb where they contribute to olfaction. In a similar fashion, neurons born in the SGZ become granule neurons that integrate into the existing circuitry of the hippocampus where they are involved in learning and memory. Consequently, the profound decline in neurogenesis with age is linked to functional declines in olfaction and spatial learning and memory [124, 125]. The cause for this decline and exhaustion of stem cell activity is unknown.

Theoretically, inhibition of adult neural stem cell activity can occur due to limits in the potential number of cellular divisions, through intra- or extracellular factors that induce cell cycle arrest to maintain a pool of viable quiescent stem cells, or the molecular composition of the neurogenic niche to either enhance or mitigate cellular proliferation [126, 127]. Indeed, recent studies in the aged brain have linked the decline in neural progenitor cell function and olfactory bulb neurogenesis to $p16^{INK4a}$ [128], the polycomb gene Bmi-1 signaling pathway [129], and FoxO3a [130], for example. In addition, telomere control seems to be important in neural stem cells as mice deficient in telomerase show exhaustion of SVZ stem cells [56] and telomerase expression decreases with age [54, 55] (see also Sect. 6.1). Furthermore, adult neurogenesis is controlled by a number of epigenetic mechanisms. For example, the immediate early gene Gadd45b, which is required for neuronal activity-induced DNA demethylation, is necessary for hippocampal neurogenesis and induction of BDNF and FGF [131]. In addition, valproic acid, which blocks histone deacetylases (HDAC), promotes neuronal differentiation [132] and REST controls neurogenesis by keeping stem cells in a quiescent state [133].

Besides these intracellular factors, a number of more global extracellular regulators have resulted in changes of neural progenitor cell proliferation and neurogenesis, including insulin-like growth factor 1 (IGF-1) and vascular endothelial growth factor (VEGF) [134, 135]. Importantly, the neurogenic niche is localized around blood vessels, which allows for the possible communication with the systemic environment [136–139]. Hence, perturbations to the systemic milieu of an organism, such as those induced by exercise or dietary restriction, can increase stem cell functionality and enhance learning and memory in aging mice [140, 141] and exercise in humans has similar beneficial effects [142–144]. Using heterochronic parabiosis, Villeda et al. showed that blood-borne factors present in the systemic milieu can inhibit or promote adult neurogenesis in an age-dependent fashion in mice [136]. Accordingly, exposing an old mouse to a young systemic environment or to plasma from young mice increased neurogenesis, synaptic plasticity, and improved contextual fear conditioning, spatial learning and memory [136, 145]. In support of the existence of pro-neurogenic factors in the young circulation, heterochronic parabiosis increased neurogenesis in the old SVZ, thus enhancing olfaction in mice, and growth and differentiation factor (GDF)-11 was sufficient to mimic at least some of

these benefits [146]. Given the potent effects of growth factors on neurogenesis it is maybe not surprising that mTOR signalling holds a key in controlling proliferation of stem and progenitor cells in the brain [147]. Specifically, the mTOR complex-1 (mTORC1) is expressed in a unique pool of transiently amplifying progenitor cells and signalling via mTOR decreases with age. Surprisingly, inhibition of mTORC1 depletes this progenitor pool, inducing a quiescence-like phenotype which may be similar to the one observed with aging and which was reversible by EGF-induced upregulation of mTOR activity [147]. How the beneficial effects of rapamycin on brain function factor into this observation is currently unclear, but it is interesting to speculate whether factors associated with a young circulatory environment exert similar effects on quiescent cell populations.

Conversely, circulating factors associated with aging or inflammation are known to inhibit neurogenesis and cognitive function and non-steroidal anti-inflammatory drugs were able to prevent this [148]. For example, increased levels of TNFα [149] or IL1-β [150] resulted in reduced levels of adult neurogenesis and adult rats treated with IFN-α showed depression-like symptoms and reduced cell proliferation in the dentate gyrus [151]. Interestingly, patients treated with recombinant IFN-α as part of a therapy for hepatitis C virus infection or cancer frequently develop depression or cognitive deficits linked to the treatment [152, 153] which has been suggested to depend on binding of IFN-α to complement receptor 2 on neural stem cells [154]. In an attempt to identify age-related factors linked to reduced neurogenesis, Villeda and colleagues used a focused proteomic approach in the heterochronic parabiosis model [136]. They demonstrated that CCL11/eotaxin, a chemokine involved in allergic responses and not previously linked to aging, was sufficient to reduce neurogenesis and impair cognition when administered systemically to young mice. Interestingly, CCL11 expression is also increased in both the choroid plexus during aging [155] and in fat deposits with obesity, an important risk factor for cognitive impairment; conversely, CCL11 decreases following exercise in obese patients [156, 157].

The first study that explored neurogenesis in AD brains reported an increase in markers for immature neurons including doublecortin and NSA-NCAM, although no statistical analysis of these results was presented [158] and there is still no consensus as to the degree to which neurogenesis is altered in the disease. In mouse models overexpressing APP alone or in combination with presenilin containing familial AD mutations, neurogenesis was typically reduced when compared with wild type mice [159, 160] although some groups reported increased adult neurogenesis in certain strains [161]. Interestingly, while overexpression of wild type or mutant presenilin-1 reduced the number of neural progenitors in the mouse hippocampus, only mutant protein was sufficient to reduce the survival of newborn neurons [162]. Because APP and presenilin function in neurogenesis during development, discrepancies observed in adult neurogenesis between the various models could be a result of differences in temporal, spatial, or levels of expression of transgenes [124, 125].

In summary, there is overwhelming evidence that neural stem cells have key functions in mammalian learning and memory and that adult neurogenesis takes

place in the human hippocampus. It is also clear that with age stem cell activity declines prominently. The mechanisms behind this exhaustion and the functional consequences to AD and human cognition in general need further investigation.

6.6 Altered Intercellular Communication and Neuroinflammation

Communication between cells of the same type, the same tissue, or across tissues is governed by thousands of secreted proteins, peptides, lipid mediators, and other molecules and must be under tight regulatory control. As the organism develops, matures, and ages, there are dramatic changes in the composition of this network, and with disease, additional adaptations will occur. Proteomic, lipidomic, and metabolomic technologies are trying to characterize these changes and a growing number of studies have applied them to brain aging and AD. Because brain tissue typically cannot be studied at the molecular level in living individuals, it has been particularly challenging to study physiological changes in the human brain or chronic neurodegenerative diseases such as AD, which develop over decades and have no strong genetic component or etiology. As an alternative, and taking advantage of the concept of intercellular communication between tissues, a growing number of studies are correlating changes in blood to normal brain aging or AD, with the hypothesis that such changes mirror, in part, changes in the brain.

Age-related changes in intercellular communication have been studied as a function of changes in secreted proteins including endocrine and neuroendocrine factors [48]. Quantifiable molecular markers of intercellular communication in the blood have indeed greatly advanced the understanding and diagnosis of human disease, and recent studies in blood suggest that aging is similarly associated with changes in intercellular communication factors. Targeted proteomic studies measuring concentrations of dozens to hundreds of intercellular communication factors in plasma from AD patients using antibody-based multiplex assays described protein signatures which may be specific to prodromal stages of the disease [163], or which characterize patients who progress from a prodromal stage to AD [164–166]. Other signatures appear to correlate with APOE genotype [167] or with pathological changes such as Aβ and tau protein levels in CSF or brains of AD patients [168, 169]. While there is some general overlap between these studies (e.g. apoE, clusterin, ICAM1, RANTES, complement) most signatures have not been independently validated and their biological significance or diagnostic value is unclear. More sophisticated and unbiased methods to study the plasma proteome use mass spectrometry, often in combination with initial fractionation or selection steps such as 2D gel electrophoresis, chromatography, or antibodies. One such study found complement factor H, alpha2-macroglobulin and other proteins to be associated with AD [170]. To our knowledge, unbiased plasma proteome approaches have not

been used to study human brain aging but, as described above, Villeda and colleagues used multiplex assays to identify plasma communication factors that correlate with age-related changes in neurogenesis and which are altered in response to heterochronic parabiosis in mice [136]. They discovered proteins which increase with age in mice and humans, including in CSF, and showed that at least one of these factors, CCL11, is sufficient to induce changes in young mice that would normally occur at a much older age. Whether this represents "accelerated" aging of the brain remains to be investigated but the study demonstrated that intercellular communication factors in the circulation are not only correlated with, but also sufficient to modulate brain aging.

Interestingly, other proteins associated with brain aging and reduced neurogenesis in the above study, including the chemokines CCL2, CCL12, CCL19, as well as beta2-microglobulin and haptoglobin [136], are immune regulatory factors and are likely part of the concerted low-grade inflammatory response associated with aging, also known as "inflammaging" [171]. Searching for factors which decrease with aging, increase with heterochronic parabiosis, and benefit the old brain, Katsimpardi et al. reported that GDF-11 increases neurogenesis, improves olfaction, and exerts beneficial effects on the brain vasculature [146]. Other age-related circulatory factors with beneficial effects on the brain include Klotho, a pleiotropic protein which suppresses insulin and wnt signaling and has been shown to extend lifespan in mice. Recent studies show that a lifespan-extending version of the protein, which is expressed at increased levels in the circulation of carriers, is associated with enhanced cognition in humans, and that mice overexpressing klotho showed increased synaptic plasticity and memory function, possibly by increasing levels of the NMDA receptor subunit GluN2B [27]. Furthermore, overexpression of klotho reduced mortality and enhanced cognition in APP mice [172]. It is likely that other age-related circulatory factors with detrimental or beneficial effects on the aging brain will be discovered and it remains to be explored whether they have a role in AD.

Immune and inflammatory processes have long been suspected to have a role in AD [173, 174], and long-term use of NSAIDs, taken for several years before the onset of clinical symptoms, and ideally at younger age, is associated with reduced risk for AD [175–177]. In addition, studies in patients with mild to moderate AD have shown that systemic inflammation and the number of systemic inflammatory events (e.g. urinary tract infection) contribute to the progression and severity of AD [178, 179]. However, the link between inflammation and AD is still unclear and likely involves both systemic and central mechanisms. One idea about how brain inflammation ties in with AD is that astrocytes and microglia become senescent and assume a so-called senescence associated secretory phenotype (SASP; [180]). Indeed, some aging astrocytes appear to express the hallmarks of the SASP including increased expression of intermediate filament proteins, cytokines, and intracellular protein aggregates [181]. As discussed above, this aged phenotype could be the result of epigenetic changes or replicative senescence. In support of epigenetic factors, Gjoneska et al. find a coordinated increase in the expression of immune response genes targeted by the transcription factor PU.1, which is restricted to

microglia in the brain [75]. Alternatively, these phagocytic cells may become activated and "inflamed" as a result of protein dyshomeostasis. For a detailed discussion of microglia in brain aging and AD see [63]. Additional support for a role of immune-based mechanisms in brain aging and AD comes from recent genome wide expression studies in multiple brain regions of >1,600 brains. One study showed that activation of inflammatory genes is a hallmark of normal brain aging that likely precedes the development of AD [182]. A gene network analysis has also suggested that TYROBP/DAP12, an adaptor protein expressed in microglia, is deregulated in AD patients compared to healthy controls [183]. DAP12 binds to various receptors including complement receptor 3, one of the major phagocytic receptors expressed on microglia, as well as TREM2 [184]. In fact, the most direct evidence that altered immune function and inflammation have a role in AD comes from genetic studies which show that rare polymorphisms in the microglial gene TREM2 increase AD risk by several fold [185, 186]. Genome wide association studies (GWAS) in AD also identified a number of single nucleotide polymorphisms in immune related genes, which modify the risk of developing the disease [187–189]. In the brain, most of these receptors are exclusively expressed on microglia.

In conclusion, there is very strong genetic, transcriptomic, and proteomic evidence that altered intercellular communication and inflammation are major components of both normal brain aging and AD. It is, however, not necessarily clear whether upregulation of immune and inflammatory pathways is a driver of aging and disease or, at least in part, a reparative or regenerative process [174]. Likewise, the interplay between central and systemic inflammation is complex as age-related changes in the hypothalamus, for example, may orchestrate organismal aging [190].

7 Conclusions and Future Prospects

In this brief and somewhat selective review of brain aging and AD we conclude that all aspects of organismal aging are observed in the brain and the boundaries between aging and disease are largely fluent. As observed in other tissues, defined hallmarks of aging are strongly inter-dependent and it remains elusive whether such hallmarks are a cause or consequence of the aging process. Clearly, they are not the result of AD unless the disease starts at birth, as has been postulated by some [17], in which case aging itself would need to be called a disease. Rather, we believe there is a continuum from brain aging to AD, which is susceptible to genetic, epigenetic, and environmental influences. An overwhelming number of individuals above age 80 show signs of AD pathology and cognitive decline; still, there are extremely rare, exceptional centenarians without pathology [191] indicating that AD is not entirely inevitable – but almost. Studying these rare subjects should be a high priority.

Genetic mouse models of AD recapitulate pathological abnormalities, synaptic loss, and cognitive deficits often during young adulthood while the same manifestations in humans are not observed until much later in life. If these models are to be relevant for the understanding of disease progression, particularly sporadic AD, the

role of aging needs to be considered and aggressive models which develop disease before midlife are likely not very informative.

Given the profound role immune responses and inflammation seem to play in both brain aging and AD, it will be critical to determine which aspects of immunity are maladaptive and which ones are attempts to maintain or repair damage. Indeed, the oldest old may show gains in brain immunity [192] and rejuvenation of immunity has been proposed as an approach to treat brain aging [193]. In fact, a number of colony stimulating factors, which are mitogens for immune cells, show benefit in models of AD and some are being evaluated in patients [194].

Remarkable effects of heterochronic parabiosis in mice on multiple tissues including the brain [136, 145, 146] suggest that the brain is malleable and that a dysregulation of intercellular communication may underlie, in part, brain aging and cognitive decline. Understanding the molecular basis of these rejuvenation approaches will likely provide new insight into brain aging and plasticity.

In this vein, and maybe most importantly, neuroscientists have focused for too long on neurons alone and ignored glial cells and vascular cells, let alone systemic factors as important regulators of brain function and dysfunction. It is now evident that with age, every cell type shows signs of deterioration and dependent on a person's genetic and environmental exposure, the sequence and severity of cellular dysfunction may produce the particular manifestations of aging and neurodegeneration. By altering basic processes involved in aging, it might be possible to counteract the cellular dysfunction that leads to neurodegeneration, including AD.

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Parkinson's Disease and Aging

Julie K. Andersen and Shankar Chinta

Contents

1 Clinical Symptoms

Parkinson's disease (PD) is the most common movement disease of older adults, with an average onset of around 60 years of age [1, 2]. Life expectancy is reduced amongst patients suffering from PD, with a mortality ratio twice that of unaffected

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individuals [3]. Mortality risk factors associated with the disease include cognitive decline and age at time of onset [4]. The disease results in progressive deficits in motor movement due to the preferential loss of dopaminergic (DAergic) neurons in an area of the midbrain known as the substantia nigra pars compacta (SNpc) [5, 6]. Cardinal symptoms include resting tremor, delayed initiation and slowness of movement (bradykinesia), muscular rigidity, and impaired balance [7]. Onset of motor symptoms is often asymmetric, with the most common initial manifestation being a mild resting tremor in one arm [8]. As the disease progresses, the patient may note gait difficulties, micrographia (shrinkage in size of handwriting), and issues with axial posture [9]. Other changes related to motor function include decreased facial expression and laryngeal dysfunction resulting in difficulties with speech [10].

The involvement of several neurotransmitter systems beyond DAergic that may play a significant role in the disease has recently been recognized [11]. These include the serotonergic, cholinergic, and noradrenergic networks, effects on which may explain some of the non-motor symptoms associated with the disease [12–15]. These can occur prior to the onset of motor symptoms and are currently being tested clinically as early disease indicators, including hyposmia (reduction in olfactory function) and disturbances in REM sleep [16–18]. Other common non-motor symptoms accompanying the disease include constipation due to reduced intestinal motility, fatigue, pain, depression, anxiety, and cognitive issues [11, 19]. Patients with PD indeed have a two to six-fold increased risk for development of dementia than control populations [20]. Cause of death include pulmonary infections due to aspiration of saliva into the lungs, accidents associated with increased risks of falls due to loss in motor function, chocking due to difficulty swallowing, and blockage of blood vessels leading to pulmonary embolism and deep vein thrombosis. Individuals do not die of PD, but rather of the life-threatening complications associated with the condition [21, 22].

Progression of symptoms is also associated with the presence of abnormal intraneuronal proteinacious accumulations called Lewy bodies that occur in both neuronal cell bodies and in neurites [6, 23]. A major component of these inclusions is a protein known as α-synuclein. PD is considered part of a larger group of disorders known as synucleopathies that include other related diseases such as Dementia with Lewy Bodies (DLB) [24]. Extent and expression of the disease at defined sites within the nervous system has been proposed to track closely with the presence of Lewy bodies at these various locations and may in part explain patient-to-patient variability in symptoms [19]. According to the Braak staging scale, a means of classifying neuropathology associated with PD based on observations at autopsy, Lewy bodies first appear in enteric neurons of the gut then the olfactory bulb and lower brain stem, prior to individuals displaying any motor symptoms [25–27]. As the disease evolves, Lewy bodies spread to the SNpc and then to the neocortex. This has led to the prion hypothesis of PD that postulates that α-synuclein spreads transcellularly, temporally inducing progressive pathological aggregation within affected neurons in a pattern that tracks with resulting symptomology [28]. If correct, this could have major implications in terms of the success of proposed therapies for the disorder, including use of cellular transplantation (see below). However there are clearly caveats to the data supporting this theory that are under intense investigation [29].

2 Current Therapies

Currently, there is no cure for PD. Existing therapies are instead primarily directed towards alleviating the most disturbing symptoms in individuals with the disease, which can vary widely in terms of both presence of particular symptoms and rate of progression [7]. More than a third of individuals with PD, for example, do not display a tremor, therefore presence of tremor in an older individual alone is not necessarily a sign of PD per se. For this reason, there is no standard treatment for the disorder and treatments must be tailored to meet the needs of each individual patient.

Approaches to lessen PD symptoms include both medication and surgical therapies [10]. The most widely used therapy is Levo-Dopa (L-Dopa) [30]. L-dopa is a precursor of dopamine and acts to increase synthesis of this neurotransmitter in remaining DAergic neurons of the SNpc. It is particularly effective in reducing bradykinesia and rigidity in early stages of the disease. It is often given in conjunction with carbidopa, a peripheral dopa decarboxylase inhibitor that acts to lessen side effects due to conversion of L-dopa to dopamine outside of the brain, which can lead to nausea and vomiting [31]. Due to the rapid turnover of dopamine (DA), resulting in oscillations in its levels and difficulties in adjusting dosage to obtain ideal brain concentrations, patients often develop symptoms related to the drug itself, including dyskinesias (abnormal involuntary movements) and fluctuations in treatment efficacy causing transient freezing [32]. These side effects are controlled in many cases by addition of other drugs to the patient's medication regime [32]. While L-dopa can control PD symptoms, it does not however halt the progression of the underlying disease. As neurons continue to be lost, L-dopa efficacy becomes diminished due to loss of striatal DAergic terminals necessary for neurotransmitter storage and buffering [33]. An alternative or complement to treatment with L-dopa is the use of dopamine agonists, which act as a sort of pseudo-DA to activate DAergic receptors in the brain [34, 35]. These include Requip, Mirapex, and Neupro that are taken either alone or in combination with Sinemet (L-dopa/carbidopa) [36]. Due to better tolerance and reduced long-term risk of complications, these are now often the first choice of treatment for PD. They however have their own side effects, including increased risk for drug-associated psychosis [37]. Other alternatives include the use of anticholinergics (Artane, Cogentin) which balance levels of acetycholine in the brain and Eldepryl and Azilect (selegeline, rasagaline) which prevent breakdown of dopamine via inhibiting the enzyme monoamine oxidase B (MAO-B) [38–40]. A newly described MAO-B inhibitor, safinamide, is currently undergoing clinical trial [31, 41]. Another strategy to prolong DA response is extracellular inhibition of its degradation by the enzyme catechol-o-methyl-transferase (COMT) via administration of entacapone or related agents. Given that PD can occur in conjunction with several other age-related comorbidities, it is particularly important for patients to check with their physicians about potential negative interactions between drugs used to treat PD and other age-related conditions. Sometimes there are additionally dietary considerations due to interactions of medications with specific foods [38]. The American Academy of Neurology has recently released a

series of recommendations for the treatment of non-motor symptoms including erectile dysfunction, constipation, night-time sleep disturbances due to uncontrolled limb movement, daytime somnolence, and fatigue, all of which may cause considerable pain and discomfort and affect quality of daily life (American Academy of Neurology, 2010).

At the point in time at which medicinal therapies lose their effectiveness, surgical interventions such as deep brain stimulation (DBS) may be tried [42, 43]. DBS involves electrical stimulation of the thalamus and subthalamic regions, aiding in the attenuation of tremors and freezing by blocking certain electrical signals within the brain contributing to these symptoms [9]. It is important to emphasize that although all of these therapies can act to reduce motor symptoms and therefore increase the longevity of PD patients, they do not result in a halting of the underlying neurodegeneration associated with the disease.

Other recommended evidence-based treatment approaches include general lifestyle modifications such as rest and exercise for improvement of motor function and reducing stress, and speech therapy to aid with losses in communication abilities [44–46]. Use of alternative medicinal therapies including herbs, vitamins, and supplements have in the majority of cases not been thoroughly studied scientifically in terms of either efficacy or safety nor vetted by the FDA for use in the disease. In the few cases in which such agents have undergone more rigorous study (vitamin E, coenzyme Q), their beneficial effects have not held out under clinical trial conditions, although they may be of benefit to certain patients [31, 47]. Creatine is currently under a phase III clinical trial for efficacy [48]. A meta-analysis of vitamin E intake in the food suggests a relationship between high intake and reduced risk for development of PD [49, 50]; however, vitamin E did not show efficacy in a previous clinical trial (DATATOP). Other over-the-counter nutriceuticals (low-dose lithium, vitamin D, nicotine, etc.) have shown efficacy either in pre-clinical or in epidemiological studies but have yet to be thoroughly assessed in human clinical trials, although some trials are being initiated or are ongoing [51]. Other clinical trials involve repositioning drugs already approved for treatment of other diseases for use in PD, including pioglitazone (BioPEP), normally prescribed for type II diabetes, and the calcium channel blocker isradipine (STEADY PD III), used to treat high blood pressure. Results from recent studies suggesting that statins may lower the risk for PD have also peaked interest in initiating longer-term studies to verify these effects [52].

Over the last decade, there has been a large amount of research put into novel restorative therapies including gene therapy and cellular replacement via transplantation. Based on pre-clinical data, for example, clinical trials were performed infusing the neurotrophic factor glial-derived neurotrophic factor (GDNF) directly into the brains of PD patients as a means of protecting against additional DAergic cell loss [53]. However, results from various clinical trials proved to be contradictory, perhaps due to differences in trial design. Other confounding factors include heterogeneity of the patient population and the difficulties inherent in teasing out symptomatic versus drug-mediated effects. Recruitment for phase I clinical trials is currently underway to test the safety and tolerability of adeno associated virus

(AAV)-mediated delivery of GDNF [54]. A previous AAV phase II trial delivering the GDNF analogue neurturin (NTN) showed some negative side effects and a lack of overall improvement in symptoms [55, 56]. A subsequent phase II trial utilizing higher viral dosages and longer follow-up times is currently underway [57]. Some success has been met in the use of gene therapy involving the AAV-mediated delivery of glutamic acid decarboxylase (GAD), an enzyme that acts to modulate GABA production in the subthalamic nucleus. This was reported to result in improved motor function in PD patients following a 6-month observational period, with minimal side effects (64, 65).

As with gene therapy, several clinical trials using cellular transplantation have been undertaken, as early as the 1980's. The goal has been to replace lost DAergic neurons, utilizing DAergic fetal cells [58, 59]. Study results have been variable, but more detailed placebo-controlled double-blind trials (versus open-labeled) generally report lack of significant overall improvement in motor function and the induction of drug-related side effects such as dyskinesia. Recent excitement has been engendered by the ability to produce inducible pluripotent stem cell (iPSC) populations from a patient's own tissues which can be differentiated towards a DAergic fate for transplant, particularly in combination with gene therapy to promote their survival in vivo [60–62]. However, excitement has been somewhat tempered based on evidence suggesting that transplanted cells have limited success in clinical trials and may actually take on the same fate as affected endogenous neurons [28]. More studies are undoubtedly required before moving such studies towards new clinical trials, including how to deal with reduced transplant efficiency in the older brain. In other words, not only do we need to consider the cells themselves, but also the environment into which they are placed.

3 Known Molecular or Cellular Underpinnings

Although familial forms of the disease account for roughly 5–10 % of PD, it is largely considered a sporadic condition arising from a combination of genetic susceptibility and environmental exposures across the lifespan [63]. These vary from individual to individual, which may to some degree help explain disparate disease presentation. This has led to the notion that PD may constitute not a single disease entity, but rather a syndrome.

Aging is the number one risk factor for the disorder and factors common to both normal brain aging and selective cell death in PD merit special consideration in terms of overlapping factors that may be causative in both conditions [64–66]. Is PD a case of accelerated aging in a specific population of cells particularly sensitive to certain genetically induced phenotypes and/or environmental exposures? Advanced age is certainly directly linked to a more rapid disease progression and older individuals are more refractory to medical treatments for the disorder, suggesting that there is an important interplay between the two [67].

Through studies of both the post-mortem PD brain and genetic pathways responsible for familial forms of the disease, much has been gleaned about the cause of cellular dysfunction in PD. Some of the pathways implicated so far include elements identified as also important for basic aging including mitochondrial dysfunction, oxidative stress, calcium mishandling, inflammation, and effects on proteostasis [68, 69]. Identification of these molecular targets has led to exploration of interventions designed to prevent or reverse their detrimental effects as a means of slowing or reversing the course of the disease.

3.1 Mitochondrial Dysfunction

Neurons are particularly dependent on mitochondrial oxidative phosphorylation as a source of ATP, with a very limited capacity for glycolysis [70, 71]. Since the discovery of defects in mitochondrial complex I activity in postmortem brain samples and increased levels of mtDNA mutations in patients with the disease, mitochondrial dysfunction has become a major focus of research [69, 72]. As a consequence, models based on both mitochondrial neurotoxins (MPTP, paraquat, rotenone) and gene defects that impact on mitochondrial function identified in rarer familial forms of the disease (α-synuclein, parkin, PINK, LRRK2) have been established [70]. These have been helpful in the segmental dissection of different aspects of disease pathology, including the role of mitochondrial defects in neuropathological features associated with the disease [73].

The master transcription factor peroxisome proliferator-activated receptor γ (PPARγ) coactivatory-1α (PGC-1α) is an important regulator of mitochondrial biogenesis and function. Its dysregulation has recently been identified as a key factor in PD (138). Activators of PGC-1α including resveratrol (acting via SIRT1) and diabetes drugs such as pioglitazone (currently in clinical trial for early PD, BioPEP) have been shown to be neuroprotective in animal models of the disease (139). However, use of agents that act to increase mitochondrial biogenesis will need to be balanced with those which increase lysosomal turnover of defective mitochondria so as to not increase the build-up of the latter. Interestingly, recent data in a Huntington mouse model has demonstrated that the neuroprotective effects of increased PGC-1α result in up-regulation of the master transcriptional regulator of lysosomal biogenesis, transcription factor EB (TFEB), suggesting the existence of a tight regulation between mitochondrial biogenesis and turnover [74]. Enhancement of fission-fusion events in early stages of the disease may also be effective in repairing damaged mitochondria. However, as levels of damaged mitochondria increase, these processes lose their effectiveness and are replaced by removal of dysfunctional mitochondrial via lysosomal degradation. Genes associated with familial forms of PD including parkin, PINK, and LRRK2 have suggested that defects in mitochondrial turnover or mitophagy contribute to neuropathology and interventions that up-regulate these processes are likely to improve neuronal function [69]. TFEB has itself been recently been identified as a potential target for intervention in PD,

although primarily as a means of removing defective protein aggregates (see below) [75]. Ability to remove both defective proteins and organelles suggests that TFEB induction could have dual neuroprotective benefits [76].

3.2 Oxidative Stress

Mitochondrial dysfunction itself can result in production of ROS that in turn can cause further damage to the mitochondria, resulting in a destructive feed forward cycle, ultimately resulting in neuronal cell death. Oxidative stress is another feature of normal brain aging that has been widely implicated in select cell death associated with PD (86–87). Whether or not age-related oxidative stress actually contributes to brain aging is controversial; however, DAergic neurons are particularly sensitive to the effects of oxidative stress due to several factors including their high number of synapses, their undermyelinated axons requiring large numbers of mitochondria to support their activity and the ability for dopamine to be oxidized to toxic by-products that can impact on mitochondrial function [77, 78]. This can be exacerbated by the relatively low levels of antioxidants such as glutathione within these cells, high levels of the ROS-producing enzyme monoamine oxidase B (MAO-B) within neighboring astrocytes, and regionally high levels of reactive iron [79]. Due to its proximity to mitochondrial ROS-production, lack of protective histones, and its high replication rate, mitochondrial DNA (mtDNA) is particularly prone to the deleterious effects of oxidative modification [80]. Mitochondrial damage is another feature shared by the normal aging brain and DAergic cells within the PD SNpc [81, 82].

As mentioned previously, reductions in oxidative stress via vitamin E did not show efficacy in earlier clinical trials (DATATOP), although whether this was due to inappropriate dosing regimes or lack of targeting to the most vulnerable cellular locations is unclear. A recent clinical trial using mitochondrially targeted CoQ10 (MitoQ) also failed to demonstrate slowing of clinical progression of the disease [83].

3.3 Calcium Mishandling

A loss in cellular calcium homeostasis is another potential contributor to the pathogenesis of PD that is also altered in the normal aging brain [84]. Losses in mitochondrial function can affect the ability of the organelle to sequester calcium and this in turn can result in the generation of mitochondrial-mediated oxidative stress and subsequent damage to the organelle that can further affect its function [85].

A growing body of evidence suggests that with age, nigral DAergic neurons become more reliant on L-type calcium channels to maintain pace-making activity and that agents that block channel activity such as isradipine (currently under

clinical trial, STEADY PD III) may slow or halt PD progression [79, 86]. A recent study reported that DAergic SNpc neurons in slices isolated from older wild type mice (25–30 months) show significantly lower spontaneous firing rates, shorter spike widths, and lower pacemaking ability, as compared to younger animals (2–7 months) [87]. This effect is due to the impact of L-type Cav1.2α and Cav1.3α channels on pacemaker firing. This was speculated to be due to changes in calcium stores resulting in effects on calcium-activated potassium channels, suggesting that age-related alterations in the function of these channels may be an important underlying cause of PD [86, 87].

3.4 Inflammation

Immune cell activation likely evolved as a means of removing foreign pathogens or damaged cells, the latter as a consequence of acute cellular stress or injury. However, in the face of chronic age-related neurodegeneration, inflammatory responses may actually contribute to neuronal loss via the ongoing release of damaging inflammatory agents, including reactive oxygen and nitrogen species (ROS/RNS), inflammatory cytokines and chemokines, and matrix metalloproteinases (MMPs) [88, 89]. Several lines of evidence suggest that inflammatory processes within the central nervous system (CNS) may contribute to neuronal cell loss associated with PD [89, 90]. The SNpc itself contains a particularly high density of immune cells such as microglia, even in the normal young brain. Post-mortem studies in the PD SNpc demonstrate the presence of markers of both innate and adaptive immunity. Activated microglia, astrocytes and $CD4^+$ and $CD8^+$ T lymphocytes have been detected in affected brain regions, along with increased expression of pro-inflammatory mediators [91–94]. In addition, preclinical studies in various animal models strongly suggest the involvement of inflammatory processes in associated neuronal cell death [95]. Neuroinflammatory processes may contribute to deleterious events leading to neuronal degeneration. Results from epidemiological studies in humans have suggested that use of anti-inflammatory drugs reduces the risk for development of PD, although reports have been somewhat mixed [96–99]. Possible factors involved in neuroprotection may include inhibition of cyclooxygenase 2 and reduced production of prostaglandins [100, 101]. Interestingly, ibuprofen, which shows the strongest effects in a prospective meta-analysis study, is a known inducer of PPARγ [102]. A genome-wide association study (GWAS) has confirmed the involvement of various immune-related factors [103]. Neuroinflammatory processes represent an attractive therapeutic target for slowing progression of the disorder.

During aging, astrocyte numbers increase and a greater proportion become activated (astrogliosis) [104, 105]. This is correlated with an increase in levels of glial fibrillary activated protein (GFAP). While microglial cell numbers do not appear to be significantly increased in normal aging as they are in PD, the cells that are present

are more likely to be in an activated state, known as 'microglial priming', an enhancement of the innate immune response [106].

It is important to note that in addition to involvement in neuroinflammatory events, astrocytes also normally interact with endothelial cells to help maintain integrity of the blood–brain-barrier (BBB), remove ions and glutamate from the extracellular space following bursts of neuronal activity, and respond to the metabolic needs of neurons including via the release of supportive growth factors [107]. These physiological functions decrease with brain aging and may contribute to neuronal losses associated with PD.

3.5 Defects in Proteostasis

A common feature of neurodegenerative diseases including PD is accumulation of proteinaceous deposits in the CNS or peripheral nervous system [28]. The location of these deposits appears to be disease-specific. Unwanted proteins are cleared from the cell by the ubiquitin proteasome system (UPS) or lysosomal-mediated autophagy [108]. Defects in both of these systems have been associated with both aging and PD, and attempts to correct them by various means have been extensively studied [109, 110]. The PD-related protein α-synuclein, associated with both familial and sporadic PD, comprises roughly 1 % of proteins found within Lewy bodies and its toxicity is believed to be caused by accumulation of misfolded proteins and formation of toxic oligomers [111]. Inclusions containing α-synuclein aggregates have been found in transplanted fetal DAergic cells in PD patients. As cells were derived from unrelated donors, it is unlikely that they are the source of the pathology and this is cited as evidence, along with recent work in mouse models, backing the 'prion transmission theory' of PD progression [28, 112]. This is another potential target for therapy, but although prion-like disease spread is known to require endosomal release and uptake, much more about the molecular mechanisms involved in this process still needs to be elucidated. A recent publication suggested that one of the factors involved in removal of intracellular α-synuclein via secretion into the extracellular space is the protein ATP13A2 or PARK9 whose mutation is involved in a juvenile onset form of parkinsonism called Kufor-Rakeb syndrome (KRS) [113].

One interventional target that has been extensively explored experimentally in PD is the heat shock proteins (HSPs) [114]. This family of proteins is a group of molecular chaperones that promote either proper refolding of proteins or their transfer to the UPS and lysosome for degradation. Drugs such as the hsp70 inducer geldanamycin have shown efficacy in several animal models of PD, but unfortunately have side effects including liver damage [115]. Drugs that enhance clearance of defective proteins have also shown efficacy in animal PD models, including rapamycin that enhances lysosomal protein turnover [116]. Interventions that bind and prevent toxic oligomerization of α-synuclein including polyphenols like curcumin, immunotherapy or siRNA therapy have also been demonstrated to have some

benefits in animal models and a clinical trial of the latter (AFFiRiS) has recently been initiated [117, 118]. There is however some concern that this may prevent potentially neuroprotective aggregation of the protein, and this may actually exacerbate the condition. It is also conceivable that post-translational modifications that drive aggregate formation may evade antibody binding and may increase neuroinflammation (see above).

4 What Have We Learned and Where Do We Go from Here?

Development of PD therapeutics that halt or slow disease progression and prevent disability is the highest priority for researchers in the field. Unfortunately, attempts to intervene have to date provided little to no benefit in human clinical trials [51]. There may be several contributing factors involved, including heterogeneity of the patient populations examined, interventions attempted too late in disease stage for effect, short durations of treatments, and small group sizes. It may also reflect lack of thoroughly vetted pre-clinical studies in order to better understand dose responses, treatment pharmacokinetics, and appropriate therapeutic windows. Identification of a disease-modifying neuroprotective intervention has remained elusive to date, likely due in part to these factors. It may however also imply that exploration of novel targets far afield from those conventionally studied is needed.

5 Possible Role of Major Hallmarks of Aging

A traditional approach in age-related disease research has been to investigate single disease conditions in isolation. While this has yielded important information and enormous efforts have been expended to develop therapies based on these findings, there have been few successful interventions as a consequence. This is likely due to an incomplete picture of the nature of complex chronic disease states. Preventive and therapeutic treatments for diseases like PD will likely require the discovery of unique targets far removed from those identified to date by conventional approaches.

Recently, it has begun to be appreciated that in order to fully understand these disorders, researchers need to better understand the underlying role of aging in conditions as disparate as atherosclerosis, diabetes, cancer, osteoporosis, and neurodegenerative diseases. Studying the role of aging mechanisms across a wide variety of disease states will allow scientists to broaden the scope of research beyond traditional disciplines, towards the central concept that these multiple human disease states likely arise from a common underlying cause: aging itself. The term “geroscience” was coined by scientists at the Buck Institute for Research on Aging in 2007 as an acknowledgement and organizing principle of this scientific concept. In 2011, the Geroscience Interest Group (GSIG) was formed, a trans-NIH team interested in this concept, and geroscience as a scientific discipline was formally recognized in

the U.S. Senate Appropriations in 2013. The NIH, with support from the Alliance for Aging Research and the Gerontological Society of America, hosted a conference entitled "Advances in Geroscience: Impacts on Healthspan and Chronic Disease" in October of 2013 to examine the extent to which the physiological effects of aging represent a common major risk factor for chronic diseases. This led to a leading-edge commentary in the journal *Cell* entitled 'Geroscience: Linking Aging to Chronic Disease' [119].

PD itself falls under this rubric and potentially could be viewed as a case of accelerated aging in a particular population of cells in response to individual variation in genetic makeup and environmental exposures. Interestingly, the SNpc displays more age-related neuronal cell loss than any other region of the brain [65]. How does aging then contribute to the preferential death of these cells?

5.1 Age-Related Loss in Adaptation to Stress and Increased Macromolecular Damage

Aging leads to a decrease in the efficiency of many aspects of organismal stress-resistance [120]. This includes reductions in the ability to maintain proteostasis and function of organelles such as the mitochondria and endoplasmic reticulum (ER) due to losses in both the Unfolded Protein Stress (UPS) and autophagic functions. Cellular defenses against the effects of oxidative stress are also reduced. The decreased ability of aged organisms to deal with stressors can lead to damage to both proteins and organelles that, due to their unique cellular features, can have particularly devastating effects on DAergic SNpc neurons [121]. Many of the disease-related effects that occur within the PD SNpc also occur during normal aging, albeit to a lesser degree [87]. These include: (1) effects on mitochondrial function including reduced enzymatic activity of both mitochondrial complex I and α-ketoglutarate dehydrogenase (KDGH), reduced NAD^+ levels, and increased mtDNA deletions [122–125]; (2) reduced ability to deal with effects of increased cellular ROS including reductions in cellular glutathione content and reduced activity of the major anti-oxidant regulator nuclear factor erythroid-derived 2 (NRF2), coupled with increased levels of redox-available iron in this brain region [126–128]; and (3) increased levels of protein accumulation, including elevated levels of α-synuclein [129]. In the normal aging SNpc, α-synuclein appears to be primarily in a more soluble form rather than associated with Lewy bodies as in the PD SNpc, although in both aging and disease this appears to be associated with a decline in neuronal function [130]. This may be due to the presence of higher levels of ROS and RNS in the context of PD. Accumulation of α-synuclein has been linked to alterations in mitochondrial fission-fusion and function [131]. Taken together, these data suggests that PD may constitute acceleration of the aging process in the SNpc in susceptible individuals. Reductions in the deleterious effects of these age-related changes may therefore greatly reduce the risk of developing PD.

Conversion of α-ketoglutarate and NAD^+ to succinyl coA and NADH by KDGH is the rate-limiting step in the conversion of carbohydrates to energy as part of the mitochondrial TCA cycle. NADH and succinate feed into electron transport chain (ETC) complexes I and II, respectively, to support mitochondrial respiration. KDGH activity in the brain is reduced in both aging and in PD, likely as a consequence of increases in mitochondrial ROS and lipid peroxidation to which the enzyme is particularly susceptible [132, 133]. This is reversible by raising levels of thiols, particularly glutathione, which is normally found at millimolar levels in the cell and is reduced during normal aging and in PD [134]. Lipoic acid, via its ability to activate the NRF2 pathway, can induce phase II genes including those involved in glutathione synthesis and metabolism [135]. NRF2 is also involved in a pathway resulting in induction of the mitochondrial transcription factor A (TFAM) that controls mtDNA transcription, translation, and repair. Accumulation of mtDNA damage has been reported to be particularly high in the aged SNpc and conditionally knocking out TFAM selectively within DAergic neurons results in PD-like neuronal cell loss and formation of protein inclusions, suggesting age-related increases in mtDNA mutation load may be a contributing factor to the disease [136]. Lipoic acid has been reported to have restorative effects on mitochondrial complex I activity in both aging and PD models [66].

NRF2 is induced by PGC1α and its co-activators, coordinating in turn expression of several nuclear-encoded mitochondrial proteins including TFAM. Losses in PGC1α have been implicated in age-related reduction in muscular strength as well as cardiac and cognitive function, due to an increase in metabolic abnormalities and subsequent reduction in cellular, tissue, and organ function [137]. PGC1α has also recently been identified as a central therapeutic target for the treatment of PD [138].

In addition to PGC1α, mitochondrial biogenesis is also controlled by the NAD^+ dependent protein deacetylase sirtuin 1 (SIRT1). Sirtuins are a family of conserved enzymes whose modulation has been demonstrated to alter the course of aging in various model systems. Alterations in the activity of these enzymes have also recently been shown to have effects in neurodegenerative diseases, including PD [139]. Activation of SIRT1 by resveratrol, for example, has been shown to be neuroprotective in mouse PD models [140]. SIRT1 inactivation has been reported to result in increased activity of the heat shock factor I (HSF1) that drives transcription of a group of molecular chaperones that regulate protein homeostasis [141]. Recently, inactivation of SIRT1 via its nitrosylation, which prevents its ability to bind zinc, has been demonstrated to result in enhanced inflammation including in a mouse model of PD, contributing to neurodegenerative effects. This was discovered to be due to activation of p53 and subsequent induction of NFkappaB [142]. The authors point to inhibition of SIRT1 by nitrosylation as a possible major target for other major age-related diseases that involve inflammatory processes, including diabetes, atherosclerosis, and Alzheimer's disease (see below). In contrast to SIRT1, SIRT2 inhibition has been reported to be protective in the MPTP model of PD, by preventing deacetylation of Foxo3a and subsequent activation of apoptosis via the factor BIM [143]. Its inhibition may also prevent formation of α-synuclein aggregates.

Aging is correlated with loss in function of various molecular chaperones normally involved in repair of conformational alterations in cellular proteins in response to stress events. This is particularly relevant in post-mitotic cells such as neurons, and chaperone deficiency has been implicated in several neurodegenerative diseases, including PD [144–146]. Loss of the ability to respond to stress can result in accumulation of age- and disease-related misfolded proteins, protein aggregation, and disruption of cellular function. The capacity to induce heat shock proteins including HSP70 and HSP90 is compromised in both the aging brain and in PD and further exacerbated in the face of age-related increases in oxidative damage. Interventions towards replacing levels of chaperone function have been proposed as a potential therapeutic for brain aging.

5.2 Systemic Inflammaging: 'Damage at a Distance'

In addition to local inflammation within the brain itself, systemic inflammation has also been suggested to play a role in PD [147]. The brain is normally protected from the effects of systemic inflammation due to the presence of the blood–brain barrier (BBB). Age-related damage, however, contributes to BBB leakiness, allowing the influx of systemic pro-inflammatory factors into the brain and resulting in the activation of microglia and astrocytes that can exacerbate ongoing neurodegeneration [148]. Recent studies suggest that activation of the peripheral immune system can elicit a pro-inflammatory response in the brain of aged subjects that does not occur in younger cohorts. For example, peripheral lipopolysaccharide (LPS) challenge in older mice results in enhanced microglial secretion of the pro-inflammatory cytokines IL-1β and IL-1 [149]. It has been suggested that this may be due to age-related microglial priming resulting in enhanced activation following entrance of immune signals from the periphery, releasing elevated levels of pro-inflammatory cytokines. Infectious diseases are associated with increased risk for development of PD, particularly in the elderly [150].

In addition to systemic pro-inflammatory factors, damage to the BBB can also allow the entrance of peripheral immune cells into the brain, including neutrophils and macrophages [151–153]. Secretion of chemokines by activated microglia can attract neutrophils and monocytes from the bloodstream. In contrast, up-regulation of anti-inflammatory factors in the periphery may act to reduce glial cell activation in the brain and therefore neuropathology. In addition to entrance via a leaky BBB, inflammatory agents may also enter the CNS via the autonomic nervous system, particularly the vagal nerve afferents which lies close to the liver and lymphatic nodes [154, 155]. Conversely, damage within the brain may trigger inflammatory effects in the periphery. For example, brain injury has been reported to result in increases in pro-inflammatory cells in the liver, resulting in neutrophil translocation in the brain [156].

5.3 Cellular Senescence

Cellular senescence is a potent anti-cancer mechanism that occurs in most, if not all, dividing cell types [157]. The senescence response arrests cell proliferation, stably and essentially irreversibly, in response to stresses that puts cells at risk for malignant transformation. Senescent cells can secrete numerous pro-inflammatory cytokines, chemokines, growth factors and proteases, a feature termed the senescence-associated secretory phenotype (SASP) [158–162]. Many SASP factors have been shown, or are suspected, to cause or contribute to the loss of tissue structure and function that occurs with age, including those associated with neurodegenerative disease. A seminal publication showed that elimination of senescent cells that accumulate in a progeroid mouse model prevents the onset of three major aging phenotypes (cataracts, sarcopenia and loss of subcutaneous fat), providing the first evidence that senescent cells play a causal role in at least some age-related pathologies in vivo [163]. While cell senescence has been causally linked to age-related pathologies in peripheral tissues, its potential role in brain aging and neurodegenerative disease has just begun to be explored. Recent findings suggest that cultured glial cells are capable of undergoing senescence and developing a SASP. In response to exogenous H_2O_2, for example, cultured astrocytes displayed numerous senescent characteristics: arrested growth, an enlarged morphology, senescence-associated β-galactosidase (SA-βgal) activity, and increased expression of p21 and $p16^{INK4a}$ [164]. In vivo and in conjunction with age-related cognitive impairment, changes in the aging brain include increased expression of astrocytic GFAP in cells with a senescent-like morphology. Telomere shortening in rat microglia both in culture following repeated cell divisions and with advancing age in vivo has been reported to lead to cellular senescence that may impact cellular function [165, 166]. In response to repeated lipopolysaccharide administration, cultured microglial cells display growth arrest, enhanced SA-βgal activity, and senescence-associated heterochromatic foci [167]. This may be what primes microglia for enhanced activation in response to systemic inflammatory stimuli. Both normal brain aging and chronic age-related neurodegenerative diseases are associated with microglial-mediated increases in components that are associated with the SASP, including pro-inflammatory cytokines such as IL-1β and IL-6. Other non-neuronal cell types in the brain may also be capable of undergoing senescence including oligodendrocytes, which could affect neuronal myelination and subsequent signaling capabilities as well as endothelial cells, resulting in breakdown of the BBB and influx of peripheral inflammatory factors [168]. Cellular senescence has been reported to occur in the vascular endothelium in the periphery, suggesting that this same cell type may be vulnerable in the aging brain. Replication-competent cell types capable of undergoing senescence and expressing a SASP could potentially affect the function of neighboring neurons and contribute to their degeneration. They could also promote an amplification of glial cell activation. A recent publication showed that both $p16^{INK4a}$ and the SASP factor matrix metalloproteinase (MMP) 3 increase in brains from AD patients compared to age-matched controls; interestingly, this

increase occurred primarily in cortical astrocytes [169]. This may be important not only in these disease states themselves, but in terms of the effective use of cellular transplantation as a therapy for these disorders (see below).

5.4 Reduced Adult Neurogenesis

Neurogenesis entails the production of new neurons from neural precursor cells (NPCs). In adult brains, NPCs proliferate in the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) [170]. They then migrate to sites of injury (in the case of PD, the nigrostriata) and differentiate into functional neuronal and glial cell types. We and others showed that the ability of NPCs to proliferate, migrate, and differentiate is significantly blunted by advancing age and the degree of this decline is exacerbated by PD-inducing agents such as MPTP [171–175]. This may be due to increased oxidative stress or neuroinflammation, resulting in neural progenitor cell (NPC) senescence or loss. It is currently not clear that induction of endogenous NPCs alone is sufficient in the context of the aging brain to restore losses in DAergic SNpc neurons. In addition to neurons, it will also likely be of import to consider alterations in the regenerative capacity and function of non-neuronal brain cells during aging and in PD.

Cellular transplantation to replace lost or damaged neurons in patients with the disease is a therapeutic option that mimics what occurs to a lesser degree during endogenous adult neurogenesis. However as mentioned previously, there are potential roadblocks. Upon extensive culturing (2 months), DAergic neurons derived from patients with sporadic PD show characteristic morphological features found in the diseased brain, including reduced neurite number, accumulation of cellular α-synuclein, and increased numbers of autophagic vacuoles [176]. This suggests that long-term cell survival may be diminished, particularly in the environment of on aging brain. It would be of interest to know whether cell survival is increased in brains made more youthful, for example following removal of senescent cells.

5.5 Beyond Mendelian Genetics: Epigenetics and the Transcriptome

Increases in DNA methylation and alterations in post-translational histone modifications including changes in histone methylation and acetylation patterns have been reported in various models of PD and observed in patient samples [177]. For example, DNA methylation has been shown to regulate expression of the gene encoding α-synuclein, SNCA, as well as others. Masliah and colleagues have reported that α-synuclein can interact with the demethylase Dnmt1 in the cytoplasm, preventing

it from translocating to the nucleus and resulting in elevations in DNA methylation [178, 179]. Alpha-synuclein has itself been reported to interact directly with histones and to inhibit histone H3 acetylation [180, 181]. Both of these can impact gene expression patterns. Our laboratory has shown that selective binding of α-synuclein to particular histone marks not only tracks with human disease and occurs in PD models, but also leads to functional effects on patterns of genome-wide transcription, including expression of PGC1alpha [181] (our unpublished data). HDAC inhibitors have been shown to be neuroprotective in various models of the disorder [182]. The histone deacetylase LSD-1 is inhibited by lithium, an agent shown by our laboratory to be neuroprotective in PD mouse models [183, 184]. LSD-1 is also inhibited by MAO-B inhibitors currently under use as treatment for the disorder [143]. A known target of transcriptional LSD-1 repression is $p57^{kip2}$, *a g*ene that aids in the differentiation and maintenance of midbrain DAergic neurons [185]. Beyond its effects on histones, LSD-1 inhibition can also impact on p53's ability to interact with 53BP1 and to induce apoptosis [186]. Its other non-histone substrates include FOXO transcription factors and NFkappaB [187]. Lithium and LSD-1 inhibition both result in increased lifespan in *C. elegans*, suggesting that they may have impacts in terms of aging itself [188].

Aging is also associated with extensive remodeling of gene expression profiles in different tissues as a consequence of epigenetic alterations. The HSPs 22 and 70, implicated in PD as well as in lifespan determination, are strongly regulated by histone modification [189]. Changes in mitochondrial function that increase cellular ROS and affect NAD^+/NADH ratios in PD and aging may also impact nuclear DNA methylation and histone modifications [190, 191]. Alterations in epigenetics as a consequence of aging and PD are likely impacted by individual environmental factors over the lifespan, which may in part explain individual variability in the presentation of both conditions.

6 Needed Research in This Area

There are several areas of research needed in order for us to achieve a better understanding of the interplay between aging and PD. These include a better understanding of the dual protective roles of autophagy in turnover of damaged proteins and organelles like the mitochondria, the precise sources of inflammation (glial cell activation, cellular senescence), and causes of lost neurogenesis in adult neural stem cells (e.g. whether cellular senescence is a factor). We also need to better understand the difference between transient stressors that allow adaptation versus chronic stressors which result in damage—retrograde mitochondrial signaling including brief elevations in mitochondrial ROS is a good example of the former [192]. More work needs to be devoted to linking findings in cellular and animal models to humans. This include the development of working mouse models of PD that take aging into consideration, as well as the use of patient iPSC-derived DAergic neurons, human autopsy tissues, and epidemiological/clinical studies. Undoubtedly this

would be aided by an increased use of non-human primate models in both PD and aging. Studies in rhesus monkeys show a strong correlation not only between the presence of α-synuclein in aged versus MPTP-treated monkeys, but also of other disease hallmarks including selective reductions in DAergic SNpc cell number, defects in UPS and lysosomal activities, markers of oxidative and nitrosative stress, and neuroinflammation [193]. Marmosets are shorter-lived and have also been used in PD research, so they represent an attractive alternative model [194].

An especially vital area of research is obtaining a better understanding of the interrelationship between genes and environment (the 'exposome') on PD and aging [195]. Dietary fat and exposure to heavy metals and pesticides are all examples of environmental factors linked to increased risk for PD [196–198]. The relationship between dietary restriction (DR), the gut microbiome, and aging may also have important implications for mechanisms underlying PD. Intermittent fasting (every other day fasting) has been proposed to have an effect on brain function [199]. DR has been linked to prevention of aging in several model systems as well as beneficial effects in PD mouse models [200]. PD patients show elevated heart rate and impaired cardiovascular stress response due to effects on the autonomic nervous system preceding DAergic SNpc loss correlating with α-synuclein accumulation [201]; DR resulted in prevention of these effects in a mouse model of the disease [202]. Production of ketone bodies during periods of fasting may also have beneficial effects in the brain due to inhibition of histone deacetylases, impacting on the transcription of genes like neurotrophic BDNF, reduction of pro-inflammatory cytokines, enhancement of neurogenesis, and perhaps even induction of mitochondrial biogenesis via SIRT1-related elevation in PGC1α levels [203].

The diversity and make-up of the gut microbiome has been shown to change with age, coinciding with inflammaging [204]. These alterations have been demonstrated to be involved in risk for chronic age-related diseases including cardiovascular disease, inflammatory bowel syndrome, metabolic disease, and cancer [205]. This is alterable for better or worse by lifestyle and diet, and as a consequence the gut microbiome has been identified as a target for improving overall health in the elderly population [206]. Scientific evidence for an involvement of the gut microbiome in brain function has recently begun to gain ground for disorders such as autism and depression [207]. Given its role in inflammaging, composition of the gut microbiome could potentially also play a role in neurodegenerative diseases like PD. The gut microbiome is responsible for the production and processing of micronutrients such as folate, thiamine, riboflavin, and biotin. Deficiencies in these have all been linked to PD as well as to aging in some cases [208]. Pyroxidine is also produced via activity of gut microbes and is known to accelerate the rate of conversion of L-Dopa in the periphery, which can be slowed by inclusion of carbidopa [209]. Disruptions in circadian rhythms have recently been linked to alterations in the gut microbiome [210]. Both are associated with similar chronic age-related diseases [205, 211]. Mice with genetically altered circadian rhythms were found to have significantly altered gut microbiota when fed a high-fat, high-sugar diet [212, 213]. These data suggest that age-related changes in the circadian clock can drive changes in the microbiome and impact chronic disease states, perhaps including PD. Indeed

loss of midbrain DAergic neurons in the Mitopark mouse has recently been found to be associated with disruption of circadian rhythms [214].

Recent animal studies have also shown that gut microorganisms can activate the vagus nerve via immunomodulatory effects and that this plays a critical role in mediating brain function [215, 216]. The vagus nerve connects the enteric nervous system to the brain and is considered a possible pathway for transmission of α-synuclein [217]. Understanding the role of the vagus nerve may have important implications for the development of microbial- or nutrition-based therapeutic strategies for PD and related disorders.

7 Conclusions

The most recent scientific evidence in the field suggests that PD is one of several disorders for which aging is not merely a risk factor, but an underlying cause of disease. This suggests the exciting possibility that by seeking to prevent or slow basic processes that drive aging, we will uncover potent new therapies for PD and related neurodegenerative conditions as well as other age-related disorders. This enterprise will involve additional research in order to identify the most promising potential therapeutic directions.

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Aging and the Bone-Muscle Interface

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Contents

1 Skeletal and Muscle Compartments: Relationship to the Geroscience Pillars

The skeleton is one of the largest systems in the body consisting of a mineralized matrix and a highly active cellular remodeling unit, composed of osteoblasts, osteoclasts, osteocytes and bone lining cells [1]. The most obvious function of the skeleton is to provide structural integrity for the organism while maintaining a degree of

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elasticity that allows for a broad range of locomotor activities. In addition to its structural function, the skeleton also serves as a mineral depot insuring the maintenance of serum calcium and phosphate levels through normal remodeling processes and by secretion of peptide factors such as FGF-23. Every 10 years the human skeleton is completely remodeled. Just as importantly, the skeleton encases the bone marrow and maintains a niche along trabecular bone elements for active hematopoiesis. The marrow niche consists of osteoblasts, adipocytes, reticuloendothelial cells, sinusoids, and mesenchymal stromal and stem cells. These progenitors respond to injuries at various sites, not restricted to bone, providing new cells for critical repair processes [2]. Remarkably, the adult skeleton also harbors a huge adipose depot, comprising 10–15 % of all fatty tissues in the body [3]. With aging that percentage increases, particularly as peripheral adipose depots shrink. Whether this is a response to chronic inflammation, metabolic changes from insulin resistance, disuse because of muscle atrophy or molecular drivers related to senescence within the marrow is currently being debated. Regardless, alterations in either the structural or metabolic functions of the skeleton, a key component of the aging process, have tremendous implications for the overall health of the organism.

Muscle represents the single largest component of the body, representing ~40 % mass by weight in a typical individual. There are more than 600 muscles in the body, with distinct differences in metabolism, cellular makeup, the ability to generate force, as well as specificity for workload. Skeletal muscles are composed of individual fibers, anchored to the skeleton through tendons, and each fiber is essentially a single cell, containing multiple nuclei throughout its length. Muscle fibers can be generally broken down into two key types, fast twitch, and slow twitch, with a number of further subtypes dependent on species. Fast twitch fibers are primarily glycolytic, and generally are associated with explosive energy demands such as sprinting. Slow twitch fibers are primarily oxidative, and are typically associated with more sustained workloads such as long distance running, and certain muscles can be predominantly comprised of only one type, while other muscles have mixed fiber types. Muscle is one of the major sites of metabolism in the body, and is responsible for more energy consumption than fat and bone combined. Although we do not yet have a complete picture of age-related atrophy with regards to each distinct muscle in any species, it is generally agreed that there is a loss of muscle mass with age. The exact extent of loss is not yet clear, and may vary depending on environment, lifestyle, and genetics. Importantly, accompanying the loss of muscle, there is a corresponding decline in function. Initially, the loss of muscle mass with age was thought to be due to a loss of type II fibers, however recent studies have suggested that the loss in muscle mass can be almost entirely explained by atrophy, rather than loss, of type II fibers alone. Therefore, changes in metabolism, mitochondrial autophagy, enhanced reactive oxygen species and impaired stem cell regenerative capacity, all could lead to muscle atrophy, disuse and ultimately to a failure in loading [4]. Thus musculoskeletal aging must be considered within the context of extending healthspan since general mobility is critical for a good quality of life. Muscle and bone are intimately associated with each other, yet we typically study each in isolation, and there are few studies examining them together as a functional system.

There are three skeletal compartments with unique functional characteristics, trabecular bone, cortical bone and the periosteum. Each compartment is com-

posed of nearly identical cells that arise from the same precursor but functionally may be very different. Not unexpectedly, these cells and their compartments differ in their response to the stressors of aging, including changes in metabolism, reactive oxygen species, and inflammation. Trabecular bone comprises about 20 % of the human skeleton in young adults and is present primarily in the axial bones. In rodents it comprises less than 5 % of bone mass and cross sectional studies to date have suggested that it is lost much earlier during the aging process relative to cortical bone. Its large surface area provides a framework for skeletal remodeling and calcium homeostasis, ensuring adequate calcium for the body while also providing the elasticity necessary for bi- and quadra-pedal locomotion.

It is estimated that every 10 years, the human skeleton is remodeled, with the greatest frequency of turnover in trabecular bone [1, 5]. There is no evidence that remodeling slows with age, and indeed, there may be increased bone resorption during the latter decades of life. This may be associated with enhanced reactive oxygen species generation, epigenetic changes, or enhanced expression of RANKL from osteocytes that leads to greater bone resorption.

The cortical compartment surrounds the trabecular elements and is composed of a solid layer of calcified matrix in lamellar bone. Cortical bone is a hard tissue with elastic properties that enhance its strength. It is also very dynamic. The endocortical surface of cortical bone is subject to remodeling due to the presence of osteoclasts and osteoblasts, as well as its proximity to the marrow space, where progenitor cells reside, although the rate of turnover is much lower than in the trabecular skeleton. The periosteum is the outer layer of the skeleton and serves several functions but does not remodel, even though it is a major source of progenitor cells for fracture healing and for endochondral bone formation. Importantly, the periosteum contributes to skeletal growth, the response to mechanical loading, the injury and fracture response, and the compensatory mechanisms associated with aging that attempt to preserve strength in the face of trabecular and cortical loss. Importantly, despite several determinants directly related to aging (i.e. geroscience pillars) including metabolic, inflammatory and epigenetic processes, the periosteum appears to respond appropriately to changes with the trabecular compartment even at advanced ages. However, few studies have addressed why a highly innervated and vascular tissue (i.e. the periosteum) can withstand the stressors of aging that have deleterious effects on other hard and soft tissues.

Like bone, there are substantial changes in the musculature with age. Between the forth and fifth decade of life, homeostatic control of muscle mass declines, resulting in an overall loss of muscle mass in later years of life – termed sarcopenia ("poverty of the flesh"). This loss of muscle mass due to intrinsic aging has been estimated to be of the order of 1 % a year from ~50 years of age, and can result in as much as a 30 % loss of muscle mass by the mid-80s. Although sarcopenia appears to be a universal attribute of aging, which has been documented in multiple mammalian species [6–9] as well as invertebrate models of aging [10, 11], comparatively little work has been done in determining how sarcopenia dynamically affects the more than 600 different muscle types in aging mammals [7, 9, 12–14]. Even less has been reported about the effect of sarcopenia on different compartments of the skeleton. Most studies to date have focused on large muscle groups where it is possible to obtain sufficient material to enumerate the number and type

of fibers present at specific ages, and nearly all data collected to date is cross sectional in nature. Common methods to measure atrophy in the fibers and subsequent loss of muscle mass include non-invasive techniques such as MRI (for evaluating cross sectional area of the thigh for example), as well as muscle biopsies, used to enumerate the total number of fibers present in the biopsy as well as defining the level of atrophy and the corresponding fiber type (myosin heavy chain specific isoforms distinguish fiber types). The mechanisms through which muscle mass is lost with age are currently unknown, but are likely multifactorial. Factors that have been implicated in sarcopenia include lifestyle, hormonal milieu, mitochondrial dysfunction, nutritional status, protein synthesis, stem cell decline, loss of neuromuscular junctions, and fiber atrophy [4, 15–22]. Mitochondrial dysfunction could result from enhanced ROS production, epigenetic changes due to environmental determinants, metabolic load (particularly glucose) and inflammation. Since both bone and muscle share common regulatory features such as cytokines, neurotropic factors, and the sympathetic nervous system, it seems likely that with aging there are concomitant changes in both tissues that ultimately result in falls and fractures.

Skeletal aging begins after the rapid phases of bone modeling and growth during adolescence. The acquisition of peak bone mass is related to a complex series of hormonal changes associated with matrix biosynthesis and mineralization. This is followed in humans by a plateau phase of variable duration in early adulthood, followed by loss of bone mass from two of the three skeletal compartments. During growth, bone modeling occurs through endochondral bone formation (i.e. requiring cartilage) by longitudinal expansion of the growth plate from the secondary and primary spongiosa [23]. Membranous bone formation without the need for chondrocytes occurs primarily in the craniofacial bones. After peak bone acquisition (ages 12–16), skeletal remodeling balances resorption with formation by a general maintenance phase that may last from 5 to 25 years in humans. Ultimately bone loss occurs from the trabecular and cortical skeleton although there is debate about the timing and magnitude as well as the compartmental specific effects [24]. In general, trabecular bone is lost first and may begin as early as the third decade of life in humans [25]. By the nineth decade of life, there is a marked diminution in trabecular bone in the distal extremities with even less trabeculae in the vertebrae. In female C57BL/6J mice, trabecular bone from the distal femur and tibia begins an incessant decline by as early as 8 weeks of age, whereas spongy bone loss from the vertebrae doesn't start until nearly 16 weeks [26]. These changes are relatively similar with regards to the biological age of human bone loss. However, unlike humans, trabecular bone in the femur/tibia of mice is essentially absent after ~12 months of age, although further studies are needed to longitudinally follow bone with age. In addition, both in humans and mice there is a much slower rate of loss of trabecular bone in males than females. It is still uncertain how age-related trabecular bone changes impact, if at all the muscle bone interface.

Cortical bone remodels in humans and in rats but less so in mice. Conversely, bone loss from this compartment starts much later on the endocortical surface than

on the endosteal perimeter, and is incessant [27]. Cortical thickness declines with aging at a relatively constant rate although it can be accelerated by hormonal imbalances (e.g. estrogen loss at menopause, hypogonadism in males, glucocorticoid excess in both sexes) [28]. The stressors that compose the pillars of Geroscience and impact soft tissue also play a major role in cortical bone changes. However, less work has been done in determining the relative magnitude or the sum of these effects. Traditionally, hormonal imbalances have been ascribed as the major mechanism for bone loss. But emerging studies suggest that accumulation of toxic substances, mitochondrial dysfunction, metabolic stress, and impaired stem cell responsiveness also play critical roles.

Cortical bone can become porous with age, although the mechanism for the development of these pores within the matrix remains uncertain. One possibility is that cortical bone becomes trabecularized by higher rates of resorption and this leads to areas of porosity that are imbedded within the cortex. Seeman has proposed the notion that most age-related osteoporosis represents disease of the cortical skeleton [29]. Hence around midlife, in women, remodeling balance becomes negative; less bone is deposited than it is resorbed by each bone's basic multicellular units (BMUs), and remodeling rate increases; there are more BMUs removing bone upon intracortical, endocortical, and trabecular surfaces. Canals enlarge and coalesce creating giant pores. Remodeling upon trabecular surfaces removes canals, whereas intracortical and endocortical remodeling fragments the cortex. Seeman proposes that bone loss becomes almost entirely cortical as trabeculae disappear [29]. Overall, remodeling removes more bone from a diminishing total mineralized bone matrix volume so that by old age, total mineralized bone matrix volume is halved; but 70 % of all bone loss is cortical because 80 % of the skeleton is cortical; a third of all the bone loss arises from the 20 % of the skeleton that is trabecular. Hence most of the fractures occurring with aging are non-vertebral (hip, humerus, tibia, fibula, radius) and predominantly cortical whereas 20 % are vertebral. If indeed, cortical bone changes are the major determinants of osteoporosis, then the impact on muscle, and vice versa must be significant.

As cortical bone thins, and porosity increases, structural fragility becomes more pronounced. Intriguingly, in mammals there is a compensatory mechanism in place during aging that is activated by the rapid loss of long bone. This is termed 'periosteal expansion' and it has a potential to increase bone area and partially buffer the higher rate of endosteal and endocortical resorption with age [30]. The unique capacity of the periosteum, which is the site of insertion of tendons from large muscles, to expand, improves skeletal properties such as the polar moment of inertia, and stiffness, thereby partially preserving bone strength and reducing the risk of fracture. Males tend to have a more vigorous periosteal response to aging and injury than females; this may be due to inherent cell autonomous differences by sex. The molecular drivers of this difference have not been elucidated nor is it clear that androgens direct this process. Moreover, the signals for periosteal compensation are also not known but it is this interface between bone and muscle that provides some fascinating insights into the physiology of aging and hence can shed light on the defects inherent in osteoporosis.

2 Unique Aspects of the Bone-Muscle Interface: Relationship to Geroscience

To understand the importance of the muscle-bone interface within the broader context of geroscience, it is critical to define the anatomical relationships. Muscles insert on bone via tendons that connect to a fibrous layer on the surface of bone. The periosteal layer or membrane is contiguous with this fibrous layer and covers all bones in the body except the joints. It is made up of sensory fibers, endothelial tissue, stromal elements including mesenchymal stromal cells (MSCs) that could become osteoblasts, vascular networks and some adipocytes. The periosteum is really a dense irregular connective tissue [31]. It can be divided into an outer "fibrous layer" and an inner "cambium layer" (or "osteogenic layer"). The fibrous layer contains fibroblasts, while the cambium layer contains progenitor cells that develop into osteoblasts. These osteoblasts are responsible for increasing the width of a long bone and the overall size of the other bone types. After a bone fracture the progenitor cells can develop into osteoblasts and chondroblasts, which are essential for fracture healing. Bone has very few long track sensory nerves beyond the innervations to osteoblasts whereas the periosteum has nociceptive nerve endings, making it very sensitive to manipulation. The nerve endings are accompanied by many blood vessels, branches of which penetrate the bone to supply the osteocytes, or older osteoblasts embedded within the cortex. These perpendicular branches pass into the bone along channels known as Volkmann canals to the vessels in the Haversian canals, which run the length of the bone.

Osteocytes are older osteoblasts that serve as mechano-sensors to modulate skeletal remodeling through the secretion of peptide factors such as sclerostin. This connection between cell surfaces (via the periosteum), which is activated by loading of the bone, can respond to fluid flux within the cortical lacunae and communicate with other cells via the canaliculi. Recent studies of the osteocyte further support its importance in regulating bone remodeling through several factors, including RANKL which can cause osteocytic osteolysis (i.e. dissolution of matrix around osteocytes) and sclerostin which by binding to Lrp5, a critical receptor for osteoblasts, can inhibit new bone formation [1, 32]. In addition, osteocytes also secrete endocrine factors such as FGF-23 which modulates phosphate homeostasis [1]. Aging bone is characterized by osteocytic drop out, or what is termed 'empty lacunae' [1, 25–27]. Apoptosis is the presumed mechanism, but the molecular drivers of that process are not known. In a recent study, Jilka et al. genetically deleted two osteocytic genes related to apoptosis and found that in aging mice, the lacunae were filled with viable osteocytes but paradoxically there was increased bone resorption and significant cortical porosity [33].

Fibrous cartilage often takes the place of the periosteum along grooves where tendons exert pressure against the bone. The periosteum itself is attached to bone by strong collagenous fibers called Sharpey's fibers, which extend to the outer circumferential and interstitial lamellae [34]. Pressure from muscle insertion on the fibrous membrane affects the mechanosensors almost certainly through growth factor

signals from the periosteum, either locally or systemically. The periosteum also produces bone when it is stimulated appropriately [31]. Practically anything that breaks, tears, stretches, inflames, or even touches the periosteum can lead to a reactive process whereby new bone is formed. This is termed a *periosteal reaction*, also known as a *periosteitis*, which is a non-specific radiographic finding that occurs with periosteal irritation. Periosteal reactions can be broken down by pattern, but in all cases the response arises from the skeletal disease itself, not in the periosteum. With slow-growing processes, the periosteum has plenty of time to respond to the process. That is, it can produce new bone just as fast as any growing lesion. This is particularly important when considering the periosteal response to bone loss and the aging process. With aging, there is some clinical evidence that fracture healing is impaired. However there is tremendous inter-individual variation and mid-diaphyseal periosteal measures including cambium and fibrous layer thickness and cellularity do not correlate significantly with age or body mass [20]. Gender certainly plays an important role in the periosteal response to aging but the cell autonomous factors involved remain unknown [35].

The *tendon* is a tough band of fibrous connective tissue that usually connects muscle to bone and is capable of withstanding tension. It is that tension which is thought to provide the initial force on the bone that leads to signals for modeling and remodeling of the skeleton. There are no studies that report on differences in mechanical forces with aging, although certainly sarcopenia must have an impact. Tendons are similar to ligaments and fasciae; all three are made of collagen. The mechanical properties of the tendon are dependent on the collagen fiber diameter and orientation. The collagen fibrils are parallel to each other and closely packed, but show a wave-like appearance due to planar undulations, or crimps, on a scale of several micrometers. In tendons, the collagen fibers have some flexibility due to the absence of hydroxyproline and proline residues at specific locations in the amino acid sequence, which allows the formation of other conformations such as bends or internal loops in the triple helix and results in the development of crimps. The crimps in the collagen fibrils allow the tendons to have some flexibility as well as compressive stiffness. In addition, because the tendon is a multi-stranded structure made up of many partially independent fibrils and fascicles, it does not behave as a single rod, and this property also contributes to its flexibility. The uniqueness of the tendon, and its transmutation of loading from the muscle must play a role in the periosteal compensation that occurs with aging [36]. However, if that signal is dampened by sarcopenia, or reduced loading, mechanically-induced expansion of the periosteum might be impaired, leading to altered biomechanical properties and ultimately skeletal fragility.

The extent of mechanical loading (e.g. through strength training) of muscle on bone via tendons and the periosteum has been strongly associated with cortical bone mass in both cross sectional and longitudinal studies [37]. Conversely, skeletal unloading due to bed rest, zero gravity states or muscle disease results in low bone mass and skeletal fragility. However, it is unclear whether all of the effects of bone unloading are mechanically mediated or if there are soluble mediators that might be released from atrophic muscles to negatively affect skeletal remodeling. Similarly,

the sarcopenia of aging is associated with falls, reduced muscle strength and fractures. Whether these age-related changes are all mechanically-mediated or are related to the intertwined factors that define aging, such as inflammatory cytokines and myokines, metabolic dysfunction, changes in innervation and/or buildup of toxic superoxides, remain to be elucidated.

3 Experimental Evidence that Periosteal Expansion Occurs During Aging

Bone loss is an inexorable feature of aging in all mammalian systems although the mechanisms are multi-factorial and species-specific. In rodents and humans, both cortical and trabecular bone are lost with advancing age although the rates differ considerably. In general, trabecular bone loss occurs first, due in part to its greater surface area compared to the cortical compartment. Inbred strains of mice lose bone from the distal femur as early as 8 weeks of age [26]. In humans, studies using quantitative CT and microCT have demonstrated trabecular thinning and slow but incessant bone loss beginning in the third decade of life in women [25]. As such, even though menopause induces an estrogen deficient state over a period of years, and this has been associated with accelerated bone loss, it is evident that the other common factors noted previously, i.e. metabolic, proteostatic, inflammatory and/or toxic, contribute to age-related bone loss. A characteristic feature of this loss, independent of gonadal steroids and in both cortical and trabecular compartments is uncoupled remodeling such that resorption accelerates beyond formation. Bone formation may increase marginally in response to the ensuing loss, but with advanced age, this response appears to be blunted. This latter feature has been variously attributed to greater reactive oxygen species, reduced stem cell pools, senescent accelerated impairment in bone formation, non-cell autonomous factors such as higher sympathetic tone and greater concentrations of toxic cytokines or ROS. Recently, Bartel et al. reported that Foxo proteins restrain age-related osteoclastogenesis by modulating H_2O_2 accumulation [38].

Mechanically, bone loss alters the skeletal microarchitecture and reduces bone strength. In the axial spine, trabecular loss leads to enhanced fragility and susceptibility to compression fractures that can have a significant impact on quality of life and morbidity [14]. In the cortical compartment, long bones undergo loss as well although it may differ by site and by the amount of trabecular bone within the appendicular skeleton. In mice, cortical remodeling is not a major feature but in rats, monkeys and humans, cortical turnover predominates in later life. The major cost to society of age-related cortical bone loss is a hip fracture. Significant architectural changes occur during the slow but inexorable process of uncoupled bone turnover. In particular, the endocortical surface of the cortex (Fig. 1) undergoes resorption, leading to an expanded marrow cavity. This loss can lead to trabecularization of the cortex and cortical porosity.

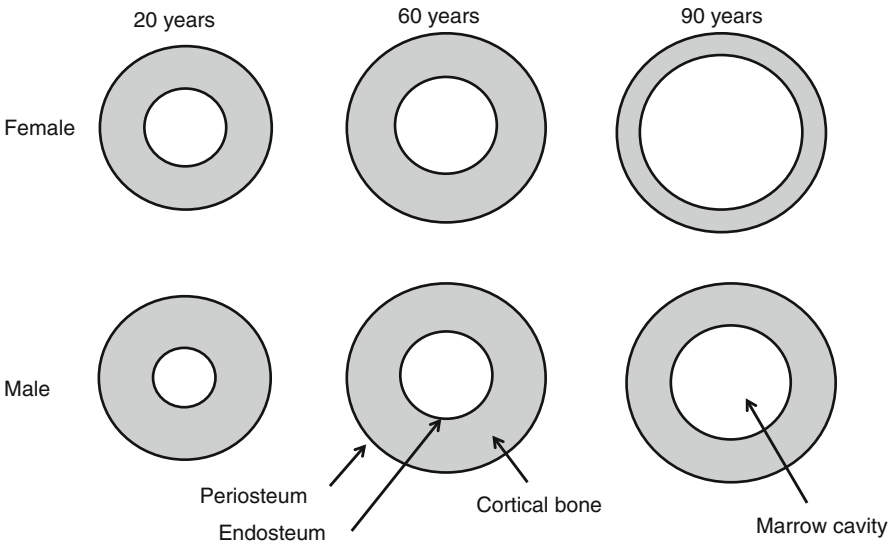

Fig. 1 Changes with aging in the endocortical and periosteal envelope by gender. A cross sectional view of a typical long bone (femur) showing different features which dynamically change with age and sex

Paradoxically, the periosteal surface of the cortex is not subject to loss and in fact expands at the same time as there is endocortical resorption [37]. Miller et al. aged genetically heterogeneous UM-HET3 mice that were produced by a cross between (C57BL/6J × BALB/cJ)F1 males and (C3H/HeJ × DBA/2J)F1 females to show that changes in bone diameter are associated with lifespan [39]. Remarkably, those mice with the greatest cortical thickness had the highest survival rate. Not surprisingly, the authors also noted that the endosteal envelope expanded by nearly 20 % due to increased bone resorption with age. Importantly, the increase in cortical area with age from 4 to 24 months was predominantly related to the increase in periosteal circumference (from 4.88 to 5.66 mm; $p < 0.01$) thereby partially maintaining cortical thickness. How periosteal expansion is related to lifespan remains to be determined in mice but may reflect the health of connective tissue or the pool of progenitors that are necessary for this compensation.

Studies in humans provide additional support for the compensation theory. Ahlborg et al. measured bone mass and the skeletal structure of the distal radius by single-photon absorptiometry every other year in 108 women, all of whom were followed from the time of menopause for a mean period of 15 years [35]. The mean (± SD) annual decrease in bone mineral density was 1.9 ± 0.7 %. The medullary bone diameter increased annually by 1.1 ± 0.9 %, and the periosteal diameter by 0.7 ± 0.3 %; while the strength index decreased by 0.7 ± 0.7 %. The expansion of the medullary diameter with a simultaneous increase in the periosteal diameter was directly correlated ($r = 0.54$, $P < 0.001$), and women in the highest quartile of medullary expansion had more loss of bone mineral density and greater periosteal

apposition than women in the lowest quartile ($P < 0.001$ for both comparisons) [35]. The strength index decreased as might be expected but might have been more severe had periosteal expansion not occurred. The factors that regulate this expansion are not clear, but interestingly, estradiol levels were inversely related to the periosteal expansion rate. In summary, aging is associated with progressive increases in medullary diameter accompanied by periosteal expansion The structural implications of this compensatory response and their relationship to progressive age-related muscle loss need further exploration. The factors that permit the periosteum to resist age-related changes in metabolism and buildup of reactive oxygen species are also unknown.

4 Cellular and Biochemical Aspects of the Periosteum in Relation to Aging

The periosteum is rich in distinct cell types, some of which may be responsive to signals from the osteocyte, or from mechanical loading of the tendon. Moreover, because it is highly vascularized, the periosteum is subject to endocrine actions of hormones such as IGF-I and PTH. But the periosteum also contains significant numbers of progenitors and mesenchymal stem cells and it is the balance between mature and progenitor cells that ultimately define the function of the periosteum [37, 31]. If one of the determinants of unhealthy aging is reduced stem cell pools or reduced stem cell function, the periosteum may be at least partially protected. Alternatively it is possible that adult periosteal cells may undergo senescence and thereby be resistant to autocrine, paracrine or endocrine signals such as inflammatory cytokines. In one report immunohistochemical detection of Ki67 and p53, Nitric Oxide (NO) production and qRT- PCR of a selected gene panel for osteoblastic differentiation, bone remodeling and 'stemness' were evaluated. The authors confirmed that both Ki67 and p53 were noteworthy indicators of senescence in human periosteal precursor cells and their expression significantly correlated with cell NO production [40]. Moreover, cell age affects genes involved in bone remodeling, with a significant increase in interleukin-6 mRNA expression and receptor activator of nuclear factor kappa-B ligand (RANKL)/osteoprotegerin (OPG) ratio [40]. O'Keefe and colleagues recently reported that reduced fracture healing in aging involves decreased proliferation and differentiation of stem cells lining the bone surface. While PTH 1–34 enhances the proliferation and expansion of the periosteal stem cell population and accelerates bone formation and fracture healing, the effects are proportionately reduced during the aging process compared to young mice [41].

Another mechanism whereby periosteal expansion may be limited during aging is through the impairment of progenitor cell recruitment from muscles. Recent studies have established that muscle-derived stem cells are able to differentiate into cartilage and bone and can directly participate in fracture healing. The role of muscle-derived stem cells is particularly important in fractures associated with more severe injury to the periosteum. Muscle anabolic agents may improve function

and reduce the incidence of fracture with aging as well as maintaining the muscle-bone interface [42].

5 Non-cell Autonomous Factors Regulating the Bone/Muscle Interface During Aging

Although the continued periosteal apposition that accompanies age-related bone loss is a biomechanically critical target for prophylactic treatment of bone fragility, the magnitude of periosteal expansion required to maintain strength and stiffness during aging has not been established. A new model for predicting periosteal apposition rate for men and women was developed by Jepsen et al. to better understand the complex, nonlinear interactions that exist among bone morphology, tissue-modulus, and aging [43]. Periosteal apposition rate varied up to eightfold across bone sizes, and this depended on the relationship between cortical area and total area, which varies with external size and among anatomical sites. There was a 65–145 % increase in periosteal apposition rate beyond that expected for bone loss alone. But periosteal apposition rate had to increase as much as 350 % over time to maintain stiffness for slender diaphyses, whereas robust bones required less than a 32 % increase over time. Small changes in the amount of bone accrued during growth (i.e., adult cortical area) affected periosteal apposition rate of slender bones to a much greater extent compared to robust bones. Thus bone growth places a heavy burden on the biological activity required to maintain stiffness with aging. Finally, sex-specific differences in periosteal apposition were attributable in part to differences in bone size. The results indicate that a substantial proportion of the variation in periosteal expansion required to maintain bone strength during aging can be attributed to the natural variation in adult bone width [43]. Clinical data to differentiate the biological responses that are attributable to size effects from other genetic and environmental factors are necessary.

6 Muscles, Myokines, and the Periosteum: Intertwining Factors During Aging

Muscles and bone are intimately linked beyond the biomechanical connection through bi-directional signals regulating gene expression, proliferation and differentiation. It is generally accepted that muscle cells secrete factors (myokines) that influence adjacent bone cells, but few myokines have been identified and characterized functionally. Importantly, the periosteum can serve as a barrier for locally secreted muscle factors unless the soluble substances are relatively small and can permeate the tissue. Lai et al. recently reported that PGE2, IGF-1, IL-15 and FGF-2 can penetrate the periosteal membrane and hence drive bone cell function in the cortex [44]. IGF-I is particularly important since it is synthesized by muscle and

bone and has a growth promoting effect on both tissues. Low IGF-I is associated with reduced bone size and periosteal circumference as well as less muscle mass in genetically modified animal models and in humans with growth hormone deficiency (or resistance) and Type I IGF receptor resistance. Importantly, with aging, growth hormone secretion declines in mammals and circulating IGF-I decreases. IGF-I is also anti-apoptotic and as such a decline in circulatory or skeletal IGF-I could contribute to a premature decline in bone cell populations.

Other myokines have recently been studied. Sims et al. demonstrated that osteocyte-specific deletion of the co-receptor subunit utilized by IL-6 family cytokines, glycoprotein 130 (gp130), resulted in impaired bone formation in the trabecular bone, but enhanced periosteal expansion, suggesting a gp130-dependent periosteum-specific inhibition of osteoblast function, potentially induced by the local muscle fibers [45]. This consistent but inverse relationship between osteoblast induced bone formation and periosteal expansion mimics conditions such as age-related and postmenopausal osteoporosis. Similarly, Sims and colleagues reported a negative relationship between ciliary neurotrophic factor from muscle and osteoblast differentiation [45].

Myostatin is a member of the bone morphogenetic protein/transforming growth factor-β (BMP/TGFβ) super-family of secreted differentiation factors. Myostatin is a negative regulator of muscle mass as shown by increased muscle mass in myostatin deficient mice. Interestingly, these mice also exhibit increased bone mass suggesting that myostatin may also play a role in regulating bone mass. Indeed, a soluble myostatin decoy receptor (ActRIIB-Fc) increased bone and muscle mass. Bone volume fraction (BV/TV), as determined by microCT, was increased by 132 % and 27 % in the distal femur and lumbar vertebrae, respectively. Surprisingly there was no effect on cortical bone or the periosteum, suggesting that one mechanism for this myokine may be more endocrine than paracrine [46].

Exercise training benefits muscle and bone by slowing age related bone loss, but also offers protection against several of the major pillars that define an impaired healthspan. For example, metabolic disorders such as obesity and diabetes, as well as an inflammatory component can be ameliorated by exercise. Exercise has been shown to reduce mitochondrial autophagy, enhance stem cell recruitment, and slow the rise in ROS from muscle cells. Irisin, a muscle cytokine produced from surface FNDC5 proteolysis, mediates a thermogenic program in adipose tissue of mice [47]. This leads to enhanced glucose utilization in adipose tissue by increasing uncoupling protein 1 and the transcriptional co-factor Pgc1α. However, it is unclear if irisin has a direct impact on either the periosteum or cortical bone, or whether these findings are relevant in humans.

7 Sarcopenia and the Bone-Muscle Interface

Although a considerable amount of work has been done in describing sarcopenia, it was only recently that a definition was agreed upon by an international working group that incorporated functional attributes as well as the loss of muscle mass in

the definition [48]. Sarcopenia is currently defined as "the age associated loss of skeletal mass and function". The coupling of the terms "function" and "loss" is critical, as an increasing body of evidence has shown that functional impairment of aged muscle is a better correlate of frailty than the amount of muscle loss alone. In addition, other conditions where substantive muscle loss occurs such as that due to cancer (cachexia) cannot be considered as the same as the muscle loss due to aging. Remarkably, aging bone and muscle both exhibit fatty infiltration although the degree to which this feature compromises musculoskeletal strength or function is not known.

More recently, one of the initial mechanistic explanations for sarcopenia is coming under question. It has long been assumed that with age, the loss of muscle mass is due to a corresponding loss of fibers (predominantly type II), and this has been reported many times, typically using methods which compare the numbers of fibers in muscle biopsies of young individuals with those from older individuals. However, recent work in humans from the van Loon group [49] has suggested that the loss of muscle mass with age can be explained entirely by atrophy of type II fibers alone. That is, muscle mass is lost with age due to a specific atrophy of type II fibers, rather than loss of fibers per se. van Loon and colleagues conclude that the number of fibers is the same between young and old age groups, but the volume occupied by type II fibers with age is much smaller, thereby accounting for the loss in muscle mass. This directly contradicts the "fiber specific loss" with age hypothesis, as an explanation for why we lose muscle mass with age [49]. Importantly, the data in this key study was concluded in part from data collected longitudinally from the same subjects over time. Further studies are needed with methods which can longitudinally profile cell number, rather than rely on cross sectional study designs to definitively answer this long standing question.

Under normal circumstances, muscle is replenished and renewed through the action of stem cells located in the basement membrane of the myofiber. These stem cells are termed satellite cells, and have long been known to be essential for maintaining muscle mass. There has been much interest over the last few years on the role that satellite cells may play in sarcopenia. There have been reports describing both a loss of satellite cells with age, as well as a decline in the niche that allows the satellite cell to replenish the myofiber [50, 51]. Data has been presented showing that the quality of muscle can be improved through systemic administration of factors present in the blood of young animals through parabiotic pairing, in which the circulatory system of young and old animals are surgically joined [52]. However, more recently, the role of satellite cells in directly affecting muscle mass in aging was addressed in an elegant series of studies in which satellite cells were genetically ablated [53]. This allowed a direct test of the role of the satellite cell in maintaining muscle mass with advancing age. Essentially, transgenic mice were created which allowed the inactivation of satellite cells in older mice. Surprisingly, animals that had their reservoir of satellite cells genetically depleted via this genetic targeting strategy showed no difference in the rate of muscle loss compared to non-treated controls [53]. These data imply that sarcopenia is not due to either a loss of satellite cells with age in mice, or alteration of the niche preventing maturation of the satellite cell. Both of these arguments have been made as causal factors involving satel-

lite cells as a key mechanism in sarcopenia. Similarly, there have been several studies concluding that satellite cells are not involved in driving sarcopenia due to a lack of significant difference in the numbers of satellite cells from younger versus older adults [54, 55]. In order to resolve such issues, further studies are needed, particularly addressing the challenging paradigm of longitudinal assessment of satellite cell number, fiber type, and the degree of atrophy. Perhaps the notion that sarcopenia is due to a simple exhaustion of the satellite cell pool or reservoir is overly simplistic. Notwithstanding, sarcopenia must be an important component of change in the bone-muscle interface.

8 CNS and the Bone Muscle Interface

Brain-derived neurotrophic factor (BDNF) plays important roles in neuronal differentiation/ survival, the regulation of food intake, and the pathobiology of obesity and type 2 diabetes mellitus. BDNF and its receptor are expressed in osteoblasts and chondrocytes. BDNF in vitro has a positive effect on bone cells; whether central BDNF affects bone mass in vivo is not known. Camarino et al. examined bone mass and energy use in brain-targeted BDNF conditional knockout mice (Bdnf(2lox/2lox)/93) [56]. The deletion of BDNF in the brain led to a metabolic phenotype characterized by hyperphagia, obesity, and increased abdominal white adipose tissue. Central BDNF deletion produced a marked skeletal phenotype characterized by increased femur length, elevated whole bone mineral density, and bone mineral content. Polar moment of inertia and cortical thickness were markedly increased suggesting a role for this neurotropic factor on the periosteum as well as the trabecular and cortical skeleton.

The effects of the sympathetic nervous system on bone have recently been explored and may be important during aging since several investigators have suggested there is an increase in sympathetic tone with advanced age [57]. Beta adrenergic activation of receptors on the osteoblast causes uncoupled bone remodeling such that formation is suppressed and resorption is increased within the bone marrow milieu and trabecular skeleton. The effects of adrenergic activity on the periosteum are not known, although nerve fibers are present in this highly vascular environment. However, in one model of chronically elevated SNS activity, the misty mouse, age-related changes in cortical bone were extremely pronounced. Cortical thickness was markedly reduced at 72 weeks vs wild type age-matched controls, as was trabecular bone volume [58]. Interestingly, periosteal expansion with aging did not occur in these mice leading to a much thinner bone during aging with enhanced skeletal fragility. Whether sympathetic tone prevents periosteal expansion as a compensatory mechanism during mammalian aging requires further investigation.

9 Research Directions: Musculoskeletal Aging as a Determinant of Healthspan

Aging is a physiologic process that affects the entire organism, including the musculoskeletal system, through the pillars related to healthspan. There is the direct impairment in bone formation and the acceleration in resorption that occurs over time in virtually all mammals primarily as a result of changes in the stem cell pool, as well as chronic inflammation, and greater accumulation of reactive oxygen species. There is a secondary increase in periosteal formation in response to bone loss albeit not to the degree that matches an increase in medullary expansion. There are also indirect cell non-autonomous effects in the aging animal including enhanced sympathetic tone, changes in the parathyroid/vitamin D axis, impaired renal function, and gonadal deficiency. Coincident with the aging skeleton, muscle mass is also declining and its function is reduced. As discussed above, the bone-muscle interface plays a critical role in modulating skeletal loading as well as cell signaling. Future research should start by more fully delineating how each of the pillars that compose the aging process affect bone, muscle and the interface between the two.

One major thrust should be in defining how the periosteum could be resistant to several of the determinants that impair healthy aging and its relationship to sarcopenia. Although the periosteal envelope can expand with aging, it is unclear whether the signals for that arise from the muscle, the bone matrix, from other bone cells or from an enhanced sensitivity to loading. One limitation is that studies of the periosteum have been relatively limited due to the difficulty in isolating the progenitor cells and studying them ex vivo. Even if models were developed to study the bone-muscle interface, we still do not know whether its expansion has any impact on muscle function. On the other hand, we know that by increasing periosteal surface tension, biomechanical properties improve or at least stabilize in the face of endosteal resorption. In that same vein, delineating the communication network between osteocytes (mechanical sensors) and the periosteum will be essential for defining age-related periosteal effects. A more important question is whether the periosteum is protected from several critical determinants that define aging; i.e. metabolic dysfunction, accumulation of ROS, excess mitochondrial autophagy, and cell senescence in the stem cell pool. A focus on the Foxo proteins during aging provides the first clues as to some of the protective mechanisms inherent within the cell that may be operative during aging.

Another important aspect of the bone muscle interface lies in the remarkable gender differences in the periosteal envelope across all ages. This parallels the differences in muscle mass and bone size that is observed between males and females, suggesting that there is always a factor based on size that determines the musculoskeletal mass. But it is not clear whether periosteal osteoblasts differ between males and females, and if aging has a selective effect (positive or negative) on the ability of these bone-forming cells to expand and lay down collagen.

Sarcopenia is a huge clinical problem because of the falls that result from muscle weakness. It is uncertain how progressive but modest muscle loss directly affects the skeleton and in particular the periosteum. Targeted therapy with myokine agonists or antagonists are soon to be developed for frailty, yet we know little about the mechanisms at the bone-muscle interface. Finally, the CNS plays a critical role in the maintenance of both bone and muscle. How the SNS modulates periosteal tone and muscle mass is a major question. Understanding the role of neuropeptides at the bone-muscle interface provides another targeted area for research, particularly with aging. For example, Linder et al. have shown that loss of a peptide Cthrc1, causes accelerated age-related bone loss, low energy expenditure and reduced muscle strength in C57BL6J mice (V Lindner, 2015, personal communication). Remarkably, Cthrc1 is highly expressed in the pituitary and hypothalamus and circulates in measureable quantities. Hence, CNS signals, whether they be neurotropic or hormonal, can profoundly affect the muscle-bone interface.

The new discipline of Geroscience attempts to merge the physiology of aging with an understanding of the pathophysiology of age-related diseases and the delineation of the pillars that define age-associated disorders. We can no longer afford to study major organ systems in isolation with age, and a major thrust for future studies will be in defining regulation of the bone-muscle interface and the downstream consequences that result from impairment in either tissue.

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Osteoporosis and Mechanisms of Skeletal Aging

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1 Clinical Aspects of Osteoporosis

Osteoporosis is a generalized skeletal disorder in which decrease in bone mass and deterioration of bone quality lead to bone fragility and increased risk of fracture. Osteoporosis is primarily a disease of the elderly, with more than 70 % of fractures being sustained by those 65 or older [1]. In fact, the risk of fractures increases exponentially with age [2] (Fig. 1). Fragility fractures, also termed osteoporotic fractures or low trauma fractures, occur when falling from a standing height during usual physical activity [3]. Fractures result from an interaction between bone strength and the mechanical force applied to it, usually during a fall. Younger individuals may experience fragility fractures when they have diseases or take medications that have harmful effects on bone. However, bone strength is influenced by bone quantity (mass) and bone quality, both of which decrease with age, thus leading to an increase in fragility fractures among the elderly. In addition, elders have an increased risk for falls, which further contributes to increased fracture incidence.

1.1 Scope and Significance of the Problem

In 2005 there were more than two million fractures in the US [1] and this number is likely to increase due to the increasing age of the population. Because the risk of osteoporotic fractures increases with age [2], this population growth will likely result in increased numbers of fractures and associated health care costs. Osteoporotic fractures result in significant morbidity, mortality, and reduced quality of life [3]. The three most common osteoporotic fractures are hip, vertebral, and wrist. [3] Hip fractures are the most devastating type of fracture in terms of both personal suffering and healthcare costs. Hip fractures are associated with increased mortality, loss of independent living, and decline in functional status [4–6]. Vertebral fractures are also associated with increased mortality as well as kyphosis, impaired breathing and digestion due to reduction in thoracic and abdominal cavities, loss of self-esteem due to change in appearance, depression, fatigue, and decreased activity [7–10]. A study of US Nationwide Inpatient Sample between 2000 and 2011 reported that among women aged 55 and over there were 4.9 million hospitalizations for osteoporotic fractures, compared with 2.9 million hospitalizations for myocardial infarctions, three million for stroke, and 700,000 for breast cancer [11]. Osteoporotic fractures accounted for nearly 50 % of hospitalizations among women 75 years and older. Although the hospitalization rates for all other diseases declined during this 11 year observation period, the rate of hospitalization for non-hip fractures actually increased [11].

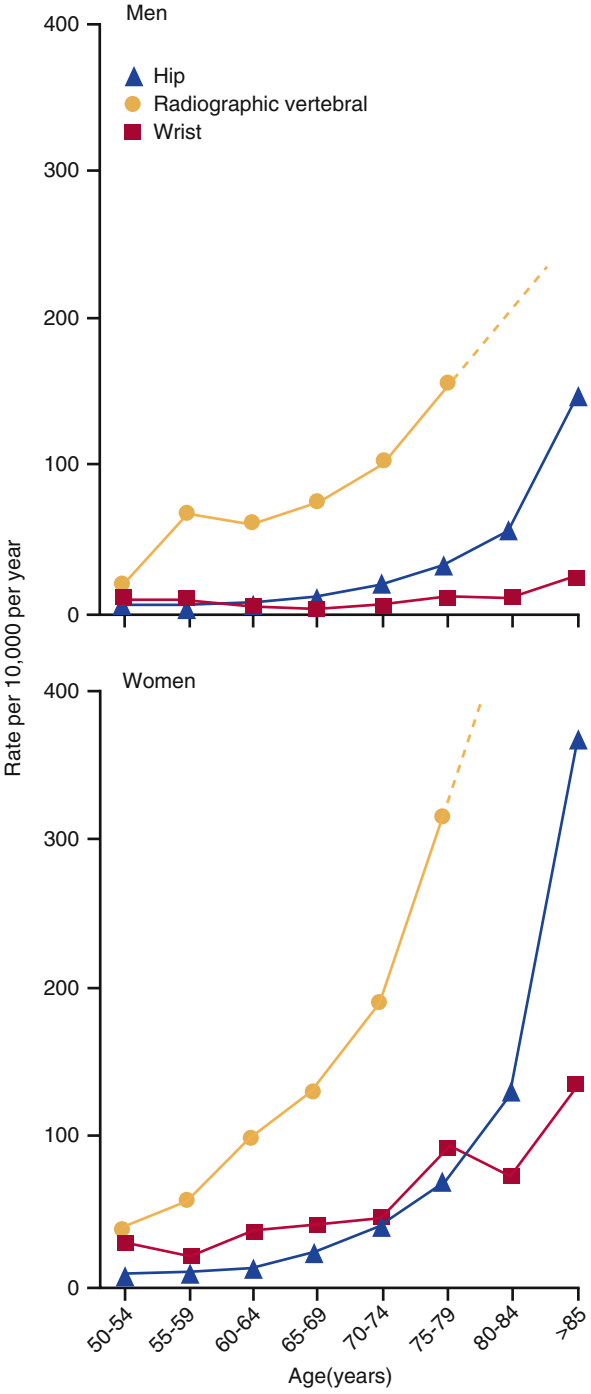

Fig. 1 Increase in fracture incidence with age for men and women. From [2]

1.2 Epidemiology of Fractures

Examining the differences in fracture rates between different populations may provide insight on biological and environmental factors influencing fragility. Fracture risk increases with age in all populations studied [2] and women have approximately twice as many fractures as men although female-to-male ratios vary depending on the skeletal site of fracture and the geographic region (Fig. 2) [12]. There are significant geographic, racial, and ethnic differences in fracture rates, the reasons for which have not been clearly identified. Although some of these differences may be due to under-reporting of fractures in countries with less developed medical care, there are probably true differences in fracture risk that are due to genetic as well as environmental factors. The best-studied geographic differences are for hip fracture rates because those fractures are most likely to be reported accurately (Fig. 2). Age-standardized rates of hip fractures reported from over 60 countries around the world vary by over 200-fold in women and 140-fold in men [12]. The highest reported rates are for Northern America and Europe, followed by Asia, the Middle East, Oceania, Latin America, and Africa. Even within the same continent there are significant differences between countries. For example, in Europe, Norway reported 532 and Poland only 173 hip fractures per 100,000 person years, a 4-fold difference [12]. Similarly, in the Middle East, the rates of hip fracture are 8 times higher in Iran than in Tunisia (Fig. 2).

In contrast to hip fractures, vertebral fractures do not show as much geographic variability. This is particularly true for morphometric (radiographic) vertebral fractures, which have similar prevalence in studies from different regions of the world [12–14].

The reasons for geographic disparity in fracture rates are not clear [12]. It is likely that genetic differences account for at least some of the observed disparity. Additional factors that may be involved relate to socio-economic status, life expectancy, health expenditures, and urbanization, all of which are higher in nations with higher fracture rates [12]. In addition, differences in physical activity, diet, vitamin D status, and hormonal factors are likely to be involved but have not been well studied to date. Finally, regional differences in fall risk have been reported and may contribute to differences in fracture rates [15, 16].

The most peculiar observation regarding geographic differences in fracture rates is a recent finding of hip fracture rates increasing in the east (China) while decreasing in the west (Western Europe, North America, and Oceania) [17]. The reasons for these trends are not clear. Decreasing fracture rates in the west may be due to increasing body weight (which is usually associated with higher bone mass and also may provide more mechanical cushioning when falling on the hip), decrease in unhealthy behaviors such as smoking, increased use of therapies for osteoporosis, or a cohort effect where later generations had better nutrition in utero and during childhood resulting in higher peak bone mass. The increasing fracture rates in China are equally puzzling. Some of the possible explanations include better reporting but also the adoption of western life-style that may lead to unfavorable changes in

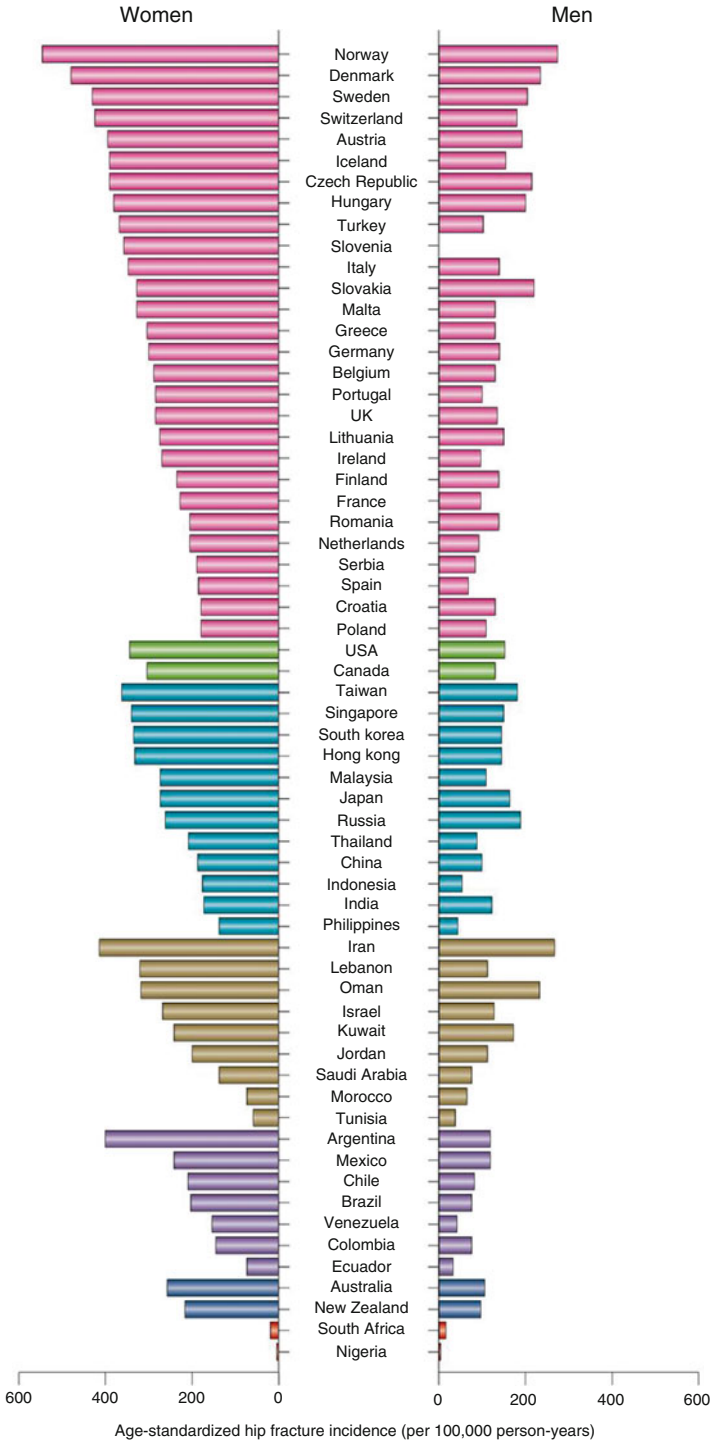

Fig. 2 Geographic differences in age-adjusted hip fracture rates. From [12]

activity level. Increasing urbanization and employment in sedentary occupations are associated with decreased physical activity, sitting on chairs rather than on the floor, use of western style toilets rather than squatting, all of which may result in decreased muscle strength and higher fall risk.

1.3 Pathogenesis and Evaluation of Osteoporosis and Fractures in the Elderly

Even in the elderly, peak bone mass is one of the most important determinants of fragility [18]. Peak bone mass is accrued during childhood and adolescence and those with low peak bone mass will be at an increased fracture risk later in life, such as is the case with those who develop eating disorders, use medications (glucocorticoids), or have diseases that affect bone during their formative years. Bone mass in the elderly also depends on the magnitude of bone loss after peak bone mass is achieved; those rates differ between trabecular and cortical bone, and between men and women [18–20]. Women have a more pronounced rate of loss during early menopause [18], which together with lower peak bone mass results in greater risk of fractures observed in elderly women [1, 6]. Compared with women, however, elderly men have worse fracture outcomes both in terms of mortality [4, 21] and recovery of their functional status [22, 23]. Thus osteoporosis in elderly men has received more attention in recent years with several professional associations providing guidelines for management of male osteoporosis [24, 25].

Assessment of bone mass is usually performed using DXA (Dual Energy X-ray Absorptiometry) of the lumbar spine and proximal femur, with osteoporosis defined by the World Health Organization as a T-score of −2.5 or below (at least 2.5 standard deviations below the mean of young Caucasian women) and severe osteoporosis as the same T-score with one or more fragility fracture [26].

Although bone mass can be estimated by DXA, bone quality, which also contributes to fragility and deteriorates with age, is more difficult to assess, the only available methods being QCT and MR. The effect of bone quality on fragility is well illustrated by the fact that at any level of bone density, fracture risk increases with age and with a history of prior fractures [27]. Fracture probability decreases in the very oldest, as a result of competing probability of death in that population, and this is one of the reasons for inaccuracies of risk estimates in geriatric studies [28]. Although bone quality cannot be directly measured in live patients, its effect on fragility can be indirectly inferred through the use of FRAX algorithm (http://www.shef.ac.uk/FRAX/). This approach has resulted in improved stratification of fracture risk by combining Bone Mineral Density (BMD) measurements with clinical risk factors (most important being age, prior fracture, and weight) [29]. Although there is universal agreement that fracture risk assessment in the elderly should include clinical risk factors, the exact method of using FRAX to derive treatment thresholds differs between countries. For example, the USA guidelines suggest pharmacologic therapy for individuals with BMD evidence of osteoporosis, or with history of hip

or vertebral fractures [3]. For those with osteopenia (intermediate risk) based on BMD, therapeutic decisions rely on FRAX-derived fracture probability [3]. In contrast, the UK approach is based on FRAX-defined treatment thresholds with BMD testing only recommended if a risk estimate is borderline [30].

Prior fracture is predictive of future fracture even when controlling for age and BMD. Vertebral fractures in particular are important in this regard because they are most strongly associated with the risk of future fractures [31–33], and their association with fragility is under-estimated by FRAX [34]. Vertebral fractures, however, are clinically apparent in less than 1/3 of subjects [35], and thus require spine imaging for their detection by radiographs or Vertebral Fracture Assessment (VFA) [36]. Because vertebral fracture prevalence increases dramatically with age [6], most of the elderly evaluated for osteoporosis would be candidates for VFA or other spine imaging [3, 36–38].

Finally, the increase in fracture risk with age is related not just to increasing bone fragility, but also to increased fall risk, which is not included in the FRAX model. Falls are common in the elderly with 30–50 % of populations over 65 falling at least once per year and 15 % falling 2 or more times per year [15, 16]. Among community-dwelling individuals over 85 years of age, annual incidence of falls is over 50 % for women and around 33 % for men [39, 40]. Because falls account for 86–95 % of osteoporotic fractures [16], understanding the determinants of falls in the elderly is very important for management of osteoporosis on both individual and population levels. Falls are at least in part due to decreased muscle function which together with weight loss, self-reported fatigue, slow walking speed, and low physical activity are components of the frailty syndrome [41, 42] (as discussed in detail in Chap. 2). Frailty is associated with increased fall and fracture risk [43–45] and thus represents an important therapeutic target in geriatric medicine. It is notable that although frailty increases with age, the effect of frailty on falls and fractures is largely independent of chronological age [45] as evidenced by the fact that the association between frailty and falls or fractures was observed within each age group.

1.4 Management of Osteoporosis in the Elderly

1.4.1 Nutrition

Dietary advice should ensure adequate caloric and protein intake, low sodium (because high sodium increases urinary calcium excretion), high intake of fruits and vegetables (because this tends to produce alkalinizing effect which decreases bone loss), and adequate amounts of calcium (usually dairy products but other sources such as fortified foods are also good choices). Nutritional deficiencies are common in the elderly [46] and particularly in women with osteoporosis. Many osteoporotic women have a life-long history of eating disorders, or at least obsession with thinness, and often limit their intake of nutrients, which is particularly detrimental in advanced age. It is important, therefore, to obtain dietary history from elderly patients, both in term of quantity of food and its composition. Protein-energy

malnutrition is a risk factor for sarcopenia and frailty [47, 48]. This effect is at least in part due to low IGF-I, a hormone with anabolic effects on multiple organs, including bone and muscle [49]. Nutritional assessment through dietary history or use of a validated instrument [46, 50] should form the basis for appropriate changes to the diet and consideration of protein supplementation [47, 51–53].

Even in the absence of nutritional deficiency, risk of osteoporotic fractures is higher in thin individuals – they have smaller bone size, lower BMD, often lower postmenopausal estrogen level, and possibly less "cushioning" that could prevent fractures when falling. More recently however, it has become clear that while being moderately overweight might be beneficial to bone, extreme obesity does not protect from osteoporosis and may actually have negative effects on bone, possibly through increased inflammation [54–57]. When controlled for BMD, obese subjects actually have a higher fracture risk compared to lean controls. Furthermore, the positive association between BMD and weight is due to lean body mass, whereas most reports indicate no association between BMD and fat mass [58]. All of these new findings about the relationship of bone to body weight and composition suggest that for bone strength, as well as for many other aspects of health, there may be an optimal weight that provides adequate skeletal loading but prevents the negative effects of excessive fat mass.

Calcium intake from food sources and supplements should also be assessed as this information may reveal the reasons for bone loss and fractures and also forms the basis for prescribing the amount and type of calcium supplements that are appropriate for each patient. Dairy products are recommended because they provide highly bioavailable calcium, as well as protein. If diet alone is not a sufficient source of calcium (1000–1200 mg/day), supplements should be used to make up the difference [3]. There has been a recent controversy regarding the association of increased cardiovascular risk with calcium supplementation [59, 60]. Although this debate cannot be easily resolved, it should be remembered that, from the bone health perspective, deficient calcium intake, particularly if coupled with low vitamin D is harmful as it leads to secondary hyperparathyroidism [61] and increased bone resorption, which is particularly detrimental in the elderly. Proper counseling regarding the importance of sufficient calcium intake is therefore an essential component of osteoporosis management in the geriatric clinic.

Vitamin D sufficiency is also essential in the management of osteoporosis and fall prevention because vitamin D plays an important role in both bone strength and muscle function. Because vitamin D is not generally sufficient from natural foods (other than the liver of cold water fish), most people need to get it from sun exposure, food fortification, or supplements. Sun exposure is frequently limited in the elderly, particularly those who are ill, homebound, or institutionalized [62]. Furthermore, the efficiency of cutaneous synthesis of vitamin D_3 from 7-dehydrocholesterol due to sunlight exposure declines markedly with age, particularly in those greater than 70 y, and this appears to be the primary reason for vitamin D insufficiency in the elderly. Thus, most elderly and particularly those living in northern latitudes need to use vitamin D supplements to achieve adequate vitamin D levels. Although there is a controversy regarding the target level for 25-hydroxyvitamin D in the serum, most agree that it should be between 20 and 30 ng/ml [3].

The required dose of vitamin D depends on the starting blood level and on body weight. Because vitamin D is a fat-soluble vitamin it is distributed throughout fat tissue. Consequently, individuals with larger fat mass will require higher doses and longer duration of vitamin D replacement to reach the target blood levels.

1.4.2 Physical Activity

Evaluating physical activity, including both activities of daily living and formal exercise, is essential for assessing the patients' fall risk as well as developing an individualized program of strengthening exercise and fall prevention. Frailty is strongly associated with fractures [44, 45], likely through an association with low bone mass as well as an increased fall risk due to loss of muscle strength and poor balance. Tools that have been proposed for assessment of frailty [63] are useful, particularly in research studies. It should be remembered, however, that careful observation of the patient while getting up from a chair, climbing onto an exam table, and walking in the hallway will reveal a lot of information about frailty, strength, vitality, and fall risk that can be used for developing the "activity prescription" for each patient. Additionally, examining for kyphosis is very informative because kyphosis may point to the presence of vertebral fractures which signify high bone fragility. In addition, kyphosis per se is associated with frailty, increased fall and fracture risk, and increased mortality [9, 10, 64, 65] and may be targeted by appropriate exercise programs [66].

A proper activity regimen will increase overall strength and fitness while at the same time decrease the risk of falling [67–71]. Walking, and in fit elderly individuals even jogging, are activities that provide gravity stimulus to the bone and also increase the overall fitness. A tailored exercise intervention should improve muscle strength and core strength. Muscle contraction also has an anabolic effect on the bone. Proximal muscle strength and core strength improve balance and decrease the fall risk. Strength and balance interventions should be aligned with the fitness and personal preferences of each individual and can include physical therapy, working with a personal trainer, strength training classes, yoga, Pilates, or Thai Chi. Thai Chi has been shown to improve balance and prevent falls in frail elderly [72, 73]. Multimodality approaches such as the go4life program from the NIA (www.go4life.nia.nih.gov) are particularly helpful as they combine endurance, strength and balance training. In addition to a proper exercise regimen, fall prevention should also include modification of the home environment, treatment of other medical conditions and elimination of medications that may increase fall risk.

1.4.3 Pharmacologic Therapy

According to the National Osteoporosis Foundation (NOF) guidelines, medications for osteoporosis should be considered for those who have a T-score at or below −2.5 or have sustained hip or vertebral fractures. For subjects with osteopenia, NOF

recommends pharmacotherapy if their FRAX-derived 10-year probability of major osteoporotic fractures exceeds 20 % or hip fracture probability exceeds 3 %. All the available agents have shown a reduction in vertebral fractures in clinical trials but only some have documented efficacy in preventing non-vertebral fractures. Due to a lack of direct comparison trials, however, and the fact that registration trials recruited somewhat dissimilar populations, direct comparison of efficacy of different agents is not possible. As all fracture trials enrolled postmenopausal women, generally with a mean age between 68 and 75 years, pharmacotherapy seems to be efficacious among the elderly. Yet in most trials, there were relatively few subjects older than 80. Nevertheless, *post hoc* analysis of the existing trials have documented good anti-fracture efficacy of pharmacotherapy in those over 75 years of age [74–79].

In general, pharmacologic agents for osteoporosis are classified into antiresorptive and anabolic agents. In fact, however, bone resorption and formation remain coupled so that use of an antiresorptive drug (bisphosphonates, denosumab) results in suppression of both bone resorption and bone formation. Similarly, the only currently available anabolic agent, teriparatide stimulates bone formation but bone resorption appears to increase as well.

Bisphosphonates have been shown to be efficacious in older populations. Information regarding efficacy of alendronate in older women comes from *post hoc* analysis of FIT1 which showed a 38 % reduction in the risk of new vertebral fractures relative to placebo in patients over 75 years of age [74], and from a pooled analysis of FIT1 and FIT2 which showed similar risk reduction in new fractures across the ages 55–85 years [80]. A *post hoc* analysis of risedronate trials (HIP, VERN-NA and VERT-MN) showed a 44 % reduction in vertebral fractures in women 80–98 years of age [77]. Finally, a *post hoc* subgroup analysis of the two zoledronic acid trials (HORIZON and HORIZON Recurrent Fracture Trial) showed anti-fracture efficacy for women age 75 and older [75].

Other classes of therapeutic agents used for osteoporosis are also effective in the geriatric population. Subgroup analysis of women ≥75 years from the teripartide trial demonstrated a reduction in vertebral fractures (by 65 %) as well as non-vertebral fractures (by 25 %) compared with placebo [76]. *Post hoc* analysis documented fracture benefit of denosumab in women over 75 [81], and an analysis of pooled data from two strontium trials showed a significant reduction of both vertebral and non-vertebral fractures in women between 80 and 100 years [79].

Despite the evidence of its efficacy, pharmacologic therapy is underutilized in the elderly, although they have the highest fracture risk and need this therapy the most. The reasons for that are not completely clear but include poly-pharmacy, erroneous belief that fractures are a natural consequence of aging rather than disease, fear of medication side effects and perhaps, an assumption that pharmacologic agents will not have enough time to exert a benefit due to limited life expectancy in the old. However, several trials have clearly documented that fracture benefit is demonstrable in 1 year or less [77, 82–88] suggesting that even those with life expectancy of just 1–2 years would benefit from therapy. Some of the novel agents or combinations being considered may be particularly useful in geriatric populations [92].

It would seem logical that a choice between an antiresorptive and anabolic agent would be based on baseline bone turnover. In practice, however, bone turnover is not routinely assessed or used in making the therapeutic decisions. Although several biochemical markers of bone turnover have been developed and approved by the FDA, current practice guidelines do not support using turnover markers for selecting the appropriate therapy [89–91]. This is due to analytic and biological variability in the levels of these markers as well as lack of data regarding the ability of the baseline marker levels to predict the response to therapy. There is no consistent effect of aging on bone turnover markers – they increase significantly with menopause but decline thereafter. Furthermore, the increase in bone turnover markers observed in the elderly in some studies may be due to decreased renal function which increases levels of the markers that are cleared by a healthy kidney [89–91].

2 Age-Related Changes in Bone Tissue

2.1 Age-Related Changes in Bone

Studies of *in vitro* mechanical properties of bone show an age-related loss in yield strength and in peak strength, increasing the risk of fracture [93]. The mechanical properties of milled samples of cortical bone decrease by 7–12 % per decade in fracture toughness [94]. Bone mass as measured by DXA declines with age and contributes to mechanical instability [18]. Other factors contribute to the fragility of bone, however, independent of bone mass or volume [95]. The aging of human bone can be described at multiple hierarchical levels, from the molecular to microarchitectural to gross changes in shape and form, each of which is detrimental to fracture resistance [96]. Bone extracellular matrix is composed of approximately 35 % organic matrix, or osteoid, by dry weight and 65 % inorganic mineral, a highly substituted carbonato-calcium phosphate. As a biphasic material, bone has tensile properties attributable to the organic collagen fibers and has compressive strength and rigidity attributable to the inorganic crystals. Each component is affected differently by aging.

2.1.1 Bone Organic Matrix

Collagen is a protein that accounts for 90 % of the organic matrix of bone tissue. The self-assembly of the linear collagen molecules into fibrils provides tensile strength to bone tissue; therefore the mechanical properties of bone are influenced by collagen biochemistry. Post-translational modifications and divalent and trivalent intermolecular crosslinks (pyridinoline and deoxypyridinoline) are important aspects of collagen synthesis in bone. Abnormalities of collagen structure can arise from genetic mutations or can be induced by lathyrogenic agents [97]. In osteogenesis imperfecta, for example, mutations in collagen's amino acids can result in the

formation of branched fibers that result in brittle bone. When the enzymatic formation of intermolecular crosslinks is inhibited by a lathyrogen, such as β-aminopropionitrile, found in sweet peas, bone strength and mechanical performance decrease.

It is known that aging bone is characterized by modifications in collagen by denaturation [98] or non-enzymatic glycation [99]. In contrast to the beneficial effects of enzymatic crosslinks on collagen structure and bone's material properties, the non-enzymatic crosslinking of collagen that occurs with aging and some diseases leads to bone's mechanical deterioration. The predominant source of glycation end products (AGEs) is endogenous, but there are also exogenous sources, such as foods and tobacco smoke. A study with healthy twins established that a genetic effect accounts for 74 % of normal variance in serum levels of a major AGE, *Nε-carboxymethyl* lysine (CML) [100].

AGEs in bone are of two types, with and without protein-protein crosslinking, and are formed spontaneously by glycation or oxidation. In spite of tissue turnover, there is an age-related increase in AGEs in most skeletal sites in humans [101]. Moreover, patients with osteoporotic fractures show significantly lower content of enzymatic cross-links and higher content of AGEs than non-fractured controls; the increase in AGEs occurs particularly in more highly mineralized, older regions of bone. In addition, AGEs are elevated in the serum of patients with osteoporosis [102].

2.1.2 Bone Mineral

The mineral phase of bone is best described as a highly substituted, poorly crystalline, carbonate-containing analogue of hydroxyapatite. Compared with pure mineral hydroxyapatite, chemical substitutions of its anions and cations in bone mineral result in a disarrayed lattice structure and a Ca/P ratio of less than 1.67. The most common ionic substitutions in bone mineral are carbonate, fluoride, citrate, pyrophosphate, chloride, magnesium, sodium, and potassium. Bone mineral is deposited as poorly crystalline carbonatoapatite and, with increasing age, there is an increase in its Ca/P ratio, an increase in crystal size, and a loss of substituting ions [103]. In ingenious studies, Boskey's team used Fourier transformed infrared microspectroscopy (FTIRM) to assess mineral composition and crystallinity within osteons of a diameter of approximately 150 μm in human cortical bone [104]. This approach provided powerful evidence of crystal maturation from the area of most recent mineral deposition adjacent to the Haversian canal to the oldest mineral on the periphery of the osteon. The data show a decrease in the Ca/P ratio and an increase in crystal size and order from the center to the periphery of an osteon. This conversion decreases the solubility of the mineral phase, a phenomenon that could have untoward consequences for mineral homeostasis if it were to continue unabated. Cement-like mineral is avoided under ordinary circumstances because of the normal turnover of bone's organic and mineral matrix that is achieved by the coordination of osteoclastic resorption and osteoblastic bone formation. Thus, bone remodeling can be

viewed, in part, as a process of matrix rejuvenation that is central for mineral exchange and homeostasis. Bone from older individuals is more mineralized than is younger bone, attributable to the incomplete remodeling of matrix and accumulation of larger, denser crystals of mineral [105]. Thus, changes in the nature of bone mineralization with age contribute to decreased fracture toughness [106]. This means that the bone becomes more brittle and less able to absorb impact.

2.1.3 Bone Microarchitecture

Human cortical bone tissue is organized as longitudinal osteons of concentric lamellae around Haversian vascular canals. Volkmann's canals connect Haversian canals and the bone surfaces. The process of internal remodeling removes portions of the matrix and lays down new generations of osteons while maintaining structural integrity, vascularization, and cellular viability within the tissue. With advancing age, there is an imbalance between the amount of bone resorbed and deposited. Thus, porosity increases as canals grow large and coalesce [107]. Mechanical strength decreases exponentially as porosity increases [108].

The age-related loss of bone mass results in loss of strength, but microarchitectural changes are additional critical determinants of bone quality and fracture risk. These changes occur in the trabecular or cancellous interior of bones and in the dense cortical shell. The fracture resistance of bone tissue depends on matrix composition and architecture, to a large degree at the levels of mineralized collagen fibrils, interconnecting trabecular plates, and cortical porosity. Histomorphometric analyses quantify parameters of skeletal architecture, such as trabecular thickness and separation of trabecular plates in cancellous bone. Investigations of bone quality in fracture and non-fracture subjects with equivalent BMD show the relative importance of microarchitecture on fracture risk. An important study of histological specimens from subjects with or without fracture but with equivalent BMD showed significantly poorer markers of trabecular connectivity in those with fractures [109]; that study also showed age-related declines in trabecular connectivity for both fracture and non-fracture groups. Non-invasive tools such as peripheral quantitative computed tomography (pQCT) have been developed to provide three-dimensional modeling of bone *in vivo*. They show sexual dimorphism in the effects of age on trabecular microarchitecture [110]. In BMD-matched women there are significant age-related reductions in trabecular connectivity; in men, there are reductions in trabecular number, spacing, and connectivity; and in both, those reductions were correlated to increased fracture risk.

2.1.4 Microcracks

Tissue fatigue is the progressive loss of strength and stiffness that results from repeated cycles of loading. It manifests as sharp-edge microcracks in Haversian bone, approximately 30–100 μm long. Microdamage accumulates in human bone

with age [111, 112]. Accumulation of even small amounts of microscopic tissue damage in human bone may have large effects on biomechanical performance [113]. There are several mechanisms that prevent microdamage from resulting in catastrophic failure; these entail crack arrest and bone turnover. The first is an advantageous feature of Haversian bone, in which crack propagation is attenuated by ultrastructural discontinuities in resorption spaces, at margins of osteons, and at lamellae. Thus, osteonal bone's microstructural features can act as barriers to arrest microcrack extension by blunting the crack tip or deflecting crack growth. The second mechanism is that bone remodeling repairs microdamage, but with aging, lower levels of turnover can retard repair and permit accumulation of microcracks [114]. Evidence indicates that microcracks in cortical bone occur in proximity to osteocyte apoptosis [115] and to sites of remodeling [116]. Although apoptosis of osteocytes is required for targeted remodeling, nearby non-apoptotic osteocytes provide RANKL to stimulate osteoclasts to initiate a resorption tunnel [117].

It is clear that linear microcracks stimulate local bone remodeling and repair by a mechanism that involves osteocytes even in rodents where cortical remodeling is typically not present. On the other hand, diffuse damage at smaller size scales, around 1 μm and less, may be repaired by a different mechanism and may not be an inevitable precursor of microcracks. With an *in vivo* rat ulnar model that introduces diffuse damage in tensile cortices without linear microcracks, Seref-Ferlenguez et al. provide convincing evidence of direct repair without remodeling [118]. This may occur by physico-chemical bridging with calcium deposition within the small gaps or with products of nearby osteocytes. The relative importance of remodeling and direct repair mechanisms in humans is uncertain in light of the fact that cortical remodeling occurs constitutively throughout the human skeleton.

2.1.5 Age-Related Changes in Bone Metabolism

Adult human bone tissue undergoes continuous renewal by a process of remodeling, in which bone-resorbing cells, the osteoclasts, degrade a quantum of mineralized matrix, after which bone-forming cells, the osteoblasts, invade and fill the voids with new organic and mineral components. This remodeling process occurs in foci and ensures the overall mechanical integrity of the skeleton while renewing the tissue, adjusting the bone architecture to mechanical forces, and repairing microdamage. These intrinsic cellular activities endow bone with the capacities of fracture healing, distraction osteogenesis (a surgical procedure to elongate bone with a device that slowly expands a healing callus), graft incorporation, implant fixation, and mineral homeostasis. By replacing mature mineralized matrix, remodeling provides new mineral that is less crystalline and more readily soluble to contribute to calcium homeostasis.

Histomorphometric evidence shows that the balance between bone resorption and formation is inadequate to conserve skeletal mass throughout the lifespan. One of the best established age-related changes in cancellous bone is the reduction in wall width [119]. The reduction is approximately one-third from young adulthood

to seniority and is the result of a reduction in bone formation rate. Changes in cortical bone are less well documented. It would be anticipated that age influences bone turnover markers, but many other factors such as immobility, fractures, renal and liver impairments, medications, and circadian and seasonal variations contribute to inconsistent observations in aging studies, especially for resorption markers [89]. In addition, those confounders challenge clinical decision-making; a better biomarker for status of bone metabolism is needed.

Nevertheless, there is evidence linking histomorphometric parameters of bone turnover to serum AGEs. In one study, dynamic parameters of bone formation and static parameters of bone resorption were determined for osteoporotic and control women and men. Multiple regression analyses revealed striking correlations between serum AGEs and osteoporosis subgroups having increased bone resorption and, more specifically, with indices of osteoclast activity [102]. This is consistent with *in vitro* and animal studies showing that AGEs enhance osteoclastic bone resorption [120].

2.2 Age-related Changes in Skeletal Stem Cells

There is a growing body of information available about the effects of age on human skeletal stem cells. Distinctions can be made between those age-related changes that are caused by intrinsic cellular factors and those induced by the extrinsic somatic environment, e.g. by declines in circulating hormones or local cytokines. Marrow cells include osteoclast progenitors in the hematopoietic fraction and osteoblast progenitors called marrow stromal cells (MSCs) or mesenchymal stem cells in the adherent fraction. With marrow cells from subjects 27–82 years old, we reported age-related increases in *in vitro* osteoclast differentiation, in expression of receptors *c-fms* and *RANK* in osteoclast progenitor cells, in constitutive expression of *RANKL* with a decrease in *OPG* by MSCs, and a resulting increase in the *RANKL/OPG* ratio in elderly subjects [121]. There is also an age-related increase in pro-osteolytic IL-6 and IL-11 secretion by MSCs [122]. All of these can mediate age-related increases in bone resorption. It is notable that marrow samples from women being treated with an anti-resorptive bisphosphonate generated only 20 % the number of osteoclasts *in vitro*, as compared with marrow obtained from age-matched controls and showed a rejuvenated *RANKL/OPG* ratio [123].

Regarding osteoblast differentiation potential in human MSCs, we [124, 125] and others [126] showed an age-related decline in their differentiation to osteoblasts *in vitro*. Other important intrinsic properties of MSCs include age-related increases in senescence-associated β-galactosidase (SA β-gal), cell doubling time, apoptosis, as well as *p53* and its target genes, *p21* and *BAX* [125]. Upregulation of the *p53* pathway with age may have a critical role in mediating the reduction in both proliferation and osteoblastogenesis of MSCs. Thus, unlike pluripotent stem cells, as multipotent MSCs from bone marrow age, their intrinsic properties gradually become compromised. Effects of age on expression of WNT genes in MSCs show

gender differences - findings that resolved some of the discrepancies in the literature concerning constitutive expression of WNTs: we found age-related decreases in expression of WNT5A and WNT13 for women and in expression of WNT7B and WNT14 for men [127]. Both marrow-derived and adipose-derived MSCs show age-related differences in microRNA profiles, especially those involved in cell proliferation and inflammation [128]. MicroRNA analysis of MSCs from children and adults revealed miR-196a upregulation in adults that was inversely correlated with MSC proliferation through HOXB7 targeting [129]. Rejuvenation of proliferation and differentiation was achieved by forced overexpression of HOXB7 [129]. Other striking effects of age relate to deteriorating MSC responsiveness to osteotropic hormones. The age-related decline in parathyroid hormone (PTH) receptors and PTH signaling may contribute to cellular and tissue aging and suggests receptor-based approaches to restore sensitivity to osteoanabolic PTH [130]. The age-related decline in vitamin D-1α-hydroxylase/CYP27B1 in MSCs accounts for an age-related decrease in stimulation of osteoblastogenesis by 25-hydroxyvitamin D (25OHD) [131]. Both of those adverse effects of age were corrected by treatment with PTH, which upregulated CYP27B1 [131]. In fact, in MSCs from elders, PTH upregulated the vitamin D receptor and 25(OH)D upregulated the PTH receptor [132]. The synergistic effects of PTH and 25(OH)D to rejuvenate osteoblastogenesis in MSCs from elders has been shown to entail an epigenetic mechanism [132]. These findings support the hypotheses that vitamin D metabolism in MSCs serves an autocrine/paracrine role in osteoblastogenesis and that vitamin D sufficiency is important for skeletal health throughout the lifespan. Further, they suggest that different clinical regimens of combined PTH and vitamin D for osteoporosis may be needed to optimize their synergy to stimulate bone formation in elders and in those with chronic kidney disease [133]. Similar age-related losses in response to bone-active agents, such as IGF-I, have been detected in human osteoblasts [134].

2.3 Significance of Age-Related Declines in Osteotropic Hormones

With aging and decreased ovarian, testicular, and adrenal production of estradiol (E2), testosterone (T), and dehydroepiandrosterone (DHEA), subsequent changes in anabolic mediators such as IGF-I, and osteolytic factors like Interleukin (IL-6) may contribute to changes in bone metabolism. These are examples of extrinsic factors that change with age and affect skeletal cells. The unfavorable skeletal effects of the menopause and of male hypogonadism are well known, but the effects of age-related declines in serum T on bone mass are unclear and may be related to conversion of T to E2. We reported that with age, serum DHEA sulfate and IGF-I decline and serum IL-6 increases (Fig. 3); we further found that serum DHEA sulfate and IGF-I were correlated with bone density and that serum IL-6 was inversely correlated with femoral neck bone density [135]. A unifying hypothesis on the possible mechanisms of bone loss associated with age-related declines in sex steroids (Fig. 4) holds that their declines lead to a decrease in IGF-I and other factors that

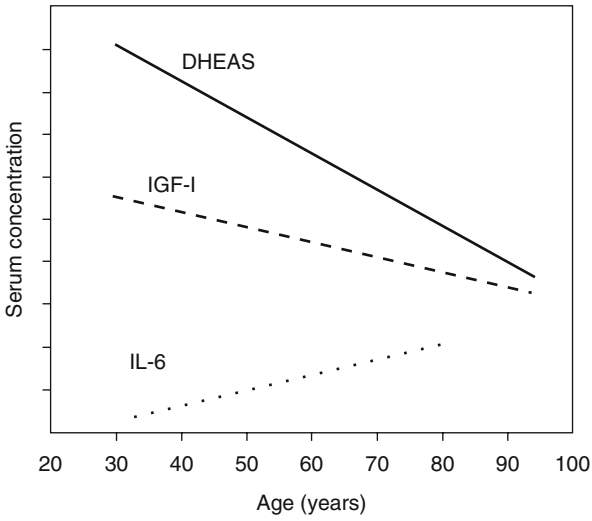

Fig. 3 Relative trajectories showing the effects of age on serum DHEAS, IGF-I, and IL-6 in healthy women (Adapted from [132])

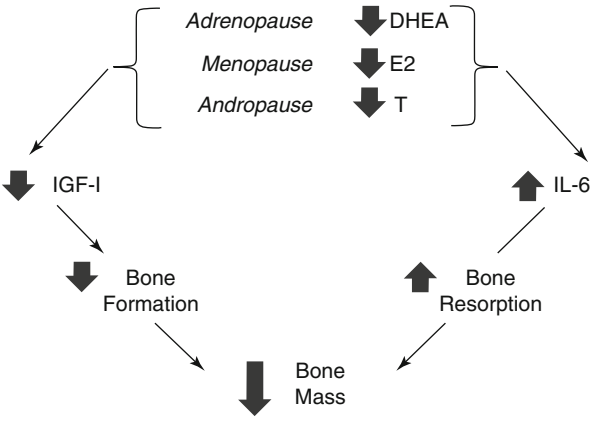

Fig. 4 An hypothesis on the mechanisms of human bone loss associated with age-related declines in sex steroids and intermediary cytokines

may contribute to decreased osteoblastic bone formation, as well as to increased cytokine mediators such as IL-6 and subsequent increased osteoclast formation and bone resorption. Each of these elements can be rejuvenated by sex steroids. For example, E2, T, and DHEA decreased secretion of IL-6 by MSCs from post-menopausal women to levels comparable to cells from young women [136].

3 Molecular and Cellular Underpinnings of Skeletal Aging – The Hallmarks of Aging

Lifespan and aging research with *C. elegans, Drosophila*, mice, and other species reveals major themes of interconnected aging processes that are common across species and organ systems and that help to establish potential interventions [137].

The themes also draw attention to mechanistic relationships between aging and certain chronic diseases. Some of these have been investigated in human MSCs in light of age-related bone loss, as discussed above, but others have received little or no attention regarding skeletal aging. The hallmarks of aging include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, inflammation, cellular senescence, and stem cell exhaustion [138]. A common theme emerges from examining these processes; they may be beneficial mechanisms that optimize normal tissue homeostasis, but under chronic circumstances may become harmful to the cells or environment. Understanding the relative roles of these mechanisms in skeletal aging may point to potential therapeutic targets [139].

3.1 Hallmarks of Aging: Genetic Mechanisms

Aging is associated with accumulation of DNA damage, as are numerous premature aging diseases, for example Werner syndrome. Because the premature osteoporosis in Werner syndrome has features that are different from osteoporosis in the general population, such as higher incidence of fractures in men than women, and earlier loss of cortical than trabecular bone [140], it may not provide a relevant model for natural skeletal aging. Progeroid syndromes are also characterized by mutations and deletions in nuclear and mitochondrial DNA; afflicted individuals tend to succumb to cancer.

Chromosomal regions called telomeres are not completely replicated by DNA polymerase and progressively become shorter because mammalian somatic cells lack the specialized enzyme DNA telomerase. Telomere attrition is linked to cellular senescence and aging of the organism. Overexpression of the human telomerase gene in human MSCs led to elongation of telomeres, extension of cellular proliferation, maintenance of stemness, and enhanced bone formation [141]. It was suggested that intermittent or transient telomerase activation may be a feasible clinical intervention.

3.2 Hallmarks of Aging: Epigenetic Alterations

Epigeneic alterations include DNA methylation, histone modifications, and chromatin remodeling. Methylation generally inactivates respective promoter regions and is maintained upon replication. Highly significant differences in methylation of specific CpG sites in homeobox genes, including *DLX5* which is involved in osteoblast differentiation, were found for MSCs from young and old donors. Both hypermethylation and hypomethylation sites were detected, findings that may explain why demethylating agents do not categorically control replicative senescence [142].

Changes in histones and chromatin occur during aging and senescence of human MSCs as well, notably in acetylation of H3, H4, and a set of miRNA regions, and in decreased HMGA2, a non-histone chromosomal structural protein that regulates cell cycle arrest proteins, $p16^{INK4A}$ and $p21^{CIP1/WAF1}$ [143]. There is rising interest in ways to reverse these changes in order to use MSCs for cell-based therapies [143, 144].

3.3 Hallmarks of Aging: Loss of Proteostasis

Many studies have demonstrated that aging is associated with disordered protein homeostasis and impaired degradation of damaged proteins by either the autophagy-lysosomal system or the ubiquitin-proteasome system [138]. Proteasome inhibitors have been shown to increase osteogenesis in mouse models [145]. There is some information about autophagy during MSC differentiation. Undifferentiated human MSCs show an abundance of undegraded autophagic vacuoles, but autophagic turnover occurs upon induction of osteogenic differentiation [146]. The relationship between senescence and autophagy is complex, with different models showing that autophagy either protects from senescence or triggers senescence. Although there is no literature on the effects of age on autophagy in human MSCs, there is some indirect information. Low dose irradiation of cultured human MSCs reduced their proliferation and upregulated SA β-gal while impairing autophagy-driven apoptosis [147]. In those cells, low dose irradiation led to only a transient rise in apoptosis, followed by senescence of the surviving cells. Because senescent cells can secrete inflammatory cytokines and other detrimental factors (the so-called Senescence Associated Secretory Phenotype, or SASP), the accumulation of senescent cells is likely to be more damaging to the tissue than their removal by apoptosis. It is not known whether similar events occur in MSCs with aging or irradiation *in vivo*. A growing body of information about the functions of autophagy in various mouse bone cells provides a basis for future aging research [148].

3.4 Hallmarks of Aging: Nutrient Signaling Pathways

Research on calorie restriction to extend the lifespan of model organisms showed the pivotal roles of energy-sensing pathways that are regulated by insulin/IGF-I, sirtuins, TOR, and AMPK signaling [138]. Those mediators also interact in complex ways and have different effects at different set points, but are likely to contribute to skeletal aging [149, 150]. The growing understanding of cross-talk between bone and fat [151] and between bone and muscle [152] may provide new approaches to improve health of the aging population.

3.5 Hallmarks of Aging: Accumulation of Oxidative Stress Damage

The mitochondrial free radical theory of aging holds that progressive mitochondrial dysfunction results in increased production of reactive oxygen species (ROS), which further damage mitochondria and tissues. Recent research challenges the role of antioxidant defenses in longevity, but highlights the positive, negative, or neutral effects of ROS, depending on context. [138] Nevertheless, considerable evidence indicates a deleterious role of ROS in skeletal aging [153]. This is likely mediated by several pathways, including activation of p53, FOXOs, and NF-κB, and downstream decreases in osteoblastogenesis with increases in bone resorption [139]. Agents as diverse as estrogens, androgens, and intermittently administered PTH exert direct antioxidant effects on bone cells, shown to contribute to their bone-protective actions [154]. In contrast, common antioxidants have not been shown to have enduring beneficial effects on aging bones, but newer agents such as mitochondria-targeting compounds may have potential [155, 156].

3.6 Hallmarks of Aging: Inflammation

"Inflammaging" is the term used to describe the chronic pro-inflammatory phenotype that accompanies aging in mammals and is responsible for altered tissue activities [138]. It has been recognized that inflammation exists as an underlying feature of aging as well as many common chronic age-related diseases such as diabetes, cardiovascular disease, obesity, and osteoporosis - disorders that are generally associated with inflammatory biomarkers like serum C-reactive protein and IL-6 [157]. Persistence of the DNA damage response contributes to systemic inflammation and activation of NF-κB. It has been shown that when AGEs, which accumulate in aging bone, bind to their receptors, RAGEs, in osteoblasts, they activate NF-κB and increase production of cytokines that inhibit osteoblast proliferation and matrix synthesis [158]. Likewise, repeated stimulation of cultured human MSCs with lipopolysaccharide induces markers of senescence, reduced somewhat by silencing $p16^{INK4A}$ [159]. Thus, when present chronically, the damage response networks that normally facilitate repair and survival can compromise tissue homeostasis and lead to cellular apoptosis and senescence.

The theme of "osteoimmunology" developed to focus on the link between bone disease and immune cell activity and concerns inflammatory cytokines such as TNFα and interleukins that stimulate osteoclastogenesis through both RANKL/RANK-dependent and -independent mechanisms. [160] Tissue macrophages, T cells, and other cells in the stem cell niche are involved in these events. With aging, the relative activities of pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages favor M1 and subsequent decrease in osteoblast differentiation and increased osteoclast differentiation [160]. This information helps to explain

how age-associated bone loss is linked to uncoupling of osteoblastic and osteoclastic activities in favor of bone resorption.

Cellular senescence is a beneficial response to damage and stress and prevents dysplasia and cancer by suppressing proliferation of compromised cells. With their persistence, however, damage and stress can alter the microenvironment by inducing the secretion of pro-inflammatory cytokines, a feature called the senescence-associated secretory phenotype (SASP) [157]. There is significant evidence that inflammation plays a key role in MSC senescence. Human MSCs display cell-intrinsic age-associated increases in pro-osteoclastogenic interleukins (IL-6, IL-11), p53, and phosphorylated NF-κB subunits p65 and p50 [122, 124, 125, 128]. In transwell experiments testing for paracrine interactions between human hematopoetic progenitor cells (HPCs) and MSCs, we found an age-related increase in gene expression of TNF-α in HPCs with no expression in MSCs [161]. In vitro treatment with exogenous TNF-α induced markers of senescence in MSCs from a 17-year-old donor, including increased SA-β-gal and reduced proliferation and osteoblast differentiation. Thus, these paracrine, or extrinsic effects of HPCs may contribute to skeletal aging by altering the environment for MSC differentiation to osteoblasts. There are no data about the SASP in MSCs except for a study with conditioned medium (CM) from early versus late passage MSCs. It was reported that tenth passage CM induced the full senescence response in early passage cells, that tenth passage CM contained more insulin-like growth factor binding proteins (IGFBP) 4 and 7, and that exogenous IGFBP4 and −7 each induced senescence and apoptosis in early passage MSCs [162].

4 Research Models for Human Skeletal Aging

Most of the information about mechanisms of skeletal aging summarized herein was obtained from human MSCs obtained from individuals representing a wide age range. Some of the research with those cells is motivated by their possible therapeutic use for age-related and other disorders [65]. To that end, ways to increase *ex vivo* proliferative and differentiation capacity of cells derived from the elderly become a paramount challenge. As anticipated from the Hayflick principle of replicative senescence, *in vitro* expansion of human MSCs activates markers of cellular senescence [144, 163, 164]. Furthermore, passaging *in vitro* is not the only way to induce cellular senescence; for example, treatment with TNF-α [161] or low dose gamma radiation [165] induces senescence in human MSCs. Although these are convenient ways to study cellular senescence, their findings need to be confirmed with cells from subjects across the lifespan.

Mouse models provide insights into lifespan research and the genetics of aging. There are several Senescence Accelerated Mouse (SAM) strains with shortened lifespan and acceleration of different age-associated disorders, including early development of osteopenia [166]. The klotho mouse displays a phenotype similar to human progeria and osteopenia, regulated by a gene called α-klotho [167]. There

are more than 700 mouse strains available for research [168], with documentation of great differences in skeletal physiology, metabolism, gender dimorphism [169], and bone healing [156]. Specific differences in skeletal metabolism between mice and human are known, including growth, architecture, regulation and, for example, the role of bone-derived undercarboxylated osteocalcin in glucose metabolism in the former [170]. It is striking that C3H/HeJ mice are characterized by the highest values for all bone parameters, like bone mineral density, while C57BL/6 J mice have the lowest. [169] Nevertheless the C57BL/6 strain is routinely used for research on skeletal physiology and aging [170]; this raises concerns about the generalizability of findings observed in this extreme case. Other concerns about the general relevance of mouse models arise from strain differences noted in calorie restriction (CR) experiments to extend lifespan. CR does not have beneficial effects on longevity in all strains of mice and, in fact, decreases lifespan in many [171–173]. A recently recognized example of species dissimilarity is the inflammatory response of mice and human to sepsis [174]. The absence of osteonal remodeling in rodent bone may limit applicability of some findings to human pathology [175]. Nevertheless, the ovariectomized rat is an often used model to screen drugs for potential efficacy for post-menopausal osteoporosis [176].

As shown for the hallmarks of aging derived from research with diverse species, it remains an advantage to use different models to develop and test approaches for reducing the disabilities associated with human aging.

5 Future Prospects

Skeletal aging has devastating consequences due to the resulting decreased mobility and increased fracture risk. Approaches to prevent osteoporosis would have significant public health benefits. A major challenge in pharmacotherapy of osteoporosis has been the inability to uncouple bone formation and resorption. Accordingly, antiresorptive agents, which are aimed at inhibiting bone resorption, eventually also suppress bone formation while teriparatide, the only anabolic agent currently in use, stimulates not just bone formation but resorption as well. Fortunately, some of the novel agents that are currently in clinical trials hold a promise of having overcome this limitation [92], and may be particularly useful in the geriatric population. Although these advances in target-specific therapy for osteoporosis are very encouraging, it should be remembered that on a societal level the reduction in fractures in the elderly require attention to skeletal health and fitness through the entire lifetime, including improved awareness, detection, and treatment of osteoporosis in the aging population, and more wide-spread interventions for fall prevention. The growing understanding of cross-talk between bone and fat [151] and between bone and muscle [152] may provide new approaches to improve health of the aging population.

As described in this chapter, there are many voids in our knowledge about the relative roles of the hallmarks of aging as they apply to age-related loss of bone

mass and increased risk of fracture in humans. It is not known how approaches designed to mitigate other chronic diseases will affect skeletal aging. It is not known how interventions designed to extend lifespan will influence skeletal aging. It is not known whether simple, inexpensive interventions like vitamin D and anti-oxidant-rich diets can diminish the rate of skeletal aging in large populations. It is not known whether or to what degree agents successful in mice are transferable to humans [141, 149, 155, 156, 177]. Several lines of evidence indicate that the decline in stem cell function during aging can involve both cell intrinsic and extrinsic mechanisms. It is not known, however, whether models of induced *in vitro* senescence or *in vivo* aging correspond with natural processes. As discoveries made from research in cell regulatory mechanisms translate to aging research, as with long noncoding RNAs for example [178], new approaches for extending the skeletal healthspan may emerge.

Research to understand the mechanistic basis for the influence of genetics on skeletal aging may yield approaches to promote healthy skeletal aging in those without the genetic advantage. Continued progress in understanding hallmarks of aging in model organisms can be tested in vertebrate species. Broader development of animal models to maximize their value for human skeletal aging research will enhance understanding and potential interventions for age-associated diseases. Use of discarded human tissue can be effective to assess clinical relevance of information gained from model species. Merging of geroscience with osteoporosis research has the potential to allow for early intervention to maximize skeletal health throughout the lifespan.

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Osteoarthritis in the Elderly

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Contents

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1 Introduction

Osteoarthritis (OA) is a quintessential condition of aging. It is a slowly progressive disease of synovial joints characterized pathologically by focal destruction of the articular cartilage, a hypertrophic response in neighboring bone that results in osteophyte formation and subchondral sclerosis, variable degrees of synovial inflammation, a thickening of the joint capsule, and damage to soft tissue structures including ligaments and, in the knee, the meniscus [1]. Gross pathological changes seen in the femoral condyles of the knee joint are shown in Fig. 1. The joint tissue changes result in failure of normal joint function that is accompanied by pain and disability. OA most commonly affects the distal joints of the hands, the knees, hips, and the spine. Often called degenerative joint disease and referred to by certain practitioners and the lay public as "wear and tear arthritis", it has been considered by some to be an inevitable consequence of aging of the articular joints. However, as with many of the other chronic conditions associated with aging, this is an oversimplification of what turns out to be a multifaceted condition that cannot be explained by simple age-related degeneration of the joints.

Recent work has determined that the pathophysiology of OA is much more complex than what might be construed by the term degenerative joint disease.

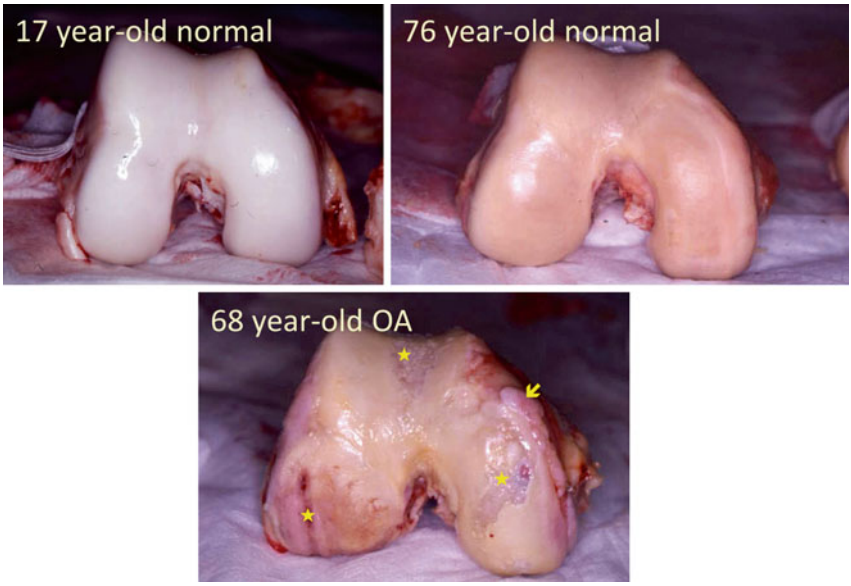

Fig. 1 OA and aging related changes in human knees. Images of the femoral condyles showing the white glass-like surface of normal cartilage in a knee from a 17 year-old. The cartilage from a macroscopically normal 76 year-old joint shows a change in cartilage color (*browning*), mainly due to advanced glycation of cartilage matrix proteins. Joint from a 68 year old shows characteristic OA-related changes, including erosions of original cartilage (*asterisks*) and formation of osteophytes at the joint margin (*arrow*)

There is evidence for the activation of a number of inflammatory pathways within the tissues affected by OA that appear to be the mediators responsible for driving joint tissue destruction [1, 2]. The finding that inflammatory factors play a major role in OA and that it is not simply a mechanical failure of the joint suggest that osteoarthr*itis*, which implies inflammation, is indeed the proper name for the condition rather than degenerative joint disease or "osteoarthr*osis*". It is also becoming clear that the multiple risk factors for OA likely follow different pathways to cause disease despite findings that end-stage OA appears to be pathologically similar in most joints.

Arthritis is a common cause of disability in older adults. Data collected from the Centers for Disease Control and Prevention indicates that arthritis and related conditions are the number one cause of disability in U.S. adults [3]. With the aging of our population, the prevalence of arthritis (of any type) in the United States is expected to rise from 47.8 million in 2005 to 67 million by 2030 with greater than 50 % of the cases of arthritis being in the 65 and older age group [4]. OA is by far the most common form of arthritis in adults affecting over 27 million Americans [5] and was recently ranked in the top ten of the diseases contributing to years lived with disability in the US [6]. OA is similarly prevalent worldwide where it is also a leading and rising cause of chronic disability [7]. Decreasing the prevalence of arthritis, including OA, was calculated to lead to a greater reduction in disability than similar reductions in coronary artery disease, stroke, cancer, diabetes, or dementia combined [8].

The prevalence of OA and its negative effects on physical function renders OA as an important co-morbidity in older adults, particularly in those who also suffer from cardiovascular disease [9]. In fact, having symptomatic OA has been associated with an increased risk of all-cause mortality with a standardized mortality ratio of 1.55 [10]. In that study, the strongest associations of OA with condition-specific mortality were with cardiovascular and dementia-associated mortality. The association with cardiovascular mortality can be attributed to the limitations in physical activity due to pain in weight bearing joints affected by OA which has been shown to contribute to reduced exercise capacity accompanied by all of the negative effects of physical inactivity [11, 12]. In addition, there is evidence that musculoskeletal pain from conditions including OA, increases the risk for falls [13] which may explain the association with dementia-associated mortality. Finally, knee OA has been associated with a greater risk for frailty in older adults [14].

These studies suggest that the development of interventions targeting common mechanisms underlying the chronic conditions of aging should include pain and disability from OA as important outcome measures. This chapter will briefly review the key risk factors and clinical features of OA, focused on the associations with age, as well as review the current management of OA, which is limited by the lack of interventions targeting the disease process. This will be followed by a more in depth discussion of the pathophysiology of OA and how it relates to the major hallmarks of aging. Future research directions and prospects for therapies will also be presented.

2 Clinical Presentation and Risk Factors for OA

The vast majority of people seeking medical care for OA do so due to joint pain. The pain most often begins insidiously and is chronic but with periods of waxing and waning. Other signs and symptoms include morning stiffness of less than an hour, impairment in function, joint crepitus with movement, and with more advanced disease, bony enlargement of the affected joints. When these signs and symptoms are present in the knee joint, close to 100 % of individuals will exhibit radiographic evidence of knee OA [15].

It is important to note that the pain associated with OA does not always correlate with the severity of disease noted by plain radiographs. Recent studies suggest that the poor correlation may be due to individual differences in central sensitization [16]. Also, standard radiographs can only detect structural changes in radiodense tissue, which is mainly the bone. The typical radiographic changes therefore include osteophytes, which are bony protuberances at the joint margins, joint space narrowing, which occurs due to cartilage loss, sclerosis, which is due to thickening of the subchondral bone, and sometimes bone cysts. Some older adults may also exhibit chondrocalcinosis which is calcification in the cartilage and, in the knee, the meniscus. Magnetic resonance imaging (MRI) is able to detect changes in soft tissues not seen by radiographs, including focal cartilage loss, synovitis, meniscal tears or degeneration, ligamentous disruption, and bone marrow lesions. Involvement of the tissues seen on MRI other than cartilage, which lacks nociceptive nerve fibers, can be an important source of pain, which also explains why standard radiographic changes do not always correlate well with symptoms.

OA occurs most commonly in the hands, feet, knees, hips or spine and may be present in a single joint (monoarticular), several joints (oligoarticular) or in multiple joints (polyarticular). Monoarticular OA is most commonly associated with a prior joint injury. Although OA of the ankle is uncommon, very severe OA can occur in ankles that have experienced significant trauma such as an ankle fracture [17]. This is also true for joints less commonly affected by OA such as the elbow and shoulder. Polyarticular OA is sometimes called generalized OA and most commonly affects the hands along with involvement of the knees and/or hips. This form of OA has been thought to have a stronger genetic component [18]. Although the genetic contribution to the overall risk of OA is estimated to be about 40 %, large genome wide association studies have found that any individual gene associated with OA has a very small relative risk in the 1.1–1.4 range [19, 20]. These studies suggest that OA may be polygenetic and that environmental factors likely interact with genetic factors to increase the risk for OA.

Clinical risk factors for OA have been well characterized (Fig. 2) with age being the strongest risk factor. Numerous studies have documented an increase in radiographic and symptomatic hand, hip, spine, and knee OA with increasing age (reviewed in [21]). The incidence of symptomatic knee OA is highest between the ages of 55–64 years where it has been calculated to range from 0.37 % per year for non-obese males to 1.02 % per year for obese females [22]. In a community-based

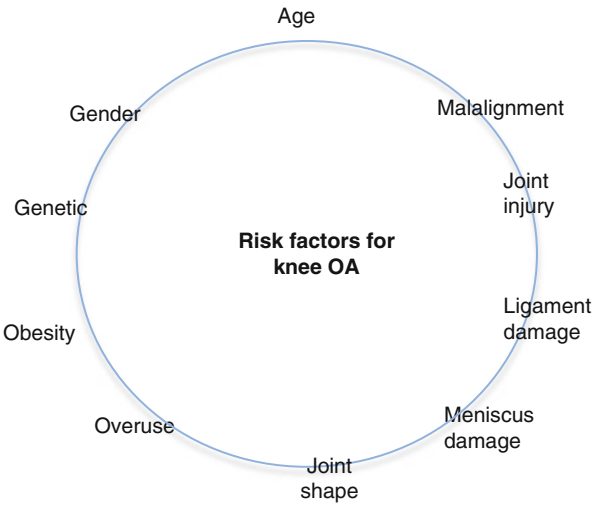

Fig. 2 Risk factors for knee OA. There are multiple predisposing factors that contribute to the development of OA either alone or in combination. These risk factors apply to other joints as well, except for meniscus damage, which is specific for OA in the knee since other joints do not contain menisci

longitudinal study in the UK, the prevalence of radiographic knee OA increased from 13.7 % at baseline, when the median age of participants was 53 years, to 47.8 % when the cohort was evaluated 14 years later [23]. Likewise, a large community based study based in the US found that about half of the participants developed symptomatic knee OA by age 85 [24]. Age is also a risk factor for a more accelerated course of OA, particularly in older adults suffering a new injury, where advancement to severe OA was seen within a year [25].

Despite the strong correlation between age and the incidence and prevalence of OA, not all older adults develop OA and not all joints are equally affected [21]. In addition to age, there are a number of other OA risk factors including obesity, prior joint injury, gender (hand and knee OA are more common in women), genetics, and anatomical factors that include joint shape and for knee OA, lower extremity alignment [26] (Fig. 2). In a meta-analysis of risk factors for knee OA in adults 50 years of age and older, the most consistent risk factors and their pooled odds ratios were obesity (OR 2.6), previous knee trauma (OR 3.86), hand OA (OR 1.49), and female gender (OR 1.84) [27]. Older age was also a risk factor but it was not possible to calculate a pooled odds ratio due to the heterogeneity in how ages were reported.

Obesity is a risk factor not only for knee OA [24, 28] but also for OA of the hips [29] and hands [30, 31]. The association of obesity with hand OA, which is not a weight bearing joint, suggests that systemic factors related to obesity and excess adipose tissue contribute to the development of OA. Adipose tissue is a source of numerous cytokines and adipokines, including IL-1, IL-6, TNFα, and leptin that have been purported to have a role in OA [32, 33]. The systemic metabolic changes associated with obesity may also contribute to OA and there is some evidence of links between OA and other obesity-related metabolic conditions including hypertension and diabetes [34–36].

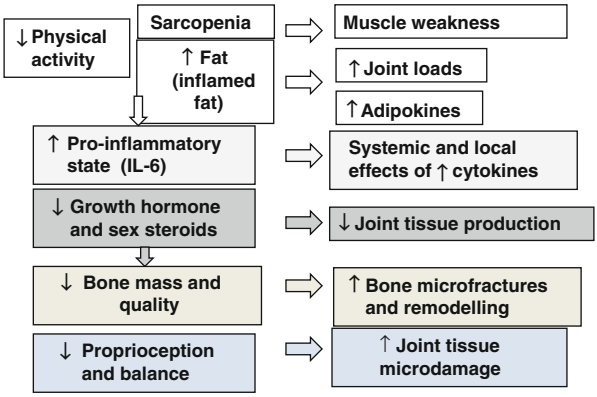

Fig. 3 Systemic aging changes that may relate to the development of OA. There are a number of changes that occur with aging, shown on the *left*, which could contribute to the development of OA. Decreased physical activity and sarcopenia resulting in muscle weakness can result in increased joint loading since muscles are important shock absorbers for the joint. The age-related increase in inflammatory fat can produce adipokines, such as leptin, and cytokines, such as IL-6, that are thought to contribute to OA. The age-related decrease in growth hormone, accompanied by a decrease in IGF-1 as well as a decrease in sex steroids, contributes to reduced anabolic activity in joint tissues. Changes in bone mass and quality can promote development of microfractures in the subchondral bone accompanied by excessive remodeling and development of bone marrow lesions seen in OA. A decrease in proprioception and balance contributes to excessive or abnormal joint loading that increases tissue microdamage (Adapted from Loeser [309])

In addition to the systemic alterations in inflammation and metabolism that result from an age-related increase in subcutaneous and visceral adipose tissue, local changes in adipose tissue could also contribute to the development of OA in older adults (Fig. 3). An age-related increase in intramuscular fat in the quadriceps muscle has been noted [37] and increased intramuscular fat in the quadriceps has been associated with knee OA [38]. This fat could contribute not only to weakness that would increase the risk of knee OA but also production of local inflammatory mediators such as IL-6 [39]. There is also evidence that the volume of the infrapatellar fat pad, which communicates with the knee joint, may increase with age [40] and could represent a local source of the adipokines, adiponectin and leptin, as well as the cytokine IL-6 [41].

Given that OA is so common in the older adult population, it is also of interest to examine factors associated with absence of OA. In a community-based study of 90 year olds living in the city of Leiden in the Netherlands, the absence of radiographic OA in the hands, hips, and knees was seen in 16 % of the 82 participants and was most strongly associated with lower BMI [42]. In addition, none of the participants who were free of OA had a family history of nodal OA of the hand, suggesting favorable genetics, and absence of knee OA was also associated with being male. Surprisingly, a history of heavy occupational work had a 7.2 odds ratio of being free from OA. The reason for this association is not clear and differs from previous studies in younger cohorts that demonstrated heavy physical labor, in particular occupations that required knee bending while lifting heavy loads, was associated with increased prevalence of knee OA [43].

3 Current Therapies

The current management of individuals with OA is mainly symptomatic and includes non-pharmacologic interventions, such as exercise and weight loss, medications to reduce pain, and, in more advanced cases, joint replacement surgery [44]. A major limitation in the management of OA is the lack of any treatment proven to slow or stop the structural progression of the disease. This is a goal of current research efforts and an area where a better understanding of the contributions of aging to OA could be of benefit, assuming the aging aspects promoting disease progression are modifiable, as proposed by the Geroscience Hypothesis.

Numerous organizations have developed OA treatment guidelines, which have recently been reviewed [45]. The guidelines stress that the management of OA needs to be individualized to each patient's needs and should utilize multiple modalities. It is generally recommended that all patients should receive non-pharmacologic modalities that include exercise, weight loss, education and self-management, and, when necessary, assistive devices such as walking aids. Pharmacologic management most often begins with the use of simple analgesics such as acetaminophen. In those that fail a trial of acetaminophen, nonsteroidal anti-inflammatory drugs (NSAIDs) can be used, although it is important to recognize individuals at risk for side-effects such as gastrointestinal bleeding. The American College of Rheumatology has recommended that since older adults are at higher risk of GI bleeding, adults 75 years and older should use topical rather than oral NSAIDs [44]. Flare-ups of joint pain can be treated with intra-articular steroids while the use of intra-articular hyaluronans has been controversial because many studies have not shown significant benefit when compared to placebo injections [44, 45]. In patients with OA of the hip or knee, joint replacement surgery (arthroplasty) is recommended when other treatments have failed and the patient continues experiencing pain and disability that interferes with their desired activities [44, 45].

Treatments that would impact the OA disease process have been referred to as disease-modifying or structure-modifying therapies. There have been several clinical trials of agents thought to have the potential for disease-modification that have failed (reviewed in [46]). Among others, these have included various inhibitors of extracellular matrix degrading enzymes, bisphosphonates used to target bone, an inhibitor of inducible nitric oxide synthase to target inflammation, and glucosamine plus chondroitin sulfate thought to target cartilage breakdown [46, 47]. A major limitation to testing potential disease-modifying drugs is that the outcome measure considered to be the "gold-standard" for efficacy is the change in joint space width on standardized radiographs as a surrogate for cartilage loss. Research is ongoing to find more sensitive outcome measures with acceptable levels of reliability, such as newer modalities of magnetic resonance imaging, which could be used to better demonstrate disease or structure-modifying effects. However, some investigators have argued that if the biomechanical aspects of OA are not addressed, such as the misalignment discussed above, then potential disease-modifying therapies will

continue to fail [48]. The advanced stage of disease and heterogeneity of patient populations in regard to risk factor profile and disease mechanisms are also potential causes for failures of prior clinical trials.

4 Age-Related Alterations in Joint Tissues Relevant to OA

In established or radiographically detectable OA, all joint tissues are affected. The earliest changes appear to occur in the articular cartilage with a very close association to changes in subchondral bone. Aging-related changes in cartilage (Table 1) thus appear to be a key event in initiation of the disease process that subsequently involves the other tissues. In trying to understand mechanisms of aging that confer OA risk, cartilage has been the focus of research on joint aging.

4.1 Cartilage

Aging-associated changes in articular cartilage include reduced cartilage thickness, reduced cell density, cellular senescence with abnormal secretory profiles, impaired cellular defense mechanisms and anabolic responses [49]. The earliest changes in cartilage are enzymatic degradation of glycosaminoglycans and cartilage proteins, and loss of cartilage cells. These changes first occur in the cartilage superficial zone [50], which is exposed to shear and compressive forces during movement [51]. Cartilage cells respond to this early tissue damage with proliferation and transcriptional activation of genes involved in extracellular matrix remodeling and inflammation. Changes in extracellular matrix and cells clearly differ between aged and OA-affected cartilage. The major differences are cell depletion, reduced biosynthetic activity and senescence in aging cartilage versus cell activation and proliferation in OA cartilage [49].

Table 1 Aging-related changes in articular cartilage

Chondrocytes	Extracellular matrix
Telomere shortening	Glycation, glycoxidation
↑SA-βgal, p53, p21, p16	Carbonylation
↑Cytokine and MMP production	↓Hydration
Mitochondrial damage	GAG depletion
Oxidative stress/damage	Aggrecan size reduced
↓Growth factor response	Collagen cleavage
↓Growth factor production	Growth factor levels reduced
Autophagy defects	Calcification
Cell death	Amyloid

OA is associated with increased proteolytic activity in cartilage and synovial fluid [52], and increased degradation of collagen molecules [53, 54]. This degradation is accompanied by the loss of intrinsic cartilage fluorescence [55], especially around cells in the superficial zone. In OA, there is also a decrease in fixed charge density, due to degradation and loss of aggrecan [56].

With normal aging, there is a marked increase in the formation of advanced glycation end-products (AGEs) (Fig. 1), including pentosidine cross-links [57]. In addition to altering the biomechanical properties of cartilage, AGEs may interact with cell surface receptors including the Receptor for Advanced Glycation Endproducts (RAGE). RAGE has been detected on articular chondrocytes and there is evidence for an increase in RAGE levels in aging and OA [58]. Activation of chondrocyte RAGE has been shown to stimulate catabolic signaling pathways that result in upregulation of MMP expression and chondrocyte hypertrophy [59–61].

A highly prevalent change in aging cartilage is deposition of calcium-containing crystals, mainly calcium pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) [62]. In the human knee this cartilage calcification is primarily an effect of aging rather than OA and represents a precursor to increased fibrillation and OA rather than a result of OA [62]. In cartilage from patients with end-stage OA, calcification correlated with increased disease severity [63, 64]. CPPD deposition is due to increased pyrophosphate production by chondrocytes from aged cartilage [64]. The presence of calcium crystals produced by chondrocytes or released into the joint space from other tissues such as the meniscus and synovium may stimulate chondrocyte production of inflammatory mediators and extracellular matrix (ECM)-degrading enzymes and thus contribute to onset and progression of OA [65].

Amyloid deposition is a prevalent and as yet underappreciated aging-related phenomenon in cartilage even in the absence of generalized systemic amyloidosis. Almost all cartilage tissues that are removed during joint replacement surgery have Congo red positive deposits [66]. In a study of autopsy cases, there was a correlation between amyloid deposition and osteoarthritic changes [67]. The protein aggregates that are present in aging cartilage and their potential effects on cells and extracellular matrix remain to be elucidated.

Aging in human and mouse joints is also associated with a reduction in cartilage cellularity (reviewed in [68]). Diverse inducers of cell death have been proposed, including acute or chronic excessive mechanical loading, certain proinflammatory cytokines, ligands for death receptors and oxygen radicals. Consequences of cell death are immediate damage to the extracellular matrix through release of matrix degrading enzymes and inflammatory mediators. The reduced cell density may also impair the tissues' ability to maintain extracellular matrix integrity. Reduced cell density is most profound in the cartilage superficial zone, which contains the highest concentration of progenitor cells. The function of these cells in tissue homeostasis, consequences of their depletion and their role in the disease process are important topics for further research.

4.2 Bone

Articular cartilage and subchondral bone function biomechanically together as the osteochondral unit and under normal conditions transmit load pressure through the joints [69]. The morphology of the cartilage–bone interface or osteochondral junction is similar between different joints and includes the deeper non-calcified cartilage, the tidemark, calcified cartilage, the cement line, and the subchondral bone plate [70, 71]. Beneath the subchondral plate is the trabecular bone of the epiphysis, containing blood vessels, sensory nerves, endothelium and bone marrow [72].

In OA-affected joints, subchondral bone turnover is increased [73, 74] and this is related to abnormal activation and differentiation of subchondral osteoblasts [75, 76] together with increased osteoclast activity [77, 78]. The consequence of the increased subchondral bone remodeling process appears to be an increase in bone volume density. However, contrary to earlier assumptions, this altered bone is not harder or stiffer but rather, it is undermineralized [79, 80]. Changes in subchondral bone may be related to the stage of the disease process. In early-stage OA, the increased remodeling may lead to bone loss whereas in late-stage OA, remodeling decreases and subchondral bone density increases [81]. Bone from severe OA patients shows an increase of bone volume density and a reduction of its mineralization content [82, 83].

In addition to the bone remodeling in OA, there is also increased angiogenesis and vascular channels and sensory nerves invade from the subchondral bone into the calcified and non-calcified cartilage [84]. Increasing numbers of vascular channels or marrow cavities break the osteochondral interface and advance towards the tidemark in the OA joints [85]. Blood flow disturbances have also been documented in OA subchondral bone

The subchondral bone remodeling process is thought to lead to changes in signaling from bone to cartilage. Even in normal joints there is a measurable transport of solutes across the calcified cartilage, suggesting a potential cross-talk between subchondral bone and cartilage [85]. The increased subchondral bone remodeling in OA is associated with production of cytokines by bone cells and, together with changes in the barrier between bone and cartilage, supports the hypothesis that cytokines and growth factors released during subchondral bone turnover diffuse into the articular cartilage and change chondrocyte functions, contributing to disease progression [86].

While OA-associated changes in subchondral bone are well characterized, changes that are associated with aging in the absence of OA are difficult to ascertain as few studies of human OA pathology included controls without arthritis that were age matched. A comparison of knee joints from aged mice with mice that had surgically induced OA revealed that aged mice had a higher prevalence of blood vessels invading the calcified cartilage, and thinning of the subchondral bone and calcified cartilage layers, which was not seen in the joints subjected to surgical OA [85].

The temporal and mechanistic relationship of bone and cartilage change is of great interest. Changes in cartilage and subchondral bone during OA pathogenesis

are closely linked [71, 87]. There is evidence at multiple scales that this is the key region in the development or progression of OA, even though the disease onset might have been triggered by an entirely different phenomenon such as by ligament or meniscus trauma or abnormal joint shape [88]. Already during early stages of OA development, morphological and functional changes in the subchondral bone occur that affect articular cartilage. It has been suggested that changes in histomorphometric parameters of subchondral bone are secondary to cartilage damage and proceed deeper into subchondral bone with increasing cartilage degeneration. However, it has also been shown that cartilage loss or further degeneration could be predicted with, or related to, increased activity within the subchondral bone [89–91]. Thus, there is substantial evidence for interactions of the two tissues in disease initiation and progression.

The relationship between OA and systemic bone mineral density (BMD), as measured at sites distant from the affected joints, has been examined in epidemiological studies which consistently demonstrated that a higher BMD is associated with a greater risk of developing subsequent radiographic OA in large joints such as knee and hip and there is also a correlation between prevalent OA and BMD [73, 92, 93]. Despite the association with incident and prevalent OA, a consistent relationship between systemic BMD and OA progression has not been found [94, 95].

The abnormal subchondral bone metabolism in OA has been targeted in animal models and clinical trials through pharmacological approaches to prevent resorption and/or to increase mineralization. Anti-resorptive drugs such osteoprotegerin, bisphosphonates, strontium ranelate, calcitonin, cathepsin K inhibitors, and estrogen were successfully tested in animal models where modulation of bone changes was associated with a reduction in cartilage damage [78, 96]. Clinical trials have been performed on a subset of these drugs including bisphosphonates, vitamin D and calcitonin and they all failed to show significant disease or structure modifying activity [78, 97]. Encouraging results from a recent clinical trial in patients with knee OA of strontium ranelate, an osteoporosis drug that inhibits subchondral bone remodeling [98] await confirmation in additional studies.

4.3 Synovium

Synovium is a membrane-like tissue that lines the inside of the joint capsule and also covers tendons and ligaments as a synovial sheath. The outer layer, also termed subintima or stroma, contains adipose and fibrous tissue that is innervated and contains blood and lymphatic vessels. The inner layer, also termed intima or synovial lining, contains a higher density of macrophages, fibroblast-like cells termed synoviocytes, as well mesenchymal progenitor or stem cells [99]. Through synovial blood vessels the joint receives oxygen and nutrients. Lymphatic vessels are responsible for fluid clearance and the transport of macromolecules to lymph nodes [100, 101]. Synovial fibroblasts are a main producer of lubricants that are secreted into the synovial fluid and mainly provide protection against shear forces [102].

Aging-related changes in the synovium that occur in the absence of diagnosed OA are poorly characterized. Amyloid deposition, which is in most cases an aging-related phenomenon, was observed in OA synovium [103] and at least some amyloid proteins can activate synoviocytes [104]. An overall increase in the thickness of the synovial membrane is typically seen in patients with diagnosed OA (reviewed in [105]). This hyperplasia is due to increased ECM formation as well as increased cell numbers. Whether this is due to proliferation of resident cells or recruitment of cells from the blood is unknown. This is also accompanied by formation of new blood vessels [70]. Predominantly perivascular leukocyte infiltrations are observed. They are usually much less intense than in rheumatoid arthritis and contain macrophages, T and B lymphocytes and plasma cells [106–108]. The significance of these synovial changes is underscored by long-term observational studies that identified the extent of synovial inflammation as a risk factor for more rapid progression of structural damage, as well as joint pain [109–111].

It is thought that these synovial changes are secondary to cartilage damage, which results in the generation of cartilage fragments, including cleavage products of extracellular matrix components such as collagen, fibronectin or cartilage oligomeric protein (COMP), which activate synoviocytes and macrophages via damage-associated molecular pattern molecule recognition receptors or Toll-like receptors [105]. Chondrocytes also produce proinflammatory cytokines and growth factors that can activate synoviocytes and recruit inflammatory cells. Calcium containing crystals, which are primarily formed in cartilage and menisci but are also produced by OA synoviocytes [112], are also potent activators of synovial fibroblasts, likely through Toll-like receptors [113].

4.4 Menisci

The menisci play an important role in both tibiofemoral compartments through load distribution and shock absorption [114–118]. Macroscopic and histopathologic analyses demonstrated a strong association between meniscus damage and OA-like changes in cartilage. Normal appearing menisci are rarely found in knees with OA [114–118].

Human meniscal degeneration in OA-affected joints has been described in detail [119–124]. However, several characteristics appear to be specific to meniscus aging in the absence of significant OA. With aging, the meniscal surface often remains intact while distinct changes in matrix stain and cellularity are observed within the meniscal substance. Tissue fibrillation and disruption is first seen at the inner rim, which spreads to the articular surfaces of the meniscus over time, and progresses to total disruption or loss of meniscus tissue, mainly in the avascular zone [125]. This is in direct contrast to degeneration in articular cartilage, which almost invariably progresses from the surface inward.

Increased Safranin O staining is observed with meniscus aging and could represent a shift from a fibroblastic to chondrocytic phenotype during early degeneration. Biochemical data [126, 127] as well as gene expression studies [128] suggest an

accumulation of water-binding proteoglycans in aging and degenerating human menisci and these changes reflect an attempt at adaption or regeneration of the menisci [129, 130].

4.5 Ligaments/Tendons

Histological abnormalities in the anterior cruciate ligament (ACL) are highly prevalent in OA-affected knees and include cystic changes, disorientation of collagen fibers and mucoid degeneration [131]. Histological changes are much more severe in the ACL as compared to the posterior cruciate ligament in the same knees [132] and such age-related deterioration was not observed in the patellar tendon [133, 134]. However, histological changes in ligaments can precede cartilage histopathology [135]. Degenerated ACL were found in knees without cartilage degeneration. Also, knees with minimal cartilage degeneration can have moderate to severe ACL damage. These findings suggest that ACL degeneration can be initiated before or progresses more rapidly than cartilage degeneration, at least in a subpopulation of individuals [135].

4.6 Common Changes and Mechanisms in All Tissues

The concept that all joint tissues are affected in OA has been firmly established based on several different types of evidence [1]. There are several mechanistic changes that appear to be involved across the different tissues. Abnormal differentiation status of mesenchymal lineage cells is seen in cartilage where chondrocytes undergo hypertrophic differentiation and also show features of immature chondrocytes. In meniscus and ligaments, cells that are normally fibroblast-like express chondrogenic genes. Abnormal cell activation is also seen across joint tissues with increased expression of proinflammatory mediators, oxygen radicals and ECM degrading enzymes. There is also cell proliferation, even in cartilage, which normally has barely detectable levels of cell division. The stem cell-like populations that are present in all joint tissues also appear to be activated but instead of contributing to a successful repair response, they appear to participate in abnormal tissue remodeling and destruction. Elucidation of signaling mechanisms that mediate changes in all tissues has the potential to deliver more promising therapeutic targets.

5 Role of Major Hallmarks of Aging in OA Biology

Seven pillars or hallmarks of aging have been proposed [136], each of which has been identified to occur to some degree in joint tissues, with most work focused on the articular cartilage. These changes are summarized in Fig. 4 and will be discussed in detail below.

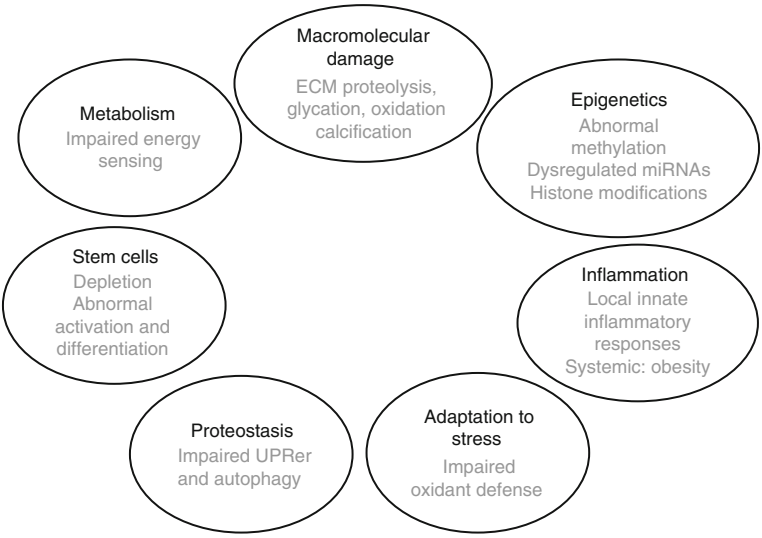

Fig. 4 'Seven pillars of aging' and the relationship to cartilage changes in osteoarthritis. Based on the recognition of conserved molecular pathways impacting aging, Kennedy at al. [136] proposed seven processes that are dysregulated in aging, emphasizing their connectedness. The figure summarizes OA and joint aging related changes in these 'seven pillars of aging'

5.1 Inflammation

A link between inflammation and OA has been made at both a systemic and a local level within joint tissues. The levels of proinflammatory cytokines, most consistently of IL-6 and TNF-α are increased in OA serum and synovial fluid and have been proposed as OA biomarkers [137, 138]. Epidemiologic studies have shown an association between serum levels of IL-6 as well as C-reactive protein (CRP) with knee OA, where higher levels correlated to risk of disease progression [139, 140]. In the Leiden cohort of 90-year-olds mentioned above, there was an association between the absence of knee OA and lower production of IL-1β and IL-6 when whole-blood samples were stimulated with lipopolysaccharide [141]. Levels of systemic markers of inflammation have also been shown to correlate with pain and function in older adults with knee OA. An increase in serum TNF-α and C-reactive protein (CRP) was associated with increased knee pain over a 5-year study [137]. Likewise, high levels of the soluble receptors for TNF-α as a surrogate for TNF activity were correlated with decreased physical ability in older adults with knee OA [142].

5.1.1 Cytokines

Cytokines are important signaling molecules that regulate cell function within and between joint tissues. Cytokines, both pro- and anti-inflammatory, anabolic and catabolic, as well as angiogenic and chemotactic have been studied for their role in

OA [143, 144]. OA-associated joint pain is at least in part mediated by cytokines such as IL-1, IL-6, TNF-α, FGF-2, NGF and certain chemokines [145, 146].

The production of cytokines by joint tissue cells is regulated by diverse extracellular stimuli, including other cytokines, enzymatic cleavage products of the extracellular matrix, and mechanical stress. Aging-related stimuli of cytokine expression in chondrocytes include advanced glycation end products [147] and amyloidogenic proteins [148]. Although some senescence markers are detectable in chondrocytes from older humans and increased expression of proinflammatory cytokines is a feature of the senescence-associated phenotype, a correlation between these phenomena in chondrocytes has not been established.

The group of cytokines that has been studied in detail for their role in OA is those that induce OA-like changes in chondrocytes. IL-1 is the prototypic catabolic and proinflammatory cytokine (reviewed [143, 144]). It affects extracellular matrix remodeling by suppressing the synthesis of cartilage-specific ECM components and by inducing the production of matrix-degrading enzymes. IL-1 also stimulates the production of other proinflammatory cytokines, prostaglandins, nitric oxide and other oxidants in chondrocytes and other joint tissue cells. Other members of the IL-1 family have similar effects, including IL-18 and members of the TNF family. Cytokines not only activate but also regulate the differentiation status of joint tissue cells. For example in chondrocytes, IL-1 suppresses Sox9, a main chondrogenic transcription factor [149].

Many proinflammatory cytokines have been implicated in causing changes in cartilage, bone and synovial tissue in spontaneous or surgically induced animal models of OA, and inhibition of these cytokines attenuated cartilage damage in animal models [144, 146]. However, attempts to modify the progression of human OA or joint pain in clinical trials with an IL-1 receptor antagonist protein (IRAP) or reduce pain with a monoclonal antibody directed against TNF-α have not been successful [150].

Anabolic cytokines, or growth factors, such as members of the bone morphogenetic proteins, transforming growth factors, insulin-like growth factors, fibroblast growth factors, platelet-derived growth factors, and connective tissue growth factor (CTGF) are produced in joint tissue cells and most are increased in OA cartilage. The function of some of these factors, such as BMP-7 or FGF-18 has been uniformly shown to promote ECM synthesis in chondrocytes and reduce the severity of OA in animal models. BMP-7 and FGF-18 have advanced to testing in clinical trials [151].

Members of the TGFβ family are important pro-chondrogenic factors. They stimulate chondrogenesis in mesenchymal stem cells, maintain the differentiation status of cultured chondrocytes and stimulate the production of ECM [152]. The chondrocyte response to TGFβ is altered in aging [153]. The abnormal TGFβ response of chondrocytes from older donors is due to changes in the ratio of TGFβ signaling components Alk1 and Alk5, which leads to a hypertrophic or pro-fibrotic phenotype and to degradation of the cartilage extracellular matrix [154, 155]. In mouse models of OA, TGFβ has been implicated in the formation of osteophytes [154].

In bone, active TGFβ1 is released during osteoclast bone resorption and recruits MSCs to form new bone at resorption sites [156]. TGFβ was elevated in OA sub-

chondral bone in human samples and various animal models [157] and was associated with early signs of OA including bone-marrow lesions [158]. High levels of active TGFβ1 induce clustering of MSCs/osteoprogenitors in the subchondral bone marrow and formation of marrow osteoid islets. Transgenic mice with expression of active TGFβ1 in osteoblastic cells developed OA, whereas knockout of the TGFβ type II receptor in MSCs reduced OA severity in a surgical model. Neutralizing antibody to TGFβ has thus been proposed as a possible therapeutic approach in human OA [158].

5.2 Oxidative Stress

Reactive oxygen species (ROS) are produced by most cell types in the body, not merely as a consequence of mitochondrial respiratory activity, but rather as a physiologic mechanism for the regulation of specific cell signaling pathways [159, 160]. The modern concept of oxidative stress emphasizes that it represents an imbalance in the production and removal of ROS that results in the disruption of normal redox signaling [161]. Chondrocytes have been shown to produce the expected reactive oxygen species (ROS) made by most cells that include superoxide, hydroxyl radical, and hydrogen peroxide, as well as reactive nitrogen species, including nitric oxide [162–164]. In the growth plate, ROS serve a physiologic function to promote chondrocyte hypertrophy [165], which may be relevant to a pathologic role in OA where chondrocytes exhibiting a hypertrophic phenotype have been noted [166].

There is evidence that oxidative stress can contribute to the development of OA, although much of the evidence to date is indirect [167]. Immunohistochemical studies of human, non-human primates, and mouse articular cartilage, have shown an increase with age and with OA in the oxidative damage marker nitrotyrosine [168, 169]. Nitrotyrosine forms when nitric oxide (NO) reacts with superoxide (O_2-) to form peroxynitrite (ONOO-), which in turn reacts with protein tyrosines to form nitrotyrosine. Increased levels of intracellular ROS have also been detected in cartilage from old when compared to young adult rats using the fluorescent probe dihydrorhodamine 123 [170]. There is *in vitro* evidence that increased production of mitochondrial ROS by chondrocytes, sufficient to promote cell death, can occur in response to excessive mechanical loading [171–173]. Given that ROS are key regulators of MMP production [174], an age-related increase in the levels of ROS may contribute to the increase in MMP activity seen in aging and OA. Indeed, ROS-mediated signaling that regulates catabolic pathways that include MMP expression has been noted in response to cytokines including IL-1β and TNFα [175–178] and to stimulation of chondrocytes by fibronectin fragments [179]. Potential mechanisms by which ROS can contribute to OA are summarized in Fig. 5.

Normally, the cellular levels of ROS are controlled by the balance of ROS production and the activity of various anti-oxidants. Glutathione is a major intracellular anti-oxidant and an age-related increase in the amount of oxidized relative to reduced glutathione was detected in chondrocytes isolated from normal human

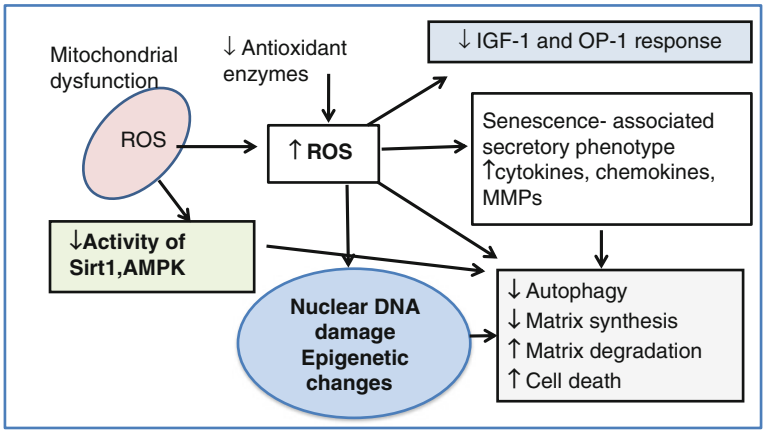

Fig. 5 Factors related to aging and oxidative stress in chondrocytes that may contribute to the development of OA. Oxidative stress is the result of an imbalance in ROS production, such as from mitochondrial dysfunction, and antioxidant enzyme function which disrupts normal cell signaling. This can contribute to reduced response to the growth factors IGF-1 and OP-1. Elevated ROS, particularly from the mitochondria, can also result in decreased Sirt1 and AMPK activity. These changes may promote the catabolic pathways associated with the senescence-associated secretory phenotype (SASP). Increased ROS can also cause nuclear DNA damage including telomere shortening as well as promote epigenetic changes. As a result of the various changes induced by elevated ROS, cells exhibit reduced autophagy and matrix synthesis and an increase in matrix degradation and cell death which all contribute to cartilage loss and OA

ankle tissue, consistent with an age-related increase in ROS [180]. The increased levels of ROS seen in aged and OA cartilage may also be due to reduced levels of anti-oxidant enzymes. Catalase and superoxide dismutase (SOD) were found to be present at lower levels with aging [170, 181] and in OA cartilage [182, 183]. Scott et al. also provided evidence for increased DNA methylation of the SOD2 promoter in human OA chondrocytes that might explain the reduced SOD2 levels [183]. SOD2 is primarily found in the mitochondria and SOD2 depletion in chondrocytes was associated with mitochondrial dysfunction [184].

Increased levels of ROS can result in DNA damage which has been noted in OA cartilage [185] including damage to mitochondrial DNA [186]. This can reduce cell viability [180] and matrix production [187] (Fig. 5). Oxidative stress can also contribute to the senescent phenotype of chondrocytes through damage to telomeres [188, 189]. Chondrocyte senescence has also been associated with increased production of oxidized low-density lipoproteins in cartilage [190]. As mentioned above, oxidative stress is associated with a disruption of normal redox signaling. Low levels of ROS are physiologic regulators of IGF-1 signaling but excessive levels may cause resistance to IGF-1, which has been noted in chondrocytes from older adults and in OA chondrocytes [191, 192]. There is growing evidence that excessive levels of ROS inhibit IGF-1 activation of the IRS-1-PI-3kinase-Akt pathway that promotes chondrocyte survival and matrix production [187, 193].

As additional support of a role for ROS in the development of OA, a low intake of anti-oxidant vitamins has been associated with OA progression in humans [194] and the use of several anti-oxidant vitamins along with selenium (a co-factor for the anti-oxidant enzyme glutathione peroxidase) was shown to reduce the development of OA in a mouse model [195]. The anti-oxidant N-acetylcysteine reduced cartilage destruction and chondrocyte apoptosis in a rat OA model [196] and in impact-loaded osteochondral explants [172] while the free-radical scavenger C60 fullerene was shown to reduce cartilage destruction in a rabbit model [197]. These studies suggest that further research into the mechanisms by which oxidative stress contributes to OA could result in the discovery of new approaches to modulate oxidative stress and slow the development of OA in older adults.

5.3 Epigenetics

Epigenetic regulation involves non-coding RNAs (ncRNAs), DNA methylation and histone modifications. As epigenetic changes are dynamic and responsive to environmental stimuli, their potential reversibility holds promise in understanding and therapeutically targeting disease mechanisms.

5.3.1 Non-coding RNAs

Among the ncRNAs, miRNAs are the most extensively studied in OA. Candidate miRs were selected based on differential expression in disease or during development for studies on cell function *in vitro* and in a limited number of cases using transgenic or knock out mice [198]. One study used a custom miR array to show that 7 of 723 miRs analyzed were differentially expressed in OA [199]. MiR-140 is the most abundant miR in cartilage [200] and its expression is reduced in OA [201]. miR-140 knock out mice showed age-related OA-like changes. Conversely, transgenic mice overexpressing miR-140 in cartilage were resistant to antigen-induced arthritis. Other miRNAs that regulate gene expression in chondrocytes are miR-125, miR-127b, miR-21, miR-148a and miR125 [202]. Upregulated miRs are potential drug targets that can be pursued by an increased availability of novel platforms to inhibit their expression or function [203].

5.3.2 DNA Methylation

OA-related methylation analyses examined a limited set of candidate genes. SOX9 expression is reduced in OA and subject to increased promoter methylation with associated repressive histone modifications [204]. A number of metalloproteinase genes including MMP3, MMP9, MMP13 and ADAMTS4 are upregulated in OA, and the promoters revealed single CpG demethylation events, possibly accounting

for the observed disease-related expression changes [205–207]. Similarly, demethylation of an NF-κB-responsive enhancer facilitates transcription of inducible nitric oxide synthase (iNOS/NOS2) in chondrocytes, a gene, which is dysregulated in OA cartilage [208]. Previously, IL-1β promoter methylation was found to inversely correlate with gene expression in chondrocytes, while leptin expression was also regulated by methylation [209, 210]. GDF5, which has a SNP associated with OA, exhibits altered methylation and reduced expression [211].

Several recent studies used methylation array technology to discover differences between normal and OA cartilage [212–214]. A large variation in differentially methylated regions was observed in these various studies and this is at least in part due to comparing OA articular cartilage from different joints without including proper control groups. Large differences were observed in methylation profiles in OA cartilage from hips compared to knees [215]. In addition, the functional consequences of differentially methylated genomes were assessed by transcriptional analysis of only a small group of genes of interest, and the resulting link between DNA methylation and transcription was not further validated.

5.3.3 Histone Modifications

Posttranslational modification of nucleosomal histones, including acetylation, methylation, phosphorylation and sumoylation, play important roles in the regulation of gene transcription through remodeling of chromatin structure [216, 217]. Histone acetylation and methylation are among the best-characterized modifications. Histone methylation occurs on both lysine (K) and arginine residues. In histone H3, different lysine residues (K4, K9, K27, K36 and K79) can be methylated. Histone lysine methylation is associated with either gene activation or repression, depending on the specific residue modified [218–220]. Methylation of histone H3 lysine 4 (H3K4), H3K36 and H3K79 is generally associated with transcriptional activation, whereas methylation of H3K9 and H3K27 is associated with transcriptional repression [218–220].

Lysine methylation is controlled by the opposing activities of lysine methyltransferases (KMTs) and lysine demethylases (LSD). The level of LSD1 expression was elevated in the superficial and middle zones of OA cartilage [221]. In cultured chondrocytes, the induction of mPGES-1 expression by IL-1β was associated with decreased H3K9 methylation at the mPGES-1 promoter. Inhibition of LSD1 prevented IL-1β -induced H3K9 demethylation at the mPGES-1 promoter and mPGES-1 protein expression [221].

Histone acetylation, which is generally associated with transcriptional activation, is dynamically regulated by histone acetyl transferases (HAT) and histone deacetylases (HDAC) which are classified into four classes; class I (HDACs 1, 2, 3 & 8), class II (HDACs 4, 5, 6, 7, 9 & 10), Class III which are also referred to as sirtuins (SirT1-7), and Class IV (HDAC11) [222]. There is some evidence of altered expression of HDACs in OA cartilage. HDAC1 and HDAC2 are increased in OA chondrocytes [223]. HDAC4 mRNA was lower in cartilage from OA patients when

compared with cartilage from healthy donors [224, 225]. HDAC4 was also lower in 40- to 60-year-old donors compared with specimens from 20- to 40-year-old healthy donors [225]. The reduction in HDAC4 was associated with increased Runx2 and other OA-related and chondrocyte hypertrophy-related genes in human OA cartilage, including MMP-13, Ihh and type X collagen [225]. One study found that HDAC4 expression was low in normal cartilage but increased in OA, most strongly in the chondrocyte clusters [226], which could explain the differences with the two prior studies.

In vitro studies using chondrocytes or cartilage explants generally showed protective effects of global inhibitors of class I/II HDACS such as trichostatin A (TSA) or sodium butyrate. These compounds inhibited metalloproteinase expression and protected against cartilage degradation [227]. Studies on individual HDACs showed that HDAC1 and HDAC2 overexpression suppressed transcription of cartilage anabolic genes such as ACAN and COL2A1 [223].

HDAC3 regulates chondrocyte hypertrophy and matrix content by inhibiting protein phosphatase Phlpp1 expression and promoting Akt activity. Chondrocytes lacking HDAC3 entered the hypertrophy stage sooner. Moreover, HDAC3 deficient chondrocytes had lower extracellular matrix production and smaller sizes than normal chondrocytes [228]. Inhibition of class I HDACs (HDAC-1, HDAC-2, HDAC-3) by the small molecule MS-275 or by specific siRNAs repressed cytokine-induced metalloproteinase expression in cartilage cells and cartilage explants [229].

HDAC4 has been extensively studied in OA, as it is a key regulator of chondrocyte differentiation during skeletogenesis, where it prevents chondrocyte hypertrophy by inhibiting the activity of Runx2 [230, 231]. HDAC4 overexpression in chondrocytes decreased the mRNA levels of Runx2, MMP1, MMP3, MMP-13, type X collagen, Ihh, ADAMTS-4 and -5, and increased type II collagen. Overexpression of HDAC4 also decreased IL-1β, Cox2 and iNOS and increased the expression of aggrecan, but also partially blocked the catabolic effect of IL-1β in human OA chondrocytes. Inhibition of HDAC4 by TSA had the opposite effect [225]. A challenge in using HDAC inhibitors is that their effects are qualitatively rather different as a function of duration of application. Short-term treatment of cells with HDAC inhibitors increased expression of cartilage ECM genes but prolonged treatment reduced expression of most of these genes [223, 232, 233]. Furthermore, toxicity of global HDAC inhibitors poses a major risk for use in chronic diseases such as OA. Specific inhibitors such as of class I HDACs may be a feasible OA treatment strategy.

In animal models, intra-articular injection of TSA in rabbits with experimental OA alleviated the extent of cartilage erosion, concomitant with reduced expression of IL-1 and matrix-degrading enzymes [234]. Systemically administered TSA also protected cartilage in the destabilized medial meniscus (DMM) model of surgically-induced OA [229].

Among the Class III HDACs, SIRT1 has been linked to aging and caloric intake, and also to various age-associated diseases such as type II diabetes, Alzheimer's disease and osteoporosis. SIRT1 activity or levels were altered in OA cartilage [235]. SIRT1 expression was clearly detected in the non-OA cartilage

while MMP-13 and ADAMTS-5 were undetectable. In contrast, in the OA cartilage, SIRT1 expression was decreased while MMP-13 and ADAMTS-5 were increased [236]. In human chondrocytes, SIRT1 plays a role in cartilage ECM synthesis and promotes cell survival, even under proinflammatory stress. Overexpression of SIRT1 significantly inhibited the up-regulation of those genes caused by IL-1β, while the inhibition of SIRT1 further increased them [237].

SIRT1 KO mice up to 3 weeks of age exhibited low levels of type 2 collagen, aggrecan, and glycosaminoglycan while protein levels of MMP-13 were elevated, leading to increased cartilage breakdown, that was evident even in heterozygous mice [236]. Additional results showed elevated chondrocyte apoptosis in SIRT1 KO mice [238]. The histological OA score was significantly higher in 1-year-old SIRT1-KO mice compared to control mice. In the surgical OA model, SIRT1-KO mice showed accelerated OA progression compared with control mice and this was associated with increases in type X collagen, MMP-13, ADAMTS-5, apoptotic markers, and acetylated NF-κB p65 {Matsuzaki, 2014 #244}. These observations indicate that SIRT1 is involved in cartilage biology and sirtuin activation could potentially serve as a drug target in treating OA even at its early stages [235]. Resveratrol, which among other effects increases the activity of sirtuins, was tested by intraarticular injection in a rabbit knee OA model. It reduced cartilage destruction, the apoptosis rate of chondrocytes and the level of NO in synovial fluid [239].

5.4 Autophagy

Autophagy is a cellular response to various types of stress and a central homeostasis mechanism to eliminate damaged organelles, long-lived or aberrant proteins, protein aggregates and superfluous portions of the cytoplasm [240]. Substrates are enclosed in a double membrane, the autophagosome, which fuses with lysosomes, allowing enzymatic substrate degradation. Cleavage products are recycled for use in biosynthesis or as energy sources [240]. Autophagy is required for lifespan extension in various organisms, and many autophagy-related proteins are directly regulated by longevity pathways [241].

Conceptually, autophagy in normal adult articular cartilage is an important mechanism for cellular homeostasis, in particular as chondrocytes in normal cartilage are undergoing very low levels of proliferation. Thus, cells in the superficial zone display a robust expression of autophagy proteins BECN1, ATG5, and MAP1LC3 [242]. When MAP1LC3 is tagged with GFP, the highest GFP signal is observed in cells present in the superficial and middle zones of the knee articular cartilage [242]. Few cells in the deep cartilage zone exhibit detectable levels of GFP-MAP1LC3 signal [242]. As with other tissues, starvation increases the number of autophagosomes in chondrocytes [243].

Cartilage aging in humans and mice is associated with reduced expression of autophagy regulators ULK1 (unc-51 like autophagy activating kinase 1), MAP1LC3, and BECN1, which is most profound in the superficial zone in the weight bearing

areas of cartilage [242]. Cartilage that is deficient in autophagy has reduced cellularity and extracellular matrix damage [242]. In the GFP-LC3 autophagy reporter mouse, basal autophagy activity was detected in young (6 months) liver and knee articular cartilage, with higher levels in cartilage than in liver in the same animals. In mice aged 28 months there was a reduction in the total number of autophagic vesicles. With increasing age, the expression of Atg5 and LC3 decreased, followed by a reduction in cartilage cellularity and an increase in the apoptosis marker PARP p85. Cartilage structural damage progressed in an age-dependent manner, subsequent to autophagy changes [244]. In contrast to the reduction in autophagic proteins in nonproliferating chondrocytes, the cell clusters in OA cartilage express high levels of these proteins [245], consistent with observations that certain chondrocytes in OA cartilage display numerous autophagic MAP1LC3 puncta [246, 247].

The reduction in autophagy protein levels and activity lends strong support to the hypothesis that basal autophagic activity decreases with age, thus contributing to the accumulation of damaged organelles and macromolecules and susceptibility to OA as an aging-related disease. Indeed, prior studies demonstrated mitochondrial dysfunction in various animal models and in human OA [248]. In addition, mitochondrial DNA mutations increase in OA chondrocytes [249]. Damaged mitochondria, producing high levels of ROS, promote pro-inflammatory signaling as they initiate formation of inflammasomes and activation of other inflammatory pathways [250]. In knee chondrocytes, IL1- or nitric oxide-dependent increase in expression of MAP1LC3 and BECN1 activates autophagy [251]. Furthermore, autophagy activation prevents IL1-mediated suppression of cartilage matrix degradation while reducing the levels of MMP13, ADAMTS5, and ROS. Given that one of the cytoprotective functions of autophagy is removal of damaged mitochondria, the IL1–induced OA-like gene expression changes might possibly occur through reduction in the intracellular ROS level via elimination of damaged mitochondria. Inhibition of autophagy caused OA-like gene expression changes, while the induction of autophagy by rapamycin reduced the MMP13 and ADAMTS5 expression induced by IL-1β [252].

These observations on autophagy defects in aging and OA-affected cartilage led to studies to test autophagy activators in OA models. A major focus of these studies was the protein kinase mammalian target of rapamycin (mTOR), which as part of the complex mTORC1, a key regulator and suppressor of autophagy. Excess mTOR activation has been linked to aging on the basis of results from genetic and pharmacological studies [253]. Major effects of mTOR are the inhibition of autophagy and the stimulation of protein synthesis. Chronic mTOR activation thus can potentially lead to increased accumulation of aggregation-prone proteins [254]. Senescent cells are enlarged or hypertrophic and these phenotypes as well as the abnormal gene expression can be reversed by the mTOR inhibitor rapamycin [255]. Abnormal mTOR signaling has been associated with autophagy to promote the secretory phenotype of senescent cells and the release of factors known to contribute to defective renewal and dysfunction of aging tissue [256].

The expression of mTOR mRNA and protein was increased in OA-affected human and mouse articular cartilage [257] (Fig. 6). The cartilage-specific mTOR

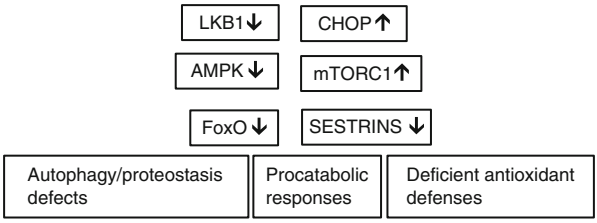

Fig. 6 Impaired nutrient and energy sensing in OA cartilage. Several recent findings on abnormal signaling mechanisms in OA appear to converge on AMPK and mTOR (mTORC1), which are central regulators of the cellular response to changes in energy and nutrient supply. Key abnormalities in OA are reduced AMPK activation and increased mTOR activation. Potential mechanisms involved in mTOR hyperactivation include deficient sestrin expression. Decreased AMPK expression and activation is related to reduced LKB1 expression and increased CHOP expression as a feature of the unfolded protein response (UPR) to endoplasmic reticulum (ER) stress. Dysregulation of these signaling mechanisms contributes to key changes in OA-affected cartilage, including defective autophagy and proteostasis, pro-catabolic (ECM-degrading and proinflammatory) responses and deficient anti-oxidant defenses

knock-out mouse showed reduced severity of OA induced by destabilization of the medial meniscus. This was associated with increased autophagy and decreased chondrocyte death [257]. Pharmacologic inhibition of mTORC-1 by rapamycin activates autophagy in chondrocytes and has protective effects against mechanical injury-induced ECM damage and cell death in cartilage explants [258]. In experimental OA in mice, rapamycin also reduced the severity of cartilage degradation and this was associated with a preservation of cartilage cellularity and a reduction in inflammatory mediators [259]. Intraarticular injection of rapamycin also reduced the severity of experimental OA in mice [236].

5.5 Damage to Macromolecules and Proteostasis

As detailed above, a hallmark of OA is degradation of extracellular matrix proteins that results in impaired joint tissue function. The most common age-related change in cartilage matrix proteins that may contribute to the development of OA is the accumulation of advanced glycation end products (AGEs) that are produced by the spontaneous nonenzymatic glycation of long-lived proteins such as collagen [57]. The role of AGEs in OA was discussed above in Sect. 4.1 on cartilage aging changes in OA.

Because articular chondrocytes are the only cell type present in cartilage and are therefore responsible for production and maintenance of the articular cartilage, they are required to synthesize large amounts of extracellular matrix proteins such as the collagens, proteoglycans, and cartilage oligomeric protein that may make chondrocytes susceptible to disruptions in proteostasis. For example, chondrocyte expression of mutant type X collagen was shown to induce the unfolded protein response

(UPR) and endoplasmic reticulum (ER) stress, resulting in pathologic changes in the growth plate including chondrodysplasia [260]. Protection from the deleterious effects of ER stress during chondrogenesis has been found to be, at least in part, due to activation of the basic leucine zipper transcription factor Bbf2h7 which up regulates Sec23a, a gene which encodes a protein that promotes protein transport from the ER to the Golgi [261]. Interestingly, the Bbf2h-Sec23a pathway was found to be under the control of Sox9, which is a master regulator of chondrogenesis [262]. Whether this pathway also may regulate ER stress responses in articular cartilage relevant to OA has not been studied.

There is growing evidence that disrupted bioenergy sensing and proteostasis may contribute to OA [263] (Fig. 6). The serine/threonine protein kinase AMP activated protein kinase (AMPK) is a master regulator of energy homeostasis. AMPK activity is constitutively present in normal articular chondrocytes, but is decreased in human knee OA chondrocytes [264]. Both IL-1β and TNFα induce a marked loss of AMPK activity in normal articular chondrocytes. Conversely, AMPK pharmacological activators attenuate cartilage explant and monolayer cultured chondrocyte procatabolic responses to IL-1β and TNFα [264]. These compounds also increased the NAD^+/NADH ratio and the expression of SIRT1, SOD2 and catalase. Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) and FoxO3A, major AMPK downstream targets, mediate the chondroprotective effect of AMPK activation [265]. Decreased AMPK activity in articular chondrocytes has the potential to disrupt cartilage homeostasis by promoting matrix catabolism, thereby contributing to progression of OA.

The presence of ER stress markers in association with altered production of type VI collagen has been noted in human OA cartilage [266] and the ER stress-induced protein C/EBP homologous protein (CHOP) has been shown to mediate chondrocyte apoptosis in a mouse OA model [267]. Increased levels of CHOP and other ER stress markers were noted in human OA cartilage [268, 269] and CHOP expression was associated with increased chondrocyte catabolic activity [269]. Chondrocyte death in response to ER stress induced by thapsigargin was found to be inhibited by the pro-survival protein Akt1 [270]. Excess CHOP activity is limited by AMPK activity in chondrocytes [269]. An inhibitor of Akt1 that is induced by ER stress, tribbles homolog 3 (TRB3), was found to be increased in OA cartilage, while overexpression of TRB3 in chondrocytes reduced matrix synthesis and promoted cell death through inhibition of chondrocyte Akt phosphorylation [271]. Thus further studies on mechanisms relevant to proteostasis in cartilage may discover important links to OA.

5.6 Stem Cells

Stem cell depletion is recognized as a hallmark of aging [272] and it has been suggested that the regenerative activity of bone marrow-derived stem cells (BMSC) declines after the age of 30 [273]. A large number of studies examined whether

aging or OA is associated with changes in BMSC [274]. The frequency of BMSC in the mononuclear cell fraction of bone marrow aspirates was reported not to correlate with the diagnosis of OA or osteoporosis [275–280] or to decline in older donors [281, 282]. The proliferation rate of BMSC during culture expansion decreased with age in some studies [277, 282–285] but not in others [286, 287]. There is also a substantial variability in proliferation rates of BMSCs in older donors, which does not appear to be related to the presence of diabetes or OA [288].

Similar to most other tissues, joint tissues in adult humans are thought to contain cell populations with certain phenotypic and functional properties of mesenchymal stem cells (MSC) or progenitor cells. Articular cartilage, synovium, menisci, ligaments, tendons [289], infrapatellar fat pad, synovial fluid [290] and periosteum [291] contain cells that express MSC surface markers and upon isolation and culture under specific conditions, they have multilineage differentiation potential towards chondrocytes, osteoblasts and adipocytes. In human articular cartilage, the highest concentration of cells expressing progenitor cell markers, including Stro-1, Notch-1, CD105, CD166 is in the superficial zone and this is reduced with donor age [292, 293].

The function of these cells in the maintenance of articular cartilage and other joint tissues under normal conditions is currently unclear. Interestingly, there is a high level of expression of these stem cell markers in the cell clusters in OA cartilage [292], a histological hallmark of OA [294]. Cells in these clusters produce a large number of mediators involved in joint inflammation and tissue remodeling. The abnormal activation and differentiation pattern of cluster cells in OA cartilage has been interpreted as chondrocyte de-dedifferentiation [295] where the differentiated articular chondrocytes change gene expression patterns in response to the different extracellular signaling environment. An alternative hypothesis is that cluster formation is the result of progenitor cell proliferation. A systematic analysis of the fate of cells expressing progenitor markers during the development of OA is required to address this hypothesis.

Surgical injury to articular cartilage is also associated with proliferation of progenitor cells that produce new extracellular matrix [293]. In addition, migratory chondrogenic progenitor cells, apparently originating from subchondral bone or bone marrow were also described in areas of OA joints where the original cartilage had been degraded [296]. It thus appears that traumatic or OA-related injury to articular cartilage activates proliferation of these mesenchymal progenitor cells.

Osteophytes are new bone tissues that are formed in OA affected joints and represent radiographic hallmarks of OA. While osteophytes most commonly form at the joint margins and originate from the periosteum, a tissue rich in stem cells, similar structures can also develop in areas of exposed subchondral bone, in ligaments and tendons [297]. The formation of osteophytes appears to be initiated by MSCs that undergo condensation, chondrogenic differentiation, and proliferation [298]. The chondrocytes then undergo hypertrophic differentiation, promoting the formation of blood vessels that allow recruitment of osteoblasts and osteoclasts that remodel the cartilaginous tissue into bone in a process similar to endochondral ossification [299].

Bone marrow-derived mesenchymal stem cells (BMSCs) have been studied extensively as a cell source for engineering of cartilage and other tissues [273]. Direct injection of MSCs into the joint can slow the degradation of articular cartilage or even regenerate it in OA animal models [300, 301]. A proof of concept study in humans who received injections of autologous adipose tissue-derived MSCs into OA-affected knees showed encouraging results [302].

For differentiation potential towards single lineages, an unaltered differentiation capacity with age was reported for adipogenesis [276, 277, 282, 303] and chondrogenesis [278, 282, 303]. For osteogenesis, reports on maintained differentiation capacity [275–277, 285, 303, 304] contrasted others showing a decline in osteogenesis with age [281, 282, 284].

Since there is no specific marker to identify MSC in bone marrow [274] and the cells are analyzed after culture, the question whether there is a change in MSC numbers and/or function with age is, therefore, an important question which still remains open. Despite these aging-related changes that were observed in some studies, bone marrow stem cells from older donors still appear to be a suitable cell source for tissue engineering, as proliferating and differentiating MSC were isolated even from very old donors, so that the ability to isolate MSC is per se not a limitation for therapeutic use [288].

6 Research Needs in Aging and OA to Meet the Goal of Developing Disease-Modifying Therapies

A major long-term goal of research in OA is to delay onset and develop disease modifying therapies that would slow or stop structural progression while also reducing pain and improving physical function. We are getting closer to that goal. When compared to the previous century of work, rapid progress in the understanding of the biology of OA has been made over the past two decades with the recognition that OA is not simply due to “wear and tear” of the articular cartilage. As detailed in the sections above, the biology of OA and the contribution of aging are much more complex than ever imagined. It includes pathological changes in all of the tissues that make up the affected joint(s) driven not only by abnormal joint mechanics that result in excessive or abnormal loading of the joint but also by the activity of a host of inflammatory mediators as well as by aging changes that promote catabolic over anabolic activity and reduced cell survival. This improved understanding of OA within the context of aging provides hope that new disease-modifying therapies can be developed but there is still much work to be done first.

A summary of the research needs in OA most relevant to aging includes:

- Determining the key pathways which lead to OA downstream from specific risk factors
- Understanding the role of systemic vs. local inflammation and determine which of the many inflammatory factors are key drivers of OA

- Determining if targeting a specific hallmark of aging will decrease the progression of OA
- Developing better age appropriate pre-clinical models of OA
- Developing clinical, biochemical, and imaging biomarkers that can accurately phenotype individuals with various forms of OA to predict those at greatest risk of progression and target therapy to a specific phenotype

A major challenge moving forward is determining which of the many pathways recently found to contribute to OA are critical to promoting disease progression. It is likely that the pathway to OA will vary for each individual based on specific risk factors in conjunction with age. For example, preventing the development of post-traumatic knee OA in a 20 year-old with an acute ACL tear will require a different strategy than for slowing the progression of knee OA in an obese 60 year-old. The former would likely require targeting an inflammatory pathway initiated at the time of ACL tear while the latter would require targeting a key metabolic aspect of obesity that contributes to OA. It is not known which inflammatory mediators are key drivers of OA and the relative role of local versus systemic inflammation associated with aging or "inflamm-aging" in the OA process is also not clear. Likewise more work is needed to determine if targeting one of the major hallmarks of aging, such as oxidative stress, epigenetic alterations, metabolism and autophagy, or ER stress will be of benefit in reducing the progression of OA. Finally, there is a need to know if protecting chondrocytes from dying and/or inducing endogenous stem cells to promote repair is feasible.

Another need to advance the field is the development of pre-clinical models of OA that better predict efficacy in humans. A number of the treatments mentioned above, such as bisphosphonates, calcitonin, and iNOS inhibitors, worked great in the pre-clinical models in which they were tested but failed in large (and expensive) clinical trials in humans [46]. A major issue is that pre-clinical studies are routinely performed in young animals in which OA is induced surgically. Using microarrays to evaluate changes in gene expression after the induction of OA in young and older adult mice, we noted significant age-related differences that suggest the genes and pathways activated as OA develops in older animals may be very different from those in younger animals [305]. Surgical induction of OA in young animals might only be a good model system for predicting efficacy in preventing post-traumatic OA in young humans, while predicting efficacy in older adult humans will require use of animals that more closely match the age of the human population for which an intervention is being developed.

There is a movement to think of OA as a condition with multiple phenotypes that might be segregated by clinical findings, including risk factor assessment, along with biochemical and imaging biomarkers to evaluate specific joint tissue involvement and disease activity [306–308]. However, we currently lack biochemical markers sensitive and specific enough to phenotype patients and although advances are being made quite rapidly in imaging, there is a lack of agreement on the most useful modalities. Clinical, imaging and biochemical biomarker data from the Osteoarthritis Initiative (OAI), an ongoing longitudinal multicenter study of close to

5000 men and women aged 45–79 years with knee OA or at risk for knee OA, is expected to provide results that can be used to better phenotype people with knee OA and predict who is at greatest risk of progression. This work and the many other ongoing studies in aging and OA provide confidence that progress in the field will continue to be made that should lead to new therapies for OA.

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Diabetes and Aging

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Contents

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1 Introduction

Age is a major risk factor for type 2 diabetes mellitus (T2DM), similarly to what is known about the etiology of most chronic diseases. Indeed, before the recent "epidemic" of childhood and adolescent obesity, T2DM was called "adult onset diabetes." The importance of chronological age as a risk factor for T2DM is only one of many links between diabetes and aging. The genetic and environmental control of the biological process of aging is intertwined with the development and consequences of T2DM in a highly complex network of interactions that are probably unique to diabetes.

Symptoms and diagnosis of T2DM reflect failure of insulin-secreting pancreatic β cells on the background of progressive increase in insulin resistance in target tissues. Insulin resistance commonly accompanies aging, although it is not clear whether insulin resistance is primarily a cause or a consequence of aging and whether it represents a protective/compensatory response to hyperinsulinemia [9]. While controversy persists about the role of aging in insulin resistance, and vice versa, it is widely accepted that maintaining insulin sensitivity through physical activity or pharmacological agents can prevent/delay the development of T2DM in high-risk young and older adults [84].

There is considerable evidence that insulin and homologous hormones in invertebrate species are intricately involved in the control of aging and lifespan [62, 124]. In experimental organisms ranging from yeast to mice, reducing (but not eliminating) insulin/insulin-like growth factor (IGF) signaling by genetic or dietary means can lead to slower aging, reduced susceptibility to age-related disease and significant, often remarkably large, extension of longevity. The increase in longevity can be quite impressive – more than twofold increase in *C. elegans* and more than 1.5-fold increase in mice. Importantly, a role of insulin/IGF signaling in the control of aging and longevity also applies to primates, including humans [106, 133]. The beneficial effects of reduced insulin/IGF signaling on longevity could be viewed as the first paradox in the relationship between insulin and aging. A severe reduction of the capacity to produce insulin leads to diabetes, a serious, life-threatening disease. In contrast, chronic hypoinsulinemia induced by calorie restriction or mutations related to growth hormone (GH) signaling are associated, most likely causally, with delayed aging and long, healthy life.

The complex interplay between insulin action, diabetes and aging does not end here. Progression of T2DM, with its practically unavoidable deterioration of glucose homeostasis, is associated with the emergence of functional deficits and pathological alterations that typically accompany aging [35]. Indeed, many diabetologists view accelerated aging as a result of T2DM (as well as type 1 diabetes) and believe that patients with T2DM are physiologically older than their chronological age. This difference may be as great as 10 years or, for new patients, approximately 1 year for each year since the diagnosis of their disease. This relationship between T2DM and aging at times has been emphasized by the simple but powerful statement that "diabetes is accelerated aging" [DeFronzo, RA, personal communica-

tion]. Although a disease (diabetes) cannot be equated with a physiological process (aging), it is indeed striking that some of their consequences are very similar. Regarding accelerated aging as one of the consequences of diabetes is consistent with many clinical and epidemiological findings. It is also important to remember that diabetes disturbs insulin secretion and its intracellular actions, which are mediated by signaling pathways known to be involved in the control of aging.

In terms of broader relationships between aging and disease as embodied in the concept of geroscience and explored throughout this volume, the suggestion that diabetes may lead to accelerated aging is extremely important. It provides a mechanistic explanation of why diabetes, similarly to chronological age, increases the risk for cardiovascular disease, cancer, frailty and dementia. Uncovering cause:effect relationships between insulin signaling, aging and diabetes is greatly complicated by the fact that circulating insulin concentration and insulin sensitivity are mutually dependent and both are altered in pre-diabetic conditions such as glucose intolerance and the metabolic syndrome, and in patients with T2DM. Although chronic insulin exposure promotes insulin resistance by well-known mechanisms, insulin resistance coexists with insulin deficiency in T2DM; meanwhile long-lived mice with GH-related mutations and offspring of exceptionally long-lived people exhibit reductions in both insulin levels and insulin resistance [8, 133]. These relationships are further complicated by differential alterations in insulin sensitivity and various steps of insulin signaling in different organs of the same individual [19], as well as by the emerging evidence that insulin resistance can be either detrimental or protective [10]. Is this another paradox related to diabetes?

Discussion of the interplay between aging and diabetes would not be complete without a reference to obesity. Aging is associated with progressive changes in the distribution and secretory activity of adipose tissue, as well as adipogenesis and adipocyte senescence, and most often also with a gradual, often very striking increase in adiposity [125]. Obesity is one of the important features of metabolic syndrome and a key risk factor for T2DM. It is also independently associated with an increased risk of cancer and cardiovascular disease, thus resembling the effects of both aging and diabetes. In turn, regulation of lipid metabolism, food intake and adiposity are disturbed in T2DM.

2 Studies in Experimental Animals Link Glucose Homeostasis and Insulin Signaling with Healthy Aging and Longevity

2.1 Animals with Reduced Longevity

Much of the evidence for causal links between glucose homeostasis and aging is derived from mice with genetic or dietary interventions that alter insulin signaling. In this species, obesity induced either by mutations or high-fat diet (HFD) leads to

a reduced lifespan, as well as insulin resistance, diabetes and functional deficits resembling those that normally occur during aging [95, 96, 121]. Mice with morbid obesity due to hereditary deficiency of leptin or leptin receptors provide a particularly striking example of these associations [67, 89, 102]. Animals heterozygous for the lethal yellow (A^Y) mutation at the agouti locus (often referred to as "agouti mice") are obese, hyperinsulinemic and hyperglycemic and more likely to develop cancer, an aging-related disease [134].

Interestingly, some genetic interventions allow dissociating abnormal glucose homeostasis and accelerated aging from obesity. Transgenic mice overexpressing growth hormone (GH) have reduced adiposity (percent of body fat) during most of their adult life [103], are insulin resistant and hyperinsulinemic, live much shorter than their genetically normal siblings, and exhibit numerous characteristics resembling aging that develop at an inappropriately early chronological age [6]. Pertinent to the subject of this chapter, these "giant mice" are more susceptible to age-related diseases including kidney inflammatory disease, glomerulosclerosis and cancer [129]. Moreover, blood pressure is elevated in these insulin-resistant transgenic mice [78].

2.2 Animals with Extended Longevity

Causal relationships between insulin signaling, glucose homeostasis, age-associated disease and longevity are strongly supported by studies in mice in which somatotropic signaling is suppressed by spontaneous mutations or targeted gene disruptions. Remarkable extension of average and maximal longevity of mice of both sexes lacking GH or GH receptors is associated with enhanced insulin sensitivity, reduced or "low-normal" levels of blood glucose, and resistance to the detrimental impact of high-fat diet on insulin signaling [8, 22, 90]. These characteristics, together with reduced blood pressure [57], could be described as a phenotype opposite to metabolic syndrome or "prediabetes." Importantly, these animals exhibit numerous features of delayed aging, including improved maintenance of cognitive, immune and neuromuscular function, collagen properties and glucose homeostasis at the age when these parameters exhibit decline in their normal (wild type) siblings [8, 22]. Incidence of cancer and various pathological changes associated with aging are delayed and/or reduced in these insulin-sensitive, long-lived mutants [73, 74].

Our hypothesis that improved insulin signaling/action is one of the key mechanisms responsible for extension of longevity in GH-related mutants was supported by experiments exposing these animals to calorie restriction (CR) throughout most of their post-natal lives. In most strains of mice, CR increases insulin sensitivity, slows aging and extends longevity. In Ames dwarf mice, which lack GH, CR led to a further increase in insulin sensitivity and further extension of their already remarkably long lifespan [7]. In contrast, in GH-resistant GHRKO (a.k.a. "Laron dwarf") mice, CR did not further increase insulin action (Fig. 1), had no effect on longevity in males and caused a small increase in maximal (but not average) longevity in

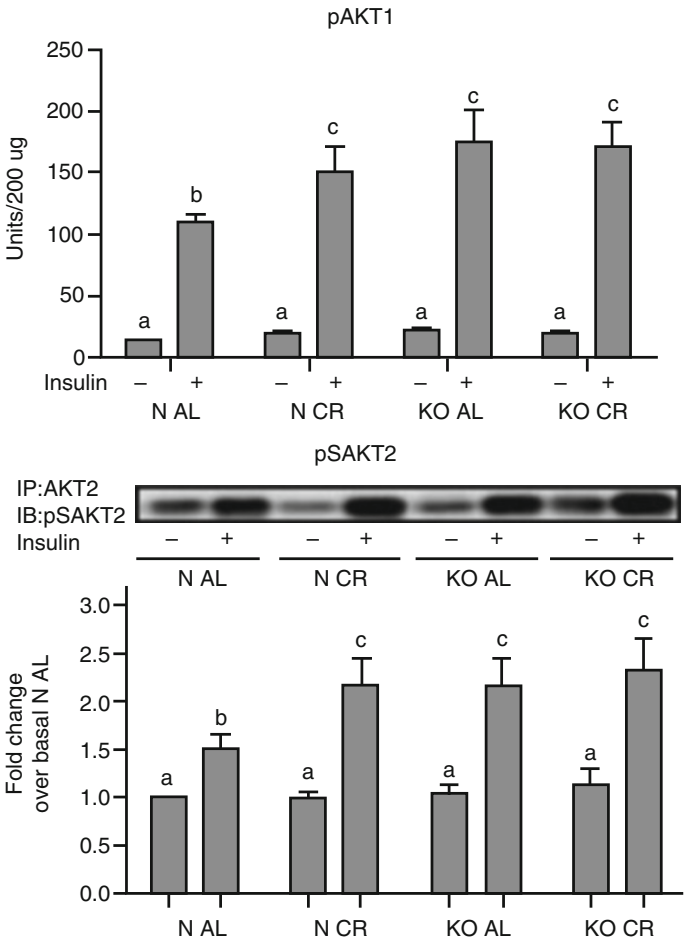

Fig. 1 Phosphorylation of Akt1 and Akt2 in response to acute insulin stimulation in the skeletal muscle of long-lived growth hormone receptor deleted (GHRKO; KO) and normal (*N*) male mice fed ad libitum (*AL*) or subjected to chronic 30 % calorie restriction (*CR*). Results indicate that GHRKO mice are more insulin sensitive than N (control animals). CR produces the expected increase in insulin sensitivity of N mice but no further increase in the GHRKO mutants. Importantly, this CR regimen extends longevity in N but not in GHRKO males [19]

females [18]. Additional evidence for a cause:effect relationship between insulin signaling and aging was obtained in a recent study in which experimental suppression of insulin sensitivity in long-lived GHRKO mice attenuated phenotypic markers of delayed aging [4]. In contrast to these observations, mice with deletion of insulin receptor substrate 1 (IRS1 –/–) and transgenic mice overexpressing klotho are long-lived in spite of enhanced rather than reduced insulin resistance [86, 116]. It was suggested that insulin resistance may act to reduce the strength of the insulin signals [86] or protect target organs from excessive insulin exposure [9]. Similarly,

fat tissue-specific insulin receptor knockout (FIRKO) mice are long-lived in spite of insulin resistance of adipocytes and perhaps also macrophages [16], and rapamycin treatment extends longevity in mice even though it can reduce insulin sensitivity [68]. However, rapamycin's effects on insulin signaling depend on the dose and duration of treatment [49]. From the data available to date, it is possible to conclude that a combination of reduced insulin levels and enhanced insulin sensitivity might emerge as one of the key mechanisms leading to delayed and healthy aging in mice with GH-related mutations as well as in various species of animals subjected to CR.

3 Role of Adiposity

Interestingly, studies in long-lived mutant mice allowed dissociation of the effects of insulin signaling on aging from the effects of obesity. In contrast to animals subjected to CR, long-lived GH-related mutants have increased rather than reduced adiposity [13]. Examination of the adipose tissue expression of pro- and anti-inflammatory cytokines and their circulating levels along with the effects of surgical removal of most of the intra-abdominal (visceral) fat in these animals provided evidence suggesting that insulin resistance is not determined by the amount of adipose tissue but by its secretory profile [94]. Long-lived mice with GH-related mutations have unexpectedly increased levels of adiponectin and reduced expression of IL-6 and TNFα in spite of their increased adiposity [91, 94]. It is well documented that in addition to its anti-inflammatory and anti-atherogenic effects, adiponectin increases insulin sensitivity [12, 127, 137]. It is possible that increased adiponectin levels, together with reduced levels of pro-inflammatory cytokines and suppressed mechanistic target of rapamycin (mTOR) signaling, provide a likely explanation for increased insulin sensitivity in corpulent or obese GH-deficient and GH-resistant mice.

4 Pathophysiology and Natural History of Type 2 Diabetes

In young and middle-aged individuals, T2DM occurs as a consequence of two pathophysiologic alterations, insulin resistance and β-cell failure. Both genetic and environmental factors (obesity, physical inactivity) contribute to the development of insulin resistance. Resistance to the actions of insulin in skeletal muscle, liver, and adipose tissue appears early in the natural history of the disease. In muscle, insulin resistance is manifested as decreased insulin-mediated glucose disposal; in the liver it is manifested as impaired suppression of hepatic glucose output; and in adipose tissue insulin resistance manifests as increased lipolysis rates, resulting in increased plasma free fatty acid concentration that further impairs insulin action in muscle and liver (i.e. lipotoxicity). During the early stages of insulin resistance, β-cells can compensate by augmenting insulin secretion to maintain normal glucose tolerance. However, in subjects destined to develop diabetes (~10–20 % of all insulin-resistant

individuals), the β-cells eventually will fail, leading to the onset of overt diabetes. The resultant hyperglycemia causes a further decline in insulin sensitivity (i.e. glucotoxicity), but it is the progressive β-cell failure that determines the rate of disease progression [44].

5 Aging as a Risk Factor for Type 2 Diabetes

Substantial evidence has demonstrated that increasing age is associated with impaired glucose homeostasis [2, 43, 107]. The Baltimore Longitudinal Study of Aging showed a progressive decline in glucose tolerance from the third through the ninth decade of life [118]. During an oral glucose tolerance test, the mean fasting plasma glucose increased ~1 mg/dl per decade, and the 2 h glucose increased ~5 mg/dl per decade. This decline in glucose tolerance was also evident in the National Health and Nutrition Examination Survey (NHANES) III, which showed that the percentage of physician-diagnosed diabetes (fasting glucose ≥126 mg/dl) is 3.9 % in subjects aged 40–49 years, whereas prevalence increases to 13.2 % in subjects ≥75 years of age [66]. The percentage of subjects with undiagnosed diabetes also increases from 7.1 to 14.1 % within these age groups. Approximately 50–60 % of subjects aged ≥65 have diabetes or impaired glucose tolerance (IGT), and ~25–50 % (depending upon the population) of subjects with IGT will ultimately convert to type 2 diabetes [47], Other than chronological age, the factors responsible for such high prevalence of IGT and T2DM in the aging population are not clear. However, age-dependent decreases in (i) insulin sensitivity and (ii) β cell function are thought to play important roles in the deterioration of glucose homeostasis that occurs with advancing age.

5.1 Molecular Basis for Peripheral Insulin Resistance

As mentioned above, insulin resistance is characteristic of peripheral tissues (i.e. muscle, adipose) from obese and T2DM subjects [43]. The first step in the insulin signaling transduction pathway is binding of insulin to the α subunits of the insulin receptor in the cell surface. The activated insulin receptor then tyrosine phosphorylates and activates downstream insulin receptor substrate (IRS) proteins, such as IRS-1 (IRS-1). Tyrosine phosphorylation of IRS-1 leads to its association with the p85 subunit of phosphatidylinositol 3-kinase (PI-3 kinase) [132]. Activation of PI-3 kinase leads to the phosphorylation/activation of a series of enzymes and proteins, such as phosphoinositide-dependent kinase (PDK)-1, protein kinase C (PKC) λ/ζ, Akt, and the RabGAP protein AS160 [50, 142, 143]. The phosphorylation/activation of these signaling intermediaries results in the translocation of GLUT4 glucose transporters to the cell membrane and the uptake of glucose [50, 142, 143]. A wide array of abnormalities distinguish insulin-resistant muscle from normal muscle, including

decreased insulin receptor tyrosine kinase activity, decreased IRS-1-associated tyrosine phosphorylation, and decreased insulin-stimulated PI 3-kinase activation [28, 41, 145]. Other defects reported in insulin-resistant muscle include decreases in insulin-stimulated PKC activity [50], and AS160 phosphorylation [80]. Insulin-mediated GLUT4 translocation also is reduced in insulin resistant subjects [54], due in part to impairments in insulin signaling described above. These molecular abnormalities are strongly correlated with decreased insulin-stimulated glucose disposal in muscle [41, 145]. Some of these cellular processes will be further discussed in Sect. 5.2

Insulin-stimulated glucose transport in muscles from nondiabetic rodents decreases with age [58, 61]. The majority of reports that have examined the effect of age on insulin sensitivity in humans also have demonstrated reduced insulin sensitivity [48]. Some studies have reported that decreased insulin sensitivity with aging is not apparent when results are expressed by lean body mass. However, when glucose disposal is measured using the hyperinsulinemic euglycemic clamp technique over a range of insulin doses, the plasma insulin concentration required to achieve half-maximal glucose disposal is considerably lower in younger compared with older subjects (shift to the right) [112]. This decrease in insulin-stimulated glucose disposal is evident whether glucose disposal rates are plotted per kg of whole body weight or lean body mass. Other dose-response studies using the euglycemic clamp also have shown impaired insulin sensitivity in older subjects [51, 105]. Petersen et al. demonstrated that, in response to physiologic hyperinsulinemia (20 mU/kg.min insulin clamp), older subjects have a ~40 % reduction in peripheral glucose disposal compared to younger subjects who were matched for body mass index (BMI) and lean body mass [105]. Importantly, the impairment in insulin sensitivity in older subjects was still evident when glucose disposal was expressed per lean mass [77]. In addition, peripheral insulin resistance in aging has been demonstrated using other techniques, such as forearm glucose uptake and the frequently sampled intravenous glucose tolerance test (minimal model) [32]. Studies in rodents and human subjects from various groups also have shown that skeletal muscle from aging animals has defects in the insulin transduction pathway as described above [55, 75, 85, 87].

The underlying pathogenic mechanism responsible for the reduction in insulin action that occurs with aging is unclear. Probable factors contributing to age-associated insulin resistance include adiposity/lipotoxicity, inflammation, and mitochondrial dysfunction. These are discussed below and illustrated in Fig. 2.

5.2 Role of Adiposity/Lipotoxicity on Insulin Resistance

Approximately 30–40 % of older U.S. adults are obese (CDC/NHANES). The high prevalence of obesity in this population is multifactorial, including decreased physical activity, lower oxidative capacity, and muscle wasting. Obesity is associated with impaired glucose metabolism, although the mechanism by which excess adipose tissue alters glucose homeostasis is unclear. Plasma concentration of free fatty

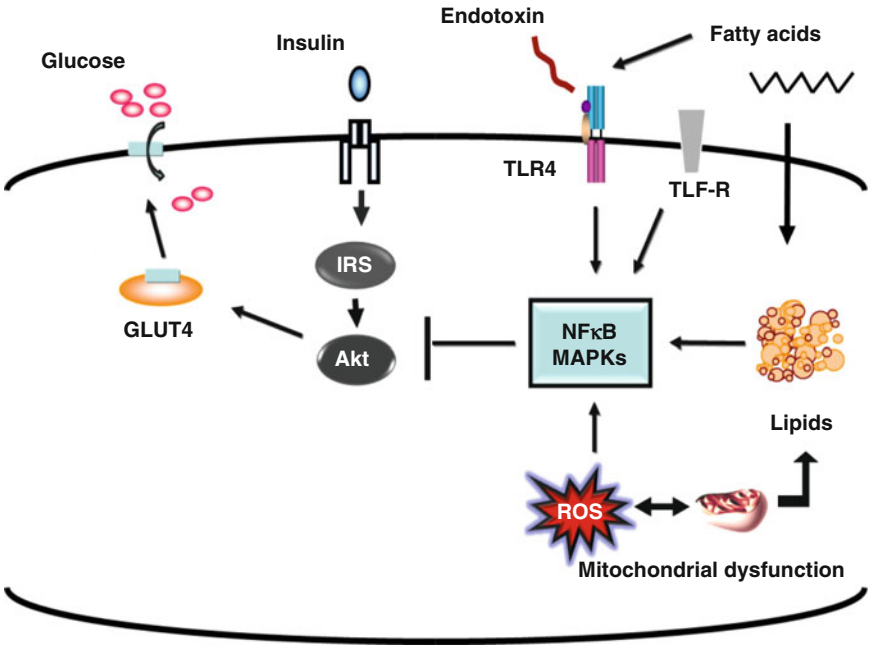

Fig. 2 Model of pathogenesis of muscle insulin resistance with aging. During aging, there is a pro-inflammatory state evidenced by increased expression and activity of mediators such as NFκB and MAP-kinases, caused by elevated levels of inflammatory stimuli, including plasma free fatty acids and endotoxin (which signal via TLR4), intracellular lipids (e.g. ceramides, diacylglycerol), cytokines (e.g. TNFα) and ROS. Decreases in mitochondrial function also contribute by promoting accumulation of intracellular lipids and increased ROS production. The activation of NFκB and MAP-kinases impair insulin action at the Akt and IRS levels, which eventually result in decreased insulin-mediated glucose disposal

acids (FFA) is commonly elevated in older subjects, and several lines of evidence implicate a deleterious effect of elevated plasma FFA level on muscle insulin sensitivity. For example, an experimental elevation of FFA induces muscle insulin resistance in normal glucose-tolerant subjects [17], whereas a reduction in plasma FFA concentrations rapidly ameliorates insulin resistance in insulin-resistant individuals [113]. In addition to the circulation, a variety of approaches has confirmed the existence of increased lipid content in insulin-resistant skeletal muscle [5, 60, 128]. Triglycerides account for most intramyocellular lipids. While triglycerides probably do not impair insulin action per se, metabolites of triglycerides/FFA, particularly diacylglycerol and ceramides, have been shown to have a deleterious effect on insulin action [63, 141]. The cause for the accumulation of intracellular lipids in insulin-resistant muscle is unknown, although one possibility is a reduction in mitochondrial oxidative capacity [105], which appears as a major hallmark of aging (see below). This elevation in intramyocellular lipids is thought to initiate a reverberating negative feedback cycle by decreasing insulin signaling and aggravating the insulin resistance that is already present. Specifically, these intracellular lipid

metabolites activate kinases, such as inhibitor κB kinase, protein kinase C, and c-jun N-terminal kinase (JNK), which in turn serine phosphorylate IRS-1, resulting in decreased activation of PI-3 kinase [1, 26, 76].

Numerous studies have examined whether intracellular lipid content varies with normal age. A study in rats found elevated intramyocellular lipid content in aging animals [126]. In humans, studies employing magnetic resonance spectroscopy (MRS) have shown that older subjects also have higher intracellular lipid levels in muscle and liver than younger individuals, and that intramyocellular lipid content correlates closely with peripheral insulin resistance [40, 105]. As in the case for insulin-resistant muscle, the molecular basis for the age-dependent accumulation of intramyocellular fat is also yet to be determined; however, it likely results from an imbalance between the rate of uptake of fatty acids and fat oxidation. Indeed, studies performed in older, nondiabetic subjects have demonstrated that aging is associated with a reduction in basal fat oxidation rates [27, 122].

5.3 Role of Decreased Mitochondrial Function

As mentioned above, aging is accompanied by alterations in various parameters of mitochondrial function and structure. In the context of metabolic diseases such as obesity and type 2 diabetes, particularly relevant are mitochondrial alterations described in skeletal muscle, which is a key tissue responsible for substrate (glucose, FFA) uptake and oxidation. Mitochondrial alterations described in aging muscle include reductions in mitochondrial number, ATP production, and respiration, abnormal structure, and, in some cases, elevated reactive oxygen species generation [reviewed in [79].

The cause for the reduction in mitochondrial function observed with aging is not clear. According to the free radical and mitochondrial theories of aging the decreases in mitochondrial function are the result of cumulative oxidative damage to mitochondrial molecules (mtDNA, proteins, and lipids) [65, 97]. Consistent with this theory, studies performed in human muscle have shown that aging is associated with oxidative damage to mtDNA and proteins [reviewed in 79]. Some [130], albeit not all [55], studies also have found increased lipid peroxidation in muscle from older subjects. Concerning the age-related reductions in oxidative capacity, various studies [88, 100, 110] have reported that aging is accompanied by a reduction in the activity of the energy-sensing enzyme AMP-activated protein kinase (AMPK). AMPK works as a fuel gauge, being activated robustly by energy-consuming stimuli such as muscle contraction, hypoxia, and ischemia [70, 135]. Upon stimulation, AMPK functions to restore cellular ATP by modifying diverse metabolic and cellular pathways, including increased fat oxidation and glucose transport. AMPK promotes fat oxidation in tissues by phosphorylating and inactivating acetyl CoA carboxylase (ACC), resulting in decreased synthesis of malonyl-CoA, an inhibitor of carnitine palmitoyltransferase I (CPT-1). The reduction in malonyl CoA relieves the inhibition of CPT-1 and promotes CPT-1 mediated transport of fatty acids into

the mitochondria for oxidation. Because the end result of AMPK activation is an increase in fat oxidation, decreases in AMPK activity, as seen with aging, could lead to an excessive accumulation of intramyocelular lipids, which in turn would impair insulin action/sensitivity. Other key regulators of mitochondrial biogenesis and oxidative capacity reported to be altered with aging are the transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator (PGC)-1α and the NAD-dependent deacetylase sirtuin-1. PGC-1α controls mitochondrial biogenesis and cellular metabolism by regulating the expression of numerous proteins involved in the Krebs cycle, oxidative phosphorylation, and mtDNA replication/transcription [64]. AMPK increases PGC-1α gene expression, and sirtuin-1 enhances PGC-1α activity through deacetylation on lysine residues. Similar to AMPK, the expression of both PGC-1α [55] and sirtuin-1 [31] are reduced in aging tissues, suggesting that a coordinated downregulation of the AMPK-PGC-1α-sirtuin 1 axis may play a role in the reductions of mitochondrial number, oxidative capacity, and cellular metabolic functions that occur with aging.

While, in general there is agreement with the notion that aging is associated with alterations in mitochondrial number, structure, and function, it is debated whether primary mitochondrial abnormalities (independent of aging) are sufficient to induce insulin resistance [59, 72]. Conflicting results about the role of mitochondrial dysfunction on the pathogenesis of insulin resistance, obesity, and type diabetes are due in part to differences in animal species, tissues, and human populations studied, and in the conditions and methods employed to assess mitochondrial function [79, 104]. Despite the ongoing debate, it is probable that increasing mitochondrial oxidative capacity via lifestyle interventions (i.e. physical activity) or pharmacological agents would result in improved metabolic outcomes during aging.

5.4 Role of Inflammation

Aging may be considered a state of low-grade "sterile" inflammation that could play a role in the high prevalence of glucose metabolism abnormalities in older subjects [24, 30, 37]. Increased low-grade inflammatory activity in older subjects could either cause age-related diseases or be a marker of diseases that occur with aging. Bruunsgaard et al. determined that low-grade increases in the levels of pro-inflammatory cytokines in older subjects were independent of the presence of medical disorders [23], although medical disorders can exacerbate this phenomenon.

The source for the elevated cytokine levels with aging is not clear. Cytokine levels in the circulation reflect production from many tissues including inflammatory cells (monocytes/macrophages, T cells, etc.), senescent cells and adipose tissue. In the young, approximately 25 % of IL-6 is derived from fat tissue [98], and adipocytes can secrete TNFα in addition to IL-6. Aging is associated with increases in abdominal fat mass and visceral obesity is associated with increased circulating levels of these cytokines [42, 52, 81]. There is some evidence suggesting that omental fat produces more cytokines than subcutaneous fat tissue [52], and this may

explain why visceral obesity has a greater detrimental effect on insulin sensitivity [53]. The production of cytokines by adipose tissue can also be modulated by the interaction between adipocytes and macrophages within the adipose tissue [82]. The number of macrophages in adipose tissue directly correlates with adiposity [131, 136] and insulin resistance [46], and adipose tissue expansion correlates with the accumulation of macrophages and the proinflammatory phenotype [20, 92]. Another potential source of inflammation with aging is the accumulation of senescent preadipocytes [125]. Senescent cells typically have a pro-inflammatory secretory profile, termed the senescence-associated secretory phenotype (SASP), that may propagate the inflammatory adipose tissue microenvironment as well as promote inflammation throughout the whole body.

In addition to cytokines produced by adipose tissue and inflammatory cells, another potential source of inflammation during aging is the microbiome and its products. Studies in flies [109], rodents [21] and humans [56] have shown that aging is accompanied by alterations in intestinal microbiota composition and intestinal barrier integrity. In line with these findings, our group recently showed that older subjects have increased plasma concentration of endotoxin (a marker of altered barrier integrity) in association with insulin resistance, sarcopenia, and increased inflammatory signaling (toll like-receptor 4, NFκB, MAPK) in muscle [56]. Thus, it is possible that endotoxin, and other yet unidentified microbial products, could be involved in the inflammatory state and consequent metabolic alterations of aging.

A potential mechanism linking aging, inflammation, and metabolic disease is immune sensing through the NLRP3 inflammasome [140]. The NLRP3 inflammasome is a multiprotein cytoplasmic complex composed of NLRP3, the adaptor molecule ASC, and the cysteine protease caspase-1. Stimulation of the inflammasome by pathogen-associated molecular patterns (PAMPs) leads to the activation of caspase-1, which cleaves the pro-forms of the cytokines IL-1β, IL-18 and IL-33 to their active and secreted forms. A role of the inflammasome in aging-related inflammation and associated pathologies is suggested by findings that NLRP3 ablation protects against glucose intolerance, bone loss, and thymic involution in aged mice [140].

6 Aging, Diabetes and Insulin Signaling in the Brain

The well-documented association of diabetes with chronic age-related disease and geriatric conditions [35] includes increased risk of cognitive impairment, dementia, brain atrophy and Alzheimer's disease [35, 38, 99]. Potential mechanisms of these associations include several well-recognized hallmarks of aging, including increased accumulation of advanced glycation end-products (AGEs), oxidative stress, inflammation, defective proteostasis and metabolic abnormalities, as well as altered insulin signaling within the brain [38, 83].

Insulin receptors, as well as various proteins involved in the intra-cellular transmission of insulin signals, are expressed in various brain regions [83]. However, the role of insulin in the control of glucose metabolism in the central nervous system has

been questioned. The controversy surrounding this issue is likely related to the fact that, in healthy subjects, glucose uptake by the brain is already maximally stimulated by normal insulin levels and therefore does not respond to further insulin stimulation [71]. In the context of Alzheimer's disease, some investigators refer to the brain insulin resistance as "type III diabetes" [34, 123]. Recent studies in obese patients suggest that iron overload in the brain may be caused by local insulin resistance and could represent yet another potential mechanism of the detrimental influence of diabetes on cognitive performance [15] and risk for Alzheimer's and Parkinson's disease [115].

Complex relationships between brain function and obesity, insulin resistance, diabetes and its complications also involve the role of hypothalamic function in the control of peripheral metabolism and aging. High-fat diet and obesity promote hypothalamic inflammation and insulin resistance [96, 111], and the hypothalamus controls multiple facets of peripheral metabolism [11, 14, 101]. Insulin signaling within the hypothalamus influences hepatic gluconeogenesis [25], lipogenesis and lipolysis in the adipose tissue [117], as well as circulating levels of branch chain amino acids, which are known to be elevated in T2DM [119]. Recent elegant studies in the Cai laboratory [144] linked hypothalamic inflammation with the control of aging. Interestingly, ongoing studies in our laboratory [69, Bartke unpublished] indicate that expression of IL-1β and other pro-inflammatory cytokines is reduced in the hypothalamus of long-lived mutant mice. Further studies will be necessary to elucidate the role of hypothalamic inflammation and insulin resistance in the development of whole-body metabolic abnormalities that lead to diabetes.

More work will also be needed to identify mechanisms responsible for the increased risk of cognitive decline and Alzheimer's disease in patients with diabetes. Epidemiological studies provide evidence that the risk of dementia in diabetic patients is reduced by treatment with metformin [33]. Metformin and related drugs have also been shown to reduce the risk of cancer and to extend longevity of experimental animals [3, 93]. These findings imply that drugs of this class can slow down and/or delay the aging process. The apparent "anti-aging" action of metformin could have contributed to its beneficial effects on cognition in diabetes patients. However, regardless of the mechanisms involved, evidence for cognitive benefits of diabetes treatment strengthens the suggestion for etiological links between diabetes and dementia. These findings also generate interest in the exciting possibility that diabetes drugs could be useful for prevention and/or treatment of Alzheimer's disease [139]. Consistent with this notion, animal studies and early clinical trials suggest that intranasal administration of insulin in order to overcome insulin resistance and enhanced brain metabolism leads to reductions in β amyoloid and tauopathy, as well as improvements in brain function and cognition in Alzheimer's disease [36, 108, 138].

7 Prevention of Diabetes Versus Anti-aging Interventions

The prevalence of pre-diabetes (IGT) among older adults is increasing [29]. Because life expectancy is also increasing, the number of older individuals with diabetes and/or at risk of developing its complications (blindness, kidney failure,

amputations, neuropathic pain, cardiovascular disease, etc.) will be substantial. Therefore, strategies for diabetes prevention are urgently needed. Since physical inactivity and obesity are common in older subjects, lifestyle interventions are a logical diabetes preventative strategy. In addition, exercise improves mitochondrial and vascular function, which are reduced/impaired with aging [55, 114, 120]. Physical activity is effective in improving insulin action in older subjects [55]. In line with these findings, the Diabetes Prevention Program (DPP) clinical trial, conducted in pre-diabetic (IGT) subjects showed that lifestyle intervention proved exceptionally effective in preventing diabetes in older individuals [39].

Pharmacological interventions with insulin-sensitizing agents also have been evaluated for diabetes prevention in older subjects. In parallel to lifestyle changes, the DPP also tested the effect of metformin on diabetes prevention. In contrast to lifestyle modification, metformin seemed to be less effective in preventing conversion to diabetes in older subjects versus middle-aged individuals [39]. Our group conducted a multi-center diabetes prevention trial in pre-diabetic subjects using pioglitazone [45], a potent insulin-sensitizer. Pioglitazone was highly effective in preventing diabetes conversion (72 % overall reduction), and it was as effective in older (mean age = 66 years) as in middle-aged (mean age = 46 years) individuals in improving insulin sensitivity and in preventing diabetes (Espinoza S, Tripathy D, Defronzo RA, Musi N, unpublished, 2015).

Since the glucose metabolism alterations seen in older subjects may be caused by "primary" aging-mediated cellular changes (mitochondrial dysfunction, oxidative damage, cellular senescence, inflammation), another strategy for diabetes prevention is to target the aging process instead of "secondary" metabolic/endocrine perturbations (β cell dysfunction, insulin resistance). This approach would have the added benefit of potentially preventing other aging-related diseases such as cardiovascular disease, cancer, neurodegeneration and arthritis at the same time. This is in fact the central tenet of the Geroscience Hypothesis, which is awaiting experimental testing. The apparent beneficial effects of metformin and physical activity on many of these diseases, exemplifies the possibility of preventing/treating them through modifying basic mechanisms of aging. As research in aging biology advances and novel molecular targets are identified, trials using agents that modify these targets should be conducted for the testing of interventions to prevent diabetes and other diseases of aging in the elderly.

8 Closing Remarks

Aging is accompanied by various changes in metabolic processes at the cellular, tissue and whole body levels, including decreases in oxidative capacity, intracellular lipid accumulation, insulin resistance, and β cell dysfunction. These metabolic changes contribute to the higher prevalence of obesity and T2DM that are important causes of disability and death in older people. A better understanding of the molecular basis for the age-induced metabolic alterations will help design strategies to

preserve metabolic homeostasis and prevent these diseases that affect millions of people around the world.

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Renal Aging and Transplantation

John P. Higgins and Stuart K. Kim

Contents

1 Introduction

The kidney plays an important role in human aging because it shows stereotyped changes in morphology and physiology beginning around age 50. Kidneys show clear changes in structure and morphology with age. Starting at about age 50, the weight and volume of the kidney shrinks by about one third [1]. The glomerulus is a network of capillaries that is located at the beginning of the nephron that filters

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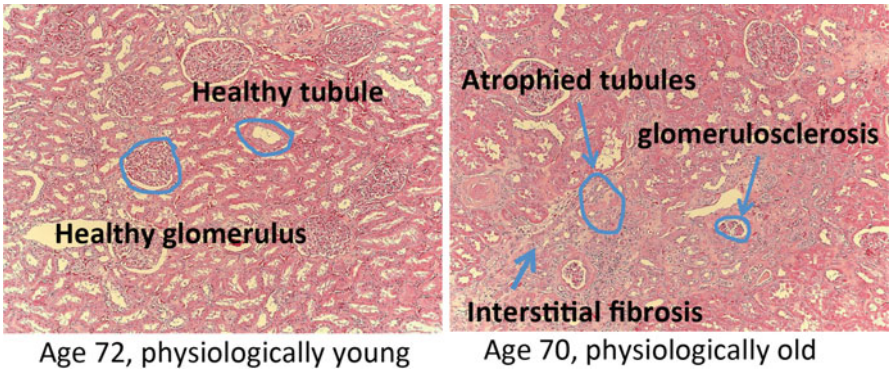

Fig. 1 Physiological kidney aging. Shown are two kidneys from elderly donors with a similar chronological age. The left kidney is physiologically younger than the right kidney. The kidney on the right shows classic signs of structural and morphological changes associated with renal aging, decreased kidney function, and poor renal transplant outcome. The glomeruli show signs of scarring (glomerulosclerosis). The cells in the interstitial space have thickened extracellular membranes indicative of fibrosis (interstitial fibrosis). The tubules are smaller, have thickened walls and have atrophied (tubular atrophy). Genetic and molecular biomarkers for physiological aging could be used to distinguish kidney donors based on physiological age. This could rescue renal organs such as the one on the left from exclusion, possibly making them eligible for renal transplant. The net effect would be to expand the pool of renal donors available for patients with end stage renal disease

blood to form urine. The number of glomeruli declines by one third to one half in old age through obsolescence or glomerulosclerosis. The tubules associated with the sclerosed glomeruli cease to function and the filtration capacity of each kidney declines. As the tubules atrophy, the tubular epithelium shrinks, the tubules contract and the basement membranes of the tubules thicken. Interstitial fibrosis increases with age, and refers to an increase in connective tissue in the space between the tubules. With age, the walls of arterioles become thick, caused by a deposition of hyaline. Hyaline is composed of plasma protein (for example C3 and IgM) that has leaked across the endothelium and accumulated in the wall of the arterioles. In Fig. 1, the kidney section on the right is from a 70 yo and shows many of the morphological hallmarks of aging: atrophied tubules, glomerulosclerosis and interstitial fibrosis.

The rate of filtration of the blood through the glomerulus (i.e. the glomerular filtration rate; GFR) is one of the primary indicators of renal function. On average, the glomerular filtration rate begins to decline at age 40, although the rate of decline is different in different individuals [1–3]. The loss of renal function due to advancing age may become clinically significant over a normal human life span. In the elderly, glomerular filtration rate often reaches levels low enough to indicate chronic kidney disease. By age 70, 35 % of people have moderate chronic kidney disease (stage 3) according to the National Health and Nutrition Examination Survey [4]. A healthy GFR is ≥90 ml/min for an adult, but when the GFR falls to <15 ml/min the patient is considered to have end stage renal disease. Patients with end stage renal disease require dialysis in order to survive as the blood no longer receives adequate

renal filtration, but simply going on dialysis doubles the 5-year risk for mortality. Renal transplantation is preferable to dialysis for end stage renal disease because the donated kidney can function at a relatively normal level and restore glomerular filtration rate. Both quality of life and survival are greatly improved by transplantation compared to dialysis [6]. The decline in glomerular filtration rate is likely caused by structural changes to the glomerulus, the interstitium and the arterioles [3, 7].

Understanding the genetic and molecular mechanisms that contribute to kidney aging will advance our basic understanding of the aging process in humans. Furthermore, aging research on the kidney could have important clinical applications. In the long run, a better understanding of renal aging could lead to strategies or treatments to delay the aging process. This could delay or prevent chronic kidney disease and reduce the number of people suffering from end stage renal disease.

In the short run, one promising opportunity is to use knowledge of aging to develop biomarkers in order to measure physiological age, as opposed to chronological age. For instance, from a cohort of elderly, it would be desirable to be able to identify those that have physiologically young kidneys. Figure 1 illustrates two kidneys from donors of similar chronological age of about 70 years. The kidney on the left retains a youthful morphological appearance, equivalent to the appearance of kidneys from middle-aged donors, suggesting that this kidney is physiologically young. The kidney on the right shows classic signs of aging. Figure 1 illustrates the concept that adding information from histopathological and molecular biomarkers to chronological age can improve our knowledge of the true age of an organ better than chronological age alone

Individuals with kidneys that are physiologically younger are likely to show a lower incidence of renal disease as they grow older. Furthermore, donor age is the major criterion for success of a kidney in renal transplantation [8, 9], which means that individuals with kidneys that are physiologically young are likely to be better renal transplantation donors than individuals with kidneys that are physiologically old irrespective of their chronological age. Instead of categorically discarding all of the organs from donors above a certain age, it may be possible to select a subset of organs that are physiologically young and suitable for transplantation (Fig. 2). Renal transplant outcome declines gradually with age, and the difference between youthful and elderly kidney donors is relative but not absolute. With elderly renal donors, the fraction of renal transplants that are successful (as measured by graft survival after 1 and 5 years) is lower than the fraction of successful transplants from youthful donors. Still, some of the transplants from elderly donors are successful. Figure 2 illustrates the concept of using physiological age to help increase the pool of renal transplant donors. Exclusion criteria based on chronological age alone become increasingly strong as the donor ages (gradient arrow). Aging biomarkers could be used to provide information about the physiological age of the tissue, which might permit certain prospective donors (dots shown in red) by expanding the criteria to include physiological in addition to chronological age. This strategy would expand the pool of kidney organs suitable for transplantation, and thereby

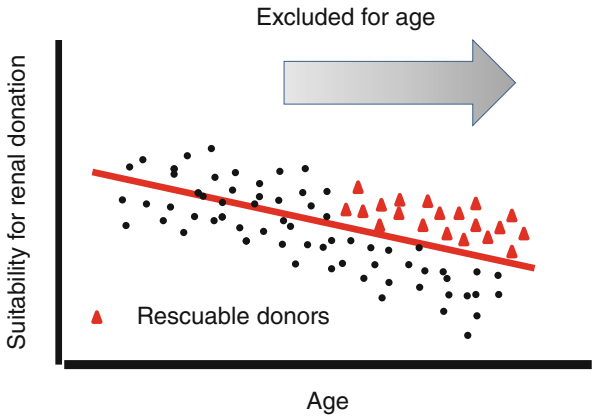

Fig. 2 Kidney aging and renal transplantation. Figure depicts how some kidneys from elderly donors may be suitable for renal transplantation. With increasing age of the donor, there is a steady decline in the percent of renal transplants that survive 1 and 5 years after transplantation. Nevertheless, there are many renal transplants from elderly donors that last for a suitable length of time. In principle, aging biomarkers could be used to identify kidneys that are physiologically young, and perhaps could be used to rescue organs that are currently discarded due to old age. The gradient *arrow* indicates how donor age becomes a stronger criterion for exclusion with increasing chronological age. The *red triangles* indicate kidney donors that may still be suitable for renal transplantation, even though their chronological age may have exceeded an exclusion criteria cut-off that is currently used. The *black dots* indicate individual donor kidneys

allow patients with end stage renal disease to receive a transplant and end their time on dialysis treatment.

2 Renal Transplantation

Renal transplantation is the best option for patients with end stage renal disease. The survival rates for kidneys in recipients following renal transplantation are 80 % after 1 year and about 60 % after 5 years [6, 10, 11]. Renal transplantation can possibly extend the lives of patients by 10–15 years compared to dialysis [6].

However, there are many more patients with end stage renal disease than there are renal transplantation donors. In 2014, there were 101,513 people in the United States on the waiting list for kidney transplantations. At the same time, there were only about 13,125 donor kidneys available [12]. Some patients with end stage renal disease receive a kidney from a living donor. In the donation process, there is a large number of volunteers that offer to donate their kidney. Most of the volunteered kidneys are excluded from becoming a kidney donor for medical reasons, including old age. In a recent study of kidney donors at Stanford University from 2007 to 2009, it was found that 92 % of potential donor kidneys were excluded from consideration, exacerbating the shortage of kidneys available for transplantation [13]. As a result

of the shortage of donor kidneys, many patients with end stage renal disease do not have the opportunity to receive a donor kidney for renal transplantation, an operation that would extend their lives. In principle, improvements in the criteria for exclusion might allow one to rescue potential donor kidneys that might be suitable for renal transplantation even though they are currently excluded from renal transplantation.

2.1 Predictors of Renal Transplant Outcome

Currently, there are three main criteria affecting the success of renal transplantation: ABO blood type and HLA histocompatibility matching, kidney preservation time and donor age. Donor kidneys that do not match the recipient for ABO blood type and HLA histocompatibility are at risk for graft rejection. Many kidneys are stored cold while awaiting the transplantation procedure, especially kidneys from deceased donors.

The third factor, age, is important for renal transplantation success as greater age of the donor diminishes the chance of success of the renal transplant. On the average, kidneys from older donors have a shorter graft survival time than those from younger donors. The short-term difference is relatively minor, but is amplified with the passage of time: About 95 % of kidneys have a graft survival greater than 1 year when the donor was younger than age 50, and about 85 % of kidneys have a 1 year graft survival rate when the donor was over age 65 [14]. At 5 years after the transplantation, there is about a 25 % increase in renal survival in kidneys from younger donors compared to those from elder donors [15]. Thus, while on average there is a drop off in graft survival from elder donors, there is also a significant number of exceptions where a kidney from an elder donor has a long graft survival time [16–21].

If we could better understand why old kidney age affects graft survival, it might be possible to identify kidneys from the elderly population that are still fit for renal transplantation. One way to do this is to develop a set of biomarkers for physiological age that could predict renal transplant outcome better than chronological age, or at least that could be used to improve transplant outcome in combination with chronological age. That is, among elderly donors of the same age, the kidney aging biomarker should be able to identify donors with a higher chance for long term graft survival. This might be one way to expand the pool of donor kidneys available for renal transplantation. Several molecular assays are being developed as biomarkers for renal graft survival.

Recent studies have begun to identify biomarkers of aging that can be used to help predict how well a kidney will perform in renal transplantation. Aging is a complex process dependent on many different mechanisms and pathways. One of the cellular pathways that may contribute to aging of the kidney is cell senescence [22–26]. Cell senescence could impact renal function if it prevented cell division necessary to replace lost or damaged cells. However, cell turnover in the kidney is

normally low compared to other tissues with high rates of cell turnover, such as the hematopoietic system or the lining of the gut [27]. Thus, it is unclear how strongly cell senescence would impact renal function under normal circumstances. However, disease or injury could result in loss of renal cells by apoptosis, and in this situation cell proliferation would be required in order to replace dead cells.

As kidneys grow old, there is an increased frequency of senescent cells. Senescence could contribute to renal aging in at least three ways. First, cell senescence may prevent new cells from replenishing cells that are lost from disease or damage. Second, cell senescence could lead to an increase of macromolecular damage. When a cell divides, there is a burst of new synthesis of all of the macromolecules needed to form the new cells (DNA, RNA, protein etc.). Conversely, in any post-mitotic cell such as a senescent cell, there is no net gain of RNA and protein, so new RNA and proteins are synthesized via transcription and translation at a much lower rate. Assuming that RNA and protein levels in senescent cells are at steady state, then the levels of transcription and translation are set to merely replace RNA as it is lost via RNA degradation and protein as it is lost by degradation via proteolysis machinery such as autophagosomes or the proteasome. As a result, macromolecules in a non-dividing cell have a longer molecular half-life than those in a dividing cell. The increased molecular half-life exposes all of the molecules to increased susceptibility for damage accumulation; for instance, macromolecules in a non-dividing cell would be expected to have higher levels of damage from reactive oxygen species. Third, senescent cells secrete a variety of signaling molecules and cytokines, a phenomenon referred to as the senescence-associated secretory phenotype [28]. The senescence-associated cytokines include factors such as interleukin-6 and interleukin-1β that can activate inflammatory signaling pathways. One possibility is increased abundance of senescent cells during aging contributes to chronic inflammation.

One marker of senescent cells is expression of the cell cycle regulator CDKN2A/p16. CDKN2A/p16 plays an important role in cell cycle regulation by decelerating progression from the G1 to the S phase [29]. In the normal cell cycle, CDKN2A/p16 acts to inhibit cell division by binding CDK4/6, which ultimately inhibits the activity of transcription factors such as E2F1 and arrests cell proliferation [30]. High levels of CDKN2A/p16 expression prevent cell division and are a hallmark of cell senescence [31]. Expression of CDKN2A/p16 increases with age in the kidney [32, 33]. Several studies have shown that expression levels of CDKN2A/p16 can be used as a biomarker of aging in order to predict renal transplant outcome. At the time of the renal transplant, a kidney biopsy was obtained and CDKN2A/p16 levels were measured [22, 34, 35]. Age, CDKN2A/p16 levels, and a combination of Age/CDKN2A/p16 levels were evaluated as predictors for renal transplant success. CDKN2A expression (biological age) was found to be better than donor age (chronological age) in predicting organ function [35]. However, CDKN2A/p16 levels combined with chronological age was found to be the most powerful predictor for renal function following transplantation [22]. The key concept is that CDKN2A/p16 levels may be measuring biological age, and that biological age may be better than

simple chronological age as a predictor of future renal function following transplantation.

Another strategy to develop biomarkers for renal transplantation is to identify gene expression signatures that can predict renal graft survival. In this approach, gene expression from the entire genome is measured from kidney biopsies at the time of transplantation using DNA chips. The renal transplants are then separated into two groups based on success of the graft, and the gene expression data are analyzed to identify differences in expression between kidneys that were or were not successful in the renal transplant. In one study, 31 renal allografts were separated into low and high GFR after 1 year following transplantation. Then, expression profiles taken at the time of transplantation were analyzed, resulting in the identification of 52 genes that showed significantly different expression profiles between the high- and low-functioning kidneys [36]. The gene expression profile of these 52 genes at the time of transplantation was able to predict the success of the renal transplantation over a medium term.

In a second study, 92 renal allografts were separated based on whether or not patients required dialysis during the first week (delayed graft function), and then gene expression data were analyzed to identify 206 genes whose expression showed a significant difference. This study suggests that preimplant gene expression profiles may be able to identify kidneys of poor quality that perform poorly in transplantation. This information may eventually improve organ allocation [37].

3 Hallmarks of Renal Aging

To better understand renal aging, it is useful to consider mechanisms that are involved in aging in other tissues, and even other species such as mice, flies, worms and yeast. The human aging process can be thought of as a clock that spans about 80 years. Aging mechanisms guide the rate at which this clock proceeds, and the most central pathways are part of the clock mechanism itself. As we grow old, aging affects many of the underlying networks in the kidney. There is accumulation of damage of diverse types to cellular components such as DNA, proteins and lipids. There are changes in gene expression and epigenetic networks with age. Cells lose their ability to divide and undergo senescence. There is a steady increase in the thickness of the extracellular matrix that is a major determinant of fibrosis. It is possible that changes in each of these networks serves as part of a molecular aging clock, that changes over time and dictates the rate of functional decline of the kidney.

A hallmark of aging not only changes as we grow old, but it also plays an important functional role in the physiological decline of the kidney with old age. Genetic and pharmacological experiments that reset the clock in old cells or organs to the young state should have a beneficial effect. By contrast, experiments in which the aging pathway has been reset in young cells to the old state should cause rapid aging to ensue.

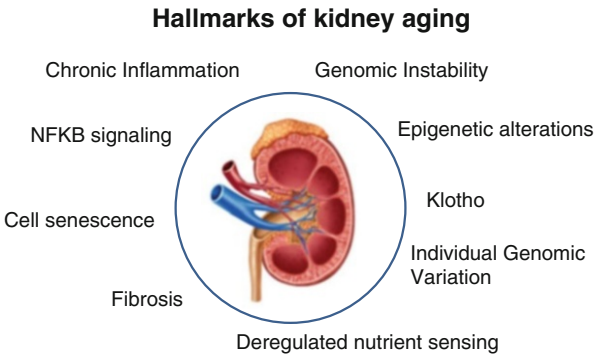

Fig. 3 Hallmarks of kidney aging. Shown are nine pathways that play important roles in renal aging

These hallmarks of kidney aging might collectively form a type of aging clock that dictate the functional and physiological state of the kidney over a lifetime. Although these pathways progress during aging in everyone, the rate of progression can vary between people. For people of the same chronological age, the aging clock might be slightly more advanced in one person than the other. With additional studies, hallmarks of aging could one day become very important because they could be used as biomarkers to report the true physiological age of a person or tissue, rather than mere chronological age. Not only would the aging biomarkers associate with the current functional state of the kidney better than chronological age, but the aging biomarkers would be better than chronological age at predicting the future trajectory of renal decay.

A recent review describes nine hallmarks of aging that form the conceptual pillars to understand changes as one grows old [38]. These hallmarks are: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. In addition to the general hallmarks, kidney aging is known to involve increased Klotho expression, chronic inflammation, and fibrosis (Fig. 3). Below, we consider each of these hallmarks and summarize what is known about how that hallmark may contribute to human renal aging.

One of the most important hallmarks of kidney aging is chronic inflammation [39]. Chronic inflammation occurs when there is an increased abundance of immune cells – B cells, T cells, neutrophils and macrophages. Low levels of activity of the immune cells lead to a low grade inflammatory response that contributes to fibrosis and tissue damage with age. One of the causes of chronic inflammation in the kidney may be increased systemic levels of inflammatory cytokines, such as IL1, IL6, and TNFα, in old age [40, 41]. The inflammatory response leads to increased production and accumulation of fibrinogen and C-reactive protein by the liver, leading to increased systemic levels of inflammatory biomarkers. The level of chronic inflammation is higher in patients with chronic kidney disease compared to healthy

age-matched controls, suggesting that chronic inflammation may play a role in the etiology of this disease [39]. However, the relationship between cause and effect between chronic inflammation and renal aging is unclear; specifically, it is unclear whether chronic inflammation causes renal damage to accumulate with age, whether age-related renal damage causes chronic inflammation, or whether both act together in a complicated feedback loop with increasingly dire consequences.

The inflammatory cytokines that are responsible for chronic inflammation could arise from several sources. One source is from immune cells (B cells, T cells and macrophages) that become dysregulated in old age [42, 43]. A second source is from adipocytes, which are known to produce many inflammatory cytokines, including TNFα and IL-6 [44]. Cytokines secreted from adipose tissue would enter the blood system and increase chronic inflammation throughout the body, including the kidney. A third source is from senescent cells, which secrete many inflammatory cytokines as part of the senescence phenotype [45]. Senescent cells increase in number in old age, leading to increased production of the inflammatory cytokines, a phenomenon termed the senescent-associated secretory phenotype [28]. A fourth source is from cells within the kidney itself. DNA microarray analysis showed that aged human kidneys have increased expression of certain inflammatory cytokines and chemokines [46]. One of the results of chronic inflammation is the recruitment of immune cells that secrete inflammatory cytokines, which may further increase chronic inflammation in the kidney as part of a positive feedback loop.

The inflammatory cytokine TNFα activates initiates a signaling cascade leading to activation of the transcription factor NFκB. The TNFα signaling pathway is induced when TNFα binds the TNF receptor (TNFR1), which leads to the degradation of IKB, a protein that normally keeps the NFκB complex inactive in the cytoplasm. Once IKB is degraded, the NFκB complex is no longer tethered in the cytoplasm and enters the nucleus where it activates expression of its target genes, which include many inflammatory cytokines and chemokines. Activity of NFκB in the kidney has been found to increase with age in the rat [47]. One of the major functions of NFKB is to mediate inflammatory and innate immune responses. Besides TNFα, the inflammatory cytokine IL-1, lipopoysacharide, and reactive oxygen species can activate NFκB.

Another hallmark of renal aging is fibrosis of the interstitium or scarring of the glomeruli [48]. The glomeruli are the functional unit of the kidney responsible for filtering the blood. The tubules and interstitium constitute 90 % of the volume of the kidney. Interstitial fibrosis and glomerulosclerosis increase with age, characterized by an increased thickening of the extracellular matrix. Extracellular matrix is composed primarily of collagen. Matrix metalloproteases are zinc-dependent endopeptidases responsible for degrading collagen and proteoglycans, and may function to help remodel the extracellular matrix. Excess production of collagen or altered expression of matrix metalloproteases could play a role in thickening of the extracellular matrix in old age. Mesenchymal cells (i.e. fibroblasts and myofibroblasts) produce extracellular matrix. Increased numbers or altered functions of these cells in old age could be responsible for increased collagen deposition and fibrosis. Activated interstitial mesenchymal cells are thought to contribute directly to renal

fibrosis by secreting fibrotic factors and extracellular matrix proteins that accumulate in the interstitial space and disrupt normal epithelial architecture [48].

Changes in DNA methylation and DNA repair play a role in fibrosis. Bechtel et al. found that increased DNA methylation plays a key role in renal fibrosis. They performed a genome-wide screen to look for DNA methylation differences between fibroblasts from fibrotic and non-fibrotic kidneys, and found 12 genes that showed increased levels of DNA methylation in the fibrotic kidneys [49]. They then inhibited DNA methylation using 5-azacytidine and found that fibrosis was lessened [49]. These results indicate that increased DNA methylation plays a causative role in causing renal fibrosis.

Another clue about the mechanisms responsible for renal fibrosis was obtained by studying a heriditary form of chronic kidney disease. Karyomegalic interstitial nephritis is a rare hereditary form of chronic kidney disease with only 12 known families worldwide [50]. Kidneys in patients with karyomegalic interstitial nephritis have enlarged hyperchromatic nuclei and develop chronic kidney disease in their third decade. The specific diagnostic traits associated with chronic kidney disease in these patients include interstitial fibrosis, tubular atrophy and microcyst formation. These symptoms are seen in normal elder subjects as part of kidney aging, suggesting that karyomegalic interstitial nephritis involves an accelerated rate of fibrosis in the kidney.

The genetic cause for karyomegalic interstitial nephritis has recently been found to be due to mutations in the FAN1 gene. FAN1 plays a role in DNA repair. Specifically, FAN1 is required to repair interstrand DNA crosslinks, so that covalently cross-linked DNA strands cannot separate during S-phase in patients with a non-functional form of FAN1 [51, 52]. In renal tissue from patients with karyomegalic interstitial nephritis, there was an increased level of double strand breaks. These results show that DNA damage caused by reduced activity of FAN1 can potentiate renal fibrosis.

Nutrient sensing is a general hallmark of aging in all tissues and most species. Caloric restriction can extend lifespan of nearly every species tested, including yeast, worms, flies, mice and primates [53]. Recent work has begun to unravel the molecular mechanisms underlying lifespan extension due to caloric restriction [54–56]. These molecular mechanisms involve key modulators of aging such as the sirtuins and mTOR.

Sirtuins are a family of protein deacetylases that are involved in a diverse array of cellular processes such as life span regulation, fat mobilization in human cells, insulin secretion and caloric restriction [57]. In mammals, there are seven sirtuin genes (SIRT1 to SIRT7). Activation of sirtuins has been shown to extend lifespan in diverse organisms including yeast, worms, flies and mice [58]. In the kidney, SIRT1 is abundantly expressed in renal medullary interstitial cells, where it is cytoprotective and participates in the regulation of blood pressure and sodium balance [59, 60]. Sirtuins require nicotinamide adenine dinucleotide (NAD+) as a cofactor, and hence could be responsive to changes in metabolic state of the cell. Levels of nicotinamide adenine dinucleotide show a marked decline in the kidney in old age, which may decrease activity of sirtuins thereby contributing to cellular dysfunction

in old age [59, 61]. Regulation of sodium balance by SIRT1 involves repression of expression of the α-subunit of the epithelial sodium channel, ENaC [59].

Genetic experiments by He et al., 2010 have shown that Sirt1 activity plays an important role in kidney function [60]. On the one hand, lowering Sirt1 activity impairs renal function. For example, reduced Sirt1 expression in mouse renal cells *in vitro* leads to reduced resistance to oxidative stress. Genetically mutant mice with reduced Sirt1 activity ($Sirt1^{+/-}$) show lower levels of cyclooxygenase-2 (COX2) expression and impaired kidney function under several conditions. $Sirt1^{+/-}$ mice show more renal apoptosis and fibrosis in mice that have suffered kidney injury by unilateral ureteral obstruction. On the other hand, increasing Sirt1 activity improves renal function. Sirt1 activity can be pharmacologically increased either by resveratrol or by the drug SRT2183. Pharmacologic activation of Sirt1 improves cell survival in response to oxidative stress, attenuates renal apoptosis and fibrosis, and increases expression of COX2 in the renal medulla.

mTOR is a conserved kinase that acts in a nutrient sensing pathway to regulate cell growth and longevity. In yeast, worms and flies, mutants with reduced TOR activity have longer lifespans [56]. In mice, inhibition of mTOR activity with the drug rapamycin results in extended lifespan [56]. During normal kidney aging, mTOR expression shows a marked increase in glomerular mesangial cells in old age [62]. This result suggests that increased levels of mTOR in the kidney may contribute to poor cell regulation and cell senescence in old age.

Klotho plays an important role in aging of the kidney [63]. Klotho is a transmembrane protein primarily expressed in the distal tubule cells of the kidney and the brain choroid plexus. Loss-of-function mutations in the Klotho gene in mice are associated with symptoms of premature aging, including hyperphosphatemia and a shorter lifespan [64]. Conversely, overexpression of Klotho extends lifespan [64]. In humans, serum levels of Klotho decrease with age after age 40 years and there are low levels of Klotho in patients with Chronic Kidney Disease [65–67]. One of the main functions of Klotho is to act as a co-receptor for Fibroblast Growth Factor 23 [68]. However, there is also evidence that Klotho can also regulate the Insulin-like Growth Factor signaling pathway, can participate in Ca^{2+} homeostasis, phosphate homeostasis and can relieve oxidative stress [69].

Changes in gene expression occur with age, and can be used to predict the physiological age of kidneys. DNA microarrays have been used to define the changes in gene expression that accompany the renal aging process. One study analyzed RNA from 74 patients ranging in age from 27 to 92 years, and found 985 genes to change expression with age [46]. Among these 74 individuals, there was a good correlation between the gene expression signature and the biological age of the kidney. For example, for some kidney samples, the gene expression signatures did not resemble kidneys of their chronological age but rather kidneys from individuals that were younger. Subsequent histological examination of these renal samples showed that they had lower levels of interstitial fibrosis, arterial hyalinosis and glomerulosclerosis than typically found in kidneys of that chronological age. That is, the gene expression signatures were able to accurately predict that these renal samples had a biological age that was younger than their chronological age. Similarly, the gene

expression signatures were also able to predict renal samples with a biological age that is older than their chronological age. A second study analyzed RNA isolated from 20 kidney samples and identified about 500 genes that changed expression with age [70]. The sets of kidney aging genes showed a large degree of overlap in the two DNA microarray studies.

What are the upstream transcription factors responsible for causing the changes in expression of the age-regulated kidney genes? In order to identify the upstream regulators, ChIP-seq data from the ENCODE consortium were examined. The ENCODE consortium has defined the in vivo binding sites for a large number of transcription factors (about 160) [71]. For each transcription factor, they used ChIP seq to first immunoprecipitate the transcription factor from tissue culture cells, and then sequenced the bound DNA in the immunoprecipitate. Each experiment resulted in a list of target genes bound by that transcription factor in specific cell lines. To find transcription factors that bind to the age-related kidney genes, a bioinformatics screen was performed to search for transcription factors that had ChIP seq datasets showing a large degree of overlap with the kidney age-related genes [72]. The top three transcription factors that showed enrichment to binding the aging-regulated genes were STAT1, STAT3 and NFκB. These three transcription factors are all known to be involved in mediating the inflammatory response.

STAT1, STAT3 and NFκB appear to mediate transcriptional changes during the kidney aging process *in vivo* [72]. All three transcription factors show higher levels of activation in old age. When the transcription factors are activated by inflammatory cytokines in human renal epithelial cells, the resulting changes in gene expression recapitulate the gene expression changes that occur during kidney aging to a large extent. These data indicate that activation of these three transcription factors during kidney aging may contribute to a large fraction of the aging transcriptional program. The finding that NFκB binds to and regulates genes during aging confirms and extends previous work showing that increased NFκB activity contributes to aging phenotypes in many tissues [73–75].

4 Genetic Differences May Explain Some of the Individual Variation in Kidney Aging

There is a great deal of variation in the rate of loss of kidney function with age between different individuals. In old age, many people have lost a considerable fraction of kidney function, leading to chronic kidney disease or end stage renal disease. Other people have relatively mild loss of kidney function. Genetic variation accounts for some of the individual variation in kidney aging. Genome-wide association studies (GWAS) have begun to define DNA polymorphisms that are associated with either reduced glomerular filtration rate or chronic kidney disease. In principle, genetic algorithms may one day be used in the young to help predict which individuals are at risk for a rapid loss of renal function as they grow old, and which

individuals are more likely to have a slower rate of loss of kidney function with age. These genetic risk scores would be able to look-up the information from each of the individual polymorphisms and create one combined score to be used to predict future renal rates of aging.

Genome wide association studies have recently been successful at identifying single nucleotide polymorphisms (SNPs) associated with a variety of renal phenotypes associated with age. An example is glomerular filtration rate, which is an estimate of renal function based on levels of creatinine in the urine. The studies are controlled for age, so DNA variants associated with GFR in these studies would identify loci that predispose one to having higher or lower GFR for a given age. There are at least two ways in which a SNP could be associated with variation in GFR. First, the SNP could be associated with a different baseline of renal function (higher or lower GFR) but not with a difference in the rate of renal aging. In this case, GFR would decline similarly in individuals that vary at the SNP, but the GFR would have a different baseline so that those with a higher baseline would be less likely to have renal disease as they grow old. Second, the SNP could be associated with a different rate of renal aging. In this case, allelic variation at this SNP may have little effect on young adults as all might have a similar starting point for renal function. However, individuals with one allele could show more rapid rates of renal decline leading to larger and larger differences in GFR as one ages.

Another powerful phenotype used in kidney GWA studies is chronic kidney disease. Chronic kidney disease is diagnosed when an individual has glomerular filtration rate below 60 ml/min/1.73 m^2 for 3 or more months, or if the individual has other signs of kidney damage. The gene association studies using chronic kidney disease as a phenotype are similar to the GWAS using glomerular filtration rate as a trait because one of the main criteria for diagnosing chronic kidney disease is glomerular filtration rate. Chronic kidney disease splits up individuals based on GFR levels above or below the 60 ml/min/1.73 m^2 cutoff and GFR assigns a continuous variable to each individual.

Kidney GWA studies have also been performed for albuminuria and diabetic nephropathy. Albuminuria refers to albumin in the urine, which is another indication of poor kidney function. Diabetic nephropathy is characterized by three related conditions: albuminuria, low glomerular filtration rate, and high blood pressure.

A total of 67,073 individuals have been examined to search for SNPs associated with GFR, chronic kidney disease, albuminuria and diabetic nephropathy [76, 77]. These studies have found 27 SNPs associated with these parameters of kidney function. These SNPs may be useful in helping to identify individuals with high and low renal function. This information could be useful to individuals when they are alive by providing information useful for precision medicine. In addition, the genetic information could be useful to help identify which kidneys from elderly donors may still be viable for use in renal transplantations.

In order to predict renal function, one would not evaluate each of the kidney SNPs one at a time, but rather one would develop an algorithm that could evaluate all of the SNPs together and provide a score that summarizes the information from all of the SNPs combined. A preliminary study was performed combining informa-

tion from 16 kidney SNPs to create a genetic risk score for chronic kidney disease [76]. The genotype score was evaluated in 2129 test subject and was partially successful in predicting chronic kidney disease risk. Carrying a high number of risk alleles was partially able to predict those at increased risk for chronic kidney disease. However, the effect of the genotype was small, and not necessarily an improvement over clinical factors such as lifestyle, blood pressure and the presence of Type 2 Diabetes. Beyond renal disease, genetic algorithms have been developed for Type 2 Diabetes mellitus, incident myocardial infarction, coronary heart disease, myocardial infarction and stroke in women [78–81]. In each case, the genetic algorithms had only a mild effect in predicting disease risk beyond current clinical tests, indicating that improved methods or more complete data will be required for these algorithms to become widely used. Future studies may be able to expand the number of SNPs known to be associated with renal function, leading to improved genetic algorithms with greater predictive power.

One study has specifically searched for SNPs that are associated with different levels of kidney function, independent of age [82]. To do this study, longitudinal data was used from the Baltimore Longitudinal Study of Aging and from the InCHIANTI cohort. Both of these cohorts have followed individuals over a number of years, so it was possible to follow the loss of renal function with age for each individual. However, the total number of people in each cohort was relatively small (1–3 thousand), precluding a straightforward GWA study for association with different rates of loss of GFR.

Instead of searching through every SNP in the genome, the study used a genomic convergence approach to systematically select SNPs that are most likely to be involved in kidney aging. The first assumption was that genes whose expression changes with age would be enriched for those with functional effect on the rate of renal aging or renal function (630 aging-related genes). The next step was to search for SNPs that are associated with differential expression of these 630 genes (expression quantitative trait loci; eQTLs) because higher or lower expression levels may affect the age at which the expression of a gene dips below a functional threshold (110 SNPs associated with differential expression in kidney aging related genes). The last step was to determine whether any of the 110 SNPs was associated with different levels of renal function independent of age in the Baltimore Longitudinal Study of Aging or the InCHIANTI cohort. Two linked SNPs (rs1711437 and rs1784418) located within the matrix metalloproteinase 20 gene (MMP20) were found to associate with loss of GFR independent of age at a significant level following correction for multiple hypothesis testing. For an individual who carries the A allele at rs1711437, his or her creatinine clearance is approximately that of someone 4–5 years younger who does not carry the A allele. In the BLSA population, the genotype of rs1711437 explains 2.1 % of the variation in creatinine clearance and in the InCHIANTI population, the genotype explains 0.9 % of the variation.

Future work will help find the missing heritability in DNA variants associated with kidney function, disease and aging. These studies may use full genome sequence rather than DNA chip analysis, enabling one to analyze rare variants. Larger cohorts may become available that will increase statistical power. Genetic

algorithms may become available that are more powerful and able to better predict renal function. These algorithms may gain effectiveness by capitalizing on the complex genetic interactions among the SNPs associated with renal aging. These interactions are termed epistatic interactions, and refer to all of the possible ways in which two SNPs may be associated with renal aging that are not merely the additive sum of each individual SNP. For instance, the two SNPs may act redundantly, such that a protective allele in one SNP is as effective as a protective allele in both SNPs. Alternatively, the two SNPs may act synergistically, such that protective alleles in both SNPs are far more effective than would be predicted by adding up the effects of each allele individually. Future work may lead to ways to use DNA information to predict current and future renal function. For the purposes of renal transplantation, this could be key as a criterion to be used to help select which potential donor kidneys are most likely to retain adequate function following renal transplantation.

5 Conclusion

As we enter the era of precision medicine, individual genetic and biomarker data can be used to provide information about the physiological age of a person, rather than simple chronological age. Recent work has begun to unravel some of the mechanisms underlying kidney aging (Fig. 3). These mechanisms include increasing levels of cell senescence, chronic inflammation, fibrosis, and transcriptional regulation of the aging gene network. A better and more complete understanding of the molecular underpinnings of aging may one day enable the development of biomarkers that are able to report true biological age, as opposed to chronological age. These aging biomarkers could be used to more accurately ascertain which kidneys are most suitable for renal transplantation. This precision medicine approach of using personalized aging biomarkers may enable one to expand the pool of available kidneys for transplantation without diminishing the length of graft survival or the quality of the transplant.

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Asthma and Aging

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1 Introduction

Asthma is a chronic airway disease, driven by complex interactions of inflammatory cells including eosinophils, lymphocytes, neutrophils and mast cells, and their mediators. Increased airway inflammation in asthma produces its characteristic features; airway hyperresponsiveness (AHR) to stimuli such as aeroallergens, histamine or methacholine and reversible airflow obstruction [1, 2]. However, in some patients, in particular those with a history of long-standing asthma, airflow obstruction may become only partially reversible. In susceptible individuals, clinical features of asthma are recurrent episodes of wheezing, breathlessness, chest tightness, and cough, particularly at night and/or in the early morning. These symptoms are usually associated with widespread but variable airflow limitation that is at least partially reversible either spontaneously or with treatment. While it is not clear whether airway hyperresponsiveness is acquired or is genetically determined to appear with the appropriate stimulus, it is thought that airway inflammation is the main stimulus as it may be induced by a number of inciting events including viral respiratory infections, allergen exposure in sensitized individuals, cold air, and exposure to noxious agents such as ozone and sulfur dioxide. Allergic or atopic reactions in the upper (nose, sinuses) and lower airways are both important in the pathogenesis of asthma in childhood and young adulthood. However, their role in the elderly is less clear. Atopy is defined by the presence of detectable IgE antibodies to environmental antigens and can be manifested as asthma, eczema and/or seasonal and perennial allergic rhinitis. In elderly patients with or without asthma, an elevated level of IgE may be an important risk factor for the development of chronic airflow obstruction [3].

Similar to other chronic diseases in this age group, asthma in the elderly population has a major impact on the patient's well-being and significantly impairs health status. Patients may consequently suffer from poor general health, symptoms of depression, and significant limitations of daily activity [4–9]. The exact prevalence of asthma in the aging population is not entirely clear as many studies do not clearly distinguish asthma from other obstructive lung diseases, but it appears to be similar to younger adults. According to the Center for Disease Control and Prevention (CDC), the overall prevalence of asthma in the US population >18 years of age is 7 % (www.cdc.gov/asthma/most_recent_data.htm) [10]. Large cross-sectional data base surveys including the National Health and Nutrition Examination Survey (NHANES) 2005–2006 and the National Health Interview Survey (NHIS) have reported that between 6.9–8 % of the U.S. population over the age of 60 years has a current diagnosis of asthma based upon a physician diagnosis [11, 12]. This translates to more than 2.7 million adults >65 age in the US with a diagnosis of current asthma. (www.cdc.gov/asthma/most_recent_data.htm) Asthma in the elderly is associated with a significant numbers of emergency department visits and hospitalizations, leading to substantial healthcare costs [13, 14]. Elderly patients with asthma are >5 times more likely to die from their disease than younger individuals and while mortality rates in some age groups have decreased, this is not true of the

elderly [13–15]. According to the CDC, asthma deaths in the elderly account for more than 50 % of asthma fatalities annually with an approximately 5.8 asthma deaths per 100,000 in this group reported in the years 2001–2003 [10, 16]. Although the majority of elderly patients with asthma have long-standing asthma that may have developed early in life, some develop asthma late in life. LOA may occur at any age, even in the eighth or ninth decades of life and when it does, moderate to severe symptoms are more likely [17]. Despite the frequent occurrence of asthma in the elderly, it is a diagnosis that has been frequently overlooked and even when discovered it is often under treated [5, 18–22]. There are a number of important reasons that may explain the under diagnosis and under treatment of asthma in the elderly and these will be discussed in this chapter.

2 Pathophysiology and Risk Factors

2.1 Airway Inflammation and Aging

The immune system is complex and comprised of an innate arm, providing an initial defense against pathogens, and the adaptive arm, a subsequent antigen-specific response. Non cellular components including C-reactive protein, mannose-binding protein and complement, and a cellular component including phagocytic cells (neutrophils, macrophages/monocytes), epithelial cells and natural killer cells comprise the innate side. Cells of the innate arm use pattern recognition receptors (PRR), such as Toll-like receptors (TLR), to identify conserved pathogen associated molecular profiles (PAMPs) expressed on the surface of pathogens. The actions of the innate response are not long-lived, but are an important initial event, triggering activation of antigen-specific responses of the adaptive immune response which include humoral immune defenses (mediated through B cells) and cellular responses (mediated by T cells).

With increasing age, there are alterations in both the innate and adaptive immune responses. One phenomenon is termed “immunosenescence” in which the adaptive arm of the immune system response is “blunted” after a pathogenic threat or tissue injury. Cellular senescence is due to an irreversible loss of cellular replication and eventually results in impaired tissue repair. Senescence either develops via a continued stepwise accumulation of DNA damage occurring during replication (replicative senescence), or abruptly by activation of oncogenes (typically associated with premature aging) [23, 24]. However, despite an inability to proliferate, senescent cells remain alive, but function at a diminished or altered capacity. This may include an increased low-grade basal systemic inflammation (characterized by increased IL1-β, IL-6 and TNF-α) in the absence of an overt infection, referred to as “inflammaging.” [25]

Immunosescence research has focused primarily upon cancer and autoimmunity, but not asthma. The underlying mechanisms of immunosenescence and inflammaging are complex, and a consequence of several processes, including both “random” (e.g., environmental exposures, accumulation of reactive oxygen species

(ROS) from metabolic activity, mutagenesis) and "regulated" (e.g., genetic) aspects. Alteration and loss of mitochondrial function plays a key role in cellular changes with aging. Decreased mitochondrial function increases generation of ROS resulting in accumulation of oxidative products [26]. Furthermore, damage of mitochondria leads to release of mitochondrial damage-associated molecular patterns which are similar in structure to many of the conserved patterns on bacterial surfaces (PAMPs), which in turn, activate the innate immune response [27]. A loss of mitochondrial function alters protein synthesis and protein folding, necessary for proteostasis. Additionally, accumulation of damaged cellular and organelle components and macromolecules may induce ongoing low-grade systemic inflammation. These products can be subsequently recognized as "danger" signals, initiating ongoing inflammation [25]. Shortening of telomeres (necessary to protect the chromosomal ends) may signal cell cycle arrest or apoptosis [28, 29] or replicative senescence, which in turn induces the release of pro-inflammatory proteins [26]. Shortening of telomere length in peripheral blood mononuclear cells (PBMCs) has recently been demonstrated to correlate with increased asthma severity in older patients with asthma [30]. Mechanistically, microRNAs (miRs, regulatory elements which can either suppress or activate gene expression, usually at the post-translational level) have been implicated in immunosenescence and inflamm-aging, increasing the expression of pro-inflammatory proteins [31], and decreasing cell proliferation and function [31, 32].

Older individuals with fewer features of immunosenescence may have a prolonged lifespan [33]. Conversely, specific features of immunosenescence are associated with increased morbidity and mortality [34], and low-grade systemic inflammation with more clinically "frail" individuals [35, 36]. However, how the effects of immunosenescence translate to airway inflammation and its regulation in older patients with asthma is not well established. Additionally, whether asthma is a distinct inflammatory phenotype in older patients is unknown, important and unclear, yet it may alter treatment of the disease. The following section will address what is known about the effect of increased age on the innate and adaptive immune responses and how these changes may alter airway inflammation of asthma in older adults.

2.2 Aging-Related Changes in Cellular Components of the Innate Immune Response

2.2.1 Epithelial Cells

The respiratory epithelium is an important component of the innate immune system, and is composed of ciliated and secretory cells. Ciliated cells propel inhaled antigens and irritants trapped in mucus produced by goblet cells, proximally up the tracheobronchial tree via mucociliary clearance. Additionally, airway epithelial cells produce nitric oxide, secrete cytokines [e.g. type I and III interferons (IFNs)],

growth factors (e.g. granulocyte/macrophage colony-stimulating factor), IgA and antimicrobial proteins, which act as an initial immune clearance mechanism. Adjacent epithelial airway cells are connected by tight and adherent junctions, which form a physical barrier against entrance of microbes and antigens. In younger patients with asthma, airway epithelial cells have disrupted tight cellular junctions, an increased susceptibility to apoptosis and an impaired production of interferons [37]. Although changes in the airway epithelial cells with aging in individuals *without* airway disease have not been investigated in detail, there is evidence that there are alterations. In non-smoking healthy individuals, ciliary beat frequency and clearance decrease with age [38, 39]. Additionally, aged airway epithelial cells have a decreased barrier function [40]. In aged mice, repeated inhalation of toxicants decreased airway epithelial cell repair and exacerbated p38 MAPK-dependent pro-inflammatory cytokine expression [41]. These age-associated changes may enhance alterations in airway epithelial cells characteristic of some younger patients with asthma or increase the risks of respiratory tract infections, thus exacerbating underlying asthma. Additionally, it is possible that individuals with asthma are at increased risk of "accelerated" cellular aging, as has been suggested with chronic obstructive pulmonary disease (COPD) [42] (see chapter "Aging in COPD and idiopathic pulmonary fibrosis" by C. Sanchez in this volume).

2.2.2 Neutrophils/Polymorphonuclear Cells

Neutrophils or polymorphonuclear cells (PMNs) have several important functions in the initial clearance of pathogens including phagocytosis, recruitment and maturation of dendritic cells, transport of antigens to lymph nodes and secretion of neutrophil extracellular traps (NETs), which immobilize bacteria. With increasing age, neutrophil recruitment is altered, preventing chemotaxis of PMNs to the site of inflammation [43]. Additionally, neutrophils from aged mice produce fewer NETs after either infection or non-specific cellular activation [44]. Aged neutrophils also have a decreased capacity for phagocytosis [45, 46]. Potential mechanisms include a decreased surface expression of CD16 [47] (necessary for Fc-mediated phagocytosis) and decreased downstream signaling of TLRs [48, 49]. Normally, PMNs produce superoxide anions, which are converted to reactive oxygen species (ROS), for intracellular killing after phagocytosis. With advanced age, there is a higher constitutive ROS expression by neutrophils which may be due to increased cellular activation (suggested by increased extracellular CD11b and HLA-DR expression) and altered regulation of cGMP and cAMP for ROS production [50–53]. Increased production of free radicals is a theory of aging and damages local tissues [54]. Despite increased basal production of ROS, induction of ROS in response to a bacterial infection is suppressed in older compared to younger patients [50, 55]. This may occur secondary to a decreased glucose accumulation (required for ROS production) in aged PMNs [56].

Despite diminished anti-pathogen activity, the number of neutrophils in the airway of aging individuals without airway disease increases [57, 58]. Whether this is

secondary to decreased neutrophil apoptosis [59] or to increased systemic inflammation with aging is not clearly established. Additionally, the impact this has on asthma in aging is not known. Emerging data suggests that older patients with asthma may have increased airway neutrophils (measured from induced sputum) compared to younger patients [60, 61]. Furthermore, increased airway neutrophilia in older asthma subjects corresponds to increased levels of sputum neutrophil mediators including MMP-9, neutrophil elastase and IL-8. This resembles changes seen in a phenotype of severe asthma noted in some younger adults [61] . Determining underlying airway inflammation in older adults with asthma is important as neutrophilic asthma is often less responsive to corticosteroid treatment [62, 63], therefore alternative therapies may be indicated for older patients with asthma.

2.2.3 Eosinophils

There is a limited understanding of the age-associated changes in eosinophil number and function in older patients with asthma. In younger patients with asthma, in particular those with an "allergic phenotype," the airway eosinophil plays an important role in airway inflammation and AHR [64] . However, aged eosinophils may have decreased effector functions and be less important for AHR. In a study examining age-related changes in eosinophil function, peripheral eosinophils from subjects with asthma (55–80 years of age) exhibited decreased degranulation in response to IL-5 stimulation and a trend for decreased superoxide production, when compared to cells from patients 20–40 years of age [60]. However, study of another in vitro eosinophil effector function, leukotriene C4 (LTC_4) production, revealed no difference between older and younger subjects with asthma [65]. The role of the eosinophil in AHR in older patients with asthma is not well established. In a mouse model of asthma, antigen sensitized and airway challenged aged mice developed greater bronchoalveolar fluid (BALF) eosinophilia in comparison to younger mice, however AHR was lower in the former, suggesting that increased airway eosinophilia was not correlated to AHR [66]. However, the development of AHR later in life was associated with elevated peripheral blood eosinophil counts in males (mean age 60 years) enrolled in the Normative Aging Study [67]. Thus, eosinophils may be less functional in the elderly; their role in AHR, however, requires further investigation.

2.2.4 Dendritic Cells

Dendritic cells (DCs) lie at the interface between the innate and adaptive immune responses and play key roles in antigen presentation. There are 2 main subpopulations of DCs, plasmacytoid (pDC) and myeloid (mDC). Both DC populations express TLRs and produce type I and type III interferons (IFN), necessary for anti-viral responses, although pDCs produce greater amounts and more rapidly. The impact of age-related changes in DC in asthma is not clear. However, there are several alterations of DC function with aging which may alter asthma in older patients.

The increased production of IL-6 and TNF-α by DCs during aging might contribute to the observed inflammaging [68–70]. Additionally, there are several changes associated with DCs during aging which may increase susceptibility to respiratory infections and asthma exacerbation, including decreased IFN production [71], phagocytosis [68], migration [72, 73] and TLR function, as well as a poor antibody response to influenza immunization [70]. Although there are likely several mechanisms underlying alteration of DC function with aging, epigenetic modifications of DNA producing hypomethylation may contribute [74].

2.3 Aging-Related Changes in the Adaptive Immune Response

In contrast to changes in immune function of the innate system with aging, changes in adaptive immunity are better defined. With aging, the ability to mount an adaptive immune response declines [75]. These changes produce several clinical outcomes including a reduced ability to produce specific and long-lasting antibodies to vaccinations [76, 77] and a lack of a rapid immunologic response when encountering pathogens. Similar to the innate immune response, the effects of age on the adaptive response and asthma in older patients is not well characterized.

2.3.1 T Lymphocytes

T cells play a key role in the adaptive arm, in particular, the cellular immune response, necessary for eradication of viruses and intracellular pathogens and cytokine production. T cells are identified by surface expression of CD3 and are further categorized into CD4 or CD8 T cells which have distinct functions. CD8+ T cells recognize class I MHC-associated peptides on infected cells, releasing granular contents to promote their cytolysis. In contrast, naïve CD4+ T cells recognize MHC II peptides on antigen presentation cells, and after activation, differentiate into subsets including: Th1 (characterized by secretion of interleukin (IL)-2 and interferon (IFN)-γ), that function to increase macrophage phagocytosis and CD8+ T-cell proliferation; Th-2, which produce IL-4, −5, −13, stimulating eosinophil activation and production of IgE antibodies by B cells; and Th-17 (characterized by secretion of IL-17a, IL-21 and IL-22 which recruit and active neutrophils).

With increased age, the number of circulating naive T lymphocytes significantly decreases. Reduction of naïve T cells has been largely attributed to thymus involution with aging and replacement of tissue with adipose, which produces additional pro-inflammatory mediators affecting thymopoiesis [78]. However, recent work suggests that a decrease in naïve T cells with aging may be instead due to alterations of peripheral cell division [79], secondary to lower systemic levels of IL-7, which normally protects against telomere loss and apoptosis [80], thereby shortening the survival of naïve T cells [81–83]. Although the number of naïve T cells decreases with aging, the total number of circulating T cells remain relatively constant due to the survival and increased resistance to apoptosis of memory T cells [84, 85]. The

increased survival of memory CD4+ was reported to be due to changes in the aged mouse microenvironment [84], and survival of memory CD8+ T cells to chronic viral stimulation, in particular Cytomegalovirus (CMV) [86].

With aging, there are several alterations which affect the T cell receptor (TCR). Aging T cells in humans lose surface CD28, necessary for TCR signaling. Loss of CD28 expression may occur from repeated antigen stimulation over time, producing a cellular stage of replicative senescence as demonstrated by shortened telomers and reduced proliferation [87–89]. Furthermore, type I interferons and TNF-α can decrease CD28 expression [90, 91]. The consequences of loss of CD28 include decreased IL-2 secretion and cellular proliferation, and increased resistance to apoptosis [92–94]. In addition, TCR diversity decreases with age [95, 96], which likely decreases viral clearance [97].

2.3.2 Th17 Cells

Th17 cells are a subgroup of T cells which secrete IL-17A, IL-17F and IL-22, which in turn stimulates IL-23 production. Th17 cells are differentiated from naïve T cells when stimulated by IL-6 (increased expression associated with aging) and TGF-β, which upregulate Th17 transcription factors retinoid acid-related orphan nuclear hormone receptor-γt (ROR-γt) and STAT3 [98]. The frequency of Th17 cells most likely increases with aging [99, 100], which clinically may translate to decreased viral clearance, increased neutrophilic inflammation increased transplant rejection [101], increased risk of autoimmune diseases and cancer [102] and mortality during a viral infection [103].

Recent data in aged mouse models of asthma also demonstrate an increased expression of airway Th17 expression [104, 105]. Murine models of allergic asthma suggest that IL-17A and IL-17F are important in airway inflammation, particularly neutrophilia [106, 107], enhanced Th2 associated eosinophila [108], increased AHR and airway mucus gene expression [109, 110]. In humans, IL-17A and IL-17F expression is increased in the airways of patients with asthma [111, 112]. Increased IL-17 in older patients with asthma likely contributes to increased airway neutrophilia and may be an additional target for therapy of asthma in this group of patients with difficult-to-treat asthma and increased morbidity and mortality from their disease.

2.3.3 Regulatory T Cells

Regulatory T cells (Tregs) suppress several effector functions of Th1, Th2 and Th17 cells. Tregs may protect against autoimmune diseases such as multiple sclerosis and Type I diabetes, but excessive numbers and activity may lead to increased susceptibility to infection and cancer. Treg cells found in the lung are in the CD4+ $CD25^{hi}$ population. Natural Tregs (nTregs) are derived from the thymus, express the transcription factor Foxp3, and mediate suppression primarily via cell contact. Peripheral

antigen-induced adaptive Tregs (iTregs) are either Foxp3+ or Foxp3-, and their major suppressive action is mediated via IL-10 and TGF-β.

The effect of age on the numbers and function of Tregs is less clear. Numbers of cord blood CD4+ $CD25^{hi}$ are elevated at 2.3–9.5 % of the total CD4+ T-cells (suggested to support fetal development of the immune system); they decline within the first 36 months of life, and then remain stable in young and middle aged healthy adults [113]. Some investigators have shown an increase in CD4+ CD25+ and CD4+ Foxp3+ T cells [114, 115] in healthy elderly compared to younger subjects, whereas other groups have found no significant differences in numbers of CD4+ Foxp3+ T cells [116]. The proportion and numbers of CD4+ Foxp3+ cells have been shown to be increased in lymphoid organs, but not in lungs or blood of aged (>20 month) C57BL/6 mice compared to younger mice [114, 117]. Earlier studies suggested that the suppressive activity of Treg cells declines with increasing age [118], but more recent studies have suggested that it is preserved or even enhanced [115–117].

The precise role of nTregs and iTregs in asthma is not clearly established, but most studies in murine models and in humans with asthma suggest a suppression of airway inflammation and AHR [119–121]. Peripheral numbers of Treg cells are lower in younger patients with asthma compared to age-matched subjects without airway disease [122]. Antigen tolerance in murine models of asthma, induced by inhalation or low-dose oral feeding of allergen to young mice suppressed several features of allergic asthma and increased Treg cell numbers [123–125]. On the other hand, aged mice fed low-dose antigen prior to antigen sensitization and challenge developed decreased AHR, antigen-specific serum IgE, BALF eosinophilia and mucus hypersecretion compared to age-matched non-tolerant mice. Additionally, the percent of lung tissue containing CD4+ CD25+ Foxp3+ cells was increased, but not to the same extent as in young mice, in antigen-tolerant aged mice suggesting that with increased age, Treg cells may continue to suppress AHR [126]. Similar to younger patients with asthma, one study reported that older patients with asthma had decreased peripheral Treg cell numbers compared to age-matched normal controls [127]. However, the importance of Treg cells in airway inflammation in older patients with asthma has not been widely investigated.

2.3.4 B Cells

B cells produce antigen specific antibodies. With increased age, the potential for the bone marrow to generate new naïve B cells from hematopoietic stem cells (HSC) diminishes [128, 129]. Although the ability of B cells to produce antibodies remains intact with aging [130], the quality of antibodies produced decreases, as exemplified by lower antigen affinity and avidity [131]. This is likely due to several alterations during aging, including deficient somatic hypermutation, responsible for the enhancement of antibody specificity for antigens [132], altered cytokine production by aged T cells and decreased expression of CD154 (CD40L), required for class switching of antibody production [133]. Therefore, the age-related decline in

diversity and quality of antibodies, particularly in the setting of new antigen exposure, may impact the ability of the elderly to clear pathogens and provide protection against a repeat exposure to the same pathogen.

2.3.5 Cytokine Secretion

Cytokine secretion by the adaptive immune system is altered with aging, although study results are varied. Some studies suggest increased IFN-γ secretion by activated peripheral CD4+ and CD8+ cells [134] due to increased methylation of the IFN-γ promoter [135], whereas several others have shown a shift towards Type 2 cytokine production [136]. In a study of healthy subjects in three different age groups (between 21–30 years, 80–81 years, and 100–103 years), flow cytometry determined CD4+ and CD8+ T cell populations with respect to intracellular IL-4 (Th2) and IFN-γ (Th1) expression upon PMA (phorbol myristic acid) stimulation [137]. When the ratio of IFN-γ/IL-4 producing cells was examined, there were no age-related differences in the CD4+ T cells; however, the IFN-γ/IL-4 ratio in the CD8+ T cells was lower in both the older age groups, suggesting an age-related shift from Th1 to Th2 cytokine profiles. Additionally, aging has been associated with a decreased CD8+ IFN-γ response to virus and cytolytic activity [138]. The underlying effects of cytokine secretion changes with aging and asthma are not clear at this point; however, allergic disorders are usually secondary to increased Th2 cell expression.

2.4 Role of Allergic Sensitization and Aging

Atopy is defined as the predisposition towards developing an IgE-mediated hypersensitivity reaction. It is documented by the presence of at least one detectable antigen-specific IgE to a common environmental (e.g. pollens, dust mites, animals with fur, cockroaches) or food antigen [139]. Antigen-specific IgE is produced by B cells and binds to the high-affinity IgE receptors on mast cells and basophils. Once re-exposed, the specific antigen binds to the IgE molecules, causing these cells to cross-link. This process initiates cellular degranulation with the release of preformed mediators including histamine, leukotrienes and the synthesis of cytokines which contribute to allergic symptoms and airway bronchospasm. Detection of antigen-specific IgE is done either through skin prick testing (SPTs) or measurement in serum. Although SPTs are easily performed and offer results quickly, atrophy of the skin and declining skin mast cells with aging may decrease their sensitivity in older patients [140].

Total IgE declines with increasing age in the general population, including individuals with and without atopy [141–143]. Cross-sectional studies including the Tucson Epidemiological Study and the National Health and Nutrition Examination Survey (NHANES 2005–2006) demonstrate that total serum IgE peaks by 20 years of age and is lowest after 70 years [11, 141]. However, some studies have not supported a trend of decreased total IgE with increased age [144–147]. Allergen-

specific IgE also decreases with aging. Studies including healthy subjects *with and without asthma and other atopic diseases*, suggest that approximately 40–50 % of subjects under the age of 50 years and approximately 35 % of subjects older than 50 are IgE sensitized to at least one allergen [143, 145, 147–149]. The increase in total and allergen-specific IgE production with age is most likely due to multiple factors such as alterations in cytokine profiles, changes in B cell antibody production, and DC and T lymphocyte function with aging, as described previously.

For many years, asthma in older patients was characterized as non-atopic or intrinsic [150, 151]. However, over the past two decades, it has been demonstrated that atopy is not uncommon in older patients with asthma. The reported percentage of older patients with atopic versus non-atopic asthma varies and may depend upon the characteristics of the population investigated. Studies examining the presence of IgE-sensitization in older patients with asthma in the United States describe sensitization rates between 23.9 % to as high as 74 % in one study [152–155]. Multicenter studies from the Netherlands [156] and France [157] reported that approximately 35 % of older patients with asthma were sensitized to at least one allergen. The most common aeroallergen to which older patients are sensitized is not consistent among studies, but typically includes cats [154], dust mites [155, 158], and cockroaches [159].

Atopy in children with asthma increases disease morbidity [160, 161]. Whether this relationship occurs in older patients with asthma is not known. Studies including older subjects with asthma, suggest that an atopic history may increase disease severity [162, 163]. In a study of 45 patients > 65 years of age recruited from an asthma clinic in New York City, cockroach sensitization was associated with more severe asthma as measured by airflow limitation and hyperinflation [159]. Data from NHANES 2005–2006 suggested that the relationship between antigen sensitization and asthma outcomes (e.g. hospitalizations, health care use) were not different in subjects 20–40 years old vs. ≥55 years old [164]. However, no studies have been conducted to date with antigen challenge of older patients with asthma and measurement of subsequent airway function.

Some studies suggest that antigen sensitization plays a role in late onset asthma (LOA) [153, 165]. In the Normative Aging Study, men developing airway hyperresponsiveness after 49 years of age were more likely to be sensitized to cat (23.9 % versus 4.4 %) compared to age-matched controls [153]. Nearly 50 % of the 40 patients in the Tucson Epidemiologic study of obstructive lung diseases who developed asthma after the age of 60 years were skin prick positive to at least one antigen, compared with 26 % of the age-matched control population without asthma [152]. In a study of 21 patients with asthma onset after 65 years of age, 81 % demonstrated a positive skin prick test to at least one allergen compared to a group of 14 patients developing asthma at <65 years, and in whom 57 % were allergen sensitized [165]. However, some studies suggest that IgE Ag sensitization may play a less significant role in LOA. For example, a French study recruited 1,485 patients (mean age 73 years) with a diagnosis of asthma from a total of 379 lung specialists to examine disease characteristics. Only 14.7 % of those developing asthma after 65 years of age were sensitized to at least one antigen by skin prick testing, whereas 60.1 % of those developing asthma prior to 21 years of age were antigen sensitized [157].

Although there are several unanswered questions regarding the roles of allergen sensitization in older patients with asthma including its role in disease inception, disease progression, trigger and management, allergen sensitization evaluation should be a component of asthma management and evaluation in this group, in particular patients with persistent asthma. Additionally, some older patients with asthma have enjoyed clinical improvement in their asthma after treatment with anti-IgE therapy [166, 167], emphasizing that allergen sensitization should be evaluated in older patients with asthma.

2.5 Role of Cigarette Smoke and Irritants

Exposure to cigarette smoke increases the risk of developing asthma in children [168]. Although first and second-hand cigarette smoke exposures are traditionally associated with the development of COPD in older adults, it also increases the risk of developing asthma later in life. Between 1/3 to ½ of older adults with asthma report a current smoking history [5, 19, 169]. In the Normative Aging Study, a history of current smoking and presence of atopy was associated with increased airway hyperresponsiveness to methacholine challenge [170]. Additionally, in 48 patients over the age of 70 years with late onset asthma (mean onset 58 years), those with a smoking history had increased frequency of antigen-specific IgE to common aeroallergens and increased generation of LTB4 by peripheral leukocytes suggesting that cigarette smoking may enhance IgE production [171]. Furthermore, cigarette smoke is a common trigger of exacerbation in adults with asthma and exacerbates the rate of lung function decline in both young and old patients with asthma [172, 173].

Exposure to biomass fuels, cleaning products, food preparation and chemicals increase the risk of developing asthma in younger adults [174]. Many of these exposures occur in the work-space, which may not be applicable to older patients if they have retired. Although the effect of irritants on the development of asthma in older patients is less well characterized than in younger patients, exposure to dust particles, art supplies, and cleaning products has been reported to induce asthma in older patients [175]. Additionally, outdoor air pollution, especially increased ozone, NO_2 and SO_2 may exacerbate and induce asthma in older patients; however, this needs further study [176]. Diesel exhaust is another potential environmental issue and it increases pulmonary neutrophilia in aged compared to younger mice with a prolonged pulmonary inflammation [177].

2.6 Role of Infections

Immunosenescence increases susceptibility to infections in older patients. Respiratory infections in children and young adults (e.g. rhinovirus, Respiratory Syncytial Virus (RSV), influenza, parvovirus) can affect asthma at several points, including disease inception (particularly in the presence of allergen sensitization),

exacerbation, and worsening asthma control [178–186]. However, the effect of infection in older patients with asthma on these stages is less defined. Observational studies have reported that nearly 50 % of subjects with asthma onset after the age of 60 years reported a prior respiratory infection [19]. To address age-related differences in the effect of a particular respiratory infection, influenza A, on antigen sensitization and its effect on subsequent allergen-induced AHR and inflammation, aged and young mice were sensitized to Ag after an acute viral infection. There were significant age-associated differences in the response to infection and sensitization with greater antigen-specific IgE production in aged infected mice, but no alteration of AHR [104]. Detection of respiratory infections is more difficult in older patients as viral culture and rapid antigen testing, which are used frequently in the pediatric population are less sensitive in the older population. In a prospective cohort study among healthy older adults and older adults at high-risk (those with chronic heart or lung disease), RSV infection was seen annually in 3–7 % of health patients and 4–10 % in high-risk subjects [187]. Additionally, RSV was noted by RT-PCR detection in 7.2 % of older adults hospitalized with asthma in this study. Although viral infections have been traditionally investigated as an asthma trigger, the role of *Chlamydia pneumonaie* and other atypical infections has recently come into focus, in particular with adult-onset asthma [188].

Vaccination is an appropriate measure to attempt to alter the immune response and decrease the risk and progression of infectious diseases in older patients. Although vaccination of older patients decreases the progression of many infectious diseases, the immunologic protective response is decreased in the aged [77, 189]. In children and young adults, both influenza and pneumococcal vaccines reduce respiratory infections and rates of asthma exacerbation in patients with asthma [190, 191]. The effect of influenza vaccination or pneumococcal vaccination has not been evaluated in reduction of asthma symptoms in older patients. Recently, high doses of trivalent inactivated influenza vaccine have been shown to increase the antibody response and increase protection against influenza in older patients [192], which may offer some protection against asthma exacerbations secondary to viral infections in older patients.

3 Diagnosis and Clinical Assessment

3.1 Clinical Phenotypes

Data on the clinical phenotypes of asthma in the elderly have been derived from both longitudinal community surveys and case studies [5, 19, 150, 152, 193–195]. Two clinical phenotypes have been reported based on the onset and duration of the disease [150, 193, 196]. Patients with late-onset asthma (LOA) first develop asthma symptoms after the age of 65 years, although particular studies define LOA as first asthma diagnosis after the age of 40 years. Some studies of elderly asthmatics have shown that as a group, as many as 40 % will have their first attack after the age of 40 years [150, 152, 197]. Patients with LOA tend to be less atopic, have a higher

baseline FEV_1, and a more pronounced bronchodilator response than those with long-standing asthma (LSA). Patients with LSA develop asthma symptoms early in life, typically before the age of 12 years of age, and tend to have higher incidence of atopic diseases, more severe and irreversible or partially reversible airway obstruction, and greater airway hyperinflation. The duration of the disease in this group is an important determinant of severity and of development of irreversible airflow obstruction [198].

3.2 Clinical Presentation

3.2.1 Symptoms

The onset of wheezing, shortness of breath and cough in an elderly patient is likely to cause concern. Symptoms of asthma that are common in younger patients, including episodic wheezing, shortness of breath, and chest tightness, are also characteristic of asthma in the elderly. Symptoms are generally worse at night and with exertion. Dyspnea is a common symptom of many other chronic disorders in older patients such as cardiac or other lung diseases, therefore asthma as an etiology of these symptoms may be overlooked. Many elderly patients limit their activity to avoid getting dyspneic, and others assume that their dyspnea is resulting from their aging process and, thus, neglect seeking medical attention early in their disease process. However, aging per se does not cause dyspnea, and an etiology needs to be always pursued in assessing an elderly patient who complains of breathlessness. Cough is a prominent symptom of asthma and may occasionally be the only presenting symptom [199]. Wheezing, on the other hand, may not be as prominent, and its presence is not very specific and does not correlate with severity of obstruction.

3.2.2 Triggers

Asthma symptoms are often precipitated by an upper respiratory tract infection. However symptoms of asthma in the elderly are non-specific and may be caused by a variety of other conditions. Symptoms can also often be triggered by medications, such as aspirin, non-steroidal antiinflammatory agents, or beta-blockers, commonly used by this population. History of atopy is a strong predictor of asthma in this age group, and allergic rhinitis, sinusitis, and nasal polyps are not uncommon.

3.2.3 Signs

Physical examination in elderly patients with asthma is usually nonspecific and may misguide the diagnosis. Physical examination should focus on ruling other causes for respiratory symptoms such as cardiovascular disease and identifying comorbid conditions such as rhinitis/sinusitis and atopic dermatitis.

3.2.4 Physiologic Measurements

The diagnosis of asthma in the elderly should be confirmed by objective measures which are often also helpful in staging the severity of the disease. Lung function testing is especially important in this age group since there is an age-related reduction in the perception of dyspnea seen in the elderly [200]. Unfortunately, objective measures of lung function such as spirometry and peak flow measurements are generally underutilized in elderly patients and this also contributes to the delay in diagnosis [19, 201]. Furthermore, spirometry may be difficult to perform in some situations, because of physical and poor cognitive impairment. In addition, a major problem is the difficulty in defining the lower limits of predicted normal values in this age group, which may vary in different patients. Elderly patients with asthma may also demonstrate an impaired acute bronchodilator response, which can lead to a misdiagnosis of COPD. This poor response may result from the decreased number of β-adrenergic receptors on smooth airway muscles that has been described with the aging process. Airway obstruction may be absent at the time of testing in approximately 8 % of elderly and further testing which may include methacholine challenge testing or even cardiopulmonary exercise stress testing may be needed to facilitate the diagnosis. Measurement of airway hyperresponsiveness to methacholine may not be an accurate test in the elderly [202], although a few studies suggest a higher prevalence of airway hyperresponsiveness in elderly asthmatics than younger populations. Peak expiratory flow variability may also be helpful in the diagnosis and follow-up of patients with obstructive airway diseases, but poor coordination and muscle weakness in some patients may lead to an inaccurate reading [203, 204]. However, a prospective study failed to demonstrate any advantage of peak flow monitoring over symptom monitoring as an asthma management strategy for older adults with moderate-severe asthma when used in a comprehensive asthma management program [205]. Other tests such as measuring the carbon monoxide diffusing capacity of the lung (DLCO) may help distinguish between asthma and COPD (emphysema).

3.2.5 Diagnostic Challenges

Studies have consistently shown that symptoms caused by asthma are frequently overlooked by elderly patients and their physicians. Several factors contribute to the under diagnosis and misdiagnosis of asthma in this age group. A major factor is that symptoms of asthma are also common to other diseases seen in this age group. The hallmark symptoms of asthma, including shortness of breath, wheeze and cough, are non-specific in the elderly and are mimicked by and often confused with such diseases as congestive heart failure, emphysema and chronic bronchitis (COPD), chronic aspiration, gastroesophageal reflux (GERD), and tracheobronchial tumors. Since smoking is an important risk factor for asthma-like symptoms of wheeze, cough and sputum production, asthma is frequently confused with COPD. Lung function is generally lower in those who smoke compared with those who do not smoke due to concomitant COPD [152]. Distinguishing between COPD and asthma

in older patients may be very challenging and in some patients asthma cannot be distinguished from COPD with our current diagnostic tests [206–208]. This group of patients has recently been classified as "Asthma-COPD overlap syndrome" or (ACOS). Symptoms of ACOS include variability of respiratory symptoms, and airflow limitation which is not fully reversible. Although there is not a distinct definition for ACOS, such patients tend to have a poorer quality of life, frequent exacerbations and a more rapid lung decline than patients with asthma or COPD alone [207–209].

It has been known for more than a century that early morning wheezing is a prominent symptom of congestive heart failure; it has been called cardiac asthma as it can mimic the clinical picture of asthma. Typical symptoms of gastroesophageal reflux in the elderly, such as vomiting and heartburn, may be absent. In a study of elderly patients with esophageal reflux proven by intraesophageal pH monitoring, chronic cough, hoarseness and wheezing were present in 57 % of patients [210, 211]. In addition to causing asthma-like symptoms, there is also evidence that GERD may be a cause of worsening asthma.

Unlike younger adults with asthma, a family or personal history of atopy is usually absent in older patients. Blood and sputum eosinophilia are common, but not universal. Since large community studies have shown that most patients first develop asthma in childhood or adolescence, many physicians have had the misconception that asthma is a childhood disease.

In addition, elderly patients have been shown to have a reduced perception of bronchoconstriction that further delays medical intervention. In fact, many elderly patients are fearful of having an illness and dying and are therefore reluctant to admit they are having symptoms. Even when they do so, they may underestimate them or consider them a result of normal aging. Underreporting of symptoms in the elderly may have many causes including depression, cognitive impairment, social isolation, denial, and confusing symptoms with those of other comorbid illnesses.

3.2.6 Assessing Asthma Severity and Control

Evidence-based guidelines for asthma advocate that assessing severity and control of the disease should be based on assessing current impairment and future risk. Assessing impairment includes questions about day symptoms, night symptoms, activity limitation, use of rescue medication and lung function measures. In addition the use of asthma questionnaires such as the Asthma Control Test (ACT) [212] or the Asthma Control Questionnaire (ACQ) is recommended in assessing asthma control. In patients who are newly diagnosed and who have not yet been started on controller medication, assessing severity of the disease is recommended at first encounter. Asthma severity is classified into either *intermittent* (no interference of daily activity, and symptoms occur less than twice per week during the daytime or less than two nights per week) or *persistent* (limitation of normal activity and more frequent symptoms). Persistent asthmatics are further classified into mild, moderate or severe based upon increasing impairment [213]. In patients who are already receiving controller medication, assessing of asthma control is recommended on a

periodic basis. Asthma control level may be well controlled to not well controlled or very poorly controlled based on the above criteria. Assessment of asthma control is essential to define management strategies described below.

4 Management

The goal of asthma therapy is to reduce symptoms and impairments in function imposed by the disease and to improve the quality of life. There are two sets of published guidelines for the management of asthma, one by the National Asthma Education and Prevention Program (NAEPP) [213] and the other by the Global Initiative for Asthma (GINA) [214]. However, these asthma guidelines are based upon studies done in patients younger than 60 years of age, and extrapolated to patients over the age of 60 years. As asthma in a 12 year old is likely to be very different from a 65 year old patient, future guidelines should include studies done specifically on older patients and may need specific age-appropriate modifications.

4.1 Non-pharmacologic Management

Non-pharmacologic components of management include asthma-education and control of environmental factors. Educating patients with asthma about their disease and how to assess and manage exacerbations reduces urgent care visits, asthma-related health care costs, and improves health status and quality of life and adherence to medication regimens medication in both younger and older patients [214–217]. Instructions, or "action plans" for routine asthma care should be easy to read and understand for the patient.

4.2 Pharmacologic Management: Challenges

Treatment of asthma in patients of all ages should address the severity of the patient's disease and how well it is controlled. Treating patients to control their disease and allow for increased quality of life, while minimizing potential medication side effects are a major goal, particularly in older patients who often receive multiple medications and therefore are at a potentially greater risk for side effects. Older patients frequently have a poor inhaler technique, due to decreased cognitive function [218, 219], or a physical impairment, and while some breath-activated medication devices may be easier for elderly patients to self administer [220], some still may have difficulties with its administration [221]. For these patients, a spacer can be attached to an HFA pressured metered dose (MDI) or some medications may be delivered by nebulizer (including corticosteroids for daily controller use, although this is not currently an FDA indicationfor older patients with asthma). There are

several medications used to treat congestive heart failure and glaucoma, such as non-selective β-blockers [222], aspirin and NSAIDs which can worsen asthma in some patients.

4.2.1 Bronchodilating Medications

All patients with asthma should have a prescription for a "rescue medication" to treat acute symptoms. Rescue bronchodilators are either short-acting beta 2-receptor agonists (SABAs) or anticholinergic agents. SABAs are relatively safe in the elderly if used on an as-needed basis to treat exacerbations, though mild systemic absorption can produce tachycardia and tremor. The combination of diuretics that do not spare potassium (e.g. thiazides) and overuse of β2-agonists may produce significant hypokalemia and hypomagnesemia, increasing the risk of cardiac arrhythmias [223]. One report suggested that ipatrotropium in elderly asthmatics was associated with a slight increase in mortality, which the authors concluded was secondary to these patients having more severe asthma than those patients not receiving ipatrotropium [224]. However, anticholinergics, due to their atropine-like effects, may produce adverse side effects in the elderly including a dry mouth, urinary hesitancy, constipation and exacerbation of glaucoma. Long-acting β2-agonists should only be used as an add-on therapy in patients who have properly used inhaled corticosteroids (ICS) without relief, as they are not effective monotherapy for asthma [225].

4.2.2 Anti-Inflammatory Medications

Patients with persistent asthma should receive a daily anti-inflammatory medication, such as an ICS to suppress airway inflammation [226, 227]. Elderly patients with asthma are often under-prescribed ICS [5, 22, 228, 229]. ICS may produce local side effects including hoarseness or oral candidiasis, which can be prevented by using a spacer or by rinsing the mouth after use. A concern when prescribing ICS to older patients is increasing the risk of osteoporosis and bone fractures, especially for women and if the dose of ICS is >1000mcg/day budeosonide equivalent [230–233]. Therefore, patients should be given an ICS with the lowest dose to control their disease,[234] and one with a lower oral bioavailability. To decrease the effects of corticosteroids on bone resorption, patients should be encouraged to exercise, avoid excess alcohol intake, and use daily supplemental calcium with vitamin D. Observational studies in the elderly have suggested that ICS have a small, but significant risk of subcapsular and nuclear cataracts [235–237]. Observational studies have also suggested that elderly patients treated with ICS may have a small risk for developing glaucoma; however, further studies are needed [238–240]. Therefore, patients receiving ICS should have yearly ophthalmologic exams.

Corticosteroids improve asthma control and symptoms in some, but not all older patients. A large database review of patients 65 years or older hospitalized at least once for asthma and followed 12 months after discharge, demonstrated that those

given ICS had a 29 % reduction in asthma readmission and a 39 % reduction in all-cause mortality in the subsequent 12 months [241]. However, some older patients with asthma may have a component of fixed airway obstruction [150]. The NAEPP has recommended for some older patients, that a 2 week trial of oral corticosteroids (0.3–0.5 mg/kg) be administered with repeat lung function measurement after the course to assess for possible reversal of obstruction and clinical benefit [242].

Leukotriene modifiers are a class of anti-inflammatory agents that inhibit the effects of leukotrienes, which are potent bronchoconstrictors, recruit inflammatory cells to the airways and induce mucus hypersecretion. When compared with ICS, leukotriene receptor antagonists generally do not have as favorable an outcome in improving FEV_1, symptom scores and other outcome measures [213]. Two studies have investigated the role of leukotriene modifiers in patients of different ages with asthma, and have concluded that their effectiveness may be limited in the elderly patients compared with younger counterparts, but continue to improve asthma symptoms without reducing the need for rescue therapy [243, 244].

4.2.3 Methylxanthines

Methylxanthines (e.g. Theophylline) increase intracellular cyclic adenosine monophosphate which bronchodilates the airways, and in lower doses have anti-inflammatory properties [245]. Its use in asthma, especially in an older group, is limited by its relatively weak bronchodilator properties and many side effects and drug interactions [246].

5 Future Research Needs

In 2008, the National Institute on Aging and the National Heart, Lung, and Blood Institute co-sponsored a conference on asthma in older individuals [247]. This program explored data from animal models and human studies of asthma in older patients and concluded that airway inflammation in asthma and its clinical response to therapy in older patients likely differs from younger patients. Since this conference, there are still several remaining unanswered questions regarding asthma in older patients. For example, how do age-related changes in the innate and adaptive immune responses impact airway inflammation in older patients with asthma and does it differ from younger patients with asthma? Understanding the pathophysiology and underlying airway inflammation in older adults with asthma and the different phenotypes and endotypes of asthma in this population is a major unmet need as this group of patients has high rates of morbidity and mortality. Furthermore, with the expected increase in the elderly populations, including elderly asthmatic patients in clinical trials is essential, and particular attention should be paid to also address how differences in inflammatory mechanisms affect responses to therapy.

6 Summary

Asthma is a major public health problem which is frequently overlooked in the geriatric population. While much has been uncovered about the pathogenesis, course and outcomes of asthma in children and young adults, studies in the aging population have been scarce or non-existent. Asthma in the elderly has at least two distinct phenotypes based on the onset of the disease. While the characteristics of long-standing asthma that starts early in life may be similar to the general asthma population, more studies are needed to uncover details about asthma that develops late in life which can have distinct clinical features and may have different course of response to therapy. Our knowledge about management of asthma in this population is based on extrapolation from studies in the younger population. Although future studies are needed to investigate the response to existing and novel interventions in the elderly, current guidelines recommend that management of asthma in this population should not differ from that of younger patients. Careful monitoring of compliance with therapy and of adverse events to medication is essential in this population. Despite severe symptoms and physiologic impairment, most elderly patients with asthma improve with therapy and can lead active productive lives.

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Aging in COPD and Idiopathic Pulmonary Fibrosis

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Contents

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1 Clinical Aspects and Pathophysiology of COPD

The term Chronic Obstructive Pulmonary Fibrosis (COPD) refers to two distinct disease entities previously considered separate: pulmonary emphysema and chronic (obstructive) bronchitis, both of them may be present in the same individual to different degrees. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) defines chronic obstructive pulmonary disease (COPD) as "a preventable and treatable disease, characterized by a persistent airflow limitation that is progressive and associated with an abnormal inflammatory response of the lungs [1]. COPD is the fourth leading cause of death in the world and will rise to the third leading cause of death by 2030 [2, 3]. COPD itself is a risk factor for other age-related disorders including cardiovascular disease, type II diabetes and other functional deteriorations [4–6]. Importantly, COPD is reported to be two to three times more prevalent in persons over 60 years of age [7].

The histopathological characterization of COPD includes the loss of lung elasticity due to emphysema and small airway obstruction due to inflammatory narrowing and fibrosis. The clinical manifestations include dyspnea, chronic cough, low exercise capacity, wheezing, and frequent or longer-lasting bronchial infections. The airway obstruction progresses with time and exacerbations of the disease tend to arise about once per year [8].

Smoking is the greatest risk factor for COPD [1, 7]. But COPD can occur in nonsmokers, in which other risk factors include asthma, advanced age, low educational level, occupational and domestic (from cooking and heating) exposure to toxins, a history of airway infections in childhood and genetic predisposition [9–11]. The understanding of COPD pathogenesis and the factors that influence its heterogeneity is a complex and evolving area of research.

1.1 Pathogenesis

It is well accepted that impairment in lung function is the result of chronic inflammatory processes, many of which are initiated years before the initial symptoms are apparent. At the molecular level, the pathogenesis of COPD includes a proteinase-antiproteinase imbalance, immunological mechanisms including systemic inflammation, oxidant-antioxidant imbalance, increased apoptosis and ineffective repair. At the cellular level, COPD represents a complex interplay among lung epithelial cells, endothelial cells, neutrophils, macrophages, and multiple subpopulations of both $CD8^+$ and $CD4^+$ T cells [12–15] (Fig. 1).

The combination of tissue damage, release of inflammatory mediators, cytokines, and chemokines leads to the activation of epithelial cells and endothelial cells. These cells are a source of chemoattractant cytokines, including CXCL1, CXCL8, CXCL10, CCL2, and CCL5 [16]. The expression of chemokines and adhesion molecules by activated epithelial and endothelial cells drives the accumulation of inflammatory infiltrates consisting of neutrophils, macrophages, and $CD8^+$ T

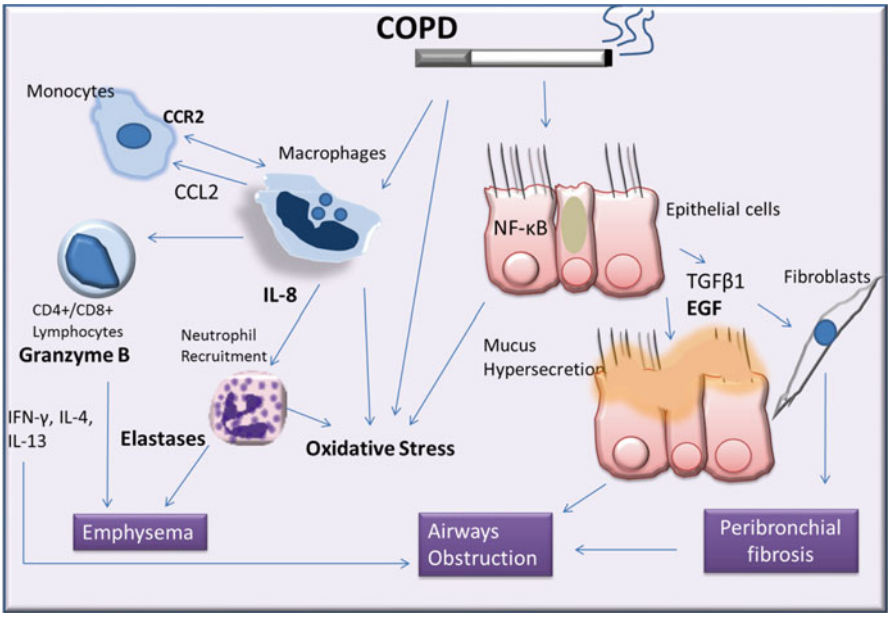

Fig. 1 Schematic representation of the main contributing factors for the development and progression of COPD. Cigarette smoking causes airway inflammation and remodeling. The inflammatory infiltrate is composed of macrophages, CD8+lymphocytes, and neutrophils. In addition, goblet cell hyperplasia, squamous metaplasia, peribronchial fibrosis, and smooth muscle hypertrophy is observed

cells [16–18]. In addition, the disease is correlated with accelerated apoptosis of alveolar and pulmonary vascular endothelial cells [18] (Fig. 1).

Evidence for a prominent role of aging in COPD progression is growing and cell senescence is one of the possible molecular pathways for development of COPD [2]. In spite of these advances, there is still a fundamental lack of knowledge about the cellular, molecular, epigenetic and genetic causes of COPD, as well as the role of aging.

2 Clinical Aspects and Pathophysiology of Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive and irreversible scarring of the lungs of unknown origin that causes tissues' inability to transport oxygen to the blood. An estimated 100,000 people in the U.S. have IPF and the mean survival is approximately 3–5 years from the time of diagnosis with a very heterogeneous rate of progression [19–23]. In 2030, there will be more than 70 million people in the U.S. aged 65 and older, and because IPFis strongly associated with advanced age, they will be at risk for pulmonary fibrosis.

The disease, which affects primarily elderly adults (mainly male smokers and ex-smokers) is limited to the lungs and is characterized by patchy interstitial fibrosis with alternating areas of normal lung, temporal heterogeneity of fibrosis characterized by clusters of actively proliferating fibroblasts/myofibroblasts, "fibroblastic foci", and honeycomb structures [19]. IPF displays a pattern of fibrotic interstitial pneumonia similar to that found in other clinical settings, including collagen vascular disease, chronic hypersensitivity pneumonitis, asbestosis, and Hermansky-Pudlak syndrome [19]. Three different forms of IPF can be identified clinically: slowly progressive, rapidly progressive, and relatively stable with peaks of rapid disease acceleration (exacerbation).

2.1 Pathogenesis

The mechanism of fibrosis in IPF remains elusive; current concepts suggest that the disease results from an aberrant reparative response to alveolar epithelial cell injury characterized by migration, proliferation, and activation of fibroblasts, as well as secretion of extracellular matrix components, leading to scarring, architectural changes, and irreversible loss of lung function (Fig. 2). Key players in pulmonary fibrosis include:

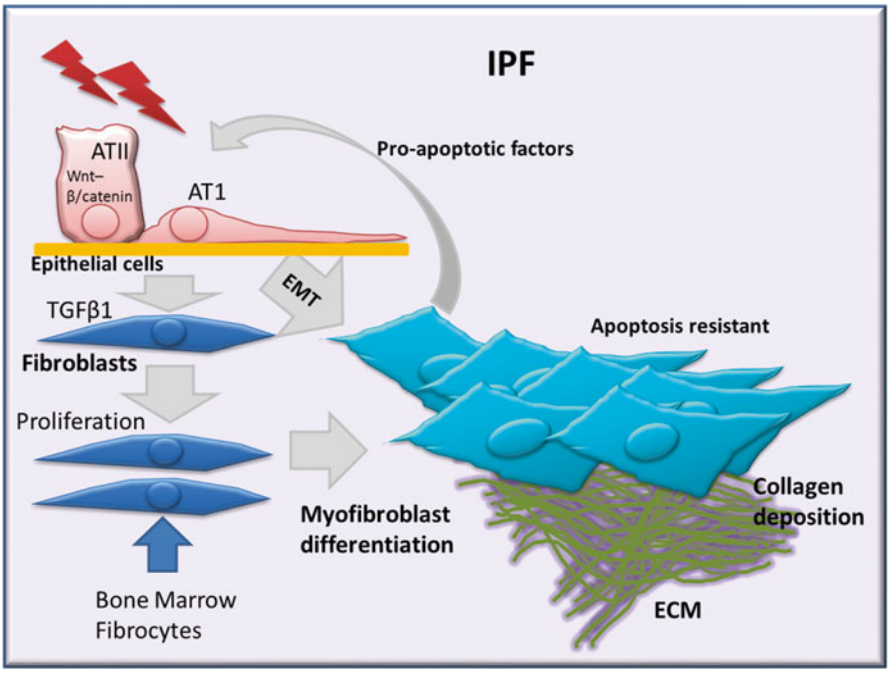

Fig. 2 Schematic representation of key players involved in the development and progression of Idiopathic pulmonary fibrosis

Epithelial Injury It is proposed that microinjuries to alveolar epithelial cells induce fibrogenesis. Abnormal or injured epithelial cells secrete growth factors that favor the recruitment of resident fibroblasts and fibrocytes that differentiate into myofibroblasts [24]. Those epithelial cells also release inflammatory mediators that initiate an anti-fibrinolytic coagulation cascade and trigger platelet activation and blood clot formation. This process is followed by activation of leukocytes at the site of tissue injury.

IPF Fibroblasts and Myofibroblast Differentiation IPF fibroblasts are characterized by excessive proliferation possibly linked to decreased PTEN expression and aberrant activation of PI3 kinase. Fibroblasts can trans-differentiate to a myofibroblast phenotype, which are major producers of excessive extracellular matrix. Continuous accumulation of myofibroblasts prevents proper re-epithelialization. Myofibroblasts express features of both fibroblasts and smooth muscle cells, and they can be recruited to the lungs or they can differentiate from resident fibroblasts [25]. Myofibroblasts may also be derived from epithelial cells undergoing Epithelial Mesenchymal Transition (EMT), a process triggered in part by the Wnt–β-catenin signaling. This pathway is constitutively active in epithelial cells from IPF patients (and in mice treated with bleomycin as a model of pulmonary fibrosis).

Transforming Growth Factor Beta 1 (TGF-β1) Signaling Pathway TGF-β1 is an important profibrotic cytokine that triggers fibroblast proliferation and activation. TGF-β1 further exacerbates the inflammatory response by stimulating the differentiation of Th17 cells. TGF-β1 induces a number of growth factors and cytokines that participate in fibrosis, including, fibroblast growth factor (FGF-2), connective tissue growth factor (CTGF), insulin-like growth factor (IGF), platelet derived growth factor (PDGF) and interleukins (ILs).

Oxidative Stress and Mitochondrial Dysfunction PTEN-induced putative kinase 1 (PINK1), is a regulator of mitophagy and maintains mitochondrial integrity by regulating diverse aspects of mitochondrial function, including membrane potential, respiration, calcium homeostasis, structure, and mitochondrial DNA integrity. Recently, it had been shown that PINK1 is deregulated in the aging lung and in pulmonary fibrosis. Deficiency of PINK1 expression promotes fibrogenesis, possibly by inducing the production of mitochondrial reactive oxygen species (ROS), which perpetuate profibrotic inflammatory responses [26–29]. The activation of the Nalp3 inflammasome and IL-1β secretion is largely driven by ROS [30–32].

Recent genome-wide studies have identified several genetic variants critical for epithelial integrity as a risk factor for IPF, including genes for cell-cell adhesion and migration [33, 34]. Furthermore, polymorphisms in common variants in TERT and TERC and oligonucleotide/oligosaccharide-binding fold containing1 (OBFC1), required for telomere length, indicates telomere attrition in IPF [35, 36]. Toll interacting protein (TOLLIP), is another genetic variant, important for innate immunity and epithelial mesenchymal transition [37]. None of these associations have been investigated mechanistically, so their role in IPF remains to be studied.

The animal model most extensively used to study pulmonary fibrosis is the bleomycin model, which recapitulates several of the features seen in the IPF patients [38]. Importantly, age-related differences are noted in this model of pulmonary fibrosis. Older mice do not resolve fibrosis as effectively as do younger mice. Twenty-four-month-old mice exhibit increased fibrogenesis, collagen deposition, and activation of TGF-β signaling compared to 3-month-old mice [39].

An interesting question is the heterogeneity of outcomes: why does a patient in the 60s with a history of smoking, shorter telomeres, alveolar epithelial senescence, oxidative stress and mitochondrial dysfunction will develop pulmonary fibrosis and not COPD, or why in some cases, emphysema and pulmonary fibrosis can occur in the same patient (Fig. 3) [40–42]. One major difference is that COPD is viewed as an inflammatory disorder while IPF is considered an abnormal wound healing disorder [43].

2.2 Current Treatment

Patients with IPF are currently referred early for lung transplantation, due to the unpredictable nature and the high mortality rate of the disease. Recently, nintedanib and pirfenidone, two compounds with pleiotropic anti-fibrotic properties, have been proven effective in reducing lung function decline and disease progression in IPF [44]. Nintedanib is a tyrosine kinase inhibitor that targets receptors thought to be involved in the pathogenesis of IPF such as receptors for the platelet-derived growth factor, vascular endothelial growth factor and fibroblast growth factor [45]. Pirfenidone has anti-fibrotic and anti-inflammatory properties [46–48]. Unfortunately, neither nintedanib nor pirfenidone is a cure for IPF [44].

3 Molecular Hallmarks of Aging in the Development and Progression of COPD and Pulmonary Fibrosis

Biological aging, usually linked to chronological age, contributes to the deterioration of pulmonary function. However, features of biological aging can also occur earlier in life (“accelerated aging”) as a result of failure to maintain cellular homeostasis (among other factors), including deficiencies in cellular maintenance or repair, DNA damage, epigenetic alterations, and loss of proteostasis. Some of these are induced by environmental factors such as cigarette smoke, viruses, particles, etc., but are also likely to interact with the aging-dependent changes in these processes. Irrespective of the proximal cause (aging or environment), loss of cellular homeostasis promotes tissue injury, involving tissue remodeling, airspace enlargement, inflammation, and/or lung fibrogenesis (Fig. 2).

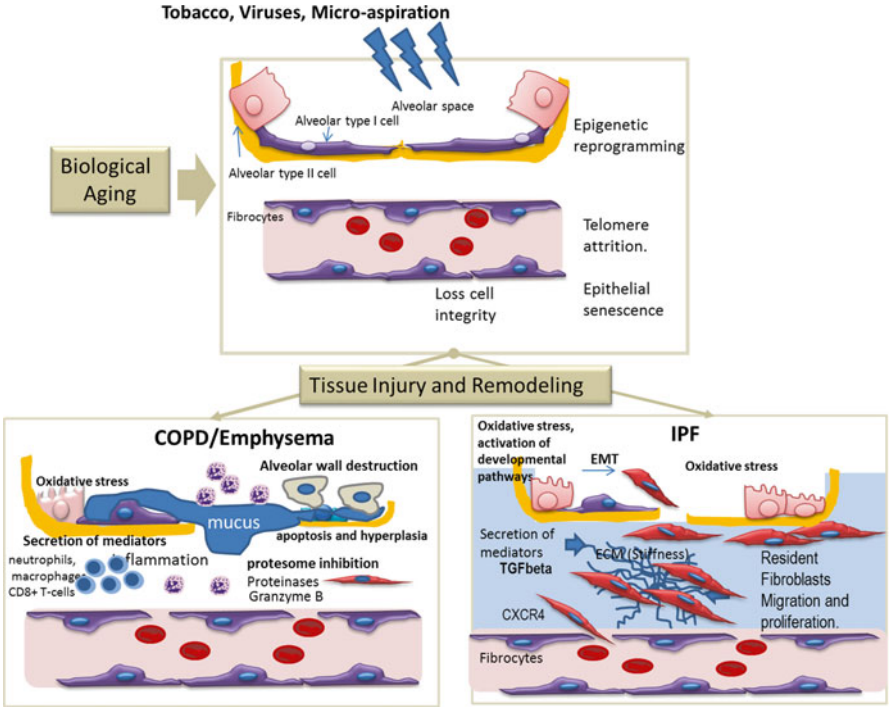

Fig. 3 Schematic representation of the key players involved in the development and/or progression of COPD/Emphysema or IPF in the elderly. **IPF** (*bottom right*)**.** In a genetically predisposed and/or pro-fibrotic lung, disrupted alveolar epithelium and basement membrane promote the release of proinflammatory cytokines and chemokines. These soluble mediators may activate residential and/or circulating cells including fibrocytes. Profibrogenic molecules such as PDGF and TGF-β1 are secreted by inflammatory, epithelial, and endothelial cells. Fibroblasts respond to these changes by proliferation and differentiation into myofibroblasts, promoting abnormal collagen deposition. The aggregates of mesenchymal cells and collagen are called "fibrotic foci". The resulting fibrotic lung has no potential for regeneration and repair**. COPD** (*bottom left*). In susceptible subjects (e.g., with severe α-1 antitrypsin deficiency), aging, cigarette smoke and other environmental factors promote the activation of the inflammatory and structural cells of the lower airways of the lungs with the release of mediators causing inflammation, remodeling and emphysema. Cigarette smoking enhances the age-related accumulation of endogenous ROS and DNA damage, NF-κB activation, and oxidative stress responses. Further amplification of inflammation, accumulation of damaged proteins, apoptosis and hyperplasia are also the result of epigenetic changes and antioxidant/nitrosant activity

There is no doubt that the pathogenesis of emphysema in COPD, as well as idiopathic pulmonary fibrosis are characterized by of the appearance of accelerated aging of the lung, including inflammatory pathways, antioxidant responses, epigenetic modifiers and proteolytic pathways [49–51]. The processes of initiation and perpetuation of COPD/emphysema and pulmonary fibrosis are not well understood, and considering the main pillars of aging might provide important new insights. Table 1 summarizes the aging hallmarks associated with COPD and IPF.

Table 1 The hallmarks of aging in COPD/emphysema and IPF described in the chapter

Hallmarks of aging		COPD/emphysema	IPF
Nutrient Sensing Systems	IGF1 (High)	Low	High
	mTOR (Activated)	Inhibited	Activated
	AMPK (Reduced)	Reduced	Reduced
	Sirtuins Reduced Sirt1 and Sirt6.	Reduced Sirt1, Sirt6	Reduced Sirt1 expression Smad3 dependent Sirt1 activation. Reduced Sirt6.
	The circadian clock (Disruption)	Disruption	Disruption
Proteostasis	Deficient autophagy	Cell type dependent Increase and decreased in autophagy reported. Increased mitophagy	Disruption Reduced macro-autophagy Reduced mitophagy
	Proteasome activity (Reduced)	Reduced	No differences in activity. Protesome inhibition as a therapeutic tool.
	ER stress and UPR response	Increased	Increased
Mitochondrial dysfunction	COX activity (reduced) mtROS (increased) Altered mitochondrial dynamics Reduced PIK1 expression	Excessive COX-2 activity Increase mitochondrial fragmentation	Reduced COX-2 activity Increase mtROS Reduced PINK1 expression
Genomic instability Microsatellite instability	Increase DNA breaks and Deficient reparative mechanisms. Excision repair (reduced) Loss of heterozygosity (LOH)	Double-strand breaks and activation of DNA damage response. LOH	Double-strand breaks and activation of DNA damage response. LOH
Telomere attrition	Short telomere length	Short telomere length	Telomere related disorder (familial cases)-TERT, TERC, OBFC1polymorphism. Short telomere length.
Cellular Senescence and SAPS	Increase	Increase	Increase

(continued)

Table 1 (continued)

Hallmarks of aging		COPD/emphysema	IPF
Epigenetic alterations	DNA methylation Increase global hypomethylation	Increase global hypomethylation DNA Hypomethylation and DNA Hypermethylation of specific genes.	No LINE1-hypomethylation Hypomethylation and hypermethylation for specific genes.
	MicroRNAs Lack of alteration in miRNA expression in the aging mouse lung.	Upregulated miRs include: miR223, 1274a, 144, 374a, 664, 17–92, 576-3p,513a-p5, 25, 99b, 125b-1, 24. Downregulated miRs include: miR21, mIr923, mIR937, miR422a.	Upregulation of miRNAs 21, 34a,145, 154, 155, 199a-5p Downregulation of miR Let7d, 17–92, 26a, 29, 200 family, and 326.
	Splicing (altered)	Splicing variants	Differential in IPF Lung Tissue.
	Histone modifications Increase (H4K16) Variations in methylation of histones H3 and H4	Reduced HDACs 2, 5, and 8, HDAC10 expression. Sirt1 downregulation. H3 and H4 panacetylation	Altered protein-levels of Class-I- (HDAC1, 2, 3 and 8) and Class-II-HDACs (HDAC4, 5, 7, 9, 10), and of the Class-III-HDAC Sirtuin-1 is significantly elevated in IPF lungs.
Intercellular communications and inflammaging	Cell-cell contacts (altered) Immunoregulation (altered) Changes in ECM	Loss of cell-cell contacts Changes in ECM Elastases Granzyme B Increase extracellular ATP levels High PDE4 Low cAMP Inflammaging/SAPS	Reduction in gap-junctions and changes intercellular communication. Changes in ECM composition. Increase elastic fibers, stiffness Increase active TGFbeta Changes in MMPs and TIMPs Inflammaging/SASP
	The microbiome (altered)		
	Hormones (disruption) 17β-estradiol/ estrogen receptor alpha signaling pathway;melatonin	Low testosterone levels Reduced melatonin levels Decrease in DHEA	Decrease in DHEA
Stem cell exhaustion	Reduced tissue regeneration, healing. Changes in stem cells	Reduced tissue regeneration, healing.	Reduced tissue regeneration, healing.

3.1 Defects in Stress Recognition and Nutrient Sensing Systems

3.1.1 Insulin-Like Growth Factor-1

Circulating IGF-1 and IGF-1 signaling is reduced in aging and reduced IGF-1 signaling increases longevity in organisms from worms to mice. Low levels of circulating IGF-I can negatively influence the progression of disease, and are common in patients with COPD/emphysema [52]. For example, exogenous IGF1 reduces diaphragm fiber atrophy in animal models of emphysema [53]. In apparent contrast in the IPF-lung, IGF-I is highly expressed by lung fibroblasts, interstitial macrophages, alveolar epithelial cells, and ciliated columnar epithelial cell [54–56]. However, it is possible that IGF-1 signaling is actually impaired due to increased expression of IGBP3 and IGFBP5, members of a family of IGF-binding proteins that bind to IGFs with high affinity and restrict access of IGF1 to the IGF1 receptor. In keeping with this, overexpression of IGFBP5 promotes fibrogenesis in lung tissues [57, 58].

3.1.2 mTOR Regulation

Reduced activity of the mechanistic target of rapamycin (mTOR)/AKT pathway increases longevity, as demonstrated both by genetic manipulations in flies and worms, and by pharmacological inhibition with rapamycin in mice. As in other tissues, activity of this pathway has been shown to be increased in the aged mouse lung, with detrimental consequences [59]. However, Rtp801-null mice exhibit increased mTOR signaling and are substantially protected against pulmonary injury from smoke exposure [60]. Rtp801, also known as Redd1, is a suppressor of mTOR signaling which is overexpressed in human emphysematous lungs and in lungs exposed to cigarette smoke. It appears therefore that the age-related increase in mTOR might be protective of the lungs against some types of injury [61].

Conversely, the mTOR pathway has been shown to be activated in pulmonary fibrosis. mTOR is mainly expressed in hyperplastic alveolar epithelial cells and in some mesenchymal cells. mTOR expression in pulmonary fibrosis patients significantly correlates with the fibrosis score and decline in lung function [62], indicating that this age-related hallmark may be associated with the prognosis of pulmonary fibrosis. Treatment with TGFβ and bleomycin also result in the activation of the AKT/mTOR pathway and the consequent phosphorylation of p70S6 Kinase, ribosomal S6 protein, and 4E-BP1. The effects of TGFβ and bleomycin on extracellular matrix deposition are reduced by pre-treatment with rapamycin, an mTOR inhibitor [63]. Taken together, it appears that the age-related increase in mTOR activity might be protective against injuries leading to COPD, but deleterious because of promoting the development of fibrosis.

Inhibition of autophagy is a downstream effect of mTOR activity, and this appears to also play a role in myofibroblast differentiation. Enhanced mTOR activity is observed in myofibroblasts within fibroblastic foci from IPF patients [64, 65].

The Akt/mTOR pathway desensitizes IPF fibroblasts from polymerized collagen-driven stress by suppressing autophagic activity, which produces a viable IPF fibroblast phenotype in collagen [66]. It is also possible that mTOR activation and the consequent blockage in autophagy promote the initial cell fate change to myofibroblasts [65, 67]. The time-dependent regulation of mTOR-autophagy during the initiation and progression of pulmonary fibrosis needs to be further evaluated [29]. It is safe to conclude that mTOR plays complex and potentially counteracting roles in lung disease.

3.1.3 Adenosine Monophosphate-Activated Protein Kinase (AMPK)

Independent of effects on the mTOR pathway, AMPK can also modulate inflammatory responses and oxidative stress. AMPK attenuates inflammatory lung injury by phosphorylating its downstream targets, including sirtuin1 (SIRT1), peroxisome proliferator-activated receptor gamma (PPARγ) coactivator-1 alpha (PGC-1α), p53, and FOXO3a. AMPK mRNA and protein are significantly reduced in skeletal muscles from rat modes of COPD, in comparison to control rats [68]. AMPKα1-deficient mice show increased fibrosis after bleomycin exposure, compared to control mice. Furthermore, metformin, an AMPK activator, decreases the expression levels of fibronectin and collagen in cultured fibroblasts and suppresses NOX4 activation [69].

3.1.4 Sirtuins

Activation of sirtuins has been shown to extend lifespan and increase healthspan in models ranging from yeast to mice. The sirtuin family of proteins contributes to interactions among autophagy, metabolism and aging (for reviews, see [70, 71]). Substrates of SIRT1 have significant roles in the formation of autophagosomes, fatty acid oxidation, glucose homeostasis, circadian rhythm and life span [72–78]. SIRT1 expression in the lung is reduced during aging and COPD [79, 80], as well as emphysema [81]. SIRT1 negatively regulates expression of the proteinase MMP9, which is increased in chronic inflammatory diseases [82]. Moreover, genetic or pharmacological activation of SIRT1 protects mice from elastase and cigarette smoke-induced emphysema in addition to attenuating stress-induced premature cellular senescence [83].

The specific role of SIRT1 in the process of lung fibrogenesis needs further studies. Recently SIRT1 has being shown to be a crucial regulator of TGFβ/Smad signaling in systemic scleroderma, whereby mice with fibroblast-specific knockdown of SIRT1 are in fact less susceptible to bleomycin- or TBRIact-induced fibrosis [84]. Another sirtuin, SIRT6, is also being studied in COPD and IPF. Reduced SIRT6 levels in COPD and IPF have been associated with cellular senescence [85–87]. SIRT6 negatively regulates cigarette and TGFβ-induced senescence [87–89], so it appears that SIRT6 may have a protective effect against COPD and IPF.

3.1.5 The Clock

Circadian rhythms are altered during aging, both at the level of the central nervous system in the suprachiasmatic nucleus, and at the individual cellular level in peripheral tissues. At the molecular level, the circadian rhythm consists of interlocking transcriptional/translational feedback loops of core clock genes and oscillatory metabolic products. The clock modulates stress responses and physiological processes unique to each organ [90–94]. Importantly, some respiratory pathologies, such as pulmonary edema, asthma, and allergic attacks, peak at certain times during the circadian cycle [95]. Animal models of pulmonary fibrosis have revealed the effects of day/night cycling in the fibrotic response, with a 'clock-gated' pulmonary response to oxidative injury. Furthermore, lungs from mice carrying a Clock gene mutation are characterized by an increased oxidative burden and increased collagen deposition around the bronchioles, even in the absence of bleomycin challenge [96]. Basal autophagy and other metabolic pathways are rhythmically activated in a clock-dependent manner [97], supporting the significance of the circadian clock as a bioenergetic regulator of human physiology and pathophysiology [98, 99].

3.1.6 Therapeutic Approaches Targeting Bioenergetics Sensors

Bioenergetic sensors have been proposed as potential targets for interventions to modulate autophagy and metabolism and to slow the progression of age-related diseases like COPD/emphysema and pulmonary fibrosis. For example, rapamycin (an mTOR inhibitor) reduced fibrosis in a mouse model of COPD [100], although it did not prevent fibrosis in the murine model using bleomycin [101]. Activating AMPK with 5-aminoimidazole-4-carboxamide-1-b-4-ribofuranoside or metformin leads to protection from airway inflammation and remodeling in a murine asthma model, at least partially through decreased oxidative stress [102].

The polyphenol resveratrol is known to activate SIRT1, inhibit phosphoinositide-3-kinase (PI3K), a pro-aging kinase, and regulate FOXO3A and mTOR signaling [103–106]. Resveratrol also promotes autophagy, and inhibits inflammation. Resveratrol reduces bleomycin-induced pulmonary fibrosis and ventricular remodeling in old rats with COPD [107–109]. In fact, resveratrol reduces the release of inflammatory mediators from human airway smooth muscle cells and relieves alveolar epithelial cells from endoplasmic reticulum (ER) stress and apoptosis in animal models of COPD [110, 111].

Melatonin, N-acetyl-5-methoxytryptamine, is a naturally occurring compound found in animals, plants, and microbes. Melatonin is produced by the pineal gland in vertebrates and it is involved in circadian rhythms through the activation of melatonin receptors. Melatonin is also known to exert a powerful antioxidant activity. Melatonin has been suggested as a 'geroprotector', as an agent to treat age-associated inflammatory diseases and to increase quality of life in elderly patients [112, 113]. Clinical studies demonstrated a decrease in oxidative stress in patients treated with melatonin, detected by 8-isoprostane levels in exhaled breath after 1, 2, and 3

months of melatonin treatment, with significant improvement of dyspnea, although there were no significant changes in lung function or exercise capacity [112]. At the cellular level, melatonin has been shown to inhibit mucin 5 AC (MUC5AC) production via suppression of MAPK signaling in human airway epithelial cells [114]. Furthermore, melatonin attenuates neutrophil inflammation and mucus secretion in cigarette smoke-induced chronic obstructive pulmonary diseases via the suppression of Erk-Sp1 signaling [115].

Finally, it is expected that more geroprotectors and "chronotherapeutic strategies" for intervention against human chronic lung diseases will be proposed in the near future, based in part on studies of stress recognition and nutrient sensing systems [112].

3.2 Loss of Proteostasis: The Role of Autophagy and Proteasomal Digestion

Aberrant proteostasis contributes to COPD, severe emphysema, and pulmonary fibrosis [116]. Quality control is maintained by the autophagy-lysosomal system and the ubiquitin-proteasomal system; both these proteolytic systems decline with age [117, 118]. In a recently published proteomics analysis of IPF, 89 differentially expressed proteins were identified, of which 51 were upregulated and 38 downregulated. Increased expression was observed for proteins involved in unfolded protein response (UPR), heat-shock proteins, and DNA damage stress [119]. In COPD, recent studies demonstrated that cigarette smoke promotes the accumulation of ubiquitinated protein aggregates in the insoluble protein fraction of cigarette smoke extract (CSE)-treated human bronchial epithelium cells. Interestingly, these aggregates disappear after induction of autophagy [120]. We review recent advances in our understanding of the contribution of proteolytic system failure to COPD and IPF.

3.2.1 Autophagy

The recycling and clearance of proteins and mitochondria by autophagy has beneficial effects on aging, including increased energy production and decreased ROS production. Nevertheless, the role of autophagy in the pathogenesis of COPD seems to be complex and cell type specific. In addition to its role in recycling proteins and mitochondria, autophagy is implicated in processes such as cilliar homeostasis and response to hypoxia.

In the lungs of patients with COPD, LC3B-II and autophagosome formation are increased [121], and increased mitophagy in alveolar epithelial cells was found to contribute to the pathogenesis of COPD [122]. Furthermore, increased autophagy also results in decreased resistance to emphysema in animals exposed to cigarette smoke [121, 123]. On the other hand, autophagic flux is impaired in alveolar macrophages, which could contribute to the deficient xenophagy seen in COPD patients

as well as accumulation of ubiquitinated aggregates in bronchial epithelial cells exposed to cigarette smoke [120, 124].

In contrast to COPD, autophagy has been shown to be reduced in IPF [64, 65], and autophagy inhibition is sufficient to induce the acceleration of epithelial cell senescence and fibroblasts-myofibroblast differentiation [64, 65]. Deficient autophagy is also associated with the anti-apoptotic features of persistent myofibroblasts and the progression of IPF [64, 65, 125]. A recent report using bleomycin animal models indicates that deletion of the essential autophagy gene ATG7 in endothelial cells leads to marked changes in the architecture of endothelial cells, and increased susceptibility to pulmonary fibrosis [126]. These findings underscore autophagic deficiency as a contributing factor in the development and/or establishment of pulmonary fibrosis. Interestingly, both upregulation and downregulation of autophagy have been associated with fibrosis in various organs, highlighting the diverse nature of the roles that autophagy may play in the various phases of the response to stress and repair in different tissues [127–129].

Mechanistically, it has been proposed that cigarette smoke induces mitophagy by stabilizing PINK1 in pulmonary epithelial cells. Genetic deficiency in PINK1 protects lung epithelial cells from cigarette smoke-induced cell death and mitochondrial dysfunction [122]. In contrast to COPD, Pink1−/− deficient mice are more susceptible to pulmonary fibrosis than are wild-type mice, PINK1 expression is reduced in animal models of pulmonary fibrosis and in biopsies of IPF patients, suggesting a key role for mitochondrial homeostasis in the pathogenesis and the progression of lung fibrosis [26]. Recent studies focused on the hSP-C^{I73T} mutation associated with interstitial lung diseases, like IPF, indicate that a disruption of autophagy-dependent proteostasis in hSP-C^{I73T} is accompanied by an increase in mitochondrial biomass, and a decrease in mitochondrial membrane potential [130]. This agrees with recent findings by our group that describe an age-dependent decline in the autophagic response to bleomycin, a decrease in PINK1 expression with aging, and deficient PINK1 recruitment to the mitochondria in a TGFβ-dependent manner, favor the profibrotic phenotype of the aging lung [29].

Recent studies also demonstrate that autophagy regulates cilia length through ciliophagy and ciliogenesis, which control the sensitivity of the cell to stressors such as cigarette smoke [131–133]. Autophagy-deficient mice are protected from cigarette-smoke-associated ciliary dysfunction [134]. Elevated expression of cilium genes is associated with more extensive microscopic honeycombing and higher expression levels of both the airway mucin gene MUC5B and the metalloproteinase MMP7, a gene recently implicated in attenuating ciliated cell differentiation during wound repair [135]. Interestingly, new findings indicate that a functional hedgehog pathway machinery is required for the effects of TGF-β1 on normal and IPF fibroblasts during myofibroblastic differentiation [136]. However, a causal role of autophagy in ciliogenesis an IPF pathogenesis remains to be determined.

In vivo studies addressing the changes in autophagy during aging and the temporal relationship between autophagy, cell fate determination and fibrogenesis are missing, in part due to the difficulties in studying autophagic flux in vivo at different time points in the fibrotic process as well as due to cell type differences. An improved

understanding of the specific mechanisms by which dysfunctional autophagy and mitophagy can promote cell type-specific features characteristic of emphysema and/or pulmonary fibrosis may lead to an understanding of this dynamic and complex process and the identification of new targets for both diagnostic and therapeutic approaches [123].

3.2.2 Proteasome Regulation

The proteasome regulatory network, a system in charge of the degradation of 90 % of cellular proteins, has been shown to be dysregulated during aging. Proteasomes can be directly inhibited by oxidative stress [137, 138] and lipofuscin, both of which accumulate with aging [139, 140]. Inhibition of proteasome activity increases senescence in fibroblasts [141, 142], and proteasome activity decreases significantly with age in the lung [143]. Protein degradation by the ubiquitin-proteasomal system plays a positive role in modulating TGF-β1 expression and signaling, a key player in pulmonary fibrosis. In fact, proteasomal inhibition is one of the approaches used in various animal models of tissue fibrosis to regulate TGF-β1 signaling and consequently fibrogenesis. This has been reviewed elsewhere [144]. However, while the IPF lung is characterized by a higher content of proteasomes, no significant differences in proteasome peptidase activity in IPF lungs compared to control lungs have been found [145]. This would suggest that if age-dependent dysregulation of proteasome activity plays a role in the etiology of IPF, it would be permissive rather than causal.

In contrast several lines of evidence support a role of proteasome dysfunction in the pathogenesis of emphysema, including the accumulation of ubiquitinated proteins and the deubiquitinating enzyme UCHL-1 [146]. Altered expression of genes involved in protein ubiquitination has been found in COPD patients [147]. For example, a high expression of the valosin-containing protein retrograde translocation complex (VCP-Rma1-gp78) correlates with the severity of emphysema in COPD and the overexpression of inflammatory, ER stress, and apoptotic mediators like NFκB, GADD-153/CHOP, and p-eIF2α in lung tissues [146]. VCP-Rma1-gp78 plays a key role in both protein extraction from the ER and ubiquitin-proteasome mediated protein degradation by ERAD [148]. Those studies propose that VCP mediates the proteasomal degradation of HDAC2 and Nrf2, as a potential mechanism for corticosteroid resistance and increased oxidative stress observed in COPD subjects with emphysema.

3.2.3 ER Stress and the UPR Response

Injury, viral infections, and defects in protein folding can promote ER stress and the consequent UPR in epithelial cells. This mechanism can promote homeostasis and cellular survival. Prolonged stress, by contrast, can contribute to apoptosis and the initiation of fibrotic remodeling [149]. UPR and ER stress are detected in alveolar

epithelial cells in lungs of patients with emphysema, COPD, and IPF. The activation of these pathways may result from altered surfactant protein processing [119, 149, 150]. Activation of the UPR system in epithelial cells may induce secretion of the profibrotic mediator, TGF-β1 [151]. Furthermore, UPR can be induced by TGF-β1, through ROS generation, promoting myofibroblast differentiation in human lung fibroblasts [152, 153].

3.3 Mitochondrial Dysfunction

Mitochondrial metabolism is known to play a central role in mediating longevity via nutrient-sensing pathways and dietary restriction [154]. Mitochondrial homeostasis controls ROS production from respiration. In consequence, mitochondrial dysfunction is often accompanied by increased ROS levels that can contribute to cellular dysfunction and disease etiology. Mitochondrial ROS can directly damage proteins, RNA, nuclear and mitochondrial DNA, and promote senescence and/or apoptosis [155]. Mitochondrial ROS is also derived from the NADH oxidase NOX4, which is important for the transformation of lung fibroblasts to myofibroblasts and consequently, collagen deposition [30, 156].

The antioxidant transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) is a "master regulator" that promotes cell survival through the coordinated induction of phase II and antioxidant defense enzymes to counteract oxidative stress and modulate redox signaling [157, 158]. Deficient NRF2 activity has been associated with the pathogenesis of chronic lung diseases such as asthma, COPD, and IPF, thus contributing to excessive oxidative stress in the lung [159, 160]. Therefore, pharmacological targeting of NRF2 is a potential therapeutic strategy under current study [161, 162].

Another potential regulator is Prohibitin-1 (PHB1), a mitochondrial protein that interacts with the NADH dehydrogenase protein complex. It is known to play a crucial role in preserving normal mitochondrial function, morphology and mitophagy, and also is linked to aging. PHB1 is significantly down-regulated in bronchial epithelial cells from patients with COPD. Deficient PHB1 is probably a contributor to oxidative stress in the COPD lung [163].

Several defects in mitochondrial dynamics, including fission, fusion, biogenesis and mitophagy have been reported in various lung diseases. Reduced expression of PINK1 is observed in the lungs of aging mice and IPF patients [26], and it is associated with pulmonary fibrosis [29]. In the absence of PINK1, cells often develop fragmented mitochondria, due to imbalanced fission/fusion dynamics. Whereas the coordinated induction of fission and autophagy is believed to facilitate the removal of damaged mitochondria through mitophagy, excessive fission may cause apoptosis [164]. Disruption of mitochondrial dynamics may also be relevant to the pathogenesis of COPD, as indicated by the exacerbation of mitochondrial fragmentation observed in Cigarette Smoke Extract-exposed bronchial epithelial cells [165]. Long-term CSE exposure significantly increased the expression of oxidative

phosphorylation proteins as well as specific markers of mitochondrial fission/fusion and oxidative stress [166]. Mitochondrial fragmentation can also be induced in human airway smooth muscle cells by CSE exposure, via the increased expression of the mitochondrial fission protein dynamin-related protein 1 (Drp1) and decreased expression of the fusion protein mitofusin2 (Mfn2). Importantly, the inhibition of Drp1 prevents the effects of CSE on mitochondrial networks and ROS generation, whereas blocking Mfn2 has the opposite effect [167]. In consequence, mitochondrial-targeted interventions are currently developed as the studies to elucidate the role of mitochondrial metabolism and recycling in lung aging, COPD, and pulmonary fibrosis.

3.4 Genomic Instability and Telomere Attrition

Smoking is reported to be one of the most important environmental factors that cause DNA damage [168]. Alveolar type I and type II cells and endothelial cells in patients with COPD showed higher levels of double-strand breaks and DNA damage response than those in asymptomatic smokers and non-smokers [169]. DNA damage and the activation of the DNA damage response are also seen in IPF [119, 170].

Telomere length has been proposed as a marker for physiological aging. Telomeres protect the DNA at the end of chromosomes from degradation, remodeling, and gene-gene fusion, and constitute a marker for aging [171]. The replicative cycle, ROS and inflammation promote telomere shortening [172]. Conversely, telomere erosion versus maintenance and repair can determine cell fate. Telomere length abnormalities and the expression of senescence markers such as p16 and p21 in alveolar cells have been demonstrated in emphysema, COPD/emphysema, and IPF [173–175]. In the case of familial IPF, 10 % of the patients have a mutation on the reverse transcriptase component of telomerase, TERT, and/or the RNA template component TERC [176, 177]. It is also known that 20 % of the patients with telomerase mutations that cause congenital dyskeratosis also develop pulmonary fibrosis [178, 179]. Interestingly, models of injury and fibrosis in mice with telomerase deficiency reveal that TERT induction is associated with the increased survival of lung fibroblasts, which favors the development of fibrosis instead of injury resolution, whereas telomerase deficiency does not result in a predisposition to enhanced fibrosis in bleomycin-treated lungs i [180, 181].

Telomere-deficient mice do not develop spontaneous lung emphysema; however, short telomere length is a susceptibility factor in COPD/emphysema; short telomeres lower the threshold of cigarette smoke-induced damage [182]. Peripheral leukocytes, alveolar epithelial cells, endothelial cells, and fibroblasts from patients with COPD contain shorter telomeres compared to cells from healthy lungs [175, 183, 184]. Interestingly, shorter telomere length in peripheral leukocytes has been associated with increased comorbidity, as well as total and cancer mortality in patients with COPD [183]. In contrast, parenchymal fibroblasts from emphysema patients

do not have altered telomere length despite showing markers of senescence such as β-galactosidase, suggesting that cell senescence in this case might be driven by stress, rather than exhaustive proliferation [185].

3.5 Cellular Senescence

Accelerated cellular senescence is considered a possible molecular pathway by which COPD occurs [2]. Pulmonary vascular endothelial cells, alveolar epithelial cells, and fibroblasts from COPD/emphysema patients show signs of premature senescence compared to controls, as determined by decreased telomerase activity, reduced telomere length, and increased expression of p16 and p21 markers. Furthermore, senescence can be induced in vitro by the exposure of fibroblasts and alveolar epithelial cells to cigarette smoke [186, 187]. It has been proposed that after cigarette smoke exposure, senescent cells may contribute to the pathogenesis of emphysema, while fibroblasts that resist cigarette smoke-induced cellular senescence may contribute to the pathogenesis of pulmonary fibrosis and possibly to fibrotic lesions through a TGF-β1-mediated pathway [188]. Nevertheless, small airways, alveolar epithelia and resident fibroblasts are characterised by different renewal strategies, and telomere dysfunction and cellular senescence could be expected to act differently in these compartments and to be associated to different pathologic outcomes.

3.6 Epigenetic Alterations

3.6.1 DNA Methylation

Epigenetic drift refers to alterations in the genomic landscape of DNA methylation as a function of age. Both DNA hypomethylation of some loci and hypermethylation of others have been shown to be differentially present in sputum and small airway epithelial cells derived from COPD patients compared to healthy individuals [189, 190].

In the context of age-related lung diseases, Alu and LINE-1 hypomethylation in circulating leukocytes was found to be associated with increased age and lung function decline [191]. Methylation changes in promoter regions drive the differential expression of specific genes important for the response against inflammation and oxidative stress in COPD, such as the transcriptional downregulation of antioxidant NRF2 and PTEN, and the transcriptional upregulation of HDAC6 in COPD patients [131, 190, 192, 193].

DNA hypermethylation and hypomethylation in IPF also correlate with the transcriptional regulation of key genes, such as the hypermethylation of COX2 and prostaglandin E receptor 2 PTGER2 [194, 195]. The hypermethylation of Thy1, a

fibrosis suppressor, has been associated with the pathogenesis of IPF [196, 197]. In IPF, hypermethylation of the promoter for the miR-17–92 cluster, which is critical for lung development and lung epithelial cell homeostasis, correlates with the reduced expression of miR-17–92 and increased expression of DNMT-1, a target of miRNAs in this cluster [198].

3.6.2 Histone Modifications

In addition to DNA methylation, another major aspect of epigenetic regulation is represented by post-translational modification of core histones. Many changes occur as a function of age; for example, histone acetylation on lysine 16 of histone H4 (H4K16) increases gradually, due at least in part to a reduction of sirtuin 1 (SIRT1) deacetylase protein level [199–201]. Variations in methylation of histones H3 and H4, depending on residues, have also been also reported. The tri-methylated lysines 9 and 36 of histone H3 (H3K9me3 and H3K36me3 respectively), and the mono-methylated lysine 20 of histone H4 (H4K20me) can also change as a function of age [202–204]. In theory, the promotion of "healthy aging" could be pursued by developing epigenetics drugs able to cope with the "aged epigenome" [205].

There is evidence that the imbalance between histone acetylases (HAC) and deacetylases (HDAC) contribute to specific alterations in histone modifications and gene expression that are characteristic of COPD/emphysema and pulmonary fibrosis [206]. Indeed, cigarette smoke can modulate histone post-translational modifications through reductions in HDAC activity and expression [206, 207]. One example is the downregulation of HDAC2 by cigarette smoke in human macrophages and lung epithelial cells in vitro and in mouse lung in vivo [207]. HDAC2 activity normally delays cellular senescence by negatively regulating pro-senescent genes, such as p21 and p16 [208].

Reductions in mRNA abundance for HDACs 5, and 8, and a decrease in overall HDAC activity are also observed in COPD lung tissue and macrophages [51]. Decreased expression of HDACs correlates with increased H4 acetylation at the IL8 promoter and consequently, elevated expression, promoting inflammation [51]. In concordance, elevated H4 pan-acetylation, a marker of "permissive chromatin", is observed in lung tissue and airway smooth muscle cells from patients with COPD [206, 209]. Elevated H4 pan-acetylation in the vascular endothelial growth factor (VEGF) promoter correlates with reduced VEGF expression in airway smooth muscle from patients with COPD [209].

Sirtuins (see Sect. 3.1.4) are histone deacetylases and reduced SIRT1 activity due to cigarette smoke exposure correlates with increased H4 pan-acetylation and MMP9 expression in COPD [82, 210]. Cigarette smoke-driven reduction of SIRT1 expression in lung epithelial cells promotes FOXO3 and p53 acetylation, regulators of cell proliferation and cellular senescence (see Section 3.5) [210, 211].

Protein levels of Class-I HDACs (1, 2, 3 and 8), Class-II HDACs (4, 5, 7, 9, 10), and of the Class-III HDAC Sirtuin-1 were found to be significantly elevated in IPF lungs compared to healthy counterparts [212]. It has also been demonstrated that the

process of myofibroblast differentiation is HDAC4 dependent and requires the phosphorylation of Akt [213]. Histone modifications are involved in the activation of some pro-fibrotic and repression of anti-fibrotic genes, while modifications at the Fas promoter are responsible for decreased Fas expression and apoptosis resistance in fibrotic lung fibroblasts [214]. COX-2-deficient mice are more susceptible to pulmonary fibrogenesis than are wild-type mice [215, 216], and a deficiency in COX2 expression is associated with deficient histone H3 and H4 acetylation, with a consequent increase in the recruitment of histone corepressor complexes to the COX-2 promoter [197]. Furthermore, recent studies demonstrated a marked increase in histone modifying enzymes and their respective binding proteins at the COX-2 promoter in lung fibroblasts from IPF patients, compared to those from nonfibrotic lungs [195]. Importantly, treatment with histone deacetylase inhibitors promotes fibroblast apoptosis and ameliorates pulmonary fibrosis in mice, inhibits the expression of fibrotic markers in IPF lung fibroblasts and restores cytokine-induced COX-2 mRNA and protein expression [217, 218]. These studies suggest that histone deacetylase inhibitors may offer a new therapeutic strategy in IPF.

3.6.3 MicroRNAs

While alterations in micro-RNA expression could contribute to the age-associated impairment of lung function, a lack of alterations in miRNA expression in the "normal aging lung" was initially described [219]. The total level of miRNA expression is reduced in smokers compared to non-smokers, probably due to the reduced activity of the endonuclease Dicer following cigarette exposure [220]. Reduced miRNA expression has been detected in whole lung tissues, airway epithelia, and alveolar macrophages of smokers. Furthermore, differential expression of miRNAs are detected in whole lung tissue, lung fibroblasts, cells from bronchoalveolar lavage, sputum, serum, and plasma samples from patients with COPD [220–224]. miRNA and mRNA expression profiles enriched for biological pathways that may be relevant to the pathogenesis of COPD including TGF β, Wnt and focal adhesion pathways have been described. For instance, differentially expression of miR15b in COPD regulates Smad7 in bronchial epithelial cells [224]. COPD patients have an abnormal repression of miR-199a-5p compared to unaffected smokers, probably contributing to the adaptive immune balance favoring a Th1 and Th17 profile [222]. The increase in miR-101 in COPD correlates with reduced CFTR expression, which may contribute to mucus accumulation, chronic infection and inflammation [225]. Importantly, recent studies demonstrated that the expression of specific microRNAs such as miR-638 correlates with emphysema severity and specific gene expression networks related to the oxidative stress response in aging emphysematous tissue as well as lung fibroblasts [226].

Fibroblasts from IPF patients with a highly progressive disease exhibit reduced expression of Dicer1 and Argonaute compared to patients with a slowly progressive disease. As Dicer and Argonaute are involved in miRNA biogenesis and silencing of gene expression, it is expected that miRNA biogenesis may contribute to the

progression of IPF [227]. Forty-three microRNAs were found deregulated in samples from IPF patients [228]. Several specific miRNAs have been implicated in fibrogenic processes [198, 229–232]. The correlation between the findings in IPF patient samples and in the bleomycin-treated animal models indicate a miRNA profibrotic signature that includes the upregulation of miR 21, 145, 155, and 199a and the downregulation of miR Let7d, 17–92, 26a, 29, 200 family, and 326 (for a review see [233]); many of these altered miRNAs regulate TGF-β signaling, inflammation, and tissue remodeling [231, 232, 234].

An important connection between microRNAs and the pathogenesis of IPF is centered in the abnormal activation of epithelial cells and fibroblasts by reactivation of developmental programs such as Wnt/B-catenin and Sonic hedgehog signaling pathways. For example, miR-154 causes activation of the WNT pathway in normal human lung fibroblasts, regulating cellular migration and proliferation, and its levels are increased in IPF fibroblasts and lung fibroblasts treated with TGF-β. Upregulation of the Wnt/B-catenin signaling has also been associated with a decrease in miR-375 and miR487b [228, 235].

Contrary to the situation of IPF, a decrease in Wnt/B-catenin signaling contributes to parenchymal tissue destruction and impaired repair capacity in emphysema. In fact, activation of the Wnt/B-catenin pathway attenuates experimental emphysema [236].

3.6.4 Long Non-coding RNAs

Multiple functions are attributed to lncRNAs, such as regulation of transcription, mRNA splicing, mRNA decay, and gene neighborhood localization [237–239]. The expression profile of lncRNAs is significantly altered in fibrotic lung tissue, as demonstrated in bleomycin animal models of pulmonary fibrosis [240]. Until now, no specific lncRNAs have been associated with the pathogenesis of COPD/emphysema or pulmonary fibrosis. In consequence, the pathobiological relevance to lung remains to be established.

3.6.5 Differential Splicing

As a major source of protein diversity, alternative splicing plays critical roles in differentiation, development and disease. RNA-Seq technology allows us to understand how alternative splicing might affect the structure of the final protein products [241]. Splicing becomes less tightly controlled in aged individuals, based on data from the InChianti study [242]. Some splicing variants are known to occur in conjunction with fibrosis. For example, the inclusion of at least one of two extra exons, termed Extra Type III Domain A (EDA) and Extra Type III Domain B (EDB), is a feature of cellular fibronectin in IPF [243, 244]. While this is a nascent field, further RNA-seq experiments might identify additional splicing variants associated with lung disease and prognosis [241, 245].

3.7 *Inflammation*

3.7.1 Immunosenescence and Inflammaging

The immune system undergoes profound transformations with age, and strong similarities in inflammation are evident among aging, COPD and IPF, including lymphocyte senescence, neutrophil accumulation, NF-κB activation, and an increase in IL-6/IL-8/TNFα levels [246]. Disruption in the balance between inflammation and immune activation after local and systemic insults contributes to increased morbidity and mortality in the elderly [247–249], and older lungs respond to insults in a different manner from that of younger counterparts. For instance, the expression of cytokines interleukin IL17A, IL6, and CXCL is induced in older lungs to a greater degree than in young lungs after house dust exposure [250] and sputum from older asthmatics reveals higher numbers of neutrophils, IL-8, and neutrophil elastase [251] [252]. However, the source of inflammatory factors involved in the pathogenesis of either COPD or pulmonary fibrosis is unknown. Alveolar macrophages, lymphocytes, senescent epithelial cells, and mesenchymal cells constitute potential candidates.

Chronic inflammation in the elderly can exacerbate responses to lung injury, promoting alveolar destruction, tissue remodeling and the development of chronic inflammatory lung diseases such as COPD, interstitial pneumonia, persistent lower respiratory tract infection, and pulmonary fibrosis [85, 253–257]. Notably, chronic lung injury, inhaled gases, and particles from cigarette smoke mimic the effects of inflammaging [258–264] (Fig. 4).

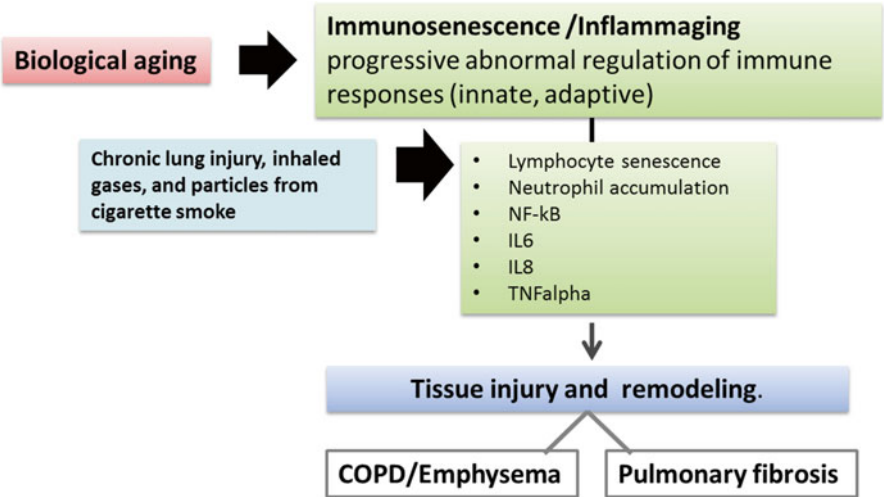

Fig. 4 Schematic representation of the relationship between immunosenescence induced by biological aging and environmental exposures in the development of pulmonary fibrosis and/or COPD and emphysema

Systemic immune-related defects described in COPD/emphysema and IPF patients include the following: ineffective phagocytic removal of apoptosed cells bodies [265–267], defects in innate immunity toll-like receptor sensors and a marked downregulation of CD28 in circulating CD4 T cells [268–270]. The downregulation of CD28 is a marker of CD8 T cell replicative senescence.

In spite of the observations cited above, the role of inflammation in the progression of pulmonary fibrosis is challenged by the lack of response to anti-inflammatory treatments and immunosuppressants. In addition, pulmonary fibrosis can be developed in mice by the overexpression of the profibrotic mediator, TGF-β1, without a significant inflammatory component [271]. Nevertheless, a pathogenic role for inflammation cannot be excluded in the initiation of the disease. In fact, the inability to identify the initiating inflammatory process in human IPF may explain the failure of the anti-inflammatory therapies.

3.7.2 Macrophages

Macrophages are essential components of innate immunity, contribute to acute and chronic inflammatory responses by releasing both pro- and anti-inflammatory mediators, as well as angiogenic, mitogenic, and under pathological conditions, profibrotic proteins [272–274]. M2-activated macrophages secrete cytokines that stimulate collagen production in lung fibroblasts. Moreover, monomeric collagen type I favors the shifting of alveolar macrophages to the profibrotic M2 type, which may perpetuate fibrosis [274–277]. The M2 phenotype is predominant in the lungs of IPF and COPD patients, with higher levels in patients who were smokers [278–280].

3.7.3 Lymphocytes

CD8+ T cells are frequently found in the airways of patients with COPD and are considered relevant for the pathogenesis of emphysema [281, 282]. Indeed, overexpression of T cell cytokines such as IFN-γ and IL-13 induce emphysematous changes in mice [283–285] and CD8+ T cells are required for cigarette smoke-induced emphysema. It is therefore suggested that CD8+ T cells induce the production of macrophage elastase, which degrades elastin, directly causing lung destruction [286].

Different types of CD4+ T cells also appear to be associated with COPD/emphysema [287]. IFN-γ–producing Th1 cells are increased in the airways and parenchymal tissues of patients with COPD [288]. IL17-producing Th17 cells accumulate in the bronchial mucosa and submucosa of patients with COPD [289, 290]. Furthermore, the expansion of CD28− CD4+ T cells, seen during aging [291], is found in patients with COPD, and correlates with impaired lung function. Moreover, these cells exhibit increased expression of perforin, and granzyme B (GzmB) [287, 292, 293]. GzmB released from cytotoxic lymphocytes protects against viral infection and it also contributes to extracellular matrix degradation and remodeling and, consequently, the emphysematous phenotype [294, 295].

3.7.4 Airway, Alveolar Epithelial and Mesenchymal Cells

These cells may also be an important source of inflammatory mediators by several mechanisms (for a review, see [296]). Clara cells are found in the epithelium of bronchioles and secrete surfactants; they may also have functions in maintenance of the bronchiolar epithelium. Loss of Clara cells in the small airways of smokers leads to decreased production of anti-inflammatory Clara cell protein or secretoglobin 1A1 [297], while squamous cells in the airway epithelium of individuals with COPD produce IL-1 alpha and IL-1 beta [298]. In COPD, airway fibroblast express high levels of IL-6, IL-8 [296, 299–301] and integrin alphavB8, which activates TGFβ, in turn stimulating CCL1 and CCL20 production in airway fibroblasts in an autocrine manner [302]. In pulmonary fibrosis, epithelial cells release chemokines that recruit inflammatory monocytes and neutrophils. Furthermore, a subpopulation of fibroblasts promotes inflammation, survival, and fibrosis through the constitutive activation of signal transducer and activator of transcription (STAT) 3 [303]. STAT3 mediates fibroblast chemotaxis through the induction of oncostatin M [304, 305].

3.7.5 TGF-β Signaling

Sequestration of latent TGF-β1 in the extracellular matrix is crucial for proper mobilization of this cytokine and its subsequent activation. TGF-β expression, activation and signaling have been shown to increase in several tissues, including the lung, as a function of aging [39, 87, 306–308]. Older mice (24 months old) are more susceptible to bleomycin exposure and develop extensive fibrosis, showing high levels of Smad3 phosphorylation (a consequence of canonical TGF-β signaling) [39]. In concordance, Smad3 null mice, deficient in TGF-β signal transmission, are resistant to bleomycin- and TGF-β-mediated fibrosis. However, the role of TGF-β1 seems to differ in emphysema and pulmonary fibrosis as Smad3 null mice develop spontaneous age-related airspace enlargement, consistent with emphysema. Furthermore, $\alpha_v\beta_6$ integrin null mouse, which is compromised in the ability to activate latent TGF-β, develops an emphysema-like response [41, 81, 309, 310]. Taken together, TGF-β and Smad3 seem to play a key role in the transition from inflammation to chronic fibrosis and/or from inflammation to emphysema. This can explain why both pathologies can appear within the same lung specimen [81].

3.8 Altered Intercellular Communications

Age-related alterations in the communication among resident cells, immune cells, and the lung microbiome promote susceptibility to age-related diseases [311, 312]. Changes in intercellular communication can occur due loss of cell-cell contact, changes in the extracellular matrix, hormones, electrical and chemical signals. Changes at many of these levels have been described in lung pathology. For

example, age-related lung fibrosis worsens after lung injury in mice deficient in CD151, a regulator of cell-cell adhesion [313].

3.8.1 The Extracellular Matrix

Alterations in the extracellular matrix (ECM) protein profile and architecture are detected in several organs and tissues as a function of physiological aging. Initial studies in the lung from aged rats demonstrated elevation in TGF-β1 protein level, increase in the expression of some metalloproteinases and decreased expression of TIMPS [314].

Interestingly, emphysematous and fibrotic changes in the ECM, induced by elastase and bleomycin respectively, can be preserved in decellularized lungs and used to evaluate the behavior of other cells, such as engraftment of epithelial cells. It appears, therefore, that decellularized emphysematous scaffolds lack the necessary extracellular matrix architecture to support sustained cell growth [315]. Furthermore, IPF scaffolds support fibroblast survival, proliferation, and more importantly, differentiation to a myofibroblast phenotype, characteristic of fibrotic tissue [316].

Metalloproteinases The increased expression of matrix metalloproteinases (MMPs) MMP2/9 and the decreased mRNA expression of tissue inhibitors of metalloproteinases (TIMPS) are characteristic of the aging lung [314]. These proteases and anti-proteases secreted into the extracellular milieu are characteristic features of the pathogenesis of COPD and IPF [39, 317–320]. The relevance of protease imbalance for COPD pathogenesis is reflected by hereditary deficiency of α_1-antitrypsin or α_1-antichymotrypsin, which drive emphysematous ECM remodeling in patients [321]. In IPF, the mechanism involves changes in expression, turnover and/or deposition of ECM components and promotion of tissue remodeling, apoptosis, migration, proliferation, and angiogenesis. For a review, see [322].

3.8.2 Hormones

Hormones can also define intercellular communications. Disruption of the 17β-estradiol/estrogen receptor alpha signaling pathway is observed in aging. Animal models for acute lung injury and inflammation treated with 17β-estradiol exhibit reduced lung inflammation in a gender-independent, age-dependent manner [323]. Testosterone levels have been found to be low in COPD patients, with a prevalence of hypogonadism in men with COPD between 22 and 69 % [324]. However, the therapeutic efficacy of testosterone replacement therapy in COPD patients remains controversial [325–327]. The most abundant steroid in humans is dehydroepiandrosterone (DHEA), and patients with moderately severe COPD have lower concentrations of DHEA than smokers with chronic bronchitis or mild COPD [328]. Both DHEA and its sulfated form (DHEA-S) have been previously linked decreased function of the immune system observed with aging and this decline was

also observed in IPF patients [329]. DHEA decreases lung fibroblast proliferation, increases apoptosis, reduces the fibroblast to myofibroblast differentiation and collagen production mediated by TGF-β1 or PDGF. In consequence, DHEA represents a putative therapeutic option and a strong support of the concept that IPF may be a disease of accelerated aging [329].

As discussed in Sect. 3.1.5, circadian rhythms play an important role in COPD and fibrosis. Melatonin is a key regulator of circadian rhythm homeostasis, and in consequence, melatonin was found to be significantly reduced during the exacerbation period in patients with COPD [330]. A low daily dose of melatonin has been shown to protect lungs from histopathological changes in rabbits exposed to smoke [331]. In animal models of pulmonary fibrosis, melatonin significantly attenuates bleomycin-induced myofibroblast differentiation, and alleviates ER stress and the ER stress-mediated epithelial-mesenchymal transition [332].

3.9 Stem Cell Exhaustion

Age-related defects in epithelial precursors act in concert with environmental toxic exposure to promote the breakdown of epithelial regeneration, leading to the chronic and irreversible alveolar loss characteristic of chronic lung injury, emphysema, and pulmonary fibrosis. Pathogenic models of COPD and IPF propose that premature cellular senescence leading to stem cell exhaustion likely affects distinct progenitor cells, such as mesenchymal stem cells in COPD, and alveolar epithelial precursors in IPF [179]. Unfortunately, our knowledge regarding lung resident stem cells is still emerging, and the changes in these cellular populations during aging and/or age-related diseases of the lung remain to be elucidated. Nevertheless, several studies provide evidence for the existence of human lung epithelial stem/progenitor cells [333–336]. Furthermore, convincing evidence for the existence of resident stem cells comes from a case study that reported compensatory lung growth with an increase in alveolar number in a 33-year-old woman, 15 years after a right-sided pneumonectomy for the treatment of lung adenocarcinoma [337].

The regenerative potential of pulmonary and extra-pulmonary stem and progenitor cells raises the hope for successful treatment options against pulmonary fibrosis, as shown by studies using human amniotic epithelial cells and bone marrow-derived epithelial progenitors cells [338, 339]. Bone marrow mesenchymal stem cells (B-MSCs) protect against the progression of emphysema and pulmonary fibrosis by increasing epithelial cell regeneration and reducing alveolar apoptosis [340, 341]. Furthermore, B-MSCs suppress the inflammatory response [342]. Currently, several studies are listed on the www.clinicaltrial.gov website using different MSC preparations and registered MSC products in patients with COPD and IPF [343, 344].

Nevertheless, it is important to consider that in addition to their reparative properties, MSCs can be a critical factor in the development of dysfunctional lung remodeling [345–349]. Furthermore, resident tissue-specific mesenchymal progenitor cells can eventually contribute to fibrogenesis in human lung allografts [349].

4 Conclusions and Future Directions

The lung is a unique organ that is directly exposed to high levels of oxygen and other reactive compounds. It will be important to further understand the interaction between the hallmarks of aging and the environment in the development of age-related lung diseases. Two generalizations can be taken from the findings reviewed in this chapter. First, the diverse pillars of aging appear to be more permissive than causal in the development of COPD and IPF. If borne out by future research, this would identify an important intersection between the biology of aging and environmental risk-factors for the prevalence of lung diseases among the elderly. Second, some aspects of aging biology may be protective in one disease but permissive in the other, as illustrated by the relatively different prevalence of fibrosis in IPF versus COPD (e.g., cellular senescence as described in Sect. 3.5)

Here we described major similarities as well as differences in the aging features in two age-related lung diseases, COPD/emphysema and IPF. Several preclinical studies using modulators of proteostasis, selective epigenetic modifiers as well as hormetic compounds to promote restoration of some of the aging hallmarks were presented. Modulation of endogenous stem cells may also help restore normal regenerative processes and correct the cellular and structural architecture of the lung and provide immunomodulation and trophic support for epithelial regeneration. It is expected that future studies will provide additional interventions to promote healthy lung aging and prevention of the onset of age-related lung diseases such as COPD and IPF.

We expect that future studies on molecular hallmarks of aging in young, middle age, and old age will provide a better understanding of the progressive decline of lung function in "normal aging" and to separate normal compensatory mechanisms occurring during aging from pathologic changes. Studies that also consider the diversity of cell types present in the lung may lead to an improved understanding of the dynamic and complex process of aging and the identification of new targets for early diagnostics, interventions and therapeutic approaches against age-related lung diseases.

The discoveries of gene variants and changes in gene expression in COPD and IPF that reliably predict outcomes have the potential to revolutionize the prognostic perspective and impact on therapeutic approaches. Emerging approaches to studying genetic/epigenetic/environment interactions, which impact disease pathogenesis are promising leads for novel biomarkers. By adding the perspective of the major pillars of aging, geroscience approaches will add a physiological layer to the efforts in personalized medicine currently focused on gene × environment interactions. This should incorporate aging as an essential parameter to match subjects with optimal therapeutic regimens while minimizing side effects.

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Age-Related Macular Degeneration and Vision Impairment

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Contents

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1 Introduction

The eye is the organ that allows for vision, the ability to see the world. For a person to be able to see, light must enter through the transparent cornea in the front of the eye, be focused by the lens, and detected by the light-sensitive retina in the back of the interior of the eye. Photoreceptors, cells in the outer retina that contain the light-sensitive opsin proteins, transmit the visual signal through the inner retina, where the signal is amplified, parsed, processed, and finally sent through the optic nerve to the brain for higher visual processing and image interpretation. In humans, photoreceptors can be divided into two primary cell types: rods and cones. Rod photoreceptors, which greatly outnumber cone photoreceptors, are incredibly sensitive to light and are primarily responsible for vision in dim light conditions. Cone photoreceptors, on the other hand, operate primarily under bright light conditions and are responsible for providing color-rich and detailed vision. Rods and cones are not evenly distributed throughout the eye; rods are found throughout the entire eye, with the exception of the very center of the retina. In humans, cone photoreceptors are predominantly found in a structure called the macula, which is located in the center of the retina and where light is most focused from the lens. For healthy vision, each component of this pathway must work in concert, and degeneration or injury to any one of these anatomical structures can lead to visual impairment or blindness. The retinal pigmented epithelium (RPE) is a cell monolayer underlying the retina that is responsible for several important functions, one of the most important being the maintenance of photoreceptor health. As individuals age, they can sometimes experience a progressive loss of RPE cells in the macula, and this loss of RPE cells compromises the overlying photoreceptors and can result in severe visual impairment or blindness. This age-associated degeneration of cells in the macula is known as age-related macular degeneration (AMD).

2 Clinical Aspects

2.1 Clinical Presentation

The initial form of AMD (known as early AMD) is marked by the presence of yellowish or whitish punctate extracellular deposits (drusen) that are found between the RPE and the underlying basement membrane known as Bruch's membrane, and that can be seen during a fundus examination. The presence of drusen alone does not necessarily indicate AMD, other diseases can also present with drusen distinct from those noted in AMD patients [1–3]. For drusen to be considered pathognomic of AMD, they must be >63 μm in diameter and be "soft" in nature (i.e., have poorly-defined edges and cluster together in the fundus) [4]. By commonly-accepted clinical diagnostic criteria [4], individuals who present with these drusen (along with Bruch's membrane thickening) without associated pigmentary disturbances are

classified as having early AMD. At these early stages of the disease, patients commonly have no reported visual deficiencies. Focal hypo- and hyperpigmentation of the RPE are also found in the fundus with drusen [5] in patients with intermediate AMD [4]. Typically, visual deficits are not noted until more advanced stages of AMD, which can be divided into two separate categories: (1) the non-exudative (or "dry") form of the disease known as geographic atrophy (GA) that presents with large regions of pigmentary abnormalities in the fundus and cell death in the RPE, without blood vessel infiltration, and (2) the exudative (or "wet") form of the disease known as neovascular AMD (nvAMD) that presents with infiltration of blood vessels into the retina that have a high likelihood of leakage [4]. The severity of AMD can be graded on a classification system [6] developed by the Age-Related Eye Disease Study (AREDS) group, which is based on the previously published Wisconsin age-related maculopathy grading system [7]. The advent of spectral domain optical coherence tomography (SD-OCT) imaging has further aided in the monitoring of AMD [8–10].

2.2 Visual Impairment

Unlike the earlier stages of AMD, in which visual impairment is not detectable or not noticeable by the patient, later stages of AMD are associated with profound visual deficits. Progression from early AMD to one or both of the advanced forms of AMD may take 10 years or more. For patients with AMD, the amount of visual acuity loss correlates with the percentage of the fovea (a particularly cone photoreceptor-dense location in the macula) affected [11]. Once patients develop GA, individuals with <10 % foveal involvement and 20/20 visual acuity (by Snellen chart) may take as little as 5 years to have >80 % foveal involvement, at which point visual acuity may be worse than 20/200 [11]. Likewise, patients with untreated nvAMD have a poor prognosis with respect to visual function: 3 years after diagnosis with nvAMD, >75 % of patients who receive no treatment will have 20/200 or worse [12]. Despite the devastating visual outcomes for many late-stage AMD patients, it should be noted that patients with untreated nvAMD typically have a worse visual prognosis than their GA counterparts, so although nvAMD patients account for only 10–15 % of all AMD cases [13], 75 % of all blindness resulting from AMD can be attributed to the neovascular form of the disease [14].

2.3 Known Risk Factors

Although the exact etiology of AMD is not known, many studies have been performed to identify factors that correlate with increased incidence of the disease in the population. The greatest risk factor for AMD is age [15]; individuals 65 years or older are more likely to be diagnosed with AMD, and individuals 85 years or older

are more likely to have advanced forms of the disease [16]. Race also appears to be a risk factor. Individuals who identify as non-Hispanic Caucasian are more likely to develop AMD than non-Hispanic African-American or individuals of Hispanic descent [17]. Also, individuals who have a family history of AMD are more likely to develop the disease [18]. In addition to these risk factors (i.e., age, race, and family history), other environmental risk factors have been shown to increase risk for AMD development and progression. After age, smoking is widely considered to be the next strongest risk factor for developing the disease. Individuals who have smoked at one point or who currently smoke are more likely to develop the disease and to have advanced forms of the disease as compared to individuals who never smoked [16]. Finally, dietary fat intake and obesity are also known to associate with AMD, and some reports indicate that individuals with healthy diets and who regularly exercise may experience lower incidence of the disease [18].

2.4 Prevalence

Because age is the risk factor that is reported to best correlate with AMD development and progression, it follows that AMD prevalence increases in older populations. Several studies have been done to estimate the prevalence of AMD in the general population, such as the Beaver Dam Eye Study [19], Rotterdam Eye Study [20], and Blue Mountains Eye Study [21]. In individuals 18 years of age or older, AMD patients only comprise approximately 1 % of the population, but in adults 65–74 years old, the prevalence of AMD approaches 9 % [22]. Of the patients afflicted with more advanced forms of AMD, nvAMD appears to be more common than GA [16]. Depending on the age group and population being surveyed, nvAMD can be twice as common as GA [16]. Because a relatively large percentage of the aging population suffers from AMD as compared to other age-related diseases, millions of people worldwide are affected. The number of affected individuals is expected to rise as the aging also comprise the fastest growing segment of the general population.

2.5 Current Treatment Options—Dry AMD

Currently, the only treatment for nonexudative AMD is the AREDS vitamin supplement formulation. Based on reports published beginning in the late 1980s [23–26], it was hypothesized that dietary supplementation with a mixture of antioxidants, vitamins, and zinc could aid in the prevention or slowing of AMD progression. A large-scale clinical trial was conducted at the turn of the century with the goal of determining whether AMD patients who took a formulation of high-dose vitamin C and E, beta carotene, and zinc would benefit from a slowing of AMD progression and visual acuity loss [27]; this study became known as the AREDS1 study [28].

The AREDS group concluded that this combined vitamin supplement (i.e., the AREDS formulation) slowed the progression of dry AMD in patients. Later, another large-scale clinical trial was performed in the AREDS2 study, which added lutein, zeaxanthin, and omega-3 fatty acids to the AREDS1 formulation to test whether these additional supplements could further slow visual acuity loss and AMD progression [29]. It was found that the AREDS2 supplement formulation did not significantly improve upon the efficacy of the AREDS1 formulation [29]. Furthermore, the AREDS2 formulation did not significantly improve upon the AREDS1 formulation in patients harboring mutations reported to confer AMD risk when patients that did not harbor these mutations were excluded from statistical analysis [30].

This is not to say that the AREDS1 formulation could be considered a success in preventing AMD progression and vision loss, however. Almost immediately after the findings of the AREDS1 formulation were published [27], the AREDS1 study's conclusion was challenged. Careful examination of the AREDS1 dataset revealed that there was no statistically significant effect of the AREDS1 formulation on visual acuity, and even whether there was any effect on the progression of early and intermediate AMD to late-stage dry AMD was questioned [31]. Other independent large-scale studies found that patients taking AREDS1 formulation were at higher risk for developing choroidal neovascularization (CATT & CAPT) [32, 33]. Meta-analysis of other small-scale studies also indicate no significant therapeutic benefit from the AREDS vitamin formulation [34]. A 10-year follow-up of patients included in the original AREDS1 study found that the progressive visual loss of AMD patients continued unabated [35], casting further doubt on the efficacy of the AREDS vitamin formulation in treating the disease. In light of these results, many AMD physicians and researchers now believe it should not be recommended that AMD patients take the AREDS supplement [34, 36]. In addition to being of questionable efficacy, it was argued that the AREDS vitamin supplement could also carry the risk of being harmful to AMD patients' health, because of the association of some of these vitamins with exacerbated lung cancer progression [37, 38]. The fact that smoking is a risk factor for AMD patients [16] has led many physicians to feel that not only are the AREDS vitamins ineffective at treating dry AMD, they may also predispose patients to a greater risk of potentially life-threatening off-target effects. In summary, there is no safe and effective treatment approved for the treatment of dry AMD.

2.6 Current Treatment Options—Wet AMD

Unlike dry AMD, there has been considerably more success with respect to treatment development for nvAMD. In decades past, destructive treatments such as thermal laser photocoagulation and photodynamic therapy with verteporfin were employed for nvAMD [39–41]. Over the past decade, the U.S. Federal Drug Administration (FDA) and regulatory bodies in other countries have approved several molecular therapies targeting vascular endothelial growth factor A (VEGF-A)

[42], a potent pro-angiogenic factor that has been implicated in the formation of the unstable, leaky blood vessels that invade the retina in nvAMD [43]. There are currently four anti-VEGF-A treatments used to treat nvAMD patients in the United States: bevacizumab (Avastin) [44], ranibizumab (Lucentis) [44], pegaptanib (Macugen) [44], and aflibercept (Eylea) [42]. Bevacizumab and ranibizumab are both derived from antibodies raised against human VEGF-A; bevacizumab, originally approved as a therapy for colorectal cancer, is the full-length anti-VEGF antibody that has been used in an "off-label" fashion to treat nvAMD, while ranibizumab was specifically approved for use in nvAMD patients and only consists of the Fab portion (the region that recognizes the human VEGF-A epitope) of the antibody [45]. Pegaptanib sodium is a pegylated aptamer (i.e., a nucleic acid designed to bind to a specific biological target) that specifically binds VEGF-A_{165}, one of the many isoforms of VEGF-A [44]. Aflibercept is a fusion protein that consists of the VEGF binding region found on VEGF receptors-1 and −2 fused to the Fc portion of human IgG1 immunoglobulin [42]. These drugs are injected into the vitreous humor of nvAMD patients on a monthly basis or as needed [45]. Extensive off-label use of bevacizumab instead of ranibizumab because of lower cost prompted the National Institutes of Health (NIH) to fund the Comparison of Age-Related Macular Degeneration Treatment Trials (CATT) Study to test whether bevacizumab was comparable to ranibizumab at preserving or improving visual acuity in nvAMD patients [45]. CATT, and subsequently similar studies in other countries, reported that bevacizumab and ranibizumab have clinically similar effects on visual acuity in nvAMD patients [45]. Although these studies indicate bevacizumab has safety and efficacy profiles similar to ranibizumab, bevacizumab is not currently approved for the treatment of nvAMD. Despite the success of treating a subset of nvAMD patients with these anti-VEGF-A therapies, many nvAMD patients do not experience significant reductions in blood vessel penetration or visual improvement [46]. Treatment options for anti-VEGF-resistant nvAMD are currently limited. For patients that do respond well to treatment, repeated use of these drugs is associated with the development of GA [47–50], which is currently untreatable.

3 Cell Types Implicated in AMD Pathology

3.1 Retinal Pigmented Epithelium (RPE)

Both late stage forms of AMD, GA and nvAMD, are ultimately diseases of RPE dysfunction and degeneration. The RPE serves multiple roles in the maintenance of healthy vision, including maintenance of photoreceptor cell health (e.g., phagocytosis of shed rod and cone outer segments [51], transport of nutrients from the choriocapillaris to the metabolically-demanding photoreceptors [52]) and the formation of the outer blood-retinal barrier separating the intraocular tissues from the systemic blood circulation that aids in maintaining the eye as an "immune privileged" site [53]. In the case of GA, the advanced form of dry AMD is marked by large,

confluent regions of RPE atrophy [13]; this suggests that one of the primary RPE functions, maintenance of photoreceptor cell health, is compromised. Likewise, another primary function of the RPE layer, formation of the blood-retinal barrier, is compromised in nvAMD [50]. Because patients can simultaneously present with both GA and nvAMD [16], the loss of both of these critical functions should not be considered to be mutually exclusive from one another. Compromised RPE cell function ultimately associates with the deterioration of other tissues on either side of the RPE, such as the retina and choriocapillaris [13].

3.2 Immune Cell Involvement

To date, there has been little evidence in the literature suggesting immune cell (e.g., macrophage) infiltration in individuals with early or intermediate AMD or in GA patients [50, 54]. For individuals with nvAMD, though, immune cell infiltration is particularly important with respect to angiogenesis. Of particular interest are macrophages, which have been implicated in multiple studies of nvAMD [55–59]. Macrophages, involved in the clearance of cellular detritus and foreign matter, have been proposed to play a role in AMD pathogenesis for quite some time—but whether their presence is pro- or anti-angiogenic is still a matter for debate. Evidence for both pro- and anti-angiogenic roles of macrophages abound in the literature. Macrophage depletion by genetic ablation of the chemoattractant *Ccl2* or *Ccr2*, necessary for macrophage recruitment to the retina, has been shown to promote angiogenesis in mouse models [58]. On the other hand, genetic ablation of the cytokine IL-10 has also been shown to decrease macrophage activity and this inhibits angiogenesis instead [60]. Other data indicate that macrophages may also have a potent pro-angiogenic role in nvAMD, as shown in numerous studies of human neovascular membranes and in mouse choroidal neovascularization (CNV) models [55–57, 59]. Because of the potential dual-role of macrophages in nvAMD, many researchers hypothesize that macrophage polarization may be important in modulating angiogenesis in nvAMD. Macrophages can interchangeably adopt either one of two polarization states, M1 or M2, which determine their activity in tissue. M1 macrophages are typically understood to assume pro-inflammatory roles in tissue, while M2 macrophages are involved in wound repair activities [50]. With respect to AMD, pro-inflammatory M1 macrophages are thought to be anti-angiogenic, and M2 macrophages are thought to be pro-angiogenic. Direct evidence for macrophage polarization modulating angiogenesis in nvAMD is sparse at the moment. There is some evidence to suggest that IL-10, which is known to be involved in switching macrophages from M1 to M2 polarization, may be upregulated in mice with laser-induced CNV [61]. Future studies will hopefully resolve current questions concerning macrophage polarization with respect to nvAMD.

The retina also contains resident macrophages called microglia that are involved in spontaneous CNV formation in mice. Genetic ablation of the macrophage-associated *CX3C chemokine receptor 1* (*CX3CR1*) results in accumulation of retinal

microglia in the subretinal space, and these mice develop spontaneous CNV [62]. *Ccl2*- and *Ccr2*-deficient mice also develop spontaneous retinal lesions in addition to spontaneous CNV formation [63]. Despite the associations of these genes with microglia involvement in spontaneous CNV formation in mice, there is still no clear evidence to date of microglia accumulation in the subretinal space of human neovascular membranes [64]. At this time, it is unclear what role microglia play in nvAMD progression, but future work may be able to explore microglia in further detail.

Work in animal models of nvAMD implicated neutrophil involvement in CNV formation, but there is also compelling evidence to indicate that neutrophil infiltration of CNVs may be the result of experimental artifact. Studies in mouse nvAMD models have reported that neutrophils may be involved in disruption of Bruch's membrane integrity following laser-induced CNV [65] and that neutrophils may directly promote CNV in laser-induced mouse models of nvAMD [66]. Other studies, on the other hand, indicate that neutrophils may only be peripherally involved in CNV formation and that even if neutrophils are associated with CNV formation in the mouse, this finding is not relevant with respect to human nvAMD cases. For example, laser-induced CNV in mice with impaired macrophage infiltration exhibit minimal neovascularization [67]; this suggests neutrophil involvement in CNV formation is minimal. Furthermore, histological analysis of human neovascular tissue indicated minimal neutrophil intrusion into the retina [68] as compared to mouse laser-induced CNV tissue. Because of the known role neutrophils play with respect to injury response, it is possible that the reported neutrophil involvement in nvAMD mouse models is a result of the injury incurred by laser rather than angiogenesis.

4 Aging Processes Contributing to AMD

4.1 Inflammation

Evidence now indicates that AMD has an important inflammatory component to its pathology, as do many aging-related diseases. The RPE expresses the components of many different pro-inflammatory pathways, including Toll-like receptors (TLRs), inflammasome-associated proteins, complement cascade proteins, and other pro-inflammatory cytokines. These pro-inflammatory components are currently an active topic of research, and although the relative contributions of each pro-inflammatory pathway on AMD progression in patients is still unclear, many lines of evidence indicate that age-related inflammatory dysregulation is involved in disease development and progression. These pro-inflammatory mediators are thus attractive targets for future therapeutic approaches. TLR, NLRP3, and complement cascade pathways are currently targets of active investigation.

TLRs are a class of transmembrane receptor proteins that are a primary component of the innate immune system. These TLR proteins are classified as pattern recognition receptors (PRRs), where each TLR is capable of recognizing a type of

pathogen-associated molecular pattern (PAMP) and inducing a signal transduction pathway to initiate an immune response [69]. TLR signal transduction pathways have been well-characterized and have also been implicated in retinal degeneration. One such TLR, TLR3, is best known for its role in recognizing double-stranded RNA (dsRNA) [70]. Unlike other TLR proteins, which rely upon Myeloid differentiation primary response gene 88 (MyD88)-mediated intracellular signaling, TLR3 relies upon TIR-domain-containing adapter-inducing interferon-ß (TRIF) for downstream intracellular signaling [69]. dsRNA-induced TLR3 signal transduction ultimately results in activation of the transcription factor Interferon response factor 3 (IRF3) for expression of type I interferon (IFN) and other pro-inflammatory cytokines [69].

In addition to TLR signaling, recent evidence of the involvement of the NOD-like receptor family, pyrin containing domain protein 3 (NLRP3; also known as the NACHT, LRR, and PYD domains-containing protein 3, or NALP3) inflammasome gives further credence to the role of age-related inflammation in AMD progression. Some TLRs, such as TLR4, which senses extracellular lipopolysaccharide (LPS) found on Gram-negative bacteria, lead to downstream inflammasome activity. The NLRP3 inflammasome, a protein complex that contains NLRP3 protein, Apoptosis-associated speck-like protein containing a CARD (ASC; encoded by the *PYCARD* gene), and cleaved Caspase-1, acts as a platform for interleukin (IL)-18 and IL-1ß cytokine maturation [71]. These cytokines can then be secreted; extracellular IL-18 is known to then bind and activate IL-18 receptor (IL-18R) to induce either pyroptotic or apoptotic cell death pathways [72]. Inflammasome activity is the result of two distinct phases: the first step is priming, in which inflammasome-associated gene products (e.g., NLRP3, Pro-Caspase-1, Pro-IL-18, etc.) are upregulated, and the second step is activation, in which the inflammasome protein complex assembles and begins producing mature, cleaved proteins capable of producing a biological response (e.g., cleaved Caspase-1, cleaved IL-18, etc.) [71]. Despite the potential overlap between TLR signal transduction pathways and the NLRP3 inflammasome, recent evidence suggests the RPE degeneration can proceed via the NLRP3 inflammasome independently of TLR receptors. Also, unlike TLR signal transduction, which typically requires a specific ligand to initiate downstream inflammasome or interferon secretion pathways, NLRP3 inflammasome activators are varied. To date, mitochondrial toxins, reactive oxygen species (ROS), various organic molecules (e.g., nigericin, LPS, etc.), and ions (e.g., potassium) have all been demonstrated to induce NLRP3 inflammasome activation [73].

The complement cascade is a central component of the innate immune response in humans; although the complement cascade can be initiated by multiple different pathogenic insults, the end result is the creation of the membrane attack complex (MAC) which is used to lyse invading pathogens [74]. The MAC complex can be assembled via activation of either the classical, alternative, or lectin pathways; all pathways converge upon C3 activation for assembly of the C5b-9 protein complex (i.e., MAC) [15]. The classical pathway requires antibody binding to antigen for activation by the C1 protein complex [15], and this same set of complement proteins (C2, C4, etc.) is activated through recognition of mannose residues on pathogen

surfaces by Mannose-binding lectin-associated serine proteases (MASP) 1 and MASP2 [75]. Unlike the classical and lectin pathways, the alternative pathway makes use of a different set of complement activating proteins (e.g., CFB, CFD) for C3 activation [15].

4.1.1 TLR Signaling

TLR3 signal transduction triggered by dsRNA has been shown to be activated in a sequence-independent manner by dsRNAs that are 21-nt in length or longer [76]. dsRNA is found in large quantities in the drusen of patients with GA, and consistent with this observation, RPE degeneration occurs in mice following subretinal injection of 21-nt [77]. Furthermore, TLR3 SNPs have been associated with a protective effect against GA in human patients [78], suggesting that TLR3 signaling may play a role in AMD progression, although these findings are not universal among studies. Paradoxically, TLR3 signaling that exacerbates one form of advanced AMD (GA) may also inhibit angiogenesis in nvAMD, the other form of advanced AMD. TLR3 signaling has been shown to inhibit both angiogenic and lymphangiogenic events [79, 80], but despite the potential utility of the TLR3 signaling pathway with respect to angiogenic inhibition, the parallel apoptotic events associated with TLR3 signal transduction have largely precluded this pathway as a viable therapeutic target. At least one TLR3 polymorphism may be protective in patients [81], but its veracity is still controversial; this is discussed later in this chapter. In spite of unclear genetic data, however, multiple labs have independently shown that TLR3 activation is capable of inducing RPE cell death [82–84].

4.1.2 Inflammasome

The NLRP3 inflammasome has been implicated in AMD under the effect of various stressors. Carboxyethylpyrrole (CEP)-adducted proteins, the result of free radical-catalyzed oxidation of docosahexaenoate (DHA) that causes damaging covalent modifications to proteins [85], have been shown to prime the NLRP3 inflammasome [86]; these CEP adducts have also been shown to accumulate with age in the retina [85]. In addition to CEP protein adducts, certain drusen components are known to be capable of inducing NLRP3 inflammasome activation. Unfortunately, aside from the fact that drusen correlates with AMD, it is not known whether drusen are directly capable of inducing pro-inflammatory events. The complement cascade intermediate C1q has been shown to activate the NLRP3 inflammasome [86]; other complement cascade proteins have also been implicated in AMD and will be discussed in further detail below. Recently, it has also been shown that DICER1, a protein normally associated with microRNA (miRNA) biogenesis, may also play a role in GA via NLRP3 inflammasome activation because of its ability to degrade cytotoxic *Alu* RNA transcripts [77]. *Alu* elements are retrotransposons that are found interspersed throughout the genome (over 1 million copies are present) [87]

and were until recently thought to be mostly transcriptionally inactive. GA patients have reduced DICER1 levels in the retina and RPE, and the resulting accumulation of cytotoxic *Alu* RNA transcripts results in inflammasome activation in an NF-κB-dependent [88], TLR-independent manner [89]. Mature IL-18 production by *Alu* RNA-mediated insult then causes MyD88- and Caspase-8-dependent RPE apoptosis [90]. Interestingly, elevated circulating levels of IL-18 have been reported in patients with dry AMD [91, 92]. Most likely, given the diverse range of substances capable of inducing the NLRP3 inflammasome, more inflammasome agonists will be found to associate with AMD progression.

4.1.3 Complement Pathway Activation

In addition to the potential involvement of C1q in inflammasome activity, the complement cascade may play a role in AMD. Of the three complement activation pathways (classical, alternative, and lectin), the alternative pathway may be associated with AMD progression. Recognition of the complement system as playing a potential role of AMD came in 1992 with the finding that subretinal membranes surgically removed from AMD patients contained complement proteins C1q, C3c, and C3d [93]. It was also found later that C5 and C9 complement components could be found in both hard and soft drusen in AMD patients [94] and that immunohistological sections of AMD patient samples were positively labeled for C3, C5, and C5b-9 [95]. The potential role of the complement system, particularly the alternative pathway, was further highlighted by a genome-wide association study (GWAS) that found roughly 50 % of the heritability for AMD could be associated with a polymorphism in the complement factor H (CFH) gene (Y402H); individuals with the CFH^{Y402H} variant were found to be significantly more likely to develop AMD [96–98]. In the alternative complement activation pathway, CFH plays an inhibitory role by regulating C3 convertase (C3bBb) activity [15]. Putative pathogenic CFH variants have been found to have reduced binding affinity for proteins found in the retina [99–101], including C reactive protein (CRP), a protein that has increased expression in response to inflammation and that is known to activate the complement system via C1q [102]. Other polymorphisms in CFH and other complement proteins will be discussed later in the chapter.

Despite reports of complement proteins appearing in drusen and statistical associations of complement gene polymorphisms with AMD in human patients, a precise mechanism for complement-mediated cell stress and cell death remains elusive. For example, C1q is capable of inducing NLRP3 inflammasome activation in cell culture, but the precise mechanism for this phenomenon remains unknown [86]. Furthermore, although complement proteins that appear in drusen may be capable of inducing an inflammasome response and causing cell death, these drusen components are insoluble; after all, drusen are deposits of insoluble cell debris. Because of this, it is still not entirely clear how insoluble materials could be capable of producing a cell response in vivo. Experiments reporting the cytotoxicity of these complement proteins are performed with soluble complement proteins and depletion of

endogenous negative complement regulators; they do not recapitulate the conditions that are present within the aging eye.

In addition to uncertainties with respect to mechanism from cell culture data, mouse models have been unable to reproduce AMD pathology when the *Cfh* gene is ablated. Despite a strong statistical association between CFH polymorphisms and AMD development in human patients, *Cfh* null mice exhibit an extremely weak phenotype. *Cfh* mutant mice exhibit no appreciable photoreceptor degeneration, even at 2 years of age [103]. Also, AMD patients have regions of RPE and retinal atrophy; there seems to be very little effect on the RPE of *Cfh* mutant mice despite the fact that they accumulate more C3 in the eye [103]; this suggests that although CFH may inhibit C3 deposition, C3 itself may not be sufficient to induce AMD phenotypes. This finding has been reproduced in humans; despite the fact that C3 deposition occurs in the eye, many humans with C3 or C5b-9 deposition never develop AMD [104, 105]. Perhaps most concerning is the fact that none of the clinical trials for inhibition of complement factors have yet met with any success in human patients. This raises concerns about the utility of the *Cfh* mutant mouse in developing treatments given the fact that the disease phenotype in these mice is very weak and therefore does not seem to accurately recapitulate the human condition. Many of these approaches have passed Phase I clinical trials without any appreciable safety concerns, but they have not shown any functional benefit with respect to inhibiting disease progression and visual function loss in patients [50]. Thus, although the *CFH* gene has been associated with AMD susceptibility in multiple studies, these genetic findings have not as of yet translated into a comprehensive molecular pathological mechanism. Much more work remains to be done to elucidate the role of the complement cascade in AMD beyond the current genetic associations [106]

4.1.4 Cytokines and Chemotactic Signaling

Increases in the expression of numerous pro-inflammatory cytokines are associated with AMD, further highlighting the importance of inflammation in disease formation and progression. In addition to specific suspected pro-inflammatory pathways involved in AMD progression that have already been discussed in this chapter, there are multiple other reports of cytokines involved in pro-inflammatory events and cytokines and chemotactic signaling factors involved in angiogenic events. Pro-inflammatory cytokines IL-17 and IL-22, for example, are recently identified cytokines that may be upregulated in patients with non-exudative AMD [107, 108]. With respect to nvAMD, multiple anti-inflammatory pro-angiogenic cytokines and chemotactic signaling proteins have been implicated. IL-10 is of particular interest as it has been shown to promote M1 to M2 macrophage polarization [108] and has been reported to be increased in the serum of AMD patients [109]. Other groups report that IL-6 may be a good marker of nvAMD progression [110]. The eotaxins CC-chemokine ligand (CCL) 11, CCL24, CCL26, CXC-chemokine ligand (CXCL) 10 and CC-chemokine receptor (CCR) 3 have been shown to be upregulated prior to blood vessel invasion in the retina [59, 111]. The increases in CCL11 and CCL24

expression do not appear to be restricted to the retina, as other studies have shown their serum levels increase in AMD patients [111, 112]. Future work will hopefully identify some as potential therapeutic targets.

4.2 Angiogenesis

Angiogenesis, the process by which new blood vessels are formed, is necessary for development [113], wound repair [114], and a number of other vital processes in healthy individuals. Given the fundamental role that angiogenesis plays in complex organisms, dysregulation of angiogenic signaling pathways can have far-reaching consequences. Inside the eye, dysregulation of angiogenic signaling events are associated not just with nvAMD but also corneal neovascularization [115] and diabetic retinopathy [116], both of which are vision-threatening. Outside the eye, dysregulation of angiogenic processes can have life-threatening consequences, such as preeclampsia [117], diabetic nephropathy [118], and cancer [119]. Like inflammation, regulation of angiogenesis is impaired with age [120]. In the context of AMD, angiogenesis plays a crucial role in nvAMD progression through the formation of new blood vessels during both CNV and retinal angiomatous proliferation (RAP) [121, 122]. Both CNV and RAP result in the formation of aberrant blood vessels in the retina, but the two terms refer to the differing origin of the blood vessels; CNV refers to blood vessels originating from the choroid that invade the retina, while RAP refers to blood vessels originating from the retina that migrate externally toward the choroid [123]. Fortunately, despite differences in the origin of these aberrant blood vessels, both RAP and CNV may respond favorably to anti-VEGF therapies [121, 122]. In light of the importance that anti-VEGF therapies have with respect to current nvAMD treatments, understanding the VEGF signaling pathway is critical to understanding nvAMD.

VEGF-A is considered the most important regulator of angiogenesis in many tissues [124], and it is expressed in a variety of isoforms [125]; with respect to angiogenesis in nvAMD, VEGF-A_{165} is considered the most biologically relevant [124]. Increased VEGF-A expression has been associated with nvAMD in human tissue samples [126]. VEGF-A is capable of extracellular binding to Vascular endothelial growth factor receptors (VEGFRs; part of a class of tyrosine kinase receptors) to induce intracellular signaling events that ultimately result in endothelial cell migration and blood vessel growth [127]. There are two VEGF-A receptors that have been shown to mediate angiogenesis in the eye: VEGFR1 (also known as fms-like tyrosine kinase 1), which binds VEGF-A with high affinity but has weak tyrosine phosphorylation upon binding, and VEGFR2 (also known as fetal liver kinase 1, or FLK1, and as kinase insert domain receptor, or KDR), which has a weaker affinity for VEGF-A but a stronger tyrosine phosphorylation in response to binding [127]. It is commonly thought that VEGFR2 is predominantly responsible for angiogenic events in a variety of tissues [127], including the retina [124]. Nevertheless, VEGFR1 is also thought to play an important role in regulating angiogenesis through the action of one of its splice variants, soluble VEGFR1 (sVEGFR1; also known as soluble

FLT-1, or sFLT-1). sFLT-1, which has been shown to have reduced expression in both the eye (where it is primarily expressed in the RPE) [128] and in the serum in nvAMD patients [129], acts as a soluble decoy receptor for VEGF-A to prevent its binding to VEGFR2 for angiogenesis [130]. In addition to VEGF-A overexpression in nvAMD patient's eyes, there are other anti-angiogenic factors with decreased expression that have been reported. One such protein is pigment epithelium-derived factor (PEDF), a potent inhibitor of angiogenesis in the eye, which is highly expressed when oxygen is abundant in the microenvironment [131]; in nvAMD, PEDF levels were reported to be decreased [132]. PEDF acts by antagonizing VEGF-A signaling through cleavage of the transmembrane domain of membrane-bound VEGFR1 [133].

Chemokine and chemokine receptors are also implicated in angiogenesis because of the roles that macrophages and microglia have in nvAMD pathology (see Sect. 3.2). CCL2, CCR2, CX3CR1 and CCR3 have all been implicated in nvAMD, and they all regulate macrophage or microglia recruitment to Bruch's membrane [58, 59, 62]. Because macrophages can have either pro- or anti-angiogenic effects with respect to AMD, the role of macrophage involvement appears to be more nuanced than in the case with VEGF-A (where increased VEGF-A expression is more easily correlated with angiogenesis). Future research is required for a more complete picture to be formed for the involvement of macrophages and VEGF-A signaling pathways in nvAMD.

The relative contributions of CNV and RAP to nvAMD are still unclear. It was originally suspected that nvAMD patients presenting with RAP account for only 12–15 % of all cases [121], but more recent work suggests that RAP may be present in as much as 1 out of every 3 nvAMD cases [134]. Given the fact that most clinical trials for anti-VEGF therapies have excluded nvAMD patients presenting with RAP and that three times as many nvAMD patients may present with this form of aberrant blood vessel growth than previously thought, more attention to the molecular underpinnings of RAP development may be warranted. Fortunately, the VEGF signaling pathway still appears to be an effective therapeutic target for a subset of nvAMD patients presenting with either RAP, CNV, or both. It is thought that VEGF is overexpressed in the retina in patients presenting with RAP much in the same way as nvAMD patients with CNV [135], thus making both sets of patients responsive to treatment. To date, multiple independent studies have indicated that different anti-VEGF therapies (bevacizumab, ranibizumab, and pegaptanib) are all effective at treating RAP lesions [136–138], although it should be noted that the reported therapeutic benefit was observed in relatively small sample sizes. Other therapies for anti-VEGF-resistant nvAMD patients are limited at this time.

4.3 Protein Homeostasis

The RPE is an extremely metabolically active cell type. Its many functions include, but are not limited to, visual cycle metabolism (the recycling of vitamin A isomers that are required for visual function) [139] and outer segment phagocytosis [140];

these processes are notoriously metabolically demanding and can lead to an overabundance of reactive oxygen species (ROS) [141]. The ROS generated by the RPE can result in significant oxidative damage to proteins and other macromolecules within the cell, and to cope with this constant onslaught of ROS damage, the cell relies on autophagy. Autophagy is the process by which cells can clear damaged proteins and organelles via lysosomal degradation [142]. Because of the constant insult from ROS on the RPE over the course of the lifetime, AMD researchers hypothesize that impaired clearance of these damaged cellular components because of impaired autophagy could lead to drusen formation [28, 143].

There is some evidence in the literature to support this hypothesis; aging patients are known to accumulate protein adducts known as advanced glycation end products (AGEs) that have been associated with some age-related diseases [144]. AGEs form under oxidative conditions through Maillard reactions, which nonenzymatically covalently glycate proteins [144]. These AGEs are capable of binding receptors for advanced glycation end products (RAGEs), and it is possible that RAGE signaling may lead to either VEGF-A upregulation that could promote angiogenesis [145] or innate immune activation (e.g., NLRP3 inflammasome) via NF-κB [146]. It is important to note, however, that these findings came primarily from cell culture data; despite a reported increase in AGEs in the serum of AMD patients [147], it is unclear if the reported observations of AGE in endothelial cell culture studies will be replicated in human tissue samples. A proteome analysis of drusen taken from human tissue samples also identified CEP-adducted proteins (formed from the oxidation of docosahexaenoic acid), which have been interpreted as being potentially relevant to AMD [148]. Furthermore, anti-CEP autoantibodies were identified in the sera of AMD patients in higher amounts than in age-matched controls [85]. Despite the potentially clinical relevance of the finding on anti-CEP autoantibodies in the sera of AMD patients, there has yet to be a defined molecular pathway leading from CEP protein adduct formation to antibody production [108]. Future research will hopefully provide the missing links to this phenomenon.

Amyloid ß (Aß) is most commonly known for its suspected role in causing Alzheimer's disease (AD), but it has also been identified as a component of drusen in human AMD patients [149]. Aß is formed from cleavage of Amyloid ß precursor protein (APP) by ß and γ secretases and accumulates in extracellular, insoluble amyloid plaques in patients with AD; the function of both Aß and the APP protein precursor are still a matter of debate [150]. Interestingly, Aß was found in drusen also positive for activated complement proteins [149], suggesting a functional link between the two with respect to AMD pathogenesis. Later work indicated that Aß could cause upregulation of complement factor B (CFB), a protein involved in the alternative complement activation pathway [151]. Further highlighting the fact that many aging processes are interrelated and can contribute to disease formation is the fact that Aß could also activate the NLRP3 inflammasome through lysosomal destabilization, in addition to being potentially linked to the alternative complement pathway [152]. Because the lysosome is central to autophagic processes, this finding further emphasizes the importance of protein homeostasis to age-related disease processes.

4.4 Metabolism

As mentioned in Sect. 4.3, one of the primary functions of the RPE is photoreceptor outer segment phagocytosis [140]. Photoreceptor outer segments are outgrowths of the plasma membrane on photoreceptors that are densely packed with the visual pigment molecules rhodopsin (in rods) and opsins (in cones) [153]. Each day, based on a circadian rhythm cycle [154], the outermost plasma membrane discs containing visual pigments (which are closest to the apical processes of the RPE) are shed so they can be phagocytized and broken down by lysosomes in the RPE [51]. The phagocytosis of shed outer segments by the RPE is compensated by the continuous renewal of new outer segment discs by the photoreceptors and allows for the removal of older visual pigment proteins that become damaged as the result of oxidative stress [51]. One of the metabolic byproducts of visual pigment activation by photons is the retinoid all-*trans*-retinal, which is typically recycled back into 11-c*is*-retinal by other visual cycle proteins for reformation of functional visual pigment in outer segments still attached to their respective photoreceptors [155]. Under oxidative conditions, two molecules of all-trans-retinal can react with ethanolamine to form N-retinyl-N-retinylidene ethanolamine (known more commonly as A2E) [156]. When A2E in outer segments is phagocytized by the RPE, it accumulates because the cell has no method for metabolizing it back into all-*trans*-retinal. A2E forms one of the primary components of lipofuscin, an autofluorescent accumulation that collects in the RPE with age [157]. Because early studies with high-performance liquid chromatography (HPLC) found that patients with AMD may have increased amounts of A2E [158], it was long thought that the age-related accumulation of lipofuscin may be a contributing factor to AMD development [157]. A proposed mechanism of action for A2E cytotoxicity was inhibition of efficient lysosomal activity that could then lead to apoptosis [156]. As more research was performed examining the A2E in AMD, though, the proposed causative role of A2E in AMD development was challenged. Initial measurements of A2E accumulation in AMD patients were performed with whole eyecups; separate HPLC measurements of macula and periphery indicated that A2E accumulated in the periphery of the eye, not in the macula [158]. Other work indicated that lipofuscin only poorly predicted the spread of atrophic regions in GA [159], and perhaps most damaging to the hypothesis that A2E contributed to lipofuscin accumulation and RPE cell death was the finding that lipofuscin and A2E distribution in the RPE did not even correlate with one another [160].

That is not to say there is no suspected role for metabolism in AMD, however. Disruptions in metal homeostasis in the aging eye may contribute to AMD [161]. Although one study has implicated reduced copper and zinc in the eyes of AMD patients as compared to age-matched controls [162], iron accumulation in particular may have a contributing role in AMD [163]. In healthy eyes, iron is an important cofactor necessary for many proteins, including proteins that perform necessary visual functions [164]. Because of the toxic nature of iron and the fact that it is required for many proteins, its transport and metabolism must be carefully regulated by the cell. Iron-induced retinal degeneration has been noted in patients and in ani-

mal models of hereditary iron overload, aceruloplasminemia, hereditary hemochromatosis, and pantothenate kinase associated neurodegeneration (PKAN) [163]. The levels of free iron (i.e., iron ions that are not bound to proteins or other macromolecules) are also known to increase with age, possibly because of reduced ability of the aging body to clear the ions from tissues [165]. This increase in free iron concentration occurs throughout the entire body, but is particularly relevant to AMD because patients were reported to have an increase of free iron in the affected macula [166] and were reported to also have iron as a drusen component [167]. Furthermore, AMD patients were found to have increased mRNA expression of the iron transporter gene transferrin (responsible for binding iron for transport into the cell), which is highly expressed in the RPE [168]. Also, mice deficient in both the iron-binding protein ceruloplasmin (Cp; also binds free iron) and hephaestin (Heph; necessary for cellular excretion of excess iron) appear to develop retinal pathologies similar to those seen in AMD patients, such as sub-RPE deposits and neovascularization [169]. Collectively, these data suggest that metabolic events favoring intracellular accumulation of iron may contribute to AMD pathogenesis. The toxicity of iron in biological systems is largely attributed to the result of Fenton chemistry, the process by which free divalent cations can catalyze the formation of hydrogen peroxide from water [170]. The resulting oxidative stress is then thought to contribute to cell death via apoptosis, but it is also possible that free divalent cations like iron could directly induce an inflammatory response. After all, it is already well known that ROS intermediates can cause NLRP3 inflammasome activation, which has been implicated in AMD by multiple different causative factors (please refer to Sect. 4.1.2). Indeed, recent evidence also suggests that iron also activates the NLRP3 inflammasome via sequestration of Poly(rC) binding protein 2 (PCBP2), which is required for efficient DICER1-mediated clearance of *Alu* RNAs, and that this mechanism is independent of ROS generation [171].

4.5 Genetic Associations

As is most likely the case with many complex diseases like AMD, a single mutation alone may not be sufficient to cause disease; genetic variants may predispose individuals to developing AMD, but other environmental risk factors are most likely necessary for interactions with those genetic variants [5]. Some of these genetic variants confer more risk for AMD development than other genetic variants. One such polymorphism, CFH^{Y402H}, has already been discussed earlier in this chapter (please refer to Sect. 4.1.3). The CFH^{Y402H} variant is thought to contribute to as much as 50 % heritability to the disease [96–98], but the exact mechanistic pathway for CFH involvement in AMD is still unclear. The relative contributions of other, less common or rare genetic variants are still a matter for further investigation. Another potential confound with respect to interpreting the genetic data in the literature is the fact that some variants found to be significant in one study may not be found to be significant in another. Reasons for discrepancies between studies include

differences in risk conferred by each polymorphism in specific populations, insufficient statistical power in the study, or the presence of false positives in the dataset. It is also worth noting that individuals of Caucasian ancestry are more likely to develop clinical features of AMD than African Americans even though the rates of late AMD do not differ significantly between the two groups, although the exact reason for this is currently unknown [172]. In this section of the chapter, known or suspected polymorphisms will be discussed with respect to the known functional roles these genes have in healthy cells, but it is important to keep in mind that many of these polymorphisms have been reported without subsequent analysis of their molecular consequences. For many reported polymorphisms, further work is needed to ascertain their functional importance in disease processes and to understand why genetic mutations present from birth do not manifest as being pathogenic until patients are older.

4.5.1 Inflammatory Pathway Genes

To date, CFH variants are the most strongly associated genetic variants with respect to AMD. The finding that the CFH^{Y402H} polymorphism could contribute to AMD [96–98] was significant because it was the first report of a strong genetic association with a complex disease [50]. Furthermore, a later GWAS study indicated that although the CFH^{Y402H} variant did strongly associate with AMD, 20 other CFH variants also showed association with AMD [173], suggesting CFH variants account for a large amount of the heritability reported in AMD patients. In addition to CFH polymorphisms, other complement pathway factors have also been associated with AMD, even though the relative contributions of these other polymorphisms is still debated [15]. $C2^{E318D}$, $C3^{R102G}$, and CFB^{R32Q} polymorphisms were each found to independently associate with AMD risk in 2011 [174]. Another CFB variant, CFB^{L9H}, was identified later [175]. The association of $C3^{R102G}$ with AMD has also been replicated in another independent study [176]. Other rare complement factor variants, such as $C3^{K155Q}$ [177, 178], CFH^{R1210C} [178], CFH^{R53C}, CFH^{D90G} [179], CFI^{G119R} [180], and $C9^{P167S}$ [181] have all been identified as being highly penetrant in their association with AMD. The exact functional consequences of these variants with respect to protein function are still being studied.

In Sect. 4.1.1, the contribution of TLR signaling to AMD was discussed; TLR3 signaling has been reported to both inhibit neovascularization [79] and induce retinal degeneration [76], but the involvement of genetic polymorphisms in TLR signaling pathway genes is less clear. Initial findings of a potential protective effect against GA conferred by a $TLR3^{L412F}$ polymorphism [78], a potential susceptibility to AMD conferred by a TLR^{D299G} polymorphism [182], and a potential association of AMD with a TLR7 polymorphism were not replicated in later studies [183, 184]. One group later reported that the $TLR3^{L412F}$ variant may have a protective effect against GA because of a reduced ability of the receptor to bind dsRNA [185], but this finding has not been independently replicated by other laboratories. Because of

the previous independent genetic studies that concluded this variant was unlikely to be significant, the contributions of TLR polymorphisms to AMD is at best uncertain.

4.5.2 Angiogenic Signaling Pathway Genes

With the recent interest in pharmacogenetics (i.e., the influence genetic polymorphisms have in the response to drug treatment), researchers have focused on the potential influence of VEGF-A polymorphisms on the nvAMD patient's response to anti-VEGF therapy. Despite the occasional report of VEGF-A polymorphism association with anti-VEGF therapy treatment outcome [186], rigorous analysis indicates that VEGF-A or VEGF-A receptor polymorphisms have no effect on treatment response [179]. Another study has implicated a CCR3 polymorphism (rs3091250) as a potential risk factor for AMD, but this result has not yet been replicated in other laboratories (it should be noted that another CCR3 variant, rs3091312, was not found to significantly associate with AMD) [187]. Stronger statistical associations of genetic variants in the *VEGFR1* gene that are predicted by computer modeling to alter splice sites and RNA secondary structure have also been reported [188] but have still yet to be examined at the protein level.

4.5.3 Genes with Other Functions

Genes with known functions in retinoid clearance from photoreceptors, lipid metabolism, and extracellular matrix maintenance have also been implicated in AMD [5, 189]. High-density lipoprotein metabolism pathways have been implicated in AMD because of an association of the hepatic lipase (*LIPC*) gene [190], and this gene association was supported by findings from another study [191]. APOE, a gene involved in low-density lipoprotein (LDL) metabolism, has also had polymorphisms associated with AMD [192]; this finding highlights the possible role that lipid dysmetabolism may have in disease development and progression. TIMP3, an inhibitor of matrix metalloproteinases [193], has also been associated with AMD along with *COL8A1*, a gene involved in the *FRK*/*COL10A1* extracellular collagen matrix pathway [194]. It is possible, but not yet clear, that these polymorphisms may affect the maintenance of Bruch's membrane with age.

4.5.4 Chromosome 10q26 Locus

Along with CFH, a genomic region with one of the strongest genetic associations with AMD is the chromosome 10q26 locus containing the two open reading frames (ORFs), *HTRA1* and *ARMS2* [195]. Because of the great degree of linkage disequilibrium in this region, genetic approaches alone have been unsuccessful in identifying the gene responsible for the strong association with AMD [195]. Studies of these two genes are further confounded by the fact that the *ARMS2* gene has a

largely unknown function because it is only present in primates and thus precludes the use of a mouse model for studies of gene function [196]. Attempts to define the cellular and tissue localization of ARMS2 protein have produced inconsistent findings [197, 198]. Because of this, ARMS2 has not been definitively implicated in AMD pathology despite the strong associations of individual gene variants with the disease. HTRA1, on the other hand, is a serine protease with a well-defined function in extracellular matrix degradation, suggesting it may have an involvement in degradation of Bruch's membrane to promote RPE atrophy. HTRA1 is also an attractive candidate for AMD pathological association because of its presence within drusen [199]. Unfortunately, HTRA1 has produced inconsistent results based on hypotheses of the proposed mechanism of action. It would be expected that HTRA1 overexpression would be connected to AMD pathology in patients because of its ability to degrade extracellular matrix, but this has not been found in humans [200]. Because of this, the HTRA1 protein remains an attractive candidate for AMD association, but like ARMS2, ultimately has an uncertain role in disease progression based on current data. Future research into the functions of HTRA1 and ARMS2 in AMD patients may resolve these questions. Again, it is worth noting that despite these strong genetic associations, it is still unclear why these genetic influences do not become relevant until old age.

4.6 Epigenetics

Currently, there are few published studies examining epigenetics and AMD risk [201, 202]. There is some evidence to suggest that DNA hypermethylation of the *GSTM5* promoter, which encodes for the glutathione *S*-transferases mu1 and mu5, may result in reduced expression of these proteins in AMD patients [203]. Future work may further explore this potential link and other possible avenues of study.

5 Future Therapeutic Prospects

Unlike nvAMD, which has multiple current therapeutic interventions approved for use in patients, dry AMD has no current FDA-approved therapies with the exception of the AREDS formulation which, as discussed, does not appear to prevent the development of geographic atrophy [35]. Multiple clinical trials with the goal of inhibiting the complement pathway have had little success in preventing or restoring vision loss [50, 204]. It is not currently clear why these complement inhibition therapies have not had any clinical success with respect to AMD, but successful interventions seen in animal models that do not translate to human patients suggests current models are not reflective of human disease pathology and that there are gaps in our understanding of the role of complement in AMD. Also, many of these therapies have been developed in younger mice, suggesting that perhaps studies in older

mice would better reflect the conditions found in aging AMD patients. The creation of better animal models will ultimately allow for better treatment validation so the field can make progress with the goals set forth in the NEI Audacious Goals Initiative [205, 206]. Nonetheless, other complement inhibition therapies, e.g., anti-complement factor D antibody (lampalizumab), are currently in clinical trials, and it is possible these new methods will be more successful than other approaches taken in the past. Because of the potential role that autophagy may play in AMD progression (please refer to Sect. 4.3), this pathway is currently a focus of interest for therapeutic intervention. Recent work indicated that RPE cell stress induced through oxidative stress may activate the Protein kinase B (AKT)/Mammalian target of rapamycin (mTOR) pathway, which could be inhibited with rapamcyin administration in a mouse model [207]. It is interesting to note that rapamycin is not only of interest in AMD research, but is also of interest in many other age-related diseases discussed in this book, because of observations that long-term rapamycin administration may promote longevity [208].

Given the advent of induced pluripotent stem (iPS) cells, cell replacement therapy is another current avenue of research that may show promise for the treatment of AMD [28]. Stem cells derived from mouse bone marrow were capable of establishing a monolayer in subretinal regions damaged by sodium iodate after subretinal injection [209]. Other work with adult hematopoietic stem cells (HSCs) indicated that these cells could assume RPE-like characteristics after transgenic expression of RPE65 [210], a visual cycle protein specific to the RPE in the eye [155]. Mice with RPE damage from sodium iodate exhibited restored visual function and prevented retinal degeneration upon subretinal injection of these HSCs [210]. Cell replacement therapy therefore offers the potential for benefit in AMD patients, but significant hurdles remain with respect to assessing its efficacy in the clinic. Despite the success of this technique in mouse models, it is unclear whether this method could rescue vision in an eye in which AMD has already caused significant RPE and photoreceptor cell atrophy. After all, restoration of the cell layer responsible for photoreceptor cell health is ultimately futile if the photoreceptors have already been lost by the time of cell replacement therapy. Furthermore, many of these proof-of-concept studies for stem cell therapies were performed in younger mice. Given the senescence of stem cells in older patients, it is currently unclear whether these therapies could be used with stem cells from aging individuals.

Recent work has also raised the possibility that nucleoside reverse transcriptase inhibitors (NRTIs) may have potential therapeutic against AMD pathology [211]. This finding is based on previous work that indicated that DICER1 deficiency could induce NLRP3 inflammasome activation and RPE apoptosis via *Alu* RNA accumulation [77, 89, 90, 212]. *Alu* RNA-induced RPE cytotoxicity requires the purinoreceptor P2X7 for cell death [88], and evidence suggests that NRTI's mechanism of action may involve blockade of the receptor [211]. NRTI treatment of AMD could offer several advantages over other therapeutic approaches currently under investigation, such as the fact that many NRTIs are already approved by the FDA for treatment of other diseases, like human immunodeficiency virus (HIV). Because AMD is a chronic disease that patients could have for several decades, drugs with long-

term safety are particularly needed. Many HIV patients take NRTIs for decades and these drugs are thus promising candidates for AMD treatment.

With respect to nvAMD, IL-18 has been the subject of recent debate as it has been proposed as a potential treatment for nvAMD, based on reports that recombinant IL-18 administration could inhibit CNV [213] and that neutralization of endogenous IL-18 by antibody administration results in increased CNV [86]. However, five independent laboratories were unable to reproduce the original findings [214]. Hence, IL-18 is most likely not therapeutic for AMD and, based on findings of IL-18 with respect to RPE degeneration induced by *Alu* RNA accumulation, will most likely prove harmful to patients already experiencing cell atrophy because of AMD.

6 Future Research Needs

Despite the advances made in the understanding of AMD pathology, biology, and treatment, much work remains because of the complexity of the disease. Treatments for nvAMD have allowed patients who would otherwise have debilitating vision loss to maintain quality of life. That being said, nvAMD treatments have much room for improvement. Even though the risk of infection with each injection of anti-VEGF-A drug is low, repeated injections of drug for several years increases the possibility of an eventual eye infection. Also, nvAMD patients who receive anti-VEGF-A therapy also have a high likelihood to develop GA, for which there is no current treatment. Because of these reasons, there is a need for future treatments that will allow sustained therapeutic benefit and that do not exacerbate the progression of the dry form of the disease. Furthermore, the pitfalls of current nvAMD therapies highlight another important need in the field today: the need for treatments for the early and intermediate forms of AMD and the advanced dry form, GA. The therapeutic benefit of the AREDS formulation, which is currently the only option available for non-nvAMD patients, is still debated and may be limited in its efficacy. It appears that the formulation may even cause more harm than good in some patients. The reasons for this dearth of options for all AMD patients are manifold but ultimately reflect the lack of basic understanding of the biology underlying AMD pathology, including the impact of aging on development of the disease. The vast majority of the data in the literature concerning AMD development and progression are correlative observations (e.g., drusen formation, complement deposition, etc.) and do not offer molecular mechanistic explanations for their actual role in the disease. For example, CFH variants associate with AMD risk but as of yet, there is little data on how these associations translate into an actual disease mechanism that could be targeted by therapy. Because of this, there is no clear demarcation between normal aging in the eye and AMD.

The first CFH variant identified was found to be highly significant, and other significant associations between individual variants and AMD have been made since then, but these variants have not allowed for the creation of an animal model

that can fully recapitulate the patient phenotype. Mouse models will always have confounding factors because of the fact that mice do not have a macula, but mice mutant for genes identified in GWAS studies have consistently failed to accurately reflect observations seen in the clinic with respect to RPE atrophy and visual function loss. Moreover, despite not having a macula, mouse models of nvAMD have been useful in their ability to advance clinical therapies for macular nvAMD in humans. This begs the question about whether the reason there is no effective treatment for early, intermediate, or dry AMD is because of the fact that current commonly accepted models do not adequately reflect the disease and the impact of aging on development of the disease has not been thoroughly tested. The number of failed clinical trials supports this hypothesis. The biggest challenge facing AMD research today is the fact that the field needs to find a way to tie clinical observations (including, but not limited to, genetic associations) to experimental models that accurately reflect the disease to allow for a comprehensive understanding of molecular disease processes so new, effective treatments can be created.

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HIV and Aging: Parallels and Synergistic Mechanisms Leading to Premature Disease and Functional Decline

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1 Introduction

Why is there a chapter on HIV in a translational science textbook on aging? Effective antiretroviral therapy (ART) has resulted in many people with HIV infection living far beyond what was thought possible just a few years ago [1, 2]. It is estimated that over half of all U.S. HIV-infected persons will be >50 years by 2015 [3, 4], and the success and availability of ART is even leading to an aging HIV-infected population in developing nations [1, 5, 6], emphasizing the need for aging-related research in those countries where HIV burden of illness is greatest [7].

There is marked debate as to whether HIV accelerates aging itself or is an added risk factor for a number of diseases and conditions that lead to an "aged phenotype." Of course, there is no single pathway that defines "aging" – in fact, two recent, excellent reviews [8, 9] emphasize a number of "hallmarks" of aging – biologic changes that accompany aging, but none is clearly "the" causal pathway. Cardiovascular disease (CVD) and many other diseases increase with age, and advancing age is the leading risk factor for CVD. But we generally don't consider CVD risk factors (e.g. hypercholesterolemia) to be conditions that accelerate aging itself. However aging with HIV is different than aging with hypercholesterolemia; a much broader array of illnesses occurs with greater frequency in people aging with HIV (PAWH). This leads not only to prematurity of a single disease, but multiple diseases, as well as decreased physiologic reserve and increased vulnerability to catastrophic illness, hospitalization and death. Functional decline – physical and/or cognitive – often accompanies multi-morbidity or may occur independently, but in either case functional decline is the strongest risk factor for disability and loss of independence, particularly when social and family support structures are lacking. This state of multi-morbidity, vulnerability, functional decline and loss of independence is what we usually view as "old" – or the aged phenotype – and there is no doubt that this phenotype occurs earlier in PAWH when compared to HIV-uninfected persons [10–14]. In this chapter, we will briefly summarize a few examples of age-related serious non-AIDS events (SNAEs) such as CVD and cancer – and geriatric syndromes (functional decline/frailty and multi-morbidity) to highlight the clinical relevance and translational opportunities to link mechanisms to clinical outcomes in PAWH.

2 Increased Prevalence of Age-Related, Serious Non-AIDS Events (SNAEs) in PAWH

While life expectancy has increased markedly for PAWH, this group experiences a greater frequency of age-associated comorbid conditions, such as CVD, non-AIDS-defining cancers (liver, lung, anal), osteoporosis/osteopenia/bone fractures, metabolic syndrome, and neurocognitive dysfunction. These events are termed SNAEs and increasingly robust data suggest they are very common in PAWH, even those

well controlled on ART [15]. CVD and cancer have the most robust database and are therefore examined in greater detail in the following paragraphs.

CVD risk factors and rates of acute coronary syndromes and heart failure are markedly increased in HIV-infected vs. age matched control subjects [16–20], and coronary artery "age" is accelerated on average by about 15 years in treated HIV-infected persons (median duration of ART ~11 years) as assessed by coronary artery calcium (CAC) score comparing PAWH to age-defined norms established in the MultiEthnic Study on Aging (MESA) cohort [21]. Higher levels of C-reactive protein, interleukin-6, and D-dimer have been shown to be significantly associated with an increased risk of all-cause mortality in HIV-infected individuals not on ART, and much of this is cardiovascular mortality [22]. Specific ART drugs also may be causally associated with early heart disease, even after controlling for age and traditional cardiovascular risk factors [23, 24]. Further, lipodystrophy and metabolic syndrome (altered body fat, hyperlipidemia and insulin resistance) are common in HIV-infected patients receiving ART [25, 26]. The redistribution of fat mass and progression to metabolic syndrome (12/100 patient-years) typically occurs within 3 years after the initiation of ART [27], when weight gain is often substantial, thus increasing cardiovascular disease risk. Enhanced cardiovascular "aging" is not limited to coronary artery disease. Left ventricular diastolic dysfunction and increased vascular stiffness [28–30] are more common in HIV-infected subjects versus uninfected, age-matched controls even after controlling for hypertension and other risk factors. Heart failure and atrial fibrillation, typically seen in older adults, is increasingly being reported in younger PAWH [18–20].

As ART use has become widespread, AIDS-defining cancers (Kaposi's Sarcoma, lymphomas) have become less common in this population, but increased survival and perhaps decreased competing causes of AIDS-defining cancer deaths have led to increased numbers of non-AIDS-Defining Cancers (NADC) [31]. A number of NADC occur more frequently in PAWH than age-matched control cohorts [32] and NADC are increasingly a cause of death in PAWH [15]. Initial reports suggested the age of onset of many NADC was much earlier than in those without HIV, but most of this appears to be a cohort effect. PAWH are a younger cohort than the general population [1] so colon, lung or other cancers may appear to only be occurring in younger adults, but there aren't many 70+ year old PAWH so this is often a false impression. As control groups and age-adjustments have been refined, it appears NADC are only minimally "accelerated" with regard to age at diagnosis – perhaps 3–5 years [33] (Table 1). It is important to note that some NADC that are most strongly related to age – breast cancer in women and prostate cancer in men – do not appear to be increased in those with HIV [33, 34], though data are sure to evolve as persons continue to age with HIV infection.

Another way to examine the question of whether HIV directly "ages" individuals or acts in parallel is to assess whether age remains an independent risk factor for SNAEs in PAWH. Within cohorts of PAWH, increased age is an independent predictor of stroke, myocardial infarction, fractures, osteoporosis, diabetes, and non-AIDS associated cancers, while controlling for CD4 count, viral load, intravenous drug use, smoking, and duration of HIV infection [35].

Table 1 Age differences between HIV-infected and HIV-uninfected for select NADC

Select NADC	Median age at diagnosis in AIDS population (years)	Median age at diagnosis in HIV⁻ general population (years)	Apparent difference (years)	Median expected age at diagnosis in HIV⁻ population if cohort limited to the same age distribution as those with AIDS (years)	Real difference (years)
Rectal	46	69	−23	51	−5
Lung	50	70	−20	54	−4
Ovarian	42	63	−21	46	−4
Myeloma	47	70	−23	52	−5

Adapted from [33]

3 Geriatric Syndromes in PAWH

3.1 Multi-morbidity

Despite the success of ART, extensive evidence suggests HIV-infected persons are more likely than their HIV-uninfected counterparts to have multiple comorbidities at a young age. This is perhaps not surprising for illnesses with overlapping risk factors (i.e. hepatitis C, human papillomavirus [HPV]-related cancers), but it is also true across organ systems where intersecting risks are not so clear; early-onset of disease in individual organ systems in PAWH has been observed (e.g. coronary artery disease, arterial stiffness, cerebral blood flow, and bone fractures) [21, 36–39]. Chronic liver and renal diseases are also more common in PAWH compared to HIV uninfected populations [40]. Although behavioral factors such as smoking and illicit drug use are more prevalent in populations of PAWH, controlled studies have shown that these factors do not fully explain the increased risk for age-related conditions such as cardiovascular and liver disease [35, 41, 42]. Where aggressive ART is widely available, 58 % of HIV-infected subjects aged 51–60 have one or more of the following: renal failure, diabetes mellitus, bone fracture, hypertension or overt cardiovascular disease vs. only 35 % of HIV-uninfected controls [10, 35]. The rate of multi-morbidity (> one major chronic illness) at age >50 years is about 2.5 times higher in HIV-infected subjects vs. HIV-uninfected controls [10, 35, 40].

On average, PAWH aged 50 and older have up to three chronic illnesses, in addition to HIV [43] (Fig. 1). Depending on the population, studies have demonstrated increased prevalence of specific comorbidities. The onset of multi-morbidity appears to be accelerated 12–15 years in those with HIV infection [10]. Further, multi-morbidity risk assessments such as the Veterans Aging Cohort Study (VACS) Index derived and validated in HIV-infected subjects correlates with mortality risk and hospitalization [44, 45]. Importantly, the VACS index has now been validated to predict mortality in HIV-uninfected populations [45] demonstrating the generalizability of this integrated measure of cumulative damage to the hematopoietic, immunologic, hepatic and renal systems.

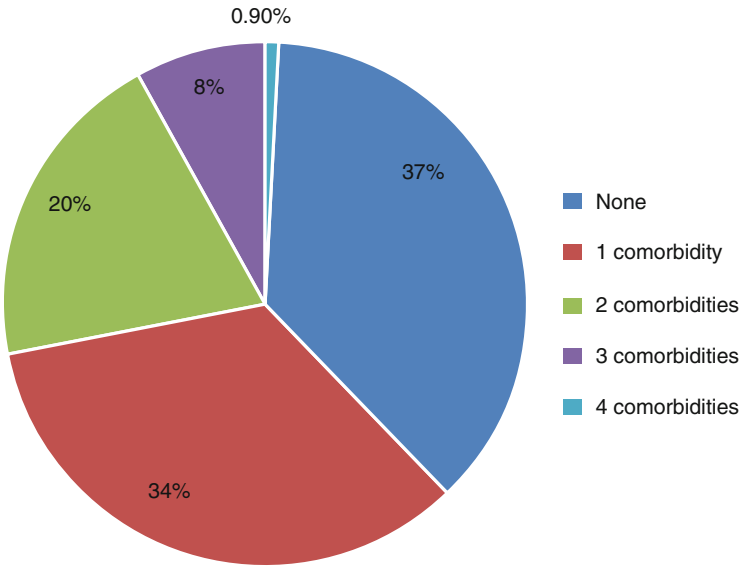

Fig. 1 Prevalence of comorbidity burden, HIV-infected persons age > 50 (Data derived from [10])

3.2 Polypharmacy

In the setting of multi-morbidity, PAWH have increased risks of developing both HIV-associated non-AIDS (HANA) and non-HIV related conditions. Consequently, polypharmacy and increased complexity of care are becoming commonplace in the health management of PAWH, noting that the disease courses may be altered depending on the patient's state of virologic suppression [46]. PAWH who are aged 50 and older are more likely to have at least one medication (in addition to ART) compared to PAWH younger than age 50 [47]. Specifically, older PAWH are more likely to take concurrent cardiovascular, gastrointestinal, and hormonal medications than younger patients [47].

The inherent complexity of polypharmacy translates into potential harm for older patients. In older adults without HIV, polypharmacy is a known risk factor for falls, adverse drug events (ADE) including drug-drug interactions (DDI), morbidity, and mortality [48]. These associations remain true for PAWH but may occur at younger ages compared to people without HIV infection [47–49]. At baseline, older patients are at increased risk for ADE, compared to younger patients. In addition to direct toxicity for the patient, ADE and DDI can mean decreased efficacy of therapy, both for HIV and other comorbidities, especially in the case of protease inhibitor (PI)-based ART [49]. The list of potential DDI is extensive and includes virtually every class of therapeutics, including cardiovascular, gastrointestinal, hematologic, anti-neoplastic, antimicrobial, psychiatric, and endocrine (including inhaled steroids which aren't typically considered to have systemic effects) [47–50]. Predicting

DDI is more challenging due to changes in drug metabolism that occur with normal aging, a process which may be accelerated or accentuated in PAWH. The known increased prevalence of liver and kidney disease in PAWH further complicates prediction and prevention of DDI and ADE [48, 49].

Adherence to ART is extremely important for PAWH and is a predictor of morbidity and mortality for these patients, but a significant challenge for many [49, 51–53]. Similar principles regarding consistent medication use can apply to other chronic illnesses, including the common HANA and non-HIV associated comorbidities which are so prevalent in this population. Recent data suggest that lower pill burden is an important factor in improving adherence and virologic suppression, making awareness (and avoidance if possible) of polypharmacy even more salient [54].

3.3 Frailty

Frailty has been defined and various measures validated in older HIV-uninfected adults, but it is generally agreed to represent increased risk and decreased ability to recuperate from illness and injury. Frailty is increased in HIV-infected vs. age-matched HIV-uninfected controls [13, 55–61]. In those PAWH, there is a high correlation between various measures of frailty validated in seniors, though definitions vary from study to study and the reader should be cautious to assess frailty definitions, cohort effects, and control group definitions when comparing individual rates of frailty between studies [62]. Early research measured the prevalence of frailty using the frailty-related phenotype (FRP) in 55 year old men with HIV infection (infected for less than or equal to 4 years) as equivalent to the prevalence of FRP in men 65 years of age or older without HIV [12]. Onen et al. measured a prevalence of 9 % for frailty in an outpatient HIV clinic (mean age of 41.7 years), which was comparable to the prevalence of frailty in Caucasian Europeans aged 65 years and older [13]. In the same study, investigators measured patient-level characteristics; frailty was associated with socioeconomic status, multi-morbidity, lower education level, longer period of HIV infection, history of opportunistic infection, as well as an increased risk of hospitalization, number of hospitalizations, and inpatient length of stay [13]. Much of the early data suggested frailty in PAWH was associated with uncontrolled HIV/weight loss/wasting, but more recent data suggest frailty in HIV has been associated with obesity and intramuscular adiposity, as seen in HIV-uninfected older persons [59, 61, 63].

Frailty is potentially mediated more by inflammation and body composition than by HIV infection itself. This is compounded by the fact that optimal immune function may be hindered by age-related changes that are independent of virologic suppression [46, 64]. In PAWH, frailty is associated with central obesity, sarcopenia, and increased muscle fat density for age [65]. Oursler et al. showed that, despite ART, physical function in PAWH aged 50 years and older was worse compared to HIV-uninfected people [60]. Regardless of age, HIV-infected patients with chronic pulmonary disease had worse physical function compared to HIV-uninfected peo-

ple, such that a 50 year old person with HIV and chronic obstructive pulmonary disease (COPD) had functional measurements approximating a 68 year old person with COPD, but without HIV [60]. Within populations of PAWH, the prevalence of frailty is increased in people who also use intravenous drugs [43]. Not surprisingly, frail PAWH have a high prevalence of comorbidities, including hypertension, COPD, viral hepatitis, dementia, and cancer; this pattern of multi-morbidity mirrors trends seen in the larger population of PAWH [11].

Beyond the effects that frailty may have on physical health and mental well-being, this phenotype has implications for healthcare delivery and models of care. Guideline-driven care may not be practical or universally applicable to PAWH if their risks of various conditions change at different age breakpoints or based on factors other than what has been measured in the foundational studies. Use of more tailored prediction tools such as the VACS Index may be more applicable due to incorporation of multiple biomarkers [46].

3.4 Neurocognitive Impairment

A full examination of the neurologic manifestations of HIV and even discussion limited to cognitive impairment is beyond the scope of this review. Briefly, 50 % of PAWH will develop an HIV-associated neurocognitive disorder (HAND) [43, 66]. HAND is a spectrum of clinical conditions ranging from asymptomatic neurocognitive impairment (ANI – least severe) to HIV-associated dementia (HAD – most severe, previously known as "AIDS Dementia Complex") [67] (Fig. 2). The symptoms can be largely reversed with ART, but the incidence of HAND is associated with worse adherence. The impact of HAND is marked with HAND being associated with decreased ability to complete daily functions, poorer quality of life, and shorter survival. While the incidence and prevalence of HAND are decreasing due to ART, the incidence and prevalence of ANI and mild neurocognitive disorder (MND) are stable to increasing, spurring a recommendation for universal neurocognitive screening of PAWH [67, 68]. Furthermore, HIV itself may alter brain structure, despite ART, thus, the full expression of HIV-related cognitive disorders may require time to become apparent [69].

3.5 Quality of Life and Mental Health

Compared to HIV-uninfected people, PAWH (ages $\geq$ 50 years) are not as happy, optimistic about aging, or resilient [43, 70]. They also experience more perceived stress, anxiety about the future, and lower quality of physical and mental health [70]. Social isolation, a common occurrence in older adults regardless of HIV status, is associated with increased risk for hospitalization and all-cause mortality. The social networks for older PAWH may shrink due to common age-related factors

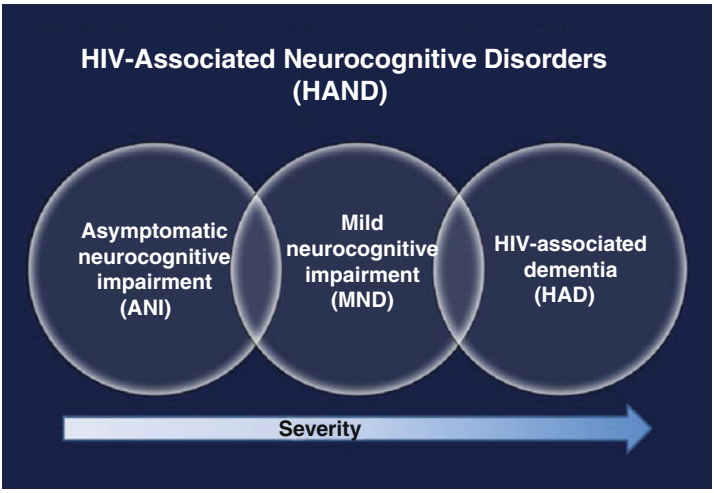

Fig. 2 The spectrum of HIV-associated neurocognitive impairment

(e.g. age-related deaths, limited transportation, geographic isolation) and/or more HIV-specific factors: loss of peers earlier in the HIV/AIDS epidemic, stigma, marginalization in the current make-up of the epidemic [43, 71, 72]. While both HIV-infected and HIV-uninfected older adults may experience social isolation to some degree, HIV infection alone is associated with increased risk and prevalence of social isolation [71].

4 Potential Mechanisms Linking Chronic HIV Infection with Age-Related Conditions

4.1 Immunological Similarities Between HIV Infection and Healthy Aging

The overlapping burden of morbidities and SNAEs in PAWH and aged individuals has led to the hypothesis that similar pathogenic mechanisms are driving the development of these diseases in both populations. Indeed there are many immunological parallels between chronic HIV infection and healthy aging which are summarized in Table 2 and discussed in detail below.

Adaptive Immune Changes

The reduced number of naïve T cells and reduced CD4:CD8 T cell ratio observed in the aged is a hallmark of T cell immunosenescence [73, 74] and also occurs in HIV-infected individuals due in part to thymic involution and reduced regenerative capacity [75, 76]. Low CD4:CD8 T cell ratio is an independent predictor of non-AIDS mortality [77] and cardiovascular disease risk [78, 79] in HIV-infected

individuals, suggesting T cell immunosenescence has important clinical implications in the context of PAWH. Importantly, the majority of HIV-infected individuals on long term ART fail to normalize the CD4:CD8 T cell ratio, despite restoration of CD4+ T cell levels [80]. Elderly HIV-uninfected and HIV-infected individuals also exhibit increased T cell activation (as measured by expression of the activation markers HLA-DR and CD38) [76, 81], an increased susceptibility to spontaneous apoptosis [82, 83] and an expansion of 'senescent' memory CD8+ T cells which lack expression of the co-stimulatory molecule CD28, contain shortened telomeres and exhibit a reduced proliferative potential [84–86]. Expansion of this cell population is thought to be largely driven by chronic antigenic stimulation by cytomegalovirus (CMV), with a large proportion of CD8+ T cells in both HIV-infected and aged individuals being specific for CMV epitopes (discussed further below). However, there are phenotypic differences in the T cells expanded due to HIV infection and those observed in CMV+ HIV seronegative individuals, in that the former show an increased number of transitional memory cells and a reduced proportion of CD28-cells expressing CD57 (a marker of reduced proliferative capacity), with low levels of this population being associated with increased risk of mortality [87]. These observations suggest that although there are many phenotypic similarities between HIV infection and aging, the mechanistic drivers, and thus immunological consequences of, senescent T cell expansion in HIV and aging may be subtly different.

While the above mentioned immunological alterations due to HIV are significantly improved by ART, they typically fail to normalize, and defects including reduced naïve T cell proportions, inverted CD4:CD8 T cell ratios and increased T cell activation largely persist in virologically suppressed HIV-infected individuals (reviewed in [88]). Furthermore, aging appears to impact negatively on the immunological benefit of ART and associated reductions in immune activation, with older HIV-infected individuals exhibiting muted naïve T cell regeneration following ART initiation [89], suggesting immunological aging may heighten HIV-related immune dysfunction in older HIV-infected individuals.

During aging, there is a reduction in the number of both total and memory B cells and defects emerge in class switching and antibody production which are thought to contribute to impaired vaccine response in the elderly [81, 90]. Viremic HIV infection is similarly associated with reduced total and memory B cell numbers together with hypergammaglobulinemia, increased cellular activation and increased susceptibility to apoptosis [91]. ART reverses many of these defects, although virologically suppressed HIV-infected individuals continue to show impaired antibody production, reduced vaccine responses and an incomplete restoration of memory B cells [92, 93]. Markers of HIV disease severity including viral load and immune activation are associated with an increased frequency of regulatory B cells (Bregs), which inhibit CD8+ T cell proliferation and function via a mechanism involving IL-10 and PD-1 [94], which may potentiate immune dysfunction in HIV. Bregs from HIV-infected individuals constitutively express higher levels of IL-6, IL-10 and cellular activation markers, suggesting increased Breg activation in vivo [95]. Interestingly, older HIV-infected individuals show an altered pattern of B cell resto-

Table 2 Comparison of immunological changes observed in HIV infection and aging

	HIV Infection	Aging
T cells	Decreased naïve and increased memory T cells Decreased CD4:CD8 T cell ratio Expansion of senescent CD8+ CD28-T cells Expansion of CMV-specific CD8+ T cells	
B cells	Decreased numbers and proportion of mature/memory B cells Impaired class switching and antibody production, leading to impaired vaccine response	
Monocytes/Macrophage	Impaired phagocytosis Increased proportion CD16+ inflammatory monocytes Increased expression of cellular activation markers Increased TLR4-stimulated cytokine/chemokine production Dysfunctional TLR-responses	
Natural Killer (NK) cells	Expansion of CD56dim population (acute HIV infection only) Impaired cytokine production (modest decline in aging)	
	Increased activation Increased spontaneous ADCC Expansion of CD56- population Impaired cytotoxic potential	
Dendritic cells	Reduced numbers in blood (pDC in HIV and mDC in aging)	
	Impaired response to TLR 7/8 stimulation Increased activation in pDC	Impaired chemotaxis and antigen uptake
Neutrophils	Increased basal activation	Decreased chemotaxis
	Impaired phagocytosis, migration, respiratory burst and intracellular killing	
Inflammation	Increased plasma levels of IL-6, TNF, hsCRP	
Soluble markers of immune activation	Increased plasma levels of CXCL10, sCD14, sCD163, neopterin	
Gut integrity	Increased plasma concentrations of LPS Increased levels of gut permeability markers I-FABP and/or zonulin-1	
Oxidative stress	Increased plasma markers of oxidative stress	
Telomere length	Shortened (PBMC, T cells, monocytes)	

Shaded cells indicate immunological changes which occur in both HIV infection and aging

ration after ART initiation, including expansion of the naïve population to levels greater than those in uninfected individuals [96], suggesting that HIV and age may potentiate immune dysfunction in PAWH. These studies collectively indicate that HIV infection induces a phenotype within the adaptive immune system which resembles age-related immunosenescence and immune dysfunction, and viral suppression associated with ART only partially improves these parameters.

4.1.1 Innate Immune Changes

Immunological similarities are also seen between HIV infection and healthy aging within the innate immune system. Monocytes from both HIV-infected individuals and the elderly show impaired phagocytic function, increased TLR4-mediated production of pro-inflammatory cytokines and chemokines and an increase in phenotypic markers of activation, including an expansion of the inflammatory CD16+ monocyte subset [97–101]. Viral suppression associated with ART appears to normalize some of these changes, such as the proportions of CD16+ subsets, whilst other markers of monocyte dysfunction persist [97, 102]. Elevated levels of soluble plasma monocyte/innate immune activation markers including the chemoattractant CXCL10 (released from IFNγ-stimulated monocytes), neopterin and soluble(s) receptors CD14 and CD163 (shed from activated monocytes/macrophages) are elevated in both the elderly and HIV-infected individuals and although ART reduces the levels of these markers in HIV infection, they fail to normalize [97, 98, 103–107].

An increase in total NK cell number, due to expansion of the $CD56^{dim}$ population occurs during aging and in acute HIV infection [74, 108]. Aging is associated with a minimal impairment of NK cell cytotoxic function and cytokine production [109–111], whilst overall cytolytic activity is impaired in HIV infection (most prominently in viremic infection) which may be due in part to expansion of an anergic $CD56^{neg}$ population in HIV-infected individuals [108]. NK cells from both viremic and virologically suppressed HIV-infected individuals show heightened basal activation [112, 113] and spontaneous ADCC activity [112], whilst cytokine production is impaired [114]. The functionality of neutrophils is similarly impaired in HIV infection as in aging, as evidenced by impaired phagocytosis and oxidative burst but a heightened basal level of activation [115]. The impact of age on the number, activation state and function of dendritic cells remains unclear due to conflicting findings (reviewed in [116]), although chemotaxis and antigen uptake are impaired in aged humans [117, 118] whilst HIV infection is associated with impaired ex vivo response of plasmacytoid dendritic cells to TLR7 ligands [119]. Taken together, these data suggest that a signature of increased activation but dysregulated function is a common effect of both HIV infection and aging on innate immune cells, although much work is required to fully define the extent of these effects. It is important to note that many of the above mentioned age-related immunological changes have been observed in cross-sectional studies of HIV-infected individuals with varying degrees of immunosuppression both prior to and following ART initiation. Future longitudinal studies are required in cohorts of individuals who initiate ART early

and maintain immunocompetence to adequately determine the impact of virologically suppressed HIV infection on age-related immune changes in PAWH.

4.2 Telomere Shortening

The presence of telomeres at the ends of chromosomes protects the DNA from damage and preserves the replicative potential of the cell. Telomere length progressively decreases with age and triggers replicative senescence, which contributes to immunosenescence and immune aging [120]. Telomere shortening is associated with risk of a range of age-related diseases including malignancies [121], cardiovascular/metabolic disease [122–124] and neurocognitive disease [125, 126] (summarized in Table 3 and reviewed in [195]) and has been linked with premature death in a large prospective study in Denmark [123]. HIV infection is associated with heightened telomere shortening within both T cells [85] and monocytes [97]. However, epidemiological links between shortened telomeres and HIV-related co-morbidities have received little investigation to date.

Telomere length is maintained within cells via the action of telomerase and premature telomere shortening in HIV infection may be due to reduced activity of this critical enzyme. The HIV proteins Vpr [196] and Tat [197] have been shown to inhibit telomerase in vitro whilst HIV-infected individuals appear to have an impaired ability to upregulate telomerase in response to cell stimulation [198]. Antiretroviral therapy may also contribute to premature telomere shortening as the nucleos(t)ide reverse transcriptase inhibitor (NRTI) drugs can inhibit the telomerase reverse transcriptase (TERT) component of human telomerase. In vitro studies have shown that even modern, relatively non-toxic NRTIs such as tenofovir and emtricitabine show inhibitory effects on human TERT [199, 200], and can accelerate telomere loss in cultured cells [199] whilst a small cross-sectional study found telomeres from individuals on NRTI-containing regimens were shorter than HIV negative controls and HIV-infected individuals taking non-NRTI containing regimens [200]. NRTIs remain the backbone of ART regimens throughout the world, but the accumulated consequences of decades of NRTI-treatment on oxidative stress and telomere shortening remain to be defined.

4.3 Oxidative Stress

An imbalance between levels of oxidants and anti-oxidants occurs during aging, resulting in increased plasma markers of oxidative stress in the elderly [201, 202] which contribute to immunosenescence and inflamm-aging (reviewed in [203]). HIV infection is also associated with increased levels of oxidative stress, with decreased plasma levels of anti oxidant factors such as glutathione and increased levels of the oxidative stress marker malondialdehyde found in both viremic and

Table 3 Associations between immunological changes occurring during aging/HIV infection and morbidity/mortality

		HIV-infected	General population
	Marker	Outcome/risk factor	
Inflammation			
Cardiovascular/metabolic disease	IL-6	Cardiovascular events [127, 128], obesity [129]	Sudden cardiac death [130, 131], cardiovascular events [131–134]
	hsCRP	Cardiovascular events [127, 128], progression of cIMT [135], metabolic syndrome [136], diabetes [137]	Cardiovascular events [131, 138], insulin resistance [139]
	sTNFRI/II	Obesity [129], diabetes [137]	Cardiovascular events [138]
	TNF	Coronary artery calcium [140]	
Neurocognitive impairment	IL-6	[141]	[132] Future cognitive decline [142]
	hsCRP		[132, 143]
	sTNFR-I/II	[144]	
	TNF		Alzheimer's disease [145, 146]
Malignancies	IL-6, hsCRP	All cancers [147]	All cancers [148]
	D-Dimer	All cancers [147]	
	TNF		All cancers [148]
Bone disease/osteoporosis	hsCRP		Bone mineral density [149], fracture risk [150], future bone mineral density loss [151]
	IL-6		Future bone mineral density loss [151, 152]
	TNF		Future bone mineral density loss [151]
Frailty/disability	IL-6	[153, 154]	[132, 155]
	TNF	[153]	
	CRP	[153]	[132, 156]
Mortality	sTNFRI, hsCRP	[157]	

(continued)

Table 3 (continued)

		HIV-infected	General population
	Marker	Outcome/risk factor	
Loss of gut integrity/microbial translocation			
Cardiovascular/metabolic disease	LPS	Progression of cIMT [158], endothelial dysfunction [159], hypercholesterolemia [160], insulin resistance [160], hypertension [161]	Metabolic syndrome [162]
	LBP		Metabolic syndrome [163]
Neurocognitive impairment	LPS	[141, 164]	
Malignancies	LPS	Non-Hodgkin's lymphoma [165]	
Mortality	IFAB-1, zonulin	[157]	
Monocyte/innate immune activation			
Cardiovascular/metabolic disease	sCD14	Increased cIMT [166], cIMT progression [158], coronary calcification [167], hypertension [161]	Diabetes [169], hypertension [169]
	sCD163	Arterial inflammation [170], non-calcified coronary artery plaques [171]	Atherosclerosis [172], insulin resistance [139, 173, 174], diabetes [175]
	MCP-1	Coronary artery calcium [140]	
Neurocognitive impairment	sCD14	[141, 144, 176, 177]	
	sCD163	[178]	
	Neopterin		Alzheimer's disease [179]
Malignancies	sCD14	Non-Hodgkin's lymphoma [165]	
Frailty/disability	Neopterin		[180]
Mortality	sCD14	[157]	
T cell activation/senescence			
Cardiovascular/metabolic disease	HLADR + CD38+ T cells	Carotid artery plaques [181, 182], carotid artery stiffness [183]	
Bone disease/osteoporosis	T cell activation	Bone mineral density [184]	
Mortality	Low CD8+ CD28- CD57+ T cells	[185]	

(continued)

Table 3 (continued)

		HIV-infected	General population
	Marker	Outcome/risk factor	
Telomere shortening			
Cardiovascular/metabolic disease	Leukocyte telomere length		Increased cIMT [122, 185], risk of myocardial infarction and stroke [122–124], Diabetes [122]
	Monocyte telomere length		Type 2 diabetes [187]
Neurocognitive impairment	Leukocyte telomere length		Dementia [125]
	Monocyte telomere length		Alzheimer's disease [126]
Malignancies	Epithelial cell telomere length		Epithelial cancer [121]
	Telomere length in mucosal tissue		Early stages of gastric carcinoma [188]
Early death	Leukocyte telomere length		[123, 125]
Cytomegalovirus (CMV) infection			
Cardiovascular/metabolic disease	CMV seropositivity		Type 2 diabetes [189], mortality in coronary artery disease patients [190]
	CMV-specific T cells responses	cIMT [191]	
	CMV IgG	Carotid artery disease [192]	
Frailty/disability	CMV IgG		[193]
Mortality	CMV IgG CMV seropositivity		All-cause mortality [193], cardiovascular related deaths [194]

virologically suppressed HIV-infected individuals [204, 205]. High intracellular levels of the antioxidant factors N-acetylcysteine and glutathione inhibit HIV replication in infected cells [206] whilst low levels of these factors are associated with increased NF-kB-mediated transcription of HIV and a heightened ability of the pro-inflammatory cytokine TNF to activate HIV transcription [207], suggesting a positive feedback loop between inflammation and HIV replication. The mechanism responsible for decreased anti oxidant levels in HIV may involve the HIV Tat protein, which has been shown in mouse models to decrease production of anti oxidants and induce mitochondrial damage [208]. Certain antiretroviral (ARV) drugs including PIs and NRTIs increase the production of reactive oxygen from cells treated in vitro [209]. Consistent with this, one study reported higher levels of oxidative stress in ART-treated individuals as compared to both untreated HIV-infected and uninfected individuals, however the HIV-infected individuals in this study had significantly higher levels of a number of confounding factors including concurrent hepatitis C infection [210]. More data from virologically suppressed HIV-infected cohorts with adequate control of variables which may potentially influence oxidative stress are required to determine the impact of oxidative stress on immune aging in the modern ART era.

4.4 Chronic Inflammation and Immune Activation

Increased inflammation is one of the cornerstones of immunological aging and geroscience, and appears to be potentiated by HIV infection. Indeed, chronic inflammation and related immune activation likely has the greatest impact on morbidity and mortality in PAWH in the post-ART era. Inflammaging is a well-documented state of chronic, low-grade inflammation occurring progressively with age and is associated with the development of many age-related morbidities and functional decline in the elderly [211]. Markers of inflammation including IL-6, TNFα and high-sensitivity C-reactive protein (hsCRP) are elevated in both HIV-infected individuals and the elderly [212, 213] and are associated with increased risk of SNAEs including CVD, frailty, malignancies, bone disease and neurocognitive decline. Inflammation is intrinsically linked with cellular activation, and biomarkers of immune activation and inflammation are increasingly being recognised as risk predictors of inflammatory diseases in HIV infection, as they are in the aged (see Table 3). Biomarkers of monocyte/macrophage activation including plasma levels of sCD163 and sCD14 are predictive of age-related diseases including neurocognitive impairment/dementia [141, 176–178], malignancies [165] and also mortality [214] in HIV infection (see Table 3). Chronic monocyte/macrophage activation appears to be particularly relevant for the development of CVD in HIV infection; biomarkers of monocyte activation including the proportion of inflammatory CD16+ monocytes, the expression of monocyte activation markers (i.e. CD11b) and the soluble activation markers mentioned above are associated with atherosclerosis and its progression [158, 159, 215], arterial inflammation [170], coronary calcium score [167] and the presence of non-calcified carotid plaques [171] in HIV-infected

individuals. Importantly, these associations have been made in cohorts of primarily virologically suppressed individuals, suggesting mechanisms other than overt HIV viremia are involved. Indeed, in the post-ART era, markers of inflammation and/or immune activation are emerging as more relevant predictors of disease outcome and death in virologically suppressed individuals than traditional HIV biomarkers such as viral load and CD4+ T cells count [157, 216]. Recent data reporting an association between sCD163 levels and telomere length [217] provide a direct link between monocyte/macrophage activation and potentiation of immunological aging. Given chronic inflammation/immune activation and resultant disease burden are similar between HIV-infected individuals and the aged, the question arises to what extent the mechanisms driving these phenomena are similar in both populations and what contributing factors may be unique to HIV infection.

5 Factors Potentiating Age-Related Changes and Morbidity in HIV-Infected Individuals

The development of SNAEs in PAWH is multifactorial, and typically results from the combined effects of traditional risk factors, HIV-specific effects, and a potentiation of age-related changes (see Fig. 3).

5.1 Traditional Risk Factors

Traditional risk factors for disease development are highly relevant for the aging HIV-infected population, not only as they are often more readily modifiable but also because they may potentiate HIV-specific factors. Many cohort studies report a higher prevalence of smoking amongst HIV-infected participants [218–220], and whilst illicit drug use is higher within certain high risk HIV-infected populations, this variable is often not adequately assessed or controlled for in HIV cohort studies. Relevant to the development of cardiovascular disease, HIV infection is associated with dyslipidemia and metabolic alterations, which are discussed further below.

5.2 Metabolic Alterations

Hyperglycemia occurs in up to 17 % of HIV-infected individuals receiving ART and diabetes mellitus is more common in HIV infected vs seronegative people [221], with some studies reporting up to a fourfold increased risk due to HIV [222]. Insulin resistance in ART-treated HIV infection is largely associated with the use of protease inhibitor antiretroviral drugs, which act to inhibit the glucose transporter Glut-4 [223], although hepatitis C virus (HCV) co-infection, inflammation and immunodeficiency also contribute to insulin resistance and diabetes in HIV infection [221].

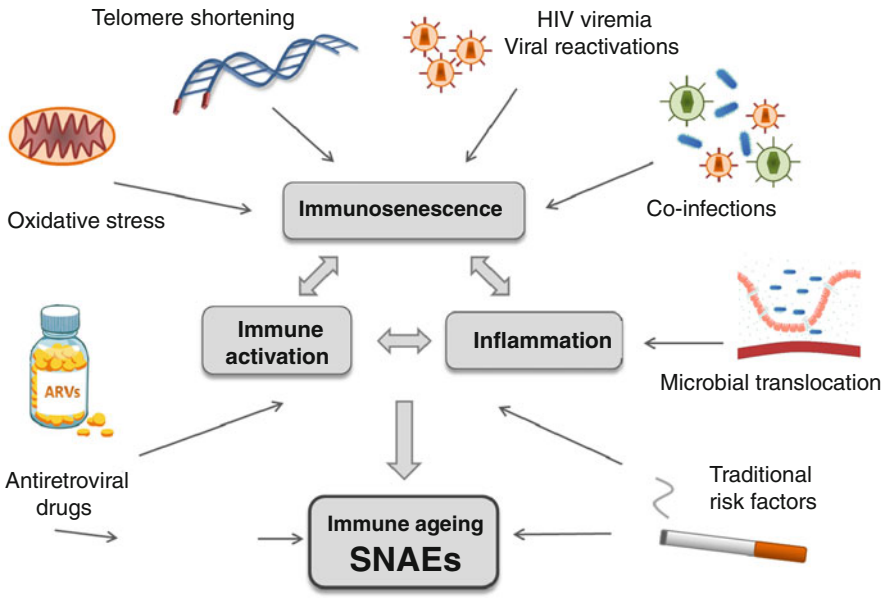

Fig. 3 Mechanism contributing to the pathogenesis of SNAEs in HIV-infected individuals

High glucose levels have been shown to increase the susceptibility of CD4+ T cells in HIV infection in vitro by upregulating the expression of the HIV co-receptor CXCR4 [224], whilst increased expression of Glut-1 on T cells from HIV-infected individuals (irrespective of ART) is associated with T cell activation and immunodeficiency [169]. Taken together, these data suggest that metabolic alterations due to both HIV and its treatment not only increase the risk of co-morbidities such as diabetes, but may also perpetuate HIV replication and immune activation to further drive immune exhaustion and senescence in PAWH.

HIV-related lipodystrophy syndrome is common in HIV infection, and includes lipoatrophy (loss of subcutaneous fat) and dyslipidemia. Lipoatrophy appears to be largely due to PI and NRTI use, particularly the NRTIs stavudine and zidovudine (reviewed in [225]). Whilst HIV infection per se is associated with lipid alterations including high triglyceride and low HDL levels (thought to be due to the effect of inflammation on lipid peroxidation, reactive oxygen species production and vascular changes [226, 227]), the majority of dyslipidemia observed in the post-ART era is due to the specific effects of antiretroviral drugs.

5.3 Antiretroviral Drugs

Although highly effective in inhibiting HIV replication and maintaining immune health, many antiretroviral drugs, particularly the NRTIs, have some degree of toxicity which is at least partially attributable to effects on the mitochondria. The

ability of NRTIs to inhibit HIV reverse transcription is due to structural similarities between NRTIs and endogenous nucleos(t)ides, and whilst nuclear DNA polymerases are not significantly affected by NRTIs, the mitochondrial replicase pol γ is inhibited by NRTIs at physiologically relevant levels, resulting in depletion of mitochondrial DNA and increased oxidative stress (reviewed in [208]). Specifically, zidovudine and stavudine have been shown to increase oxidative stress in a number of cell types including adipocytes and macrophages [228]. As discussed above, NRTIs are also able to inhibit the RT component of cellular telomerase and may potentially contribute to premature telomere shortening. Interestingly, certain NRTIs have recently been shown to be able to inhibit NLRP3 inflammasome-mediated activation of caspase-1 and subsequent production of the pro-inflammatory cytokines IL-1β and IL-18 [229], suggesting NRTIs may have an unexpected influence on cytokine production in HIV-infected individuals receiving these drugs.

HIV-infected individuals treated with ART have a relative risk of CVD of 1.52 (95 % CI 1.35–1.70) compared to untreated individuals [230], suggesting ART may contribute to the pathogenesis of CVD. Indeed, recent use of certain PIs and the NRTIs abacavir and didanosine has been associated with increased risk of myocardial infarction [231, 232] although the association with abacavir was not reproduced in a randomised control trial and remains controversial [233]. The increased risk attributable to PIs is largely due to an effect on lipid levels, as 70–80 % of HIV-infected individuals receiving PI-containing ART regimens show elevated lipid levels [226]. Most PIs (with the possible exception of atazanavir) have been shown to induce dyslipidemia involving increased plasma concentrations of triglycerides, total cholesterol and LDL [234], all of which are known risk factors for cardiovascular disease. The mechanism involves a direct effect of PIs on adipocyte differentiation and also an ability of these drugs to inhibit factors involved in lipid transport and metabolism [227].

Untreated HIV infection results in loss of bone mineral density which contributes to increased fracture risk and osteoporosis (as discussed above), but ART-initiation potentiates this effect and results in a further loss of bone mineral density of approximately 2–6 % within the first 2 years of ART initiation. This effect is thought to be due to disruption of the delicate immunological balance in the bone marrow which governs osteogenesis, and specific antiretroviral drugs including the NRTI tenofovir [235] and the protease inhibitor class of drugs have been shown to potentiate bone loss in ART-treated individuals [236].

The relatively recent introduction of ART, combined with the lengthy and multifactorial pathogenesis of many HIV-related co-morbidities, means that significant associations between specific ARVs and disease outcomes are continuing to emerge. HIV-infected individuals initiating therapy in the early days largely did so with low CD4 counts and received ARVs which have since been phased out due to side effects and toxicities. Thus, ongoing and future longitudinal studies will be critical for evaluating the long term effects of current ARVs on immune changes and the development of age-related diseases in HIV-infected individuals who avoid significant immunological damage by initiating ART at higher CD4 T cell counts.

6 Mechanisms That May Contribute to Chronic Inflammation and Immune Activation in HIV

6.1 Microbial Translocation and Endotoxemia

HIV infection is associated with increased permeability of the gut to microbial products, which translocate across the gut epithelium and eventually into the bloodstream, resulting in increased plasma levels of the bacterial endotoxin lipopolysaccharide (LPS) and bacterial DNA in HIV-infected individuals [237]. The cause of increased gut barrier permeability in HIV infection is due to immunodeficiency and structural defects within the gut-associated lymphoid tissue (GALT) resulting from HIV-mediated T cell depletion [238]. The majority of lymphocytes in the body are contained in GALT, which is an important site for both pathogenesis and persistence of HIV. CD4+ T cells are rapidly depleted from the GALT during primary HIV infection and remain depleted into chronic infection. Studies in Simian Immunodeficiency Virus (SIV)-infected macaques (a pathogenic animal model of HIV infection) have revealed that peak infection of CD4+ T cells in the lamina propria of the gut occurs within 10 days of infection, at which point 93 % of target CD4+ memory T cells are infected [239]. While effective ART suppresses viral replication and restores peripheral CD4+ T cells, gut-associated CD4+ T cells remain depleted years after ART initiation [240]. Interestingly, a subset of HIV-infected individuals who maintain high CD4+ T cells counts and low/undetectable viral loads in the absence of ART (known as long term non-progressors) maintain normal CD4+ T cell levels in the GALT [241], suggesting the importance of this compartment for disease pathogenesis. The mechanism of increased gut permeability in HIV involves epithelial disruption and decreased production of tight junction proteins in the distal portions of the colon [242] which is consistent with increased levels of intestinal fatty acid binding protein (I-FABP; a marker of enterocyte damage) and zonulin-1 (a regulator of tight junction permeability) in the plasma of HIV-infected individuals [157, 243]. The inability to fully restore GALT structure and function despite effective restoration of peripheral T cells by ART means that chronic endotoxemia (elevated levels of LPS in the blood) persists in virologically suppressed HIV-infected individuals. Lipopolysaccharide (LPS) is a potent immune activator which is recognised by toll-like receptor (TLR)-4 expressing cells such as monocytes/macrophages in an immune complex consisting of LPS-binding protein (LBP), the adaptor protein MD2 and either soluble or cell-bound CD14. LPS signalling stimulates the production of pro-inflammatory cytokines including IL-6, TNF and type I interferons. Microbial translocation is considered a significant driver of both HIV disease and related co-morbidities, with gut translocation markers such as LPS, the LPS binding protein LBP and I-FABP/zonulin-1 associated with immune activation and HIV disease progression [237, 244, 245], cardiovascular and metabolic disease [159, 160], neurocognitive impairment [141] and mortality [157, 216].

In contrast to HIV, relatively little is known regarding the effect of age on the integrity of the gut epithelium in humans [246], however work in *Drosophila* has demonstrated that loss of intestinal barrier integrity occurs with aging and is a better

predictor of age-related morbidity and death than chronological age [247]. Increased plasma levels of LPS [98] and LBP [248] in the elderly indicate microbial translocation, may also increase during aging and the inverse association between LBP levels and physical function in the aged [248] suggests it may also contribute to morbidity in this population, although this requires further investigation.

6.1.1 Alterations to the Gut Microbiome

Within the GALT, cytokines including IL-17 and IL-22 play a critical role in maintaining gut integrity and orchestrating the mucosal immune responses to gut pathogens. Depletion of CD4+ T cells from the gut during HIV infection reduces the production of these cytokines and disrupts the delicate mucosal immunological balance. The gut microbiome interacts intimately with mucosal immunity and helps educate and regulate immune cells. Significant alterations are observed in the gut microbiome of HIV-infected individuals, with sequence analysis of bacterial communities from stool/gut mucosa samples revealing an overall increase in genetic diversity, an expansion of *Prevotella* and potentially pathogenic bacteria and a reduced proportion of *Bacteroidia* species [249–252]. Importantly, these changes in microbial communities are associated with inflammation, innate and adaptive immune activation and markers of disease progression in HIV-infected individuals. ART appears to only partially normalize the bacterial composition of the microbiome in a proportion of treated individuals [251]. A higher proportion of bacteria from the order *Lactobacillales* (lactic acid-producing bacteria) in the distal gut of ART naïve individuals has been associated with more favorable immunological parameters including higher pre-ART CD4+ T cells counts and CD4:CD8 T cells ratio but lower viral loads and sCD14 levels [253]. The complex interplay between the gut microbiome, GALT immunity and systemic inflammation/immune activation continues to be elucidated but may reveal an important mechanism of persistent immune dysfunction in HIV which can be targeted therapeutically.

6.1.2 Cytomegalovirus (CMV) and Latent Viral Infections

Accumulative immune stimulation by pathogens and subsequent immune exhaustion is an integral mechanism of immune aging and heightened pathogen burden due to concurrent and reactivated viral infections may hasten this process in PAWH. While CMV-seropositivity rates vary considerably between different countries (ranging from 40 to >90 %), there is a consistent trend of increasing seropositivity with age [254] and CMV is recognized as a significant driver of immunosenescence [255, 256]. CMV infection profoundly shifts the lymphocyte subset proportions towards a differentiated memory T cell phenotype [257, 258]. In aged individuals, the proportion of CD8+ T cells specific for a small number of CMV epitopes can represent up to 27 % of the total CD8+ pool [259], with these cells typically being dysfunctional and exhibiting an immunosenescent phenotype [260]. CMV seropositivity has also been associated with an increased risk of age-related diseases such as cardiovascular disease [261].

CMV disease is a significant cause of morbidity and mortality in HIV-infected individuals with AIDS and/or severe immunodeficiency [262], while asymptomatic CMV infection also appears to potentiate immunosenescence in HIV-infected individuals. CMV infection is almost ubiquitous in the HIV-infected population with seropositivity rates of approximately 95 % [263] and the presence of IgM antibodies suggests viral reactivation/reinfection commonly occurs [264]. Levels of CMV-specific CD8+ T cells are up to twice as high in HIV-infected as in uninfected individuals and persist in ART-treated individuals despite long term virological suppression [265], which is consistent with reactivation and impaired immune control [263]. HIV-infected/CMV seronegative subjects show higher CD4:CD8 T cells ratios and less phenotypic evidence of immunosenescence than HIV/CMV seropositive individuals [266] whilst serum CMV IgG levels, which are increased in HIV-infected individuals, correlate with inflammatory markers [267]. Taken together, these observations suggest that CMV seropositivity may potentiate HIV-related immunosenescence and inflammation and hasten the aging process.

Although ART reduces HIV viral load to near undetectable levels in the plasma, residual HIV replication (up to 20 copies/mL) can be detected in the plasma of the majority of virologically suppressed individuals using ultra-sensitive assays [268]. In addition, ongoing HIV replication may persist at higher levels within anatomical sites such as lymphoid tissue where antiretroviral drugs may fail to penetrate to effective therapeutic concentrations. Reactivation/replication of other latent viruses including Epstein–Barr virus (EBV) and Herpes Simplex Viruses (HSV) also appears to be heightened in HIV-infected individuals, likely due to increased immune activation. HSV-2 reactivation occurs frequently in HIV-infected individuals, is positively associated with HIV viral load [269] and is shed more frequently in HIV-infected vs seronegative individuals [270]. EBV viral loads in HIV-infected individuals are reportedly greater than those in EBV+ HIV-uninfected individuals [271].

Human endogenous retroviruses (HERVs) are a family of replication defective viral elements which comprise up to 8 % of the human genome. Although thought to be largely silent, increased transcription of HML-2 RNA (a member of the HERV-K family) has been demonstrated in PMBCs from HIV-infected individuals [272] and has also been detected at increased levels in plasma in some [273] but not all [272] studies. Increased HERV transcription may be due to heightened immune activation and/or the ability of the HIV Tat protein to activate endogenous retroviral transcriptional elements [274]. Although cause and effect are difficult to delineate, it is clear that heightened inflammation/immune activation and reactivation of latent viral infections may constitute a self-perpetuating cycle contributing to immune exhaustion and immunosenescence in many PAWH.

6.1.3 Concurrent Infections

The development of age-related morbidities in HIV-infected individuals can be influenced by concurrent infection with a range of pathogens. Co-infection with HCV can be up to 90 % in certain high risk HIV+ groups, and is associated with an

increased risk of coronary heart disease [275], osteoporotic fracture [276], and neurocognitive impairment [277], suggesting hepatitis C infection may potentiate the pathogenesis of these conditions. The mechanism of this is unclear, although a potentiation of inflammation and immune activation is likely, and increased levels of pro-inflammatory factors such as IL-6 have been demonstrated in HIV/HCV co-infected, as compared to mono or uninfected individuals [278]. Active HCV infection is also associated with shorter leukocyte telomere length in those with HIV [279]. Taken together, these data suggest that HCV co-infection may further heighten inflammation/immune activation and associated immunosenescence in HIV-infected individuals and potentiate the development of age-related diseases.

HIV-infected individuals co-infected with tuberculosis (TB) have significantly increased pro-inflammatory cytokine production [280] and ART initiation in highly immunocompromised HIV+/TB+ individuals often results in TB-associated immune reconstitution inflammatory syndrome, which results in significant pro-inflammatory cytokine production [281]. Heightened CD4+ T cell activation and pro-inflammatory cytokine production also occurs in malaria co-infection [282]. These observations suggest concurrent infections may further potentiate inflammation due to HIV and aging in co-infected individuals, however further studies are required to elucidate the full impact of these effects on age-related disease outcomes.

7 Potential Treatments/Interventions to Alleviate the Effects of HIV on Aging/SNAEs

The immunological similarities between HIV infection and aging (particularly chronic inflammation and its consequences) suggest that addressing mechanism of aging may alleviate premature aging and disease pathogenesis in HIV-infected individuals. A large number of preliminary trials are underway to address immune activation, inflammation, microbial translocation and other mechanisms of enhanced aging in PAWH, but none has yet demonstrated efficacy in definitive clinical trials [283–287]. If this is accomplished in PAWH, it will have vast implications for aging in general and may be applicable to a much broader population.

8 Concluding Remarks

The success of antiretroviral therapy in preventing AIDS and extending the life span of HIV-infected people has revealed unexpected parallels between the impact of HIV infection and aging on immune function. Current research is only beginning to uncover how HIV may be potentiating age-related changes and the consequences of this for premature aging and increased risk of age-related comorbidities in those living and aging with HIV. It is still unclear whether HIV-associated ‘aging’ is the

result of chronic infection, or whether those infected with HIV at an older age may experience similar effects. Furthermore, the impact of long-term ARV drug use on age-related process remains to be fully elucidated. HIV infection further complicates the many health challenges experienced by aging individuals including multimorbidity, polypharmacy, impaired physical and mental health and reduced quality of life. Uncovering the critical processes which drive age-related changes and identifying therapeutic strategies to ameliorate the residual effects of HIV will be important for ongoing management of the increasingly aging HIV-infected population.

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Pain in the Elderly

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Contents

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1 Introduction

Pain, especially chronic pain, arguably represents the most prevalent and costly public health condition in the United States. The Institute of Medicine (IOM) noted that chronic pain affects 100 million U.S. adults with costs exceeding $600 billion annually [1, 2]. While pain affects individuals throughout the lifespan, older adults are at increased risk for chronic pain and pain-related disability [3]. Despite the greater prevalence and adverse impact of pain among older adults, the relationship between pain and aging remains a surprisingly underexplored area of inquiry. This chapter provides a broad overview of past and current research regarding chronic pain in older adults, including a discussion of biopsychosocial mechanisms contributing to age-related influences on pain. First, we will describe findings from epidemiologic and clinical studies examining age differences in pain prevalence and impact. Then, after briefly reviewing the impact of aging on biological processes that contribute to pain we will discuss the biopsychosocial model of pain. Next, we discuss human laboratory studies examining age-related changes in pain processing, followed by consideration of psychosocial factors that contribute to pain perception among older adults. Finally, we review the impact of aging on medical and non-medical therapies. We conclude the chapter with a discussion of future directions for pain and aging research.

2 Epidemiological and Clinical Aspects of Pain and Aging

Epidemiological studies emphasize pain prevalence in the population, which is subject to all of the methodological vagaries inherent in such research (e.g., case definition, sampling strategies). However, prevalence alone inadequately captures the overall burden of pain; and for this reason it is critical to characterize the impact of pain through measures of pain severity, impact on physical function and disability, quality of life, and psychological morbidity. Below we address the influence of aging on these issues.

Several studies have investigated the prevalence of chronic pain across the lifespan. For example, Blyth and colleagues [4] surveyed more than 17,000 Australians and found that chronic pain (i.e., pain experienced daily for three of the previous 6 months) frequency increased with age, though sex differences emerged in the pattern of pain prevalence (see Fig. 18.1). Other studies show a similar pattern of increases in chronic pain prevalence until approximately age 70, at which point pain prevalence plateaus or even declines slightly [5–7].

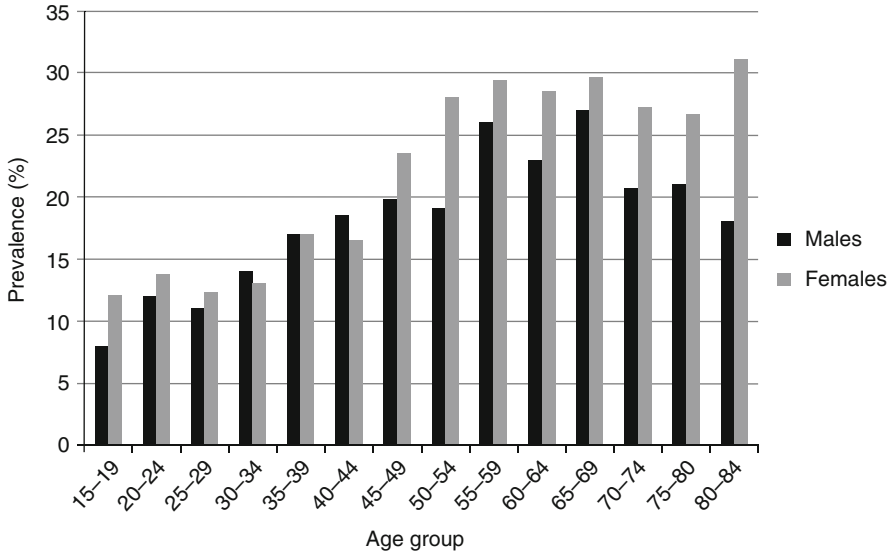

Fig. 18.1 Pain prevalence across the lifespan. Data are from interviews of 17,543 Australians. Chronic pain was defined as pain experienced every day for 3 months in the 6 months prior to interview (Adapted from Blyth and colleagues [4])

2.1 Specific Pain Conditions Associated with Aging

The findings summarized above relate to chronic pain in general; however, age-related influences on chronic pain prevalence vary across different pain conditions. Specifically, compared to younger people, older adults are more likely to experience musculoskeletal pain, including pain of the joints, lower extremities and back [7]. A recent systematic review based on data from more than 116 thousand elderly Brazilians reported that lower limb and spine pain were the most common pain conditions, reported by over half of the sample [8]. Similarly, the incidence of osteoarthritis (OA) of the knee, hip, and hand all increase through seventh decade of life and then decrease above age 80 [9]. In fact, a recent analysis from Sweden estimates that the odds of developing musculoskeletal pain become one and a half times greater for each decade of increased age [10]. In contrast, the prevalence of abdominal pain, migraine headache, and pain due to temporomandibular disorders peak in the third to fifth decade and decrease thereafter [11, 12]. Below we discuss the impact of age-related influences on pain in several specific clinical conditions.

2.1.1 Osteoarthritis Pain

OA represents one of the most common sources of pain and disability in the elderly [13]. The knee is the most frequently affected joint, with the lifetime risk of symptomatic knee OA estimated to be 40 % for men and 47 % for women [9]. In one study, the prevalence of symptomatic knee OA increased from 16 % at age

55 to more than 30 % at age 80 [14], emphasizing the importance of age as a strong risk factor for OA. The cumulative wear and tear on joints over time has been the suspected culprit in driving age-related increases in symptomatic OA. However, the poor correspondence between measures of disease severity and symptoms suggests that factors beyond peripheral tissue damage must contribute to OA-related pain [15]. Indeed, as discussed extensively in the Chapter on Osteoarthritis, many other age-related changes in physiology of joints also play a role in the age dependency of the disease. Moreover, increasing evidence implicates altered central nervous system (CNS) pain processing in symptomatic OA [16, 15].

2.1.2 Low Back Pain

Low back pain (LBP) is the leading cause of disability both in the US and worldwide [17, 18]. Conflicting data exist regarding age-related changes in the prevalence of LBP. In a study of nearly 35,000 Danish twins, LBP prevalence peaked around age 45 and declined thereafter for both women and men [19]. Others have reported that prevalence increases with age until age 60–65, gradually declining in subsequent years [20, 21]. A systematic review found that the prevalence of *severe*, but not "benign or mixed," back pain increases with age [22]. Thus, while overall back pain prevalence may decline slightly in older age groups, more severe pain increases in frequency, suggesting a greater burden of back pain among older adults.

2.1.3 Neuropathic Pain

Neuropathic pain refers to "pain arising as direct consequence of a lesion or disease of the somatosensory nervous system [23]." Common neuropathic pain conditions include diabetic neuropathy, post-herpetic neuralgia, and central post-stroke pain. Because the medical conditions producing these neuropathies are more common in older adults, the prevalence of these neuropathic pain conditions increases with age [24]. However, age can increase the risk for neuropathic pain independent of its effects on the parent medical condition. For example, among patients with acute herpes zoster, age represents a risk factor for progression to post-herpetic neuralgia [25]. Moreover, risk of diabetic neuropathy increases with age, thereby increasing the likelihood of painful diabetic neuropathy in older adults [26]. Less commonly studied neuropathic pain conditions that show increased prevalence with advancing age include trigeminal neuralgia and glossopharyngeal neuralgia, both of which show peak incidence in the seventh decade [27]. Among patients with multiple sclerosis, central neuropathic pain is also more prevalent with age, peaking around age 60 [28]. Also, older age increases risk for HIV-associated sensory neuropathy [29]. Thus, older adults are at substantially greater risk for many forms of neuropathic pain.

2.1.4 Visceral Pain

Pain emanating from internal organs (e.g., gut, heart, bladder) represents a considerable problem. Evidence suggests, however, that older adults experience reduced visceral pain relative to their younger counterparts [30]. For example, prevalence of irritable bowel syndrome declines after the fifth decade [31]. Also, older adults are more likely to experience silent myocardial ischemia and painless myocardial infarction [32]. Similarly, visceral conditions, such as peptic ulcer disease, pneumothorax, and intestinal blockage are characterized by reduced or absent pain in older compared to younger adults [33]. Although the reduction of visceral pain might be considered a positive aspect of aging, pain from internal structures often signals the presence of a potentially life threatening condition. Therefore, the decreased ability to detect visceral pain may increase older adults' risk for morbidity and mortality.

2.1.5 Pain in the Cognitively Impaired

Cognitive impairment represents an enormous public health issue in the elderly. Importantly, pain and cognitive impairment may be reciprocally related. Several studies have demonstrated reduced cognitive performance in chronic pain populations [34, 35]. There is conflicting evidence regarding whether dementia is associated with altered prevalence and severity of pain [36, 37], because the ability to self-report pain is often compromised [37]. In fact, from a clinical perspective, pain among older adults with dementia presents management challenges due to the difficulty of assessing pain and determining treatment effectiveness. Among non-demented individuals, cognitive performance is inversely correlated with pain severity and mediates the influence of pain on physical performance [38]. Thus, cognitive impairment may be a risk factor for increased pain and pain-related physical dysfunction.

2.2 Symptoms and Impact of Chronic Pain in Older Adults

The burden of chronic pain on older adults extends well beyond age differences in pain prevalence. Several aspects of the severity of pain differ across the lifespan, and multiple clinical features that are comorbid with chronic pain can disproportionately impact older adults.

2.2.1 Pain Characteristics

Chronic pain is characterized not only by the intensity of the pain, but also by other pain characteristics (e.g., pain quality, temporal pattern, locations) and interference with daily living. Conflicting evidence exists regarding age-related influences on the intensity of pain. As noted above, studies of severe LBP (e.g., LBP interfering with

daily activities) reported a higher prevalence in older versus younger adults, while studies of back pain in general (e.g., LBP lasting 1 day or longer in the past 4 weeks) reported decreased LBP frequency after middle age [22]. In contrast, in a sample of mixed chronic pain conditions, no age differences in numerical pain ratings emerged; however, older adults reported lower sensory and affective (i.e., the emotional component of pain, which includes how unpleasant or bothersome the pain is) pain scores on the McGill Pain Questionnaire (MPQ) [39]. Also, older adults with metastatic cancer reported lower pain intensity ratings than their younger counterparts [40], although a more recent study showed no age-related differences in MPQ scores among patients with cancer pain [41]. Regarding pain duration, some evidence links increased age with persistence of pain [42]; however, age appears to be protective against development of chronic postoperative pain [43, 44]. In terms of body dispersion of pain, both regional and widespread pain conditions increase from early adulthood to middle age, and prevalence seems to peak in the fifth or sixth decade, declining slightly thereafter [45, 46]. Thus, there are inconsistent associations of age with various pain characteristics, and additional research is needed to clarify these findings and elucidate their mechanisms.

2.2.2 Pain, Quality of Life, and Function

Chronic pain is commonly associated with activity limitations and symptoms that negatively impact quality of life, including fatigue and sleep disturbance, and these issues may represent particular problems in older adults. For example, pain is associated with mobility impairment among older adults [6, 47], and the association of musculoskeletal pain with activity interference and disability increases with age [48, 49]. As described below, falls are a major clinical concern among the elderly, and pain significantly increases fall risk among older adults [50].

Fatigue is associated with aging, is an important symptom of frailty, and predicts functional limitations, disability, and mortality in older adults [51]. Because pain and fatigue often coexist, fatigue is a particularly pertinent clinical issue among older adults with chronic pain. Indeed, independent of pain, fatigue is a significant negative predictor of physical activity in older adults with OA [52]. Further, sleep disturbance increases significantly with age [53], and sleep disturbance confers increased susceptibility to chronic pain, and vice versa [54]. Thus, sleep disturbance may be an age-related risk factor for development and/or exacerbation of pain. These findings suggest that while chronic pain severity is not consistently greater among older adults, pain nevertheless predicts substantially diminished quality of life and mobility and increased fall risk in the elderly.

3 Aging and Pain Biology

Aging is a complex, multifactorial process occurring across many different levels of functional organization – psychological, physiological, and molecular events that occur with the passage of time [55]. Similarly, chronic pain is recognized as a

multifactorial experience driven by complex interactions among multiple biopsychosocial processes [56]. Important characteristics of aging include a declining ability to respond to stress, increasing homeostatic imbalance, and an increase in the risk for onset of pathological changes, which parallels descriptions of chronic pain. The overlapping mechanisms contributing to aging and chronic pain provide a broad foundation for future study, as demonstrated by the examples presented below.

Multiple biological systems, including the nervous, endocrine, and immune systems, are responsible for the maintenance of homeostasis and for the activation of defense responses. Environmental exposures can produce a dysregulation of these systems, resulting in maladaptive changes that contribute to both aging and chronic pain. For example, leukocyte telomere length is a marker of cellular aging that is also associated with the intensity and persistence of stress. Shorter leukocyte telomeres have been associated with age-related diseases, as well as chronic mental and physical health conditions, while longer telomeres are associated with a healthy life [57, 58]. Recent reports have linked chronic pain with shorter telomeres, particularly among individuals with chronic pain and high stress, supporting a potential link between cellular aging and chronic pain [59, 60]. Historically, this relationship has been overlooked; recently however, it is attracting attention and providing a new perspective on the overlapping mechanism(s) of aging and chronic pain [61].

Another factor common to the biology of aging and chronic pain is the role of free radicals. Free radicals have been suggested to be involved in the development and maintenance of capsaicin-induced hyperalgesia through central sensitization and the elevation of spinal reactive oxygen species due to the increased production of mitochondrial superoxides in dorsal horn neurons [62, 63]. It may be argued that oxidative damage is linked to aging in a universal manner and that changes in endocrine function are secondary to changes in oxidant production in endocrine cells [64, 65]. Thus, similar cellular mechanisms (free radical production) may broadly affect aging and simultaneously contribute to the development of pain.

Another example of the overlap in the biology of chronic pain, and aging is in the case of central pain resulting from spinal cord injury. Specifically, neuronal injury and changes in neuronal excitability have been shown to involve the extracellular signal-regulated kinases (ERK) cellular signaling pathway [66, 67]. Similar changes in neuronal function are commonly observed in uninjured older animals, suggesting an overlap in the cellular mechanisms responsible for neuronal changes in excitability resulting from injury and aging. Similarities in cellular mechanism(s) underlying pain and aging can also be found with the results of the drug rapamycin, initially developed as an antifungal agent and found to have immunosuppressive properties [68]. Rapamycin has been shown to extend the lifespan of mice by affecting a large number of processes regulated by the Target of Rapamycin (TOR) signaling pathway [69]. A rapamycin sensitive signaling pathway was also shown to be essential for the expression of persistent pain states [70], and rapamycin reduces clinical signs of neuropathic pain in a model of experimental autoimmune encephalomyelitis [71]. These examples of overlapping mechanisms reveal the need for collaborative efforts between the fields of pain and aging research to more fully explore the biological processes that contribute to both senescence and chronic pain.

3.1 Age-Related Changes in the Somatosensory System

Understanding the impact of advancing age on pain-related biological systems represents a major challenge in the field of pain research [72, 73]. Age-related anatomical and functional changes have been described in both human and non-human studies of the somatosensory system [74]. For example, peripheral nerves show an age-related reduction of both myelinated and unmyelinated fibers [75, 76] as well as signs of Wallerian degeneration [77, 78]. The number and size of sensory neurons in dorsal root ganglia (DRG) also decreases with age [79, 80]. Reduction in the number of peripheral afferents, the presence of demyelination, together with increasing inflammation are similar to the cascade of pathological events associated with nerve and tissue injury-induced pain in younger animals, thereby suggesting a common pathway between the events associated with neuropathic pain and those common to age-related changes in nociception [81, 82].

In addition to peripheral changes, central changes in the expression of neurotransmitters and receptors may contribute to age-related alterations in somatosensation. The decreased presence of calcitonin gene-related peptide (CGRP), substance P, nitric oxide, and somatostatin in the dorsal horn of aged rats has been reported [83–87], and loss of serotonergic and noradrenergic terminals in the dorsal horn also suggests the potential for age-related changes in descending pain modulatory pathways [81, 86, 88]. A decrease in the number of opiate receptors and decreased efficacy of opiate-mediated antinociception may also contribute to age-related changes in the processing of nociceptive information [89–91]. Finally, age-related altered gene expression for trophic factors, neuropeptides, cell adhesion molecules, ion channels, and genes related to mitochondrial function and calcium handling, as well as changes in the functional state of spinal and supraspinal glial cells may drive enhanced pain sensitivity with aging [92].

3.2 Effects of Age on Autonomic Function: Impact on Pain Sensitivity

Age-related changes in autonomic function may contribute to the changing pain sensitivity in older adults [93]. Experimental evidence has shown that tonic whole-body sympathetic nervous system (SNS) activity increases with age [94]. Further, autonomic dysfunction can be instrumental in the generation and maintenance of chronic pain. Two reviews summarize an extensive literature supporting the following relationships: (1) psychological stress activates limbic structures projecting to the hypothalamo-pituitary axis (HPA), resulting in an increase in sympathetic tone; (2) activation of stress circuitry increases pain sensitivity by central actions leading to stress-induced hyperalgesia; (3) chronic sympathetic activation and associated peripheral vasoconstriction produces muscular ischemia and a microenvironment conducive to myofascial pain; and (4) nociceptors in deep tissues are particularly

sensitive to sympathetic induced ischemia and are potent generators of central sensitization when tonically active [95, 96]. Thus, older adults may experience maladaptive physiological responses to stressors, including psychosocial and environmental events, as well as inflammation and pain, which can potentiate hyperalgesia thereby exacerbating pain.

Aging is associated with changes in the sympathetic nerve supply to a number of targets, and quantitative changes in sympathetic nerve fibers result in changes in transmitter expression [97]. Although the exact relationship between pain and the sympathetic nervous system remains unclear, the sympathetic nervous system is known to be involved in maintaining protective body reactions associated with pain. Moreover, in certain pathological conditions, the SNS may be involved in the generation of pain. For example, SNS activity can elicit spontaneous pain and enhance pain evoked by mechanical and cold stimulation [98, 99]. Clinical and preclinical studies have documented that physiological activation of sympathetic neurons can enhance pain and blockade of sympathetic activity can relieve pain. The SNS also contributes to the generation of pain during inflammation [100]. Given age-related changes in SNS structure and function and that the SNS influences the generation and maintenance of chronic pain, sympathetic function represents an important potential mediator of age-related changes in pain.

3.3 Aging, Pain and Immune Function

Changes in immune function, including microglial responses, may contribute importantly to age-related increases in chronic pain. The glial response to injury contributes to neuronal hypersensitivity leading to the production of inflammatory mediators such as cytokines and chemokines. This "glia cascade" has been related to the regulation of synaptic strength and plasticity and the generation of central sensitization [101, 102]. However, the contribution of glia to the induction or maintenance of chronic pain in aged rats is unknown. Stuesse and colleagues [103] found that ligation-induced hyperreflexia was correlated with increased staining for activated microglia regardless of age. Activated microglia have also been implicated in the initiation of chronic pain via the local release of neuroactive substances, including cytokines, ATP, substance P, reactive oxygen species, nitric oxide, arachidonic acid, fractalkine, and nerve growth factors [104, 105]. Selective inhibition of activated microglia can alleviate acute and chronic pain behaviors [105], though clinical evidence of a beneficial effect of microglia inhibition in persistent pain conditions is lacking [106, 107].

The microglia-to-neuron signaling link has also been shown to involve a molecular pathway in the spinal cord that includes Toll-like receptors, phosphorylated mitogen-activated protein kinase and purinergic P2X4 receptors on microglia [108, 109]. Interestingly, the microglia-to-neuron signaling pathway involving prostaglandin E2 (PGE2) is involved in producing excitability changes underlying chronic pain following spinal cord injury [110]. This same pathway could contribute to the

emergence of age-related chronic pain conditions. Thus, substantial evidence exists that immune responses elicit a well-orchestrated temporal pattern of activation of different immune cells, including microglia and astrocytes, which may contribute to chronic pain development. At present the involvement of glia in the induction or maintenance of chronic pain in aged rats is an evolving story. However, age-related morphological changes in microglia may reflect an important mechanism mediating age-dependent increases in pain sensitivity.

3.4 Effects of Age on Pain Sensitivity: Pre-clinical Studies

Pre-clinical studies examining the effects of age on pain sensitivity have resulted in conflicting observations that include increases, decreases, or no change in nociceptive responses with advancing age [88]. It is important to point out that the majority of these studies employed reflex-based behavioral measures to determine changes in thermal and/or mechanical sensitivity. The execution of these reflex-based measures do not require cerebral processing for the conscious perception of sensory events and are subsequently thought to be less relevant to clinical pain [111]. In order to address these deficiencies an operant escape task was developed to evaluate thermal nociceptive sensitivity in awake, unrestrained rats [112]. This test overcomes the limitations inherent with reflexive responses by providing a measure of *pain sensitivity* and *affective response* to nociceptive stimuli. Use of operant (learned) tests provides a measure of pain involving neuronal pathways extending throughout the neuraxis. Importantly, reflex-based and operant assays often yield substantively different results [113–115], and the findings from operant assays are typically more consistent with predictions from available human reports than are the results from reflex-based tests [111].

3.4.1 Findings from Reflex-Based Assays

A review of studies using reflex-based strategies to examine age-related changes in pain sensitivity reveals inconsistent and confusing results. Using paw lick and tail flick latencies in young (2–3 months), adult (6–12 months), and aged (24 months) rats, Hess et al. [90] described a decrease in sensitivity to thermal and electric shock with increasing age. These results correlated with a decrease in the number of opiate receptors in the frontal poles, striatum, and hippocampus. Another evaluation of thermal response latencies showed that young mice (6–8 weeks) had significantly shorter latencies than animals 24 months of age [116]. The decreased sensitivity in older animals was greater for females and correlated with a decrease in the expression of Nav1.8 sodium channels. Similarly, Wang and Albers [92] showed that aged male and female mice had decreased sensitivity to thermal stimuli, accompanied by a decrease in receptor expression for the growth factor artemin and the ion channel TRPV1. In contrast, Chan and Lai [117] showed decreased hot plate response latencies (i.e., increased sensitivity) for 11.2 versus 1.6 month old rats.

In contrast to the above findings, age-dependent increases in sensitivity to mechanical [118] and thermal stimuli [119] in the rat have been reported. These latter results paralleled the response profiles of wide dynamic range and nociceptive specific neurons recorded in the spinal cord of adult versus aged animals [119]. Significantly lower mean background activity and after-discharge responses were recorded in adult animals compared to those recorded in aged animals. Similar increases in neuronal excitability and receptive field sizes for neurons recorded in the dorsal column nuclei in aged versus adult animals have also been reported [120]. In summary, the results of 25 studies addressing age-related changes in pain sensitivity using reflex-based behavioral responses showed decreased sensitivity (9/25), increased sensitivity (12/25), or no changes (4/25) with advancing age. In addition to the behavioral assessment strategies used to evaluate responses to different stimulus conditions, there are many possible factors contributing to the variability of results, including the role of different sexes, species, and ages of animals when tested [121].

3.4.2 Findings from Operant Assays

In a cross-sectional study with rats ranging in age from 8 to 32 months, both operant escape and reflex testing methods were used to evaluate the effects of age on thermal sensitivity [122]. Operant measures of pain assessment revealed an increase in thermal sensitivity at older ages. By contrast, reflex responding did not show any age-related differences in sensitivity to 44.5 °C. In the case of cold sensitivity, operant escape revealed increased sensitivity from 8 to 32 months. Decreased latencies for licking/guarding responses to 1.5 °C were observed for animals ranging in age from 11 to 27 months of age. Interestingly, there was an increase in lick/guard latencies (i.e., decreased sensitivity) when animals were tested at 35 months. Thus, when comparing the results obtained from different age groups using operant escape and a reflex-based assessment task, consistent changes in thermal sensitivity were not observed. These results provide additional evidence that there are significant differences when comparing results of reflex versus cortically dependent outcome measures [111].

3.4.3 Inflammatory Pain

An important factor leading to central sensitization is the change in excitability of peripheral nociceptors by chronic inflammation [123]. The influence of injury- or age-induced inflammation on pain sensitivity was evaluated by Zhang et al. [123] in a study evaluating hindpaw withdrawal latencies in adult and aged animals following injection of complete Freund's adjuvant (CFA). Aged animals had a significant increase in nociceptive sensitivity after CFA compared to adult animals. Kitagawa et al. [120] showed greater excitability of dorsal horn nociceptive neurons with advancing age, but the excitability could not be further increased by inflammation. Using a different inflammatory agent, Gagliese and Melzack [124] showed that formalin injections elicited similar nociceptive responses in animals 3 and 24 months, which were significantly lower than animals 18 months of age, suggesting that

sensitivity to inflammatory pain may peak at mid-life. Formalin injections showed a larger number of c-fos (a marker of neuronal activation) positive cells in the medullary dorsal horn of older rats compared to their younger counterparts [125], which correlated with increased nociceptive sensitivity in an older cohort of animals.

The effects of formalin injection on thermal sensitivity were also evaluated using an operant escape task over 5 weeks of testing [122]. A significant formalin-induced increase in thermal sensitivity was obtained for cold and heat stimulation in animals 16 and 24 months old, but not in 8-month old animals. Similarly, paw injections of complete Freund's adjuvant increased thermal sensitivity and expression of the peptide dynorphin (DYN), a pronociceptive peptide [126], in the spinal cords of 18 month old rats, compared to 3 month old rats [123].

3.4.4 Neuropathic Pain

Considerable evidence suggests that aging and nerve injury may elicit similar anatomical, physiological, and behavioral changes. Age-dependent changes in pain sensitivity following nerve injury were evaluated following sciatic nerve ligation in young (4–6 months), mature (14–16 months) and aged (24–26 months) rats. This study observed prolonged increases in thermal sensitivity at 3 and 21 days following injury. The effects were most pronounced in the oldest animals, lasting a period of 35 days [127]. In a comparison of chronic constriction injury (CCI) and partial sciatic nerve ligation (PSNL) Crisp et al. [128] showed aged (24–26 months) rats undergoing PSNL, but not CCI, developed a more vigorous and longer duration thermal hyperalgesia and tactile allodynia compared to their younger (4–6 months) counterparts. Chung et al. [129], using a model of L5/L6 spinal nerve ligation, showed a decrease in mechanical sensitivity in middle-aged (15 months) versus young (40 days) and mature (4 months) animals using reflex based behavioral testing. A decrease in sensitivity to neuropathic pain for senescent (37–39 months) animals compared to old (20–22 months) and young (4–6 months) animals was observed by Pickering et al. [118]. Others [130] found no differences in responses to thermal stimuli for animals 7–8 weeks versus 18 months of age following partial denervation of the tail, while older animals showed increased mechanical allodynia [130]. The variable results across studies may be due to the use of reflex-based assays, different ages of animals, and differences in neuropathic pain models.

4 The Biopsychosocial Model Applied to Pain and Aging

The biopsychosocial model has become a guiding framework for conceptualizing the experience of disease and illness, including chronic pain [131]. The biopsychosocial model recognizes that while primary biological disease processes are important in human health, our understanding of illness is enhanced by incorporating the additional contributions of psychological and social factors. Importantly, these three sets of factors interact to influence the development, manifestations, and

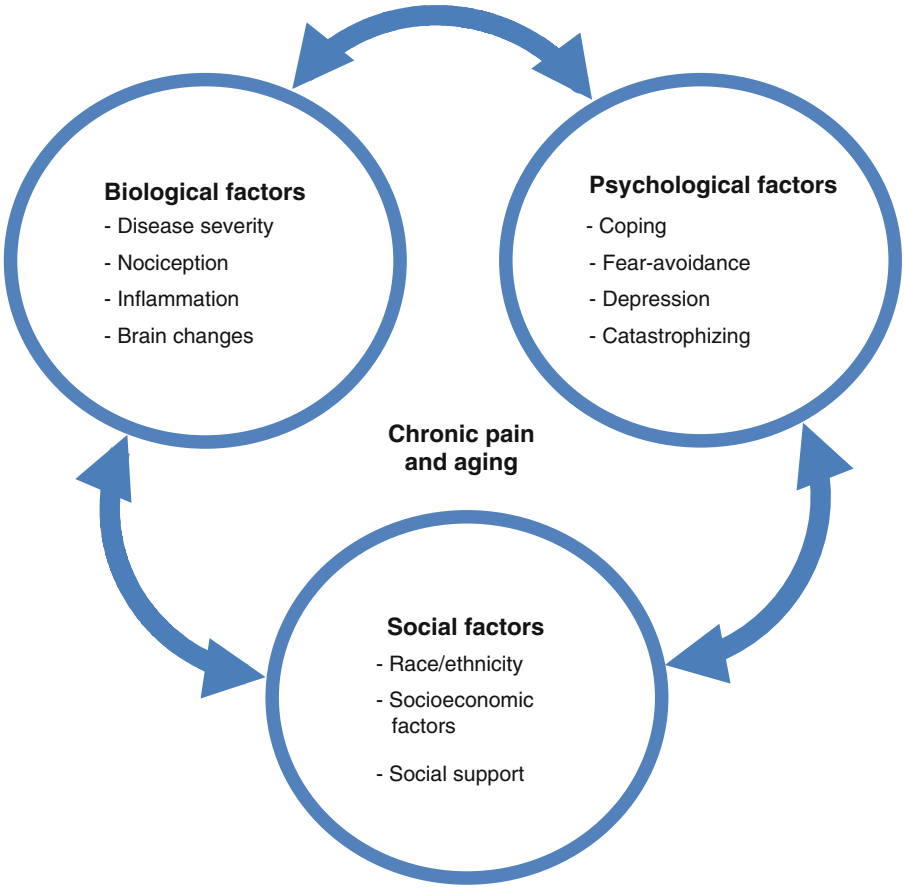

Fig. 18.2 The biopsychosocial model of pain and aging

course of clinical conditions such as chronic pain. Moreover, the biopsychosocial model represents a more comprehensive approach for understanding health and disease in aging [132, 133]. Figure 18.2 depicts the biopsychosocial model as applied to pain and aging. By way of example, while disease processes in OA, including reductions in cartilage volume, bony changes (e.g., osteophytes, subchondral bone cysts), and local inflammation (e.g., synovitis) are clearly important, measures of joint damage are at best only modest predictors of OA-related pain and disability [15, 134]. Interestingly, psychosocial factors contribute significantly to symptoms of OA. Indeed, recent systematic reviews found moderate to strong evidence that psychological factors, including depression [135] pain coping, self-efficacy, somatic symptoms, and catastrophizing, contribute to knee OA pain [136]. Further, social factors, including race and socioeconomic status, are also associated with OA-related pain and disability [137–139]. Hence, OA provides a prototypical example of the application of the biopsychosocial model's relevance to an age-related pain condition.

5 Age-Related Changes in Neurosensory Pain Processing

As noted, multiple biological and psychosocial factors conspire to influence the perception and experience of pain, and many of these pain-related biopsychosocial factors change as a function of age. Thus, older adults may perceive pain differently than younger adults. Age-related changes in pain processing have been examined using quantitative sensory testing (QST), "a group of procedures that assess the perceptual responses to systematically applied and quantifiable sensory stimuli for the purpose of characterizing somatosensory function or dysfunction [140]." QST includes multiple stimulus modalities (e.g., heat, cold, pressure, electrical), stimulation parameters (e.g., brief vs. prolonged stimuli), and measures of pain perception (e.g., threshold, tolerance, suprathreshold ratings), and the impact of age on QST responses varies importantly depending on these methodological issues.

5.1 The Impact of Aging on Pain Threshold

Pain threshold refers to the minimum stimulus intensity required to elicit pain. One meta-analysis found that regardless of stimulus modality, pain threshold increased with age, suggesting age-related decreases in pain sensitivity [141]. Also, this age-related increase in pain threshold was slightly larger among women than men. Interestingly, a study of electric shock pain reported that age-related increases in pain thresholds emerged only if the painful stimulus was of short duration, suggesting older adults are relatively more sensitive to prolonged stimuli that more robustly engage the somatosensory system [121].

5.2 The Impact of Aging on Pain Tolerance

Pain tolerance refers to the maximum stimulus intensity that an individual is willing or able to withstand. Studies of pain tolerance have revealed no age differences in response to thermal and electrical stimuli, but decreased pressure pain tolerance with age [141]. In addition, although not included in the meta-analysis, one study demonstrated dramatically lower tolerance for ischemic pain among older adults [142]. This finding is notable, because this experimental stimulus produces a sustained, deep muscle pain that is qualitatively similar to some forms of clinical musculoskeletal pain.

5.3 The Impact of Aging on Pain Facilitation

Temporal summation of pain refers to the increase in pain evoked by repeated rapid stimulation at a fixed stimulus intensity, which reflects a transient form of central sensitization and is therefore considered a measure of pain facilitation. In general,

temporal summation of heat pain is greater in older versus younger adults [141], suggesting an age-related increase in pain facilitation. Another form of pain facilitation occurs in response to capsaicin, a chemical that selectively activates peripheral nociceptive neurons (e.g., c-fibers). After the primary pain from capsaicin subsides, there remains an increased pain response to mechanical stimuli (i.e., mechanical hyperalgesia), which reflects a reversible central sensitization. Zheng and colleagues [143] reported that capsaicin-induced mechanical hyperalgesia lasted substantially longer in older versus younger adults, which may indicate prolonged pain facilitation.

5.4 The Impact of Aging on Pain Inhibition

Several QST methods have been used to investigate pain inhibitory function, the most common of which is conditioned pain modulation (CPM). CPM refers to the decrease in pain evoked by one stimulus (the test stimulus) produced by contemporaneous application of a second pain stimulus at a different body site (the conditioning stimulus). Consistent age differences have been observed in CPM, with older adults showing reduced pain inhibition [141]. In fact, some studies have found that older adults report pain facilitation in response to the conditioning stimulus. Importantly, a recent study found the age-related reduction in CPM was independent of expectations and depression [144] suggesting that other central mechanisms are involved and need to be investigated.

Another measure of pain inhibition is offset analgesia. This occurs when a prolonged heat stimulus is delivered, in which the stimulus is slightly increased in intensity and then returned to the original temperature. This slight reduction in heat evokes a disproportionate decrease in pain [145]. A recent study reported that older adults showed reduced offset analgesia compared to younger adults [146]. Riley and colleagues [147] also reported that older adults showed a reduced decay of pain following offset of a prolonged heat pain stimulus, which may reflect impaired pain inhibition.

6 Cognitive Function and Pain Perception in Older Adults

Several QST studies have examined whether age-related declines in cognitive function influence pain processing. Benedetti and colleagues [148] reported that, compared to cognitively intact older adults, those with Alzheimer's disease showed similar pain thresholds but higher tolerance for electrical and ischemic pain, and pain tolerance was positively correlated with scores on the mini-mental status exam (MMSE). They reported similar findings for patients with frontotemporal dementia [149]. Also, autonomic responses to painful stimuli were reduced in patients with Alzheimer's as well as those with mild cognitive impairment [150, 151]. In contrast, Gibson and colleagues reported that patients with Alzheimer's disease showed

similar pain thresholds and evoked potential amplitudes compared to cognitively intact older adults in response to laser-induced painful stimulation [152]. Marouf and colleagues [153] recently demonstrated that reduced CPM demonstrated by pain-free older adults was associated with poorer performance on a cognitive inhibition task (i.e., the Stroop task), suggesting that diminished cognitive function is associated with reduced pain inhibitory capacity.

The findings from QST studies suggest that aging is associated with decreased sensitivity to mild painful stimuli (e.g., pain threshold) but increased pain in response to higher intensity pain stimuli. Moreover, dynamic QST measures reveal increased pain facilitation and reduced pain inhibition among older adults. While dementia appears to be associated with attenuated pain responses, cognitive performance in cognitively intact older adults may positively predict pain inhibitory function. Yarnitsky and colleagues [154] recently suggested the concept of a pain modulation profile, which reflects an individual's balance of pain inhibition versus pain facilitation. This profile can be ascertained through QST measures such as CPM and temporal summation, and those who show a pro-nociceptive imbalance (i.e., high pain facilitation, low pain inhibition) are at increased risk for adverse pain outcomes. Based on the studies described above, aging is characterized by a pro-nociceptive pain modulation profile that may contribute to the increased risk of certain clinical pain conditions or of more severe or widespread pain in older adults. The factors driving these age-related changes in pain modulation are largely unknown, but are likely to include multiple biological (e.g., inflammation, endogenous opioid function, changes in brain structure and function) and psychosocial (e.g., mood, cognition, coping) processes and their interactions.

7 Neurobiological Factors Contributing to Age-Related Changes in Pain Processing

7.1 Brain Structure and Function

Aging is associated with global reductions in grey matter and white matter volume, though some brain regions are more affected than others [155, 156]. While these brain changes have been linked to declines in cognitive function, it seems plausible that aging effects on the brain could also impact pain processing. Indeed, chronic pain has been associated with decreases in grey and white matter volume [157–159]. Also, some evidence links structural brain changes to QST measures of pain perception. For example, total grey matter volume in fibromyalgia patients was negatively correlated with sensitivity to digital palpation [160]. Also, in pain-free adults, grey matter volume in several brain regions has been inversely associated with visceral sensitivity [161] and heat pain sensitivity [162]. While no study has yet linked changes in brain structure with age differences in pain processing, a plausible hypothesis is that age-related changes in brain morphology contribute to the enhanced pain facilitation and/or reduced pain inhibition observed in older adults.

Painful stimuli elicit patterns of neural activity in a variety of brain regions, and age differences in this pain-evoked cerebral activation could help explain age-related changes in pain perception. Two studies using heat pain showed reduced pain-evoked brain activity among older compared to younger adults. In response to painful heat older adults showed lower activation in several cortical regions, including somatosensory cortex, anterior insula, and supplementary motor area [163]. More recently, age was inversely associated with pain-related activation in somatosensory, insular, and premotor cortices [164] and grey matter volumes in the anterior and mid-cingulate cortex were positively correlated with pain ratings. Both of these studies showed decreased pain-related cerebral activation evoked by mild to moderate heat pain, which older adults typically report to be less painful. In contrast, Cole and colleagues [165] examined brain responses to pressure pain, to which older adults were more sensitive compared to their younger counterparts. While no age differences emerged in response to a mild pressure pain stimulus, younger adults showed greater activation in the contralateral putamen and caudate nucleus in response to moderate pressure pain. The authors suggested that these age differences may reflect an impairment of endogenous pain modulation among older adults.

7.2 Inflammation and Age Differences in Pain Processing

Aging is characterized by increases in systemic inflammation (i.e., "inflammaging"), which could contribute to increases in pain sensitivity. Systemic inflammation could influence pain processing via both peripheral and CNS mechanisms, as systemic inflammation can sensitize nociceptors [166, 167], and increasing evidence demonstrates that systemic inflammation can alter brain structure and function [168, 169]. Indeed, induction of systemic inflammation in humans reliably increases functional activation in several brain regions implicated in pain processing, including the brainstem, amygdala, anterior and posterior cingulate, and the insula [170]. Recent studies have reported that inducing systemic inflammation via endotoxemia in humans significantly increases pain sensitivity in response to visceral and somatic stimuli [171, 172]. Thus, age-related increases in systemic inflammation could contribute to the imbalanced pain modulatory profile that has been observed in older adults.

8 Psychosocial Factors Contributing to Pain Perception and Response in Older Adults

8.1 Beliefs/Attitudes About Pain

Regardless of age, people's beliefs and appraisal processes contribute to the exacerbation, attenuation, or maintenance of chronic pain, and to behavioral responses including affective distress, adjustment, health care seeking, response to treatment,

and disability (i.e., [173, 174]). Furthermore, people's expectations about the impact of their pain and the likelihood of recovery following a painful injury have been shown to be more predictive of long-term disability than objective levels of physical pathology [175, 176]. Consequently, pain that persists over time should not be viewed as solely physical or solely psychological. Rather, as described earlier, persistent pain is a complex, biopsychosocial phenomenon that results from the reciprocal interactions between psychological, social, as well as physiological components.

Individuals' perceptions and responses have important implications for the persistence of pain and associated disability across the life cycle. However, some beliefs, expectations, and responses are particularly prevalent and important for older adults experiencing pain. For example, community-dwelling older adults often restrict their activity in response to pain [177, 178]. People may reduce their activities as a direct attempt to diminish the pain but also may reduce activity because they believe that pain is a signal of harm and wish to prevent further tissue damage or exacerbation of their pain. For example, a study of older adults reported that *all* study participants changed their activity in response to the experience of persistent pain by deliberately substituting activities perceived as physically demanding with more passive ones or stopping certain activities altogether [178]. Activity restriction was viewed as a way of safeguarding function and avoiding conventional treatments, such as medications and surgery [178]. Paradoxically, although activity restriction was approached strategically to preserve function and avoid medical interventions, the associated physical constraints, and loss of social contact were emotionally distressing [178]. Moreover, reduction in activity may reduce muscle strength, endurance, and flexibility, thus contributing to deconditioning, increased activity-related pain, and ultimately further disability. These findings highlight the trade-off that many people with chronic pain, older persons included, face between wanting to participate in valued activities and safeguarding function through reduced activity.

8.2 Emotional Distress – Anxiety/Fear, Depression

The sequelae of chronic pain include depression, anxiety and impaired cognitive function, as well as reduced socialization, compromised sleep and ambulation [35, 179]. These consequences are particularly important for older adults as they may contribute to falls, hospitalization, and increased dependence. Emotional distress may be a precipitant of symptoms, be a modulating factor amplifying or inhibiting the severity of pain, be a consequence of persistent pain, or a perpetuating factor. Moreover, these potential roles are not mutually exclusive and any number of them may be involved in a particular circumstance interacting with cognitive appraisals. For example, the literature is replete with studies demonstrating that current mood state modulates tolerance for acute pain (e.g., [180]). Levels of pre-surgery anxiety have been shown to influence not only pain severity, but also complications and length of stay following surgery [181, 182].

Fear of movement and fear of (re)injury are better predictors of functional limitations than biomedical parameters or even pain severity and duration [183, 184]. For example, Crombez et al. [185] reported that approximately two-thirds of individuals with chronic LBP (CLBP) avoid back-straining activities because of fear of (re) injury. Further, pain-related fear was the best predictor of behavioral performance in trunk-extension, flexion, and weight lifting tasks, even after separating out the effects of pain intensity. Moreover, fear of movement/(re)injury was the best predictor of self-reported disability among CLBP patients, and sensory perception of pain and biomedical findings did not add any predictive value [186]. Interestingly, reduction in pain-related anxiety predicted improvements in functioning, affective distress, pain, and pain-related interference with activity [187]. Pain-related fear of movement can be an important issue among older adults and may be further complicated by fear of falling. Clearly, fear, pain-related anxiety, and concerns about harm-avoidance all play important roles in age-related chronic pain and need to be assessed and addressed in treatment.

Falls are a common, costly, and often devastating problem among older people, causing a significant amount of morbidity, use of health care services, and increased risk of loss of independent living status, premature nursing home admissions, and mortality [188–190]. Analysis of a large national sample of Medicare beneficiaries found the prevalence of falls and the fear of falls that limits activity are three times higher in older adults with pain than in those without pain [191], and longitudinal studies of older adults show that chronic pain is associated with decreased mobility function and increased falls over time [192]. Importantly, *concerns* about falls [193] may be a crucial determinant of activity limitations, regardless of the objective fall risk [194, 195]. Indeed, fear of falling itself may become a risk factor, as it contributes to deconditioning, loss of flexibility, weakness, and abnormal gait, and in the long run may increase risk of falls [196, 197].

Depression represents another pain-related concern among older adults. A large survey of community-dwelling older adults found that baseline depression symptoms increased the odds of disabling LBP after two years, independent of sociodemographic characteristics and functional status. Conversely, disabling LBP at baseline also increased the odds of depressive symptoms after 2 years to a similar degree [198]. Depressed mood is quite common in OA, and is associated with increased pain sensitivity and disability [199]. Williamson and Schulz [200] found that activity restriction mediated the relationship between pain and symptoms of depression, and accounted for differences in pain intensity between non-depressed people and those at risk for developing depression. More recently a longitudinal study in older adults with OA found that pain interference in activity was a risk factor for developing depressive symptomatology [201].

8.3 Family and Other Social Supports

Consistent with the biopsychosocial model, persistent pain occurs in a social context. Social support, relationships with others, and resources can be defined as the availability of tangible (e.g., help with meals, transportation, assistance with

personal care), affectionate (expressing love and affection), emotional (empathic understanding), informational (e.g., advice), and positive social interaction support (sharing of activities, companionship) [202]. Older adults with chronic health conditions often have difficulty participating in everyday activities [203, 204], thus affecting their quality of life and ability to participate in their communities.

Social isolation has an especially important impact on pain and disability in older adults. The elderly often face significant social losses (e.g., death of loved ones, reduction in social status, loss of independence) and difficulty maintaining social relationships, which can contribute to the exacerbation of persistent pain conditions and can negatively impact adherence and response to treatments [205]. In turn, persistent pain contributes to increased social isolation, as older adults with chronic pain spend less time in previous social roles and experience greater restrictions in social and leisure activities [206, 207].

Variations in the family, community, home, and healthcare environments can play important roles in how older adults adjust to pain. Significant others may express sympathy and excuse the individual from responsibilities, and encourage passivity, thereby fostering further functional impairment. Nursing homes are often perceived as coercive settings, promoting non-autonomous orientation that restricts activities. When events are objectively coercive, people may perceive a lack of autonomy and hence be at greater risk of depression. What may really be important to emotional well-being is not so much pain itself, but the way in which pain alters older people's lives.

9 Treatment of Pain in Older Adults

A substantial armamentarium is available to treat patients with chronic pain. These can be grouped into several general classes: (1) educational, (2) pharmacological, (3) activation (physical exercises), (4) psychological (e.g., cognitive-behavior therapy (CBT), (5) surgical, (6) neuroaugmentative, and (7) a variety of complementary health approaches and modalities. This chapter will focus on some of the most commonly studied and prescribed – medication, physical activation, complementary medicine, and CBT. Although we discuss these separately, they are often used in combinations (e.g., multidisciplinary programs).

9.1 Pharmacological Treatments

The first-line treatment for pain, regardless of age, is analgesic medication either over-the-counter or prescribed. The most commonly used and prescribed analgesic medications include nonsteroidal anti-inflammatory agents (NSAIDs) such as acetaminophen, opioids, antidepressants (e.g., amitriptyline, duloxetine, milnacipran) and anticonvulsants (calcium channel alpha2-delta ligands; gabapentin, pregabalin).

There are also a plethora of newer agents prescribed (sodium channel modulators [Lidocaine patch 5 %; mexiletine]; alpha$_2$-receptor agonists [clonidine, tizanidine], NMDA receptor antagonists [ketamine, memantine], cannabinoid receptor agonists, and vanilloid receptor ligands [topical capsaicin]). Each of these classes of medications may provide some level of pain relief, but often there are significant limitations and some adverse effects associated with each of them, particularly when used in older adults. For example, NSAIDS have known gastrointestinal and nephrotoxicities [208, 209]; NSAIDS and opioids have cardiotoxic and problematic hormonal effects [210, 211]; opioids, antidepressants, and antiepileptic drugs are all associated with increased susceptibility to falls and fractures [212, 213]; and opioids have potential for abuse (see Table 18.1 for a summary of the advantages and limitations of the most commonly prescribed medications).

Medical comorbidities are an important consideration in treating pain in older persons. Older adults often have several medical conditions in addition to the particular pain diagnosis (e.g., cardiac diseases, diabetes, cancer, dementia, osteoporosis). Aging itself and associated diseases affect gastrointestinal and physiological processes including motility, secretions, blood flow, and absorptive surface [214], and these changes can affect drug absorption, bioavailability, and transit time, as can reductions in plasma albumin, increased fat to lean mass ratios, and decreased total body water [214, 215]. In addition, liver mass, liver blood flow, and the glomerular filtration rate of kidneys decrease with age. Of particular clinical importance, reduced renal clearance leads to a decline in the excretion of water-soluble drugs [215]. Lowered activities of most of the cytochrome P450 enzymes reduce the drug-elimination clearance rate of the liver, especially in the presence of chronic disease [216]. The potential for drug accumulation and increased CNS sensitivity increases the risks for cognitive impairment and respiratory depression in conjunction with concomitant CNS medication or with underlying pulmonary conditions [217].

In a United States study published over a decade ago, 50 % of patients aged 65 or older consumed five or more prescription drugs and 10 % were using ten or more medications [218]. Polypharmacy can be a confounding risk factor when prescribing pain medications as there are both known and unknown drug-drug interactions that need to be considered. For example, individuals aged 60 or older are provided with an average of 40.8 prescriptions per year according to a study conducted in the United Kingdom [215]. With polypharmacy, dose-limiting adverse effects of pain-relieving medications may limit the potential achievable efficacy. Because of the increased likelihood of drug-drug and drug-disease interactions, as well as the pharmacokinetic and pharmacodynamic challenges associated with polypharmacy in older adults, frequent monitoring is critical when analgesic medications are prescribed.

Age-related changes in body composition and organ function can also alter metabolic and pharmacokinetic responses to medications. These changes along with medical and psychiatric comorbidities and concomitant polypharmacy (see also [219, 220]), suggest that conventional pharmacological therapies may not always be appropriate for older adults and should be used with caution [221–224].

Table 18.1 Common treatment options: advantages and limitations with elderly populations

Treatment	Advantages	Limitations
Pharmacologic treatments		
Analgesics in general	Pain relief	Age-related physical changes Interactions with comorbidities/frailty Drug-Drug interactions Adverse events (AEs)
NSAIDS/acetaminophen	Pain relief Reduced inflammation	AEs (e.g., Gastrointestinal, cardiac and nephrotoxicities, immune function, increased bleeding)
Opioids	Pain relief	Societal stigma Acceptance AEs (e.g., constipation, sedation, hormonal balance, dizziness, increased fall/fracture risk, cognitive impairment, cardiotoxicity) Interaction with alcohol Misuse potential
Anticonvulsants	Pain relief Improved sleep	Avoid in renally impaired AEs (e.g., sedation, cognitive impairment, weight gain, increased fall/fracture risk)
Antidepressants	Pain relief Improved mood	AEs (e.g., impact on blood pressure, dizziness, increased fall/fracture risk, nausea, sleep disruption)
Nonpharmacologic treatments		
Information/education	Prevention of falls	Cognitive limitations Sensory limitations Time intensive
Physical activation in general	Improved function	Acceptance Generalizability Adherence/maintenance Time/effort Motivation
Supervised exercise (e.g., EnhancedFitness)	Improved function Possible pain relief	Possible increased fall risk
Home exercise	Improved function Enhanced adherence	Possible increased fall risk
Tai Chi	Improved function Improved balance Reduced fall risk	Adherence/maintenance Time/effort
Psychological treatments in general	Improved mood Enhanced coping	Cognitive requirements Acceptance Motivation Time/effort
Cognitive Behavioral Therapy (CBT)	Improved mood Reduced fear of falls	Access/availability

Despite these cautions, older individuals are among the highest consumers of analgesics [225] with up to 20 % of the elderly taking analgesics for more than 6 months [226, 227].

Analgesics are often inappropriately prescribed for elderly patients, failing to follow clinical practice guidelines [228, 229]. For example, one study of community-dwelling men over age 70 noted that 8.2 % reported regular NSAID use compared to 2.9 % who reported as-needed use. Furthermore, the mean treatment duration for regular NSAID use was 4.9 years, suggesting long-term rather than short-term use as is recommended by guidelines [219]. Opioids are poorly tolerated by elderly patients [228, 230], and antidepressants and anticonvulsants are limited due to their effects on hepatic and renal function that may already be compromised because of the aging process. As noted, opioids, anticonvulsants, and antidepressants all increase the risk of falls and are major contributors to morbidity, increased hospitalization, and mortality in the elderly.

In sum, although conventional pharmacological treatments for pain can provide some relief for symptoms, they have significant hazards in older adults that need to be balanced in treatment decisions. Further, the long-term effectiveness of analgesic treatments for this population is unclear as few randomized controlled trials (RCTs) involve older adults with multiple morbidities [231, 232]. Moreover, in general, pharmacologic treatments provide only modest reductions in pain (30 % in fewer than 50 % of treated patients) and little impact on improving function [233]. There have been few studies that specifically address the issue of treatment effectiveness with older adults. In view of the limited evidentiary base and well-established adverse effects of current analgesic medications, there is an urgent need to develop both safe and effective pharmacological and non-pharmacological therapies for the rapidly growing older population. Greater emphasis on non-pharmacological approaches, alone or in combination with lower doses of pharmacological agents, may be particularly important for older adults with chronic pain.

9.2 Non-pharmacological Treatments

Clinical practice guidelines frequently recommend non-pharmacological interventions for pain in older adults. For example, in the 2013 guidance on the management of hip and knee OA, the European League Against Rheumatism (EULAR; [234]) recommended that all patients should have an individualized management plan that includes information and education on all aspects of OA management, advice on how to maintain activity, an exercise program consisting of aerobic and muscle-strengthening exercises, advice on weight loss as necessary and advice on home adaptations, among others.

Exercise is widely recognized as an approach for reducing pain and improving physical function in patients with chronic pain regardless of age [235, 236]. Despite recommendations for exercise, several studies have shown that objectively measured levels of physical activity are significantly lower in older chronic pain popula-

tions [6]. Indeed, as noted, activity restriction is a commonly reported strategy older adults use to reduce pain [177]. An important target for physical activation in older adults is improved balance [237–239]. Two examples of activity-based programs that have been specifically developed or adapted for use with older adults include – EnhanceFitness™ and Tai Chi (TC). The former is a more traditional approach to physical activity whereas TC makes use of complementary and alternative medicine modalities of relaxation and structured movements. Both approaches may be particularly useful when targeted to improve balance.

EnhanceFitness (EF) is a community-based multicomponent, supervised group physical exercise program for older adults, involving balance, strength, and endurance training that is widely disseminated throughout the United States [240]. Each EF class uses a standardized format that includes several phases: a warm-up, moderate-intensity aerobic conditioning/walking phase, progressive strength training, and a cool down. EF focuses on flexibility and static and dynamic balance exercises that are known to reduce falls risk. Two RCTs [241, 242] have shown that EF modestly improves health-related quality of life, objective measures of balance, functional and gait performance, and reduce health care costs. However, in a large RCT, adherence to EF was variable with a median of 58 % (interquartile range: 15–75 %) of exercise classes attended over 12 months [241]. Unpublished exit interview data identified pain as a leading cause of non-adherence. Greater adherence may lead to better outcomes but as noted below, adherence with any self-management regimen is a significant concern.

Tai Chi (TC) is a traditional Chinese martial art that incorporates aerobic activity, diaphragmatic breathing, relaxation, and meditation with postures that are designed to flow imperceptibly and smoothly through slow, gentle movements (low impact, low velocity) that are particularly appropriate for elderly adults. The slow speed and constant weight shifting associated with TC increases the load on the lower limbs in a gradual fashion and may have a direct effect on improving balance. TC enhances self-awareness of balance and thereby may contribute to the amelioration of fear of falling [243, 244]. It has emerged as a viable exercise intervention, and it is recommended for older populations by the American Geriatrics Society [221]. TC improved function in people with knee OA [245], CLBP [246, 247], and improved balance and reduced risk of falls in older adults [238, 248]. It has been shown to be more effective than other exercises for improving mobility and reducing fear of falling in older adults [246, 249].

In general, increasing exercise is a key challenge to address in the geriatric population as relatively few older adults use exercise and other behavioral strategies to cope with pain [177, 250]. Instead, passive strategies and avoidant behaviors are more common and associated with increased disability [250]. Thus, efforts to increase physical activity in the geriatric pain population will require restructuring maladaptive beliefs about exercise and fears of falling, which seems to occur with EF and TC, as well as addressing common comorbid symptoms that are prevalent and often contribute to activity limitation, such as difficulty sleeping and depressed mood.

9.3 Psychological Treatments

Although there are a range of psychological approaches to managing pain, Cognitive-behavior therapy (CBT) is perhaps the most commonly prescribed and thoroughly investigated [251]. CBT is a generic term; there are many components (e.g., exposure, addressing maladaptive beliefs, skills training [252]). The primary assumptions shared by all CBT interventions are that: (1) people are active processors of information; (2) people are capable of gaining control over their thoughts, feelings, behaviors, and to some extent physiological processes; and (3) there are interrelationships among thoughts, feelings, behaviors, and physiological processes; changes in one or more of these factors may result in changes in the others.

Meta-analyses and systematic reviews have shown beneficial effects of CBT for a number of pain conditions that often affect older adults (e.g., OA pain, cancer pain, CLBP) [253–255]. In general they demonstrate that CBT produce significant decreases in pain (typically small to medium effect sizes) and significant improvements in indices of adjustment to pain (e.g., activity, depression, anxiety, self-confidence, maladaptive beliefs). However the results are not consistent across studies, which may relate to the specific content, mode of delivery, duration of treatment, and extent of therapist training. In designing CBT for older patients, clinicians should consider providing rationales, guided practice, experiential learning (more active, less didactic), home practice assignments in written and verbal forms, using audio recording to guide home practice to eliminate the need for memorization, and possibly involving significant others [256]. Of particular relevance, CBT has demonstrated significant yet modest efficacy in the treatment of fear of falling in older adults [257]. The results are comparable to those reported for exercise in community-dwelling older adults [249, 258]. CBT has been well received in small samples of older adults with chronic pain [259, 260]. Tailored CBT for older populations has included a set of components including:

- Education about the long-term benefits of exercise for chronic pain
- Restructuring beliefs about activity and pain
- Teach coping skills to address pain and related symptoms including problem-solving skills to overcome barriers to exercise
- Foster activity pacing
- Set realistic goals for increasing activity incorporating any limitations in physical capacity to ensure safety and promote the adoption of a physically active lifestyle
- Expose participants to feared and avoided activities that are not restricted by physical limitations
- Address fear of falling by restructuring misconceptions to promote a view of falls risk as controllable
- Teach coping skills to address pain and related symptoms, including regulating daily activities and sleep schedule, and modifying cognitive and emotional responses to pain
- In general, emphasize self-control and self-efficacy

- Set realistic goals for activity and promoting the adoption of a physically active lifestyle
- Advise about the environment to reduce fall hazards

9.4 Mechanisms of Psychological and CAM Interventions

With the advent of sophisticated imaging procedures such as fMRI, it has become possible to identify regions of the brain associated with self-reports of pain as described above. Recent studies using fMRI have been able to document unique physiological responses in the brain that are associated with prolonged pain, emotional responses accompanying pain, attentional foci, and processing of noxious stimulation (e.g., [261, 262]).

Of particular interest is the use of neuroimaging technologies to identify changes in brain function that accompany alterations in pain perception and responses following pain treatments. Jensen [263] has hypothesized that different psychological pain treatments and changes in the psychological factors targeted by these treatments (e.g., maladaptive cognitions, reassuring thoughts) could potentially have different effects on the activity of different brain structures and processes. CBT has been shown to produce structural and functional changes in pain related regions of the brain in several populations [264–267]. Non-pharmacological therapies, including CBT, evoke increased activation of brain regions involved in executive cognitive function, while decreasing activity in regions associated with pain transmission [268]. In addition, neuroimaging studies suggest that non-pharmacological approaches may produce positive structural brain changes in areas that often decline with aging (i.e., cortical thickness in the prefrontal and right anterior insula and occipito-temoral region) [209]. Seminowicz and colleagues [269] reported increased prefrontal cortex gray matter volume following CBT for chronic pain, and greater reductions in pain catastrophizing after CBT were associated with greater increases in gray matter volume in several brain regions. The impact of CBT on brain structure and function in older adults with chronic pain represents an important yet unexplored area.

9.5 Adherence to Prescribed Treatments

The non-pharmacological treatments described above require patient understanding, motivation, and adherence. These may be of particular concern for older adults who have cognitive and sensory limitations. The impact of pain on cognitive functioning (e.g., memory) might impede following prescribed regimens. Treatments that were originally developed for younger individuals need to have appropriate adaptations and adjustments in content and format when prescribed for the elderly to accommodate any age-related limitations. Another impediment

to adherence is cost, which impacts adherence to prescription medications among older adults, especially among individuals who have poor health, multiple morbidities, and limited drug coverage through insurance [270]. Successful treatment of older people with chronic pain will require that problems associated with treatment adherence be addressed regardless of the intervention – pharmacological or non-pharmacological.

10 Conclusions and Future Directions

Age-related changes in pain are complex and remain poorly understood. Epidemiological and clinical studies demonstrate that pain prevalence and impact change with age, although patterns vary for different types of pain; some pain conditions increase while others decrease in prevalence with age. Preclinical models reveal conflicting findings regarding age-related changes in nociceptive sensitivity, likely due to methodological variations. In humans, QST studies reveal an age-related pain modulatory imbalance, as older adults tend to show increased pain facilitation and diminished pain inhibition. Additional research is needed to more clearly define the biopsychosocial factors that contribute to age-related changes in pain processing. Potential biological mechanisms include anatomical, physiological, immune, neuroendocrine, inflammatory, and autonomic changes. Likewise, multiple psychosocial factors influence pain experiences among older adults, including beliefs and perceptions, negative mood (e.g., fear, depression), and social changes and support (e.g., isolation, reduced social activity). However, limited information exists regarding the extent to which each of these factors individually contributes to age-related influences on pain, let alone their interactions. Likewise, aging complicates the treatment of pain. Pharmacologic therapies offer limited clinical efficacy and produce increased adverse effects in older adults, and non-pharmacologic treatments, while effective, are often underprescribed in elderly patients.

Based on the current state of the evidence, we recommend the following lines of investigation to move the field of pain and aging forward.

- Investigators should take a more systematic and standardized approach to characterizing chronic pain in studies of pain and aging, including: increased consistency in case-definitions for pain conditions (e.g., time-frame – pain lasting 3 months, last 30 days), assessment of pain impact, pain severity, temporal patterns, etc.
- Studies are needed to evaluate the interaction of how systems known to change as a function of age (immune, HPA-axis, autonomic nervous system) impact age-related changes in pain sensitivity and the prevalence of chronic pain conditions.
- Advancing age needs to be a relevant variable in preclinical models of pain. Specifically, additional cross-sectional and longitudinal studies comparing vari-

ous pain models and assays (e.g., operant vs. reflex-based, neuropathic vs. visceral) could help elucidate mechanisms underlying differential age-related patterns across different clinical pain conditions.
- Preclinical studies are needed in older animals to determine the efficacy and drug-drug interactions of pain medications and the potential side effect profile of drugs compared to those in younger animals.
- Studies are needed investigating how age may be protective against development or severity of certain pain conditions.
- There is a need for increasing the representation of older adults in clinical trials of pain therapies and for secondary analysis of age as a potential moderating factor for efficacy in existing clinical trials.
- Given the modest outcomes for existing treatments, and currently limited treatment armamentarium, evaluation of treatment combinations for pain in older adults requires investigation.
- Given the importance of self-management strategies in managing pain in older adults, research examining adherence and maintenance enhancement strategies is essential.
- Evaluation of changes in brain structure and function that may predict positive outcomes from non-pharmacological interventions in older adults is warranted.
- Investigators should explore adaptations to pain management approaches required to compensate for cognitive and sensory limitations associated with aging.

Addressing these issues and adopting these methodological enhancements should help reduce inconsistencies in the literature, thereby substantially improving our understanding of age-related influences on pain.

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The Way Forward: Translation

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Contents

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1 Introduction

Major age-related chronic diseases are the leading drivers of morbidity, loss of independence, hospitalization, mortality, and health costs throughout most of the world. These include atherosclerosis, most cancers, mild cognitive impairment, dementias, Parkinson's and other neurodegenerative diseases, type 2 diabetes, renal dysfunction, arthritis, blindness, frailty, chronic obstructive pulmonary disease, sarcopenia, and many others [1–4], as discussed throughout this book. For each of these conditions, chronological aging is a major risk factor and for most, aging leads all other known predictors combined. Numbers of chronic disorders *per individual* increase with aging, associated with loss of independence, frailty, and increased risk of death. Although more epidemiological research is needed to be certain about this, it seems that while many elderly individuals are healthy, those who are not have multiple comorbidities that often begin around the same time. If true, this suggests there are shared pathogenic mechanisms. Also as discussed in the preceding chapters, the major age-related disorders often share the disturbances in tissue, cellular, and molecular function that occur with chronological aging. These include chronic "sterile" inflammation, cellular senescence, macromolecular changes (DNA, proteins, carbohydrates, and lipids), and stem and progenitor cell dysfunction.

Based on these points, the "geroscience hypothesis" has been proposed: by targeting fundamental aging processes, it may be possible to alleviate the major age-related chronic disorders as a group, instead of one at a time. Therefore, as summated in the previous chapters of this book, targeting fundamental aging mechanisms may be a way to delay, prevent, alleviate, or treat chronic disorders as a group, instead of one at a time. Even if a single major chronic disease such as atherosclerosis were eradicated, as transformative as such an advance would be, it would only add 2–4 years to life expectancy, only to be followed by another potentially fatal age-related chronic disease [5, 6]. However, targeting the intersection between fundamental aging mechanisms and processes that lead to chronic diseases could alleviate multiple age-related disorders and extend healthspan.

2 Is Aging a Modifiable Risk Factor?

In the first chapter of this book, Austad argues that nature has achieved changes in longevity multiple times and seemingly by independent, distinct mechanisms. Nevertheless, and although aging is the leading predictor for chronic diseases and disabilities, it has only recently become viewed as a potentially modifiable risk factor. Supporting the contention that aging can be modified in several species in the laboratory (*i.e.*, independently from evolution) are the findings that: (1) maximum lifespan is extended and age-related diseases can be delayed in experimental animals by a number of single gene mutations [7], suggesting that pathways impacted by these mutations could be therapeutic targets. (2) Humans who live

beyond age 100 can have delayed onset of clinically overt age-related diseases, such as Alzheimer's [8], contributing to compression of morbidity and enhancing healthspan. (3) As discussed below, a number of agents, including rapamycin, metformin, acarbose, 17α-estradiol, angiotensin converting enzyme inhibitors (ACEi), senolytic agents (a new class of drugs that selectively eliminate senescent cells [9]), flavonoids, and others increase healthspan and/or lifespan in experimental animals [3, 10–13]. For example, rapamycin appears to delay age-related cognitive decline and cancers [14]. A pipeline is developing of yet more agents that show promise for enhancing lifespan and perhaps healthspan in experimental animals that have not yet been published. In general, these agents alleviate inflammation, cellular senescence, metabolic dysfunction (in some cases by targeting pathways related to caloric restriction), macromolecular damage or processing, or stem cell and progenitor dysfunction. (4) Caloric restriction, which can increase maximum lifespan in some experimental animals, is associated with delayed onset of multiple chronic diseases [15]. (5) Factors in the circulation of young animals alleviate muscle, cardiac, brain, and potentially other forms of dysfunction in older animals [16, 17]. (6) Senescent cell accumulation is associated with many age-related chronic diseases and frailty [9, 18], and genetic or pharmacological senescent cell elimination enhances healthspan and delays age-related dysfunction, at least in mice [19, 13].

Since interventions that increase lifespan and healthspan in mammals exist, it might be possible to circumvent an issue that has made studying the pathogenesis of many of these diseases in humans difficult: many of these chronic diseases, such as Alzheimer's or atherosclerosis, occur only in humans or a very limited number of species. Furthermore, many of them become manifest clinically once the disease is advanced at the molecular and cellular levels. These issues make delineation of initiating mechanisms difficult because of the impracticality of obtaining tissue samples for analysis sufficiently early during disease development in humans. By targeting upstream, fundamental aging processes that predispose to these human diseases, these difficulties could be circumvented.

3 Do We Have Interventions That May Work?

Recent, important advances have been made in our understanding of the basic biology of aging. The field has moved from an era of description to hypothesis-driven research with a focus on elucidating mechanisms and, most recently, into developing interventions that target fundamental aging processes. Modulators and interventions that delay age-related changes in experimental animals include caloric restriction, several hundred single gene mutations across species, and, most recently, drugs. Among the alterations that extend lifespan or healthspan are mutations in the growth hormone (GH)/ insulin-like growth factor-1 (IGF-1)/ insulin signaling pathway and pathways related to protein, carbohydrate, or lipid metabolism, caloric restriction, inflammation, and the renin-angiotensin system. The NIA-funded

Interventions Testing Program (ITP) has been particularly effective in identifying drugs that extend lifespan in mice. Several are beginning to show promise in extending healthspan and delaying age-related chronic diseases as well. In general, these genetic and pharmacologic interventions are related to inflammation, cell-survival, cellular senescence, macromolecular processing, fuel sensing and processing, stem and progenitor cell function, and integrity of the stem cell niche. Agents resulting from research conducted through the ITP that might be among the first to be translated into humans are considered below.

3.1 Rapamycin and Rapalogs

Rapamycin is an immunosuppressant approved by the FDA-for solid organ transplantation, while derivative "rapalogs" are approved for treating particular cancers [20]. Rapamycin inhibits the eponymous mechanistic target of rapamycin (mTOR) kinase, which comprises two distinct protein complexes. The mTOR Complex 1 (mTORC1) is an integrative node for cellular energy signaling, activated by glucose and amino acids, IGF-1, insulin, and other growth signals. Accordingly, mTOR inhibition recapitulates some of the effects of caloric restriction. Rapamycin extends lifespan in yeast, flies, and worms, as well as in C57BL/6, 129/Sv, and genetically heterogeneous mice, even when started after mid-life, as found by the ITP [10, 21]. A net effect of mTOR inhibition by rapamycin is an increase in protein quality. Rapamycin has a number of effects in mice that might explain how it extends longevity, including modulating stem cell function and inflammation, promoting autophagy and alleviating the pro-inflammatory senescence-associated secretory phenotype (SASP), thus resulting in improvements in cognitive decline, heart failure, and neurodegeneration [22]. It has side effects, including increased rates of kyphosis and cataracts in rodents and metabolic dysfunction, impaired wound healing, and aphthous ulcers in humans [22, 23]. Despite these challenges, rapamycin and rapalogs are currently being considered as an adjuvant to cardiac rehabilitation, to reduce cognitive impairment in Alzheimer's disease [24], and to enhance influenza vaccine responses in the elderly [25].

3.2 Metformin

Metformin is an approved first-line drug for type 2 diabetes mellitus (T2DM). It has been used for over 60 years with an excellent safety record. Metformin can prevent the progression of impaired glucose tolerance to overt diabetes in overweight subjects [26], including overweight subjects older than 60 [27]. Metformin has been shown to be associated with increased longevity in rodents [28–31] as well as nematodes [32], suggesting evolutionarily conserved mechanisms.

Metformin reduces oxidative stress and inflammation, with prolongation of both lifespan and healthspan in mice [11]. The mechanism of action in treating T2DM includes decreased hepatic glucose production through several mechanisms [33, 34] and improved insulin action, leading to lower glucose and insulin levels. Metformin also decreases IGF-1 signaling and inhibits the pro-inflammatory SASP [35]. Although the precise molecular target of metformin is not known with certainty, it inhibits mitochondrial complex 1, which is associated with activation of AMP-activated protein kinase (AMPK) and inhibition of mTOR. In the United Kingdom Prospective Diabetes Study and other studies, metformin was associated with a decreased risk of cardiovascular disease in human subjects compared to other anti-diabetes drugs [36–41]. In vitro studies have indicated that metformin attenuates tumorigenesis [42–48], and epidemiologic studies have suggested an association between metformin use and decreased risk of cancer and cancer mortality [49–53]. The potential protective effect of metformin against cancer is being studied intensively, with over 100 studies registered at the Clinical Trials.gov website. Data about effects of metformin on dementia are emerging, but controversial [54, 55]. Importantly, a recent observational study indicated that metformin treatment of diabetics was associated with a 15 % increase in overall survival of subjects in their 70's compared with matched control subjects without diabetes [56].

3.3 Acarbose

Acarbose has been used for treating T2DM for over 15 years. It slows processing of starch into disaccharides by inhibiting intestinal α-glucosidases in the intestine, thus reducing peaks in glucose absorption [57–59]. Acarbose has an excellent safety record, although it frequently leads to minor gastrointestinal side effects, especially on North American diets. It was tested in the ITP based on the notion that post-prandial glucose spikes might contribute to aging [60]. Acarbose increased median lifespan by 22 % in male mice, but by only 5 % in females [12]. Conversely, acarbose increased maximum lifespan by around 10 % in both sexes. Despite increased food intake, acarbose led to decreased body weight and glucose, insulin, and IGF-1 levels [12, 61]. In humans, acarbose can prevent progression of impaired glucose tolerance to T2DM [62]. It was associated with a significant decline in the risk for cardiovascular events, including myocardial infarction [63]. Acarbose was also associated with reduced incidence of hypertension and silent myocardial infarction [64]. Glucose-lowering efforts using classes of drugs (other than acarbose or metformin) in attempts to prevent cardiovascular effects associated with T2DM have failed. Thus, acarbose may impact aging processes through mechanisms other than reduced postprandial glucose spikes, perhaps including effects on the intestinal microbiome, by inducing intestinal cells to release protective peptides, or systemic absorption, perhaps leading to direct effects of acarbose on cellular function.

3.4 17-α Estradiol

17-α-estradiol is a non-feminizing endogenous estrogen that has lower affinity for estrogen receptors than its feminizing and more widely studied enantiomer, 17-β-estradiol. For many years, it has been given to post-menopausal women as a component of Premarin [65].17-α-estradiol has neuroprotective properties in animal models of Parkinson's disease through its anti-oxidative effects [66]. These effects do not seem to be mediated through estrogen receptors [67, 68]. 17-α-estradiol administration to mice in the ITP caused significant extension (12 %) in median lifespan in male mice, but not in females, with wide variations in findings among the three ITP test sites [12]. 17-α-estradiol appears to be safe in humans [66], and it is approved for topical use in Europe for treating alopecia, with few reports of side effects [69].

3.5 Growth and Differentiation Factor Analogs

Stem cell and progenitor pools can become depleted or lose functionality with aging. Cell autonomous and non-autonomous changes can occur during aging that restrict cellular replicative potential, interfering with repair or regeneration following injury or disease [70]. Non-cell autonomous changes in the stem cell niche or microenvironment can contribute to declines in adult stem cell recruitment. The chronic, low grade, sterile (non-microbial) inflammation associated with aging may result in a toxic microenvironment that leads to stem cell or progenitor dysfunction, as can dysregulated crosstalk among organ systems, for example between adipose tissue and bone [71]. Findings from parabiosis experiments, in which old and young mice are joined surgically so that they share circulations for several weeks or months, implicate age-related changes of the progenitor cell microenvironment in the observed age-related decrease of tissue repair capacity [16]. For example, following skeletal muscle injury in the older animal, circulating factors from the young animal in the parabiotic pair lead to faster repair by the older animal's progenitors than occurs in parabiotic pairs of old-with-old animals. Conversely, factors from old animals induce dysfunction and impede neurogenesis in young animals, compared to parabiotic pairs of young animals cross-circulated with young animals. Thus, tissue environmental influences, potentially including inflammation, SASP products, or circulating or paracrine factors, appear to contribute to age-related adult stem cell dysfunction, suggesting that aging progenitor cells may have at least a degree of preserved inherent function, which is suppressed by the aging environment. Provision of circulating factors from young animals to old animals, including growth and differentiation factor (GDF)-11 and oxytocin, enhances progenitor potential and repair capacity of the brain, heart, and muscle of old mice [72, 73], suggesting that pharmaceutical agents based on GDF-related factors or oxytocin might enhance regeneration in elderly humans.

3.6 Senolytics

Cellular senescence refers to the essentially irreversible cell cycle arrest induced by oncogenic and metabolic insults that appears to have evolved as a defense against tumor formation or to facilitate wound healing [18]. Senescent cells can develop a senescence-associated secretory phenotype (SASP), with release of pro-inflammatory cytokines, chemokines, thrombotic factors, extracellular matrix proteases and proteins, and growth factors [74, 75]. The pathogenic mechanisms promoted by senescence at the tissue level include inflammation, loss of functional progenitor cells, clotting, extracellular matrix dysfunction, and altered tissue architecture. Senescent cells accumulate in multiple tissues with aging [9, 18, 76–78].

Senescent cell burden is associated with lifespan. At 18 months of age, there are fewer senescent cells in fat tissue of long-lived Ames dwarf, Snell dwarf, and growth hormone receptor knockout mice than age-matched control animals, while short-lived growth hormone over-expressing mice have increased senescent cell burden [79]. Caloric restriction sufficient to increase lifespan is associated with decreased expression of $p16^{INK4A}$, a cell senescence marker, in multiple tissues of mice compared to ad libitum-fed controls [80]. Conversely, senescent cells accumulate in fat and other tissues in obesity, particularly when associated with diabetes [81, 82]. Senescent cell burden is also increased in several types of progeroid mice [13, 18, 83–85]. In longer- compared with shorter-lived mouse cohorts, senescent cell burden predicts mean and maximum lifespan [86].

Cellular senescence contributes to age-related dysfunction and is frequently evident at the sites of pathology that underlie chronic, age-related diseases, including atherosclerosis, hypertension, dementias, other neurodegenerative diseases, cancers, arthritis, osteoporosis, chronic obstructive pulmonary disease, renal dysfunction, adverse effects of chemotherapy and radiation, diabetes, and many others [4, 9, 18, 78]. The senescent cells at sites of pathology in these conditions might have systemic effects through the SASP that predispose to other pathologies. Genetically eliminating senescent cells by activating a drug-inducible "suicide" gene only in senescent cells enhances healthspan, at least in progeroid mice [19]. Genetically targeting senescent cells led to partial reversal of age-related lipodystrophy and delayed progression of frailty, sarcopenia, and cataracts [18, 19]. These findings support a link between senescent cell burden and age-related dysfunction, raising interest in developing drugs that eliminate senescent cells – *senolytics* [9]. These drugs selectively eliminate senescent cells without clearing normal cells [13]. They do so without interfering with the mechanisms that permit generation of new senescent cells when they are needed, for example as a defense against cancer or for wound healing [87]. The first senolytic agents discovered act by interfering with the pro-survival pathways that confer resistance to apoptosis to senescent cells [13]. These agents enhance cardiac and carotid vascular function in old mice, reverse gait impairment due to radiation of a leg in younger mice, and delay development of frailty, neurological dysfunction, and osteoporosis in progeroid mice. Alleviation of senescence-related dysfunction by senolytic agents is sustained for many months

even after a single oral dose. Another strategy for reducing adverse effects of senescent cells would be to target components of the SASP – *SASP protectors* [9].

For senolytic agents, SASP protectors, or other drugs that target fundamental aging mechanisms to be translated into application in humans: (1) Better animal models of human age-related diseases need to be developed. (2) Models have to be developed in which it can be tested whether any beneficial effects of candidate senolytic drugs are caused directly by clearing senescent cells or targeting the SASP, rather than "off-target" effects of the drugs on non-senescent cells. One such model has been described: gait is improved for 7 months after a single dose of senolytic drugs in mice. These agents have a half-life of <12 h. The classes of drugs that have sustained effects after a single dose modify cellular or extracellular matrix composition of tissues, and include antibiotics, chemotherapy agents, or teratogens. Few, if any, other examples exist of drugs that have sustained effects after the drug is cleared from the system. Thus, the sustained effects of senolytic drugs are consistent with senescent cell ablation. (3) Models are needed to test for possible side effects of senolytic agents. Even though continuous clearance of senescent cells from genetically-modified mice did not lead to any overt side effects in over a year of observation [19], it has been shown that cellular senescence has beneficial effects under some circumstances. Indeed, cellular senescence protects against cancer development, helps to resolve tissue fibrosis during healing, is involved in immune responses, promotes skin wound resolution, and can contribute to tissue remodeling [18, 87–89].

There are potential important advantages of senolytics over other treatment approaches. It may be feasible to administer these agents intermittently, perhaps for a day or two every few months or once a year during periods of good health. Furthermore, unlike microbes or cancer cells, senescent cells do not divide, suggesting that acquired drug resistance to senolytics is unlikely to occur. Finally, it has been shown that removing only a fraction of senescent cells can have beneficial effects. Indeed, while only 30 % of senescent cells were removed from *INK-ATTAC;BubR1$^{H/H}$* mice treated with AP20187 to activate the "suicide" gene expressed in their senescent cells, healthspan was enhanced [19]. Similar dramatic improvements in age- and senescence-related dysfunction were found in mice treated with senolytic agents that remove from 20 to 70 %, but not all senescent cells from tissues [13]. Thus, senolytic agents hold promise for treating age-related diseases and dysfunction.

3.7 Angiotensin Converting Enzyme Inhibitors and Angiotensin II Receptor Blockers

Angiotensin converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARB's) are widely used antihypertensive drugs. Independently from their antihypertensive effects, ACEi and ARB's reduce mortality due to heart disease and protect the kidneys in subjects with diabetes [90]. ARB's appear to be associated with reduced cancer risk and mortality [91, 92] and decrease incidence and

progression of dementia [93–95]. Genetic disruption of angiotensin II receptor expression prolongs mouse lifespan and variations in the angiotensin II receptor gene are associated with exceptional human longevity [96, 97]. ACEi increase lifespan in healthy as well as hypertensive rodents [98–101]. ACEi and ARB's increase lifespan and improve cardiovascular function in rats [102], as well as delaying age-related decreases in renal and cognitive function [103]. Thus, ACEi and ARB's alleviate a range of age-related disorders and phenotypes in humans and rodents, as well as increasing lifespan in rodents. How they impact molecular pathways tied to fundamental aging mechanisms remains to be elucidated.

3.8 Sirtuin Activators and Flavonoids

Sirtuins are mammalian NAD-dependent deacetylases related to Sir2, one of the first longevity genes discovered in yeast. Genetically enhancing the activity of Sir2 or its homologs increases yeast and possibly fruit fly and round worm lifespan [104–107]. There are seven mammalian Sir2 homologs. Overexpression of SIRT6 increases longevity in male mice and protects against the deleterious effects of high-fat feeding, possibly due to repression of IGF signaling [108]. Overexpressing other mammalian sirtuins has not been found to increase lifespan, but does have beneficial effects in physiology and a number of age-related diseases [109]. Mice overexpressing SIRT1 have reduced incidence of cancer and osteoporosis and enhanced wound healing and glucose tolerance [110]. Possibly, sirtuin activating compounds may phenocopy some of these effects. Resveratrol, a plant flavonoid that leads to activation of sirtuin-related pathways, extends yeast and fly but not mouse lifespan [111–113]. Newer sirtuin activators, including SRT1720 and SRT2104, extend healthspan and lifespan in non-obese mice [114, 115]. Sirtuin activators alleviate the effects of high-calorie feeding in mice, rhesus monkeys on a high calorie diet, and possibly humans [116–120]. However, it is unclear if sirtuin activators improve glucose tolerance in humans [121, 122]. Flavonoids related to resveratrol may eventually prove to be effective in targeting certain age-related disorders.

Flavonoids and pathways impacted by them are involved in cellular senescence. Interfering with sirtuin signaling by targeting the deleted breast cancer-1 gene product protects against generation of senescent cells in obesity [123]. Quercetin, a flavonoid, is senolytic, particularly in combination with dasatinib, a tyrosine kinase inhibitor [13].

3.9 Aspirin and Salicylic Acid

Aspirin is an analgesic, anti-inflammatory agent and an anti-platelet thrombosis inhibitor. Aspirin appears to reduce cancer risk [124–126], and it was found in the Intervention Testing Program to increase median but not maximum lifespan in mice

[127]. Many of its clinical effects are due to irreversible acetylation of serine residues in the active site of cyclooxygenases. Salicylic acid, which is a metabolite of aspirin as well as the related anti-inflammatory drug, salsalate, is an allosteric activator of AMPK [128]. Salicylic acid increases lipid utilization in mice through AMPK activation and also enhances glucose homeostasis through an independent, as yet uncharacterized mechanism [128]. Salsalate improves glucose control in diabetics [129]. Aspirin activates AMPK in human colorectal cancer cells [130]. It enhances lifespan though activation of AMPK and Daf-16/Foxo3 in worms [131]. These results from human and experimental animal studies indicate that aspirin and related drugs may affect fundamental aging processes and age-related diseases through several mechanisms, including inflammation-related and nutrient processing-related pathways.

3.10 Others

At least a dozen more agents that target basic aging mechanisms are being developed but are as yet unpublished. New rapalogs (analogs of rapamycin) are being developed that have lower side effect profiles than rapamycin, including less glucose intolerance and gastrointestinal irritation [132, 133]. Drugs affecting mitochondrial function, drugs that target protein synthesis or enhance autophagy, and caloric restriction mimetics are among compounds that are currently under development. No doubt, many other interventions are being developed or will be devised in the near future that will increase life- and/or healthspan in experimental animals and potentially humans. Some of the agents under development are already used clinically for other conditions. Others are new chemical entities and new classes of compounds. Pre-clinical and proof of concept clinical trials paradigms need to be developed to identify those compounds emerging from the expanding basic biology of aging pipeline that merit continued translational effort.

3.11 Combinations

Agents that target diverse aging pathways are being combined to test if their effects are synergistic in experimental animals. New combinations will probably be devised in the near future. Using drug combinations follows the path used for developing clinical interventions in other emerging fields, such as for treatment-resistant cancers or viral diseases. In these cases, combinations are often used initially in order to enhance treatment effectiveness, reduce doses of each individual drug, and thereby minimize side effect severity. As more becomes known about interventions that target fundamental aging processes, next generation single drugs that are more precisely targeted could supplant combinations of earlier generation drugs. Alternatively, combination therapy involving multiple drugs with non-overlapping

mechanisms of action has remained a mainstay for treating many common diseases, including coronary artery disease, chronic obstructive pulmonary disease, tuberculosis, and most cancers, and could remain the strategy of choice for targeting fundamental aging processes and age-related chronic diseases.

4 Translation

The promise of interventions that target fundamental aging mechanisms is that they could have a huge impact on important outcomes: independence, function, quality of life, and freedom from pain and disability through alleviating many different age-related diseases, conditions, and syndromes as a group. Data from preclinical experimental animal studies and limited human studies of the interventions described above suggest they might be effective in slowing age-related decline in multiple organs, tissues, and functions, potentially shifting the curve of aging phenotypes and chronic disease predisposition to the right. If clinical trials of these interventions broadly affect multiple age-related disorders, the implications for health care and society would be enormous [2, 9].

The first step in this path will be one or several focused, small, short term clinical trials with several objectives. Ideally, each of these trials would test proof of a principle in a specific, but generalizable group of subjects. They would provide information needed to design and scale-up for larger trials. They would also provide biological data that could spur reverse translational studies in preclinical animal models to dissect mechanisms and determine next steps in translating each aging intervention into humans. We consider here the potential proof of concept clinical trial strategies for studies of interventions that target basic aging mechanisms.

Successfully translating interventions that are effective in experimental animals into clinical application is difficult, lengthy, and expensive. It can take over 17 years to complete translation into clinical practice, even in areas with an established translational tradition, such as infectious diseases or oncology. The needed steps include pre-clinical studies to test efficacy, safety, and pharmacokinetics in mammals, usually in at least two species and following good laboratory practices, as stipulated by regulatory agencies [134]. For pharmaceutical interventions that target basic aging mechanisms and that are intended to treat age-related chronic diseases, it would make sense to conduct these safety studies in old animals, rather than the young mice that are currently commonly used.

Before preclinical animal studies begin, the nature and goals of clinical studies need to be planned so that the preclinical test program can be designed to yield data useful for designing and refining the clinical trials. Even for preclinical studies in animals, it is important to select outcome measures that have been validated, are reproducible, can be measured in a short time-frame, are as non-invasive as possible, are accepted by regulators, and are related to the outcomes to be tested in subsequent clinical trials. Iterative bench-to-bedside coupled to reverse translational bedside-to-bench developmental phases are generally necessary, particularly for

investigational new drugs (IND's). This requires a close partnership between basic biologists and clinicians with a strong basic biology background. While it is feasible to repurpose existing agents not covered by patent protection by conducting publicly funded trials in academic settings, without early attention to protecting intellectual property, the chances of getting a treatment to patients through commercial partnerships are reduced. On the other hand, commercial entities might find attractive the possibility that seeking FDA approval for adding a label indication to an existing drug might require less effort and take fewer years than a de novo drug approval. Early attention to marketing and the potential interest of payers for interventions can motivate commercialization and moving treatments into the clinic.

4.1 Potential Clinical Trial Scenarios

Elderly subjects have generally been excluded from clinical trials, especially those involving new formulations. Since several interventions that target basic aging mechanisms and extend lifespan appear to be effective in mice, there is an opportunity to select those that are more readily translatable. Some interventions, such as lifestyle modifications, are particularly challenging (e.g., caloric restriction in the face of an obesity epidemic). Desirable characteristics of interventions suitable for translation include: (1) low toxicity and few side effects, (2) effectiveness of oral as opposed to parenteral administration, (3) low dosing frequency (i.e., relatively long half-life), (4) stability, (5) scalability and low manufacturing cost, (6) detectability in blood, and (7) importantly, effectiveness if initiated in later life or once symptoms have started to develop. Interventions that need to be applied in earlier life while subjects are still asymptomatic with an expectation of affecting health much later in life would be difficult to translate into humans, particularly if they exhibit virtually any side effects.

It will of course be impractical to study the success of experimental strategies to extend lifespan in humans within a reasonable time frame. Therefore, feasible and clinically relevant trials paradigms must be devised to test if agents that target fundamental aging processes can be translated into clinical use by developing more rapidly detectable outcomes. Short-term clinical trials in symptomatic individuals are feasible, and some early clinical trials have already commenced using some of the drugs discussed above that enhance life- or healthspan in rodents. For example, trials of rapamycin in Alzheimer's disease are currently underway or about to begin and a trial of rapalogs on enhancing immune responses of elderly subjects to vaccination has already been reported [25]. Resveratrol congeners are being developed to treat type 2 diabetes [135]. Collection of ancillary biological data would help inform later studies by providing additional ways to predict risk, response to the intervention, and data examining effects on basic aging mechanisms.

At least six potential drug development scenarios can be envisaged in which agents that target basic aging processes might first be tested. These include treatment of: (1) multiple co-morbidities, (2) otherwise fatal conditions, (3) frailty and

geriatric syndromes, (4) resilience enhancement, (5) localized diseases related to fundamental aging processes, and (6) accelerated aging-like states.

4.1.1 Multiple Co-morbidities

Most clinical trials have been focused on younger subjects with a single target condition and have excluded older subjects with co-morbidities. However, multiple age-related chronic diseases often occur within the same older individuals. Therefore, agents that target basic aging processes might simultaneously alleviate several age-related chronic diseases within the same older subjects or delay the appearance of new chronic diseases in subjects who have already developed their first age-related chronic disease. Such scenarios will require novel clinical study designs. A potential scenario for initial small-scale proof-of-principle trials of candidate drugs would be to study their effect in elderly subjects with combinations of two or more of: atherosclerosis, hypertension, memory impairment, diabetes, chronic obstructive pulmonary disease, renal dysfunction, or other age- or senescence-related conditions. Outcomes could be surrogate endpoints already recognized by regulatory agencies, such as blood pressure, psychometric indices of cognitive function, fasting glucose or HbA_1C, circulating lipids, left ventricular function or hypertrophy, pulmonary function tests, etc. The endpoints could be combined into a composite score, although this carries the risk that an effective drug may appear less than effective if one of the composite endpoint components is affected in a direction opposite to that expected. For example, rapamycin may lead to improvements in several age-related measures of function, but also causes decreased glucose tolerance [136].

4.1.2 Otherwise Fatal Conditions

Another scenario for initial proof-of-concept trials of agents that target basic aging processes may be to test their impact on otherwise fatal conditions for which either very invasive or no effective treatments are available. In the case of senolytics, these include certain cancers, cancer predisposition syndromes, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and primary biliary cirrhosis, among others [3, 9, 137]. In some cancers, caloric restriction, senolytics, or other approaches that target fundamental aging mechanisms might facilitate use of higher chemotherapy or radiation doses or enhance effectiveness of these treatments.

4.1.3 Frailty and Geriatric Syndromes

Frailty is an age-related syndrome that involves weakness, loss of function, and decreased resilience [138–147]. It can be diagnosed through clinical scales that are moderately sensitive and specific, involving assessments of a combination of weight

loss, decreased activity, weakness, fatigue, and burden of chronic disease and disabilities [144, 146, 148, 149]. The prevalence of frailty increases with aging [138–149], and it is associated with chronic diseases, loss of independence, high mortality, and the "geriatric syndromes" of sarcopenia, immobility, falling, cachexia, depression, and confusion, as well as chronic inflammation.

Subjects with mild or moderate degrees of frailty or sarcopenia would likely be better candidates for initial trials of agents that modulate fundamental aging processes, especially sterile inflammation and perhaps cellular senescence, than subjects with advanced, potentially irreversible frailty. Effects of agents that target fundamental aging processes could be studied on outcomes indices that are related to frailty, predictive of mortality, and are recognized or close to being recognized by drug regulators [18, 150, 151]. These include timed walking distances, strength measurements, inflammation-associated and SASP markers such as circulating IL-6, MCP-1, or PAI-1, pulmonary function (e.g., VO_2), renal function tests, among others.

4.1.4 Resilience Enhancement

Resilience in this context refers to the ability to recover from perturbations such as surgery, anesthesia, chemotherapy, radiation, a heart attack, fracture, or stroke. Resilience declines with aging and is associated with concurrent or subsequent overt frailty. Studies involving a stressor and relevant outcome measures could be a means for testing proof of principle for interventions targeting aging processes to determine if they enhance resilience and impact multiple clinically relevant outcomes in relatively short clinical trials. The stressor tested could be major, such as hip fracture after a fall, or minor, such as routine vaccination. The stressor could be elective and planned, such as elective surgery, chemotherapy, therapeutic radiation, or immunization, or unplanned, for example an acute illness, such as pneumonia, a heart attack, or a stroke. In the case of elective stressors, the drug could be administered before the stress occurs and in the case of either elective or unplanned stressors, during recovery after the stress-inducing event.

Some of these types of clinical resilience trials have already been reported. For example, the response to influenza immunization is improved a few weeks following a brief course with low doses of a rapalog [25]. Responses to chemotherapy are enhanced and its side effects reduced if the chemotherapy is preceded by a brief caloric restriction [152]. The effects of candidate agents that target basic aging mechanisms could be tested in multiple clinical trial scenarios, for example: (1) strength, endurance, nausea, and appetite after chemotherapy; (2) time needed for return of function, wound healing, incidence of delirium, or discharge disposition after elective surgery; or (3) recovery after myocardial infarction in elderly subjects. Secondary outcomes of these types of studies could include measures of function, comorbidity, and blood or tissue biomarkers of basic aging mechanisms.

4.1.5 Localized Diseases Related to Fundamental Aging Processes

Certain localized disorders involve operation of pathogenic processes shared with those that are associated with chronological aging. These conditions might be alleviated by local administration of agents that target those basic aging processes, such as aerosols, local injections, or topical skin solutions. For example, idiopathic pulmonary fibrosis is associated with accumulation of senescent cells in the lung [153, 154]. Subjects with this potentially fatal condition that lacks effective treatment options might benefit from aerosol delivery of senolytic or SASP-protective agents. This would need to be tested first in animals, for example in mice with pulmonary fibrosis induced by aerosolized bleomycin [155] crossed with animals from which senescent cells can be cleared genetically or treated with senolytic drugs. A similar strategy could be used for chronic obstructive pulmonary disease, which is associated with senescent cell accumulation in the lungs, following cigarette smoke exposure. Another example is osteoarthritis, an inflammatory condition that can affect multiple joints and becomes increasingly common in old age, and which is at least partially associated with accumulation of senescent cells and SASP factors (inflammatory cytokines, matrix metalloproteinases) in joints [156]. Currently, this disorder is treated with oral analgesics, anti-inflammatories, and intra-articular steroid injections. The oral agents have to be administered frequently, often at least daily. Steroid injections have effects that last for weeks, but often have to be administered repeatedly. Unfortunately, repeated steroid injections eventually contribute to worsening joint damage. Potentially, injected or systemic agents that target basic aging processes would have a more sustained effect and fewer adverse effects than currently used treatments.

4.1.6 Accelerated Aging-Like States

Several conditions have features resembling an accelerated aging-like state, including obesity and diabetes, long term effects of chemotherapy or radiation, HIV or side effects of the drugs used for treating it, and progeroid syndromes [3, 9, 18]. These conditions represent a potential scenario for proof-of-principle studies of candidate agents that target basic aging processes. Obesity and diabetes are associated with accelerated onset of other age-associated conditions, including atherosclerosis, vascular dysfunction, sarcopenia, early menopause, cancers, cognitive impairment and dementia [81, 157, 158]. Survivors of cancers who were treated during childhood with chemotherapy or radiation can develop frailty and sarcopenia, diabetes, cardiac disease, cognitive impairment (“chemo-brain”), and second, unrelated cancers by mid-adulthood [159, 160]. Progeroid syndromes with phenotypes resembling an accelerated aging-like state have been associated with increased senescent cell burden and accentuation of other fundamental processes that are also associated with chronological aging [161]. Short term effects of candidate agents on muscle strength, metabolic, cardiovascular, cognitive, or other functional measures in these subjects could be tested in initial proof-of-principle trials. For example, trials of rapalogs for Hutchinson-Guilford progeria are currently being planned.

4.2 Preclinical Studies

Animal models reflective of the potential indications for agents that target aging mechanisms in humans are needed to facilitate the preclinical studies required before proceeding to proof-of-principle human trials. An advantage to the aging biology field is that because aging is not a disease, unlike disease-focused studies, it is not necessary to 'model' aging: natural aging occurs in virtually all species as a natural model that does not need to be modelled. Mortality can be followed in animals that are otherwise 'normal', not genetically or otherwise modified. For studying some age-related diseases, relevant genetically modified mice are available or disease-inducing pharmacological or dietary manipulations have been devised. However, in many cases there are either no models of age-related chronic diseases or only models that are imperfect. Some mouse models are available that are reasonably close approximations to human progerias that result from single gene mutations [18, 83–85]. Drug candidates need to be tested in such mice before human subjects with these diseases. In addition to helping in devising interventions for these diseases, such studies would indicate if the drug candidates can resolve aging-like or healthspan phenotypes, such as impaired glucose homeostasis, grip strength, exercise endurance, activity, cardiac, or neurological or cognitive dysfunction. Senolytic agents have been reported to alleviate frailty, neurologic dysfunction, and osteoporosis in progeroid mice [13].

For multifactorial polygenic human diseases that become clinically manifest in later life, animals with single gene mutations that develop superficially similar syndromes in early life have drawbacks for drug development. For example, single gene mutations that lead to phenotypes resembling Alzheimer's disease in young mice do not fully phenocopy human Alzheimer's disease. Animals with dysfunction-provoking mutations that are inducible in later life may be better for testing agents that target basic aging mechanisms. An aging tissue microenvironment would be recapitulated in such mice. Furthermore, a range of mammalian species beyond mice is needed to test generalizability and meet regulatory requirements, especially for investigational new drugs.

Manipulations can be used in experimental animals to model human age-associated disorders or clinical stresses, including high fat feeding, localized or systemic radiation, pharmacological interventions (*e.g.*, chemotherapy, streptozotocin, Parkinson's-inducing agents), localized pharmacological interventions (*e.g.*, inhaled bleomycin or cigarette smoke), cancer xenografts, skin wounding, or surgically-induced arthritis. Effects of candidate agents on a panel of such models could be helpful in selecting potential clinical applications for each new drug, as long as the test animals are of the appropriate ages. In some cases, such as Alzheimer's disease, screening for agents might be more practical in human cell culture systems that mimic disease pathology more faithfully than currently available animal models [162]. Also, for investigational new drugs, systems for medicinal chemical optimization and testing toxicology and pharmacokinetics (absorption, distribution, metabolism, and excretion) need to be developed using aging cell culture and animal models, rather than young cultures or animals.

4.3 Clinical Trials

Clinical trials are prospective human studies used to determine whether new biomedical or behavioral interventions are safe, efficacious, and effective. Clinical trials are generally categorized in phases. Phase 0 studies are used to determine if investigational new drugs act in humans as expected from preclinical animal studies, to acquire preliminary data about their pharmacokinetics or pharmacodynamics, to select the most promising lead candidates, or to determine biodistribution characteristics. Phase 1 trials are used to provide information about the metabolism and pharmacologic actions of the candidate drug in humans, side effects associated with escalating doses, and early evidence for effectiveness. These trials may include healthy participants or patients with the disease under study. Phase 2 studies are controlled clinical trials that evaluate the effectiveness of the candidate drug for a particular indication in subjects with the disease or condition under study and to identify the most common short-term side effects and risks. Some trials are combinations of Phases I and II, investigating both efficacy and toxicity. Phase 3 studies are expanded controlled or uncontrolled trials that follow the acquisition of preliminary evidence about the candidate agent in Phases 0–2, and are designed to provide additional information about overall risk-benefit relationships as well as for drug labeling. Generally, these trials compare new candidate drugs to drugs already in use for that condition. Phase 4 studies are conducted after clinical use at the population level has begun. They provide additional information about the risks, benefits, comparative effectiveness, and optimal use of the drug. These studies monitor effectiveness of the approved drug in the general population and provide data about any adverse effects that become apparent with widespread use.

Information collected during proof of concept and later phase trials can be used for following subjects to determine long-term clinical outcomes. Long-term outcomes can provide useful information about whether the agent also delays or prevents other chronic diseases, functional pre-frailty or frailty, loss of resilience, or loss of independence.

5 Biomarkers

There are three types of biomarkers that can catalyze clinical trials: surrogate endpoint biomarkers, drug activity biomarkers, and biomarkers related to mechanisms. Surrogate endpoint biomarkers are those that can be substituted for a clinical event endpoint as the outcome of a clinical trial. An example is fasting blood sugar as a surrogate outcome for a drug treating diabetes, as opposed to hard clinical endpoints such as weight loss, polyuria, or diabetic crises. Another surrogate biomarker is hypertension as a predictor for risk of stroke. These types of surrogate endpoints take years or decades to achieve acceptance by the medical community and regulators, for usage in place of the hard clinical event endpoints that the drug is expected

to affect. There is a lack of surrogate biomarkers that: (1) predict lifespan or healthspan in humans, (2) have been demonstrated to vary in the same direction as lifespan or healthspan in response to interventions, and (3) are generally accepted as potential endpoints for clinical studies. It could take decades to do the studies that would convince the medical community and regulators that a surrogate biomarker reliably predicts and tracks longevity in humans and would be an acceptable clinical trial primary outcome endpoint. Some work has begun to validate surrogate outcomes for studies of frailty or age-related disability, such as clinical frailty scales involving physical function tests or assays of circulating cytokines. Although frailty, healthspan, and resilience biomarkers are some way from gaining regulatory acceptance and need further refinement in experimental animals and human studies, they are more within reach for use in clinical trials than biomarkers predictive of human longevity.

The second types of biomarker are those that reflect drug delivery, activity, or efficacy. As an example, in the case of rapamycin or related agents, assays of blood levels of the drug would be useful to ensure compliance. Assays of cellular S6 kinase activity in blood cells or tissue biopsies could be used to follow drug delivery and mTOR pharmacodynamic activity and would be useful in adjusting dose intensity of the intervention.

The third type of biomarkers includes those that test the mechanism of action in humans. In studies of candidate agents that are intended to target fundamental aging mechanisms, it would be desirable to measure a range of parameters suspected of being associated with aging processes to test if this is really how the drug could work. These biomarkers can be helpful, but it should be noted that precise information about the mechanism of action is not always needed for drug development. Indeed, for many drugs currently in widespread use in humans, the molecular mechanism of action was not determined before clinical use. Metformin is an example of this: its molecular mechanism of action is still not precisely understood. On the other hand, knowledge of the mechanism of action might help to predict side effects, although for many side effects this knowledge has not helped. Nevertheless, markers related to molecular aspects of aging might help to define the mechanism of action of the intervention in humans. This may stimulate reverse translational laboratory studies that could inform future clinical trials, spur discovery of new drug targets and development of new agents, and lead to optimization and refinement of preclinical animal models.

6 Personnel Needed

There is a divide between biologists studying aging processes and clinicians treating the elderly. Geriatricians rarely have basic biology training. Few geriatricians have investigational new drug (IND) experience. In the US, there are 7,000 Board-certified geriatricians. Only around a dozen have R01 grants from the Division of Aging Biology at the National Institute on Aging. Worldwide, there are few

geriatricians who are also basic aging researchers. This is unlike most other biomedical fields, such as endocrinology or hematology, in which academic clinicians frequently conduct laboratory research. Few geriatricians attend meetings in the basic biology of aging. Conversely, basic biologists only rarely attend clinical geriatrics meetings. Unlike in other areas of medicine, such as infectious diseases or oncology, few geriatricians have experience in translating IND's into clinical application. Thus, there is a shortage of investigators with the combination of basic biological, geriatric medical care, and human trials skills needed to design and conduct the pre-clinical and clinical studies and navigate the regulatory framework necessary to translate recent advances from the basic biology of aging into clinical practice. A new group of investigators needs to be trained in the basic biology of aging who have a thorough grasp of translational strategies and clinical geriatrics, as well as a group of geriatricians with sufficient understanding of the fundamental biology of aging and clinical trials methodology, to spearhead the process of taking IND's through pre-clinical studies, clinical trials, and regulatory approval. This could take well over 5 years, even if we begin training these investigators right away. In the meantime, we need to encourage collaborative strategies that bring teams of basic biologists, geriatricians, and clinical trials investigators together to translate agents that target fundamental aging mechanisms into the clinic. We also need to develop clinical trials networks, perhaps emulating successful approaches taken by the networks in the cancer field.

7 Conclusions

We are on the verge of a new era in the basic biology of aging. There is tremendous potential for drugs that target aging fundamental mechanisms to prevent or treat age-related disorders as a whole, rather than one at a time. We are at the point where it seems increasingly likely that interventions targeting aging mechanisms could begin to be tested to delay, prevent, alleviate, or reverse multiple age-related chronic diseases and disabilities that afflict the elderly. If true, and if the interventions effective in targeting fundamental aging mechanisms in mice can be translated into humans, geriatrics practice and all of medicine as we know it would be transformed. Despite this, financial, infrastructure, and personnel resources are insufficient to expand or even sustain the important discovery, mechanistic, and interventional basic biology of aging pipeline, let alone to fuel translation of promising drug candidates into clinical practice. Creation of new experimental paradigms, development and validation of relevant, measureable outcomes, sufficient funding, and training of investigators with new skills are necessary. A strategy to increase resources and personnel and to optimize existing resources is needed to accelerate progress and avoid duplication without depriving the basic biology pipeline.

While it seems possible that drugs acting on basic aging processes will alleviate many age-related conditions, it is unlikely that it will be feasible to reverse every age-related change in the foreseeable future, if ever. Some age-related changes

involve progenitor cell depletion, some inflammation, others extracellular matrix and structural changes (*e.g.*, cataracts, osteoporosis), and yet others involve macromolecular dysfunction. Different fundamental age-related disruptions are implicated to different extents in different organs and cell types. This suggests that several strategies will need to be combined to be maximally effective: no one strategy is very likely to be a panacea. A process involving at least two overall steps may ultimately be needed, based on the longstanding principle of first removing damaged tissues and then replacing them with good tissue. The first step may involve eliminating damaged or senescent cells, blunting the senescence-associated secretory phenotype, reducing inflammation, and removing cytotoxic lipids and lipofuscin, protein aggregates, damaged macromolecules, and advanced glycation endproducts. The second step may involve transplanting stem cells, differentiated cells, tissues, or organs or restoring endogenous stem cell or progenitor function to repopulate damaged organs. Advances are being made in each of these areas, and effective combined approaches may one day be feasible. If any or all of this comes to fruition, if we are able to push back age-related diseases as a group and extend healthspan, and if this can be translated into clinical treatments, health care as we know it would be transformed with myriad economic and social consequences and benefits.

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