Pramod C. Rath *Editor*

Models, Molecules and Mechanisms in Biogerontology

Physiological Abnormalities, Diseases and Interventions

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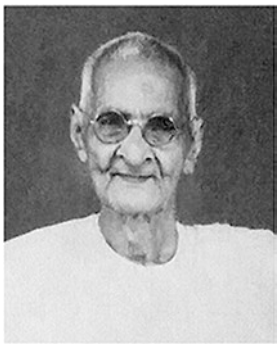

Purna Chandra Rath
(08 October 1889–01 May 1982)

This book is dedicated to Kaviraj Purna Chandra Rath (1889–1982), an exponent of Ayurveda, the ancient Indian traditional science of life, health, and medicine, who lived up to the age of 93 without any major health issue in the holy city of Puri situated in the state of Odisha on the east cost of the Bay of Bengal in India. He was educated in Sanskrit, Ayurveda, and prepared large number of Ayurvedic medicines from natural medicinal herbs and other materials according to the ancient Ayurvedic scripts at his home dispensary. He had successfully

practiced Ayurveda for 60 years for treatment of thousands of patients suffering from various diseases across the state of Odisha. He had played key role in the establishment of Ayurvedic schools and trained next generations of experts, many of them learnt the age-old science by staying with him at his home as a "Gurukul" free of cost. For this exemplary contributions in Ayurveda and public life, he was awarded the prestigious title of "Vaidyaratna" by the then Viceroy of British-India in 1938. Purna Chandra had written many books on Ayurveda and poetry in Odia, the language of Odisha later became the sixth classical language of India, for the benefit of people. His biography was written by a Professor of Odia literature, describing the journey of his life beginning as a village boy to a highly acclaimed authority in Ayurveda, who demonstrated the unmatched value and utility of this ancient Indian traditional medicine system for the society at a nominal cost. This is an ideal example of healthy aging of a socially relevant person who lived in India several decades ago.

Original Address: Late Vaidyaratna Kaviraj Purna Chandra Rath, South Gate, Puri-752001, Odisha

Preface

The most simple and elegant design of nature is DNA being the genetic material. It takes only four letters A, G, C, T to describe the entire cellular living world. We can make sense, if DNA makes a protein sequence, because proteins are directly linked to cellular functions. But only about 2% of the human genome makes all the proteins, the rest 98% is nonprotein coding in nature. Genes (DNA) are known to be associated with functions through their mutations leading to loss of function. Transmission of protein-coding genes and their mutations through generations has revealed genetic control of functions and its link to diseases. This is not yet fully explained for all RNA genes and nonprotein-coding DNA. Epigenetic regulation of chromatin through DNA methylation, histone modifications, and enzymes and cofactors regulating them has become prominently responsible for both normal health and disease phenotypes. Metabolic control mechanisms and key metabolites in turn have emerged as promising gene and chromatin regulatory agents. Thus a DNA sequence variation, a small RNA, an abnormal protein, and a dysregulated metabolite can in principle become a biomarker, a diagnostic criterion, a drug target, and a prognosis parameter in case of diseases. Strong foundations of biology and cutting-edge-technology for health together can provide cure from diseases. Therefore, biology must melt into technology to provide solutions to maintain good health and cure from diseases. Aging is not a disease by itself, but old organisms become more prone to diseases. Old age invites many diseases.

A cell born will die, a human adult will age, and aging will invite diseases. Physiological abnormalities arising in various cell types and tissues during aging of an organism can provide clues to the possible causes of the age-related diseases. Diagnostic biomarkers can help detect indications of age-related diseases at an early stage. Drug targets can help monitor prognosis of disease and measure outcome of therapy. Since prevention is always better than cure, certain interventions like dietary or caloric restrictions, regular exercise, and maintaining good lifestyle have been documented to provide significant benefits during aging. This includes reduction of oxidative stress, delayed manifestations of aging, extension of longevity or lifespan, reduction of age-related diseases, and increased healthspan. Every elderly may have some health-related difficulty, but still possesses enormous expertise, experience, and blessings to hand over to the next generation. However, physical and social isolation comes in between and often makes this a remote possibility. A database of the elderly may prove to be helpful in this direction. More research

on socio-biology and disease-biology should also be helpful for understanding both individual and population aging dynamics and networks.

This book contains twenty chapters written by authors from fifteen national/international university/institute(s) including six Indian universities, two hospital/medical institute(s), two Indian Institute of Technology/National Institute of Technology, and five university/medical institute(s) from Korea, Japan, and USA. It has two parts. Part I describes alterations in nervous system, genes, hormones, and immunity in relation to aging and age-related diseases. It has neurological problems, molecular markers for neurodegenerative diseases, oxidative stress-epigenetic modifications and neurodegeneration, polyglucosan bodies in aged brain and neurodegeneration, nociceptors and pain, REM sleep-noradrenaline, human retinal changes, DMD and BMD, mitophagy, genetics and genetic syndromes, stress hormones of hypothalamus-pituitary-adrenal (HPA) axis, sex steroids and their receptors, immunosenescence-inflammaging-cancer-anemia, and bone marrow stem cells in relation to aging and age-related diseases. Part II describes some interventions for healthy aging including calorie and dietary restrictions, regular exercise, nutrition, and care for the elderly.

I sincerely thank all the authors for contributing the chapters balancing recent information about aging and age-related diseases with an attempt to link molecules with mechanisms during aging. It is expected that the book will be helpful to graduate students, researchers, and clinicians. How cellular processes and mechanisms suffer from age-related changes and thus give way to diseases is the focus. Molecular markers and targets linking aging to diseases will facilitate our understanding and approach to intervene aging and promote healthspan. Longevity or lifespan should essentially and ideally be the healthspan. With this goal, research on aging and geriatrics should bring a smile on the elderly face. I thank all the authors who have contributed the chapters unconditionally. Apologies are due for not being able to make a mention of all the work done in this field. We expect this book will be useful to people from academic, medical, and policy-making institutions. The School of Life Sciences at Jawaharlal Nehru University, New Delhi, India, is acknowledged for all help.

Pramod C. Rath, Editor

Contents

About the Editor

Pramod C. Rath is a Professor of Molecular Biology at the School of Life Sciences, Jawaharlal Nehru University, New Delhi. He received his Ph.D. in Zoology (Biochemistry) in 1988 from the Banaras Hindu University, Varanasi, on the topic "gene expression during aging" under the supervision of Prof. M. S. Kanungo, who started research on "biology of aging" in India. He completed his postdoctoral research at the Institute of Molecular Biology I, University of Zurich, Switzerland, with Prof. Charles Weissmann, a well-known molecular biologist.

He has 28 years of teaching and research experience, having mentored 18 Ph.D. and 4 M.Phil. students. He has published his research in respected international journals, such as *Ageing Research Reviews*, *Molecular Neurobiology*, *Journal of Molecular Neuroscience*, *International Journal of Developmental Neuroscience*, *RNA Biology*, *PLOS ONE*, *International Journal of Biological Macromolecules*, *Molecular Biology Reports*, *Journal of Biosciences*, *Biochemical and Biophysical Research Communications*, *Biochimica et Biophysica Acta*, *FEBS Letters*, *Journal of Clinical Immunology*, etc. He has also published a Springer book, titled *Topics in Biomedical Gerontology*.

He teaches molecular biology, molecular genetics & genetic engineering and cell signaling to master's and Ph.D. students. Research in his laboratory is focused on cytokines, transcription factors, cell signaling and diseases, genomic biology of repetitive DNA and noncoding RNA, bone marrow stem cells, and molecular aging in mammals. He has received numerous awards and fellowships and has been the Vice-President of the Association of Gerontology (India) and Acting Dean at the School of Life Sciences. He is a Member of several national academic and scientific committees.

Part I

Alterations in Nervous System, Genes, Hormones and Immunity During Aging

Neurological Problems of the Elderly

1

Laxmi Narayan Tripathy

Abstract

Old age has various definitions, as the life expectancy advances due to improved healthcare and hygiene. But no one wants to get old! Unfortunately it dawns on everybody, you like it or not. Everything on this planet has a shelf life or 'best by date'. Although one should be graceful at ageing, most systems of the body do wear out leading ultimately to death in a natural process. Hence, there is no immortality on earth!

Keywords

Old age · Neurological problems

The brain, the crown jewel of the body, is no exception to this rule. The natural reduction in blood circulation due to narrowing of the lumen and stiffness of the arteries, diminution of neurotransmitter and hormonal content as well as loss of neuronal volume result in impairment of memory and difficulty in carrying out daily tasks which were taken for granted in younger age. In any abnormal situation, the process gets accentuated, resulting in stroke (blood circulation defects), dementia (neuronal defects), Parkinson's disease (neurotransmitter deficiency), hypopituitarism, dyselectrolytaemia (hormonal deficiency), etc.

The commonly occurring neurological problems involving the brain in old age are stroke; dementia, including Alzheimer's disease (AD) and Parkinson's disease (PD); normal pressure hydrocephalus (NPH); subdural haematoma (SDH); and delirium due to low sodium or hyponatraemia (dyselectrolytaemia). In the spine, the most common condition in this group of patients is degenerative spine, otherwise known as spondylosis.

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1.1 Stroke

Stroke is a clinical syndrome of sudden focal or global cerebral dysfunction (Cincinnati Scale) [1] lasting more than 24 h of presumed vascular origin. Stroke is classified into two types, i.e. occlusive and haemorrhagic. Occlusive stroke may result in cerebral infarction. It is also known as ischaemic stroke. Ischaemic stroke is the commonest type, accounting for about 85% of all strokes. The complete stoppage of blood flow for longer than 5 min produces irreversible damage to brain cells. The age-adjusted prevalence rate of stroke is between 250–350 and 100,000 [2]. Nowadays, acute ischaemic stroke within 4 h of onset can be treated with thrombolytic (clot dissolving) agents (e.g. alteplase, a recombinant tissue plasminogen activator) or by thrombectomy (clot evacuation) [3] by the use of devices through intravascular route with good results. Prompt prehospital and emergency management of patients affected with stroke yields good results. If untreated, the area of neuronal death enlarges to include the surrounding penumbra area.

In a study, after treatment with alteplase, 13% of the patients show very good recovery, 19% had partial recovery, 3% had complications with severe disability or death; and the rest (65%) remained stable with no major change [4].

The second type of stroke is haemorrhagic (Fig. 1.1a, b), meaning blood clot in the brain. This is much less in incidence than ischaemic stroke (15%).

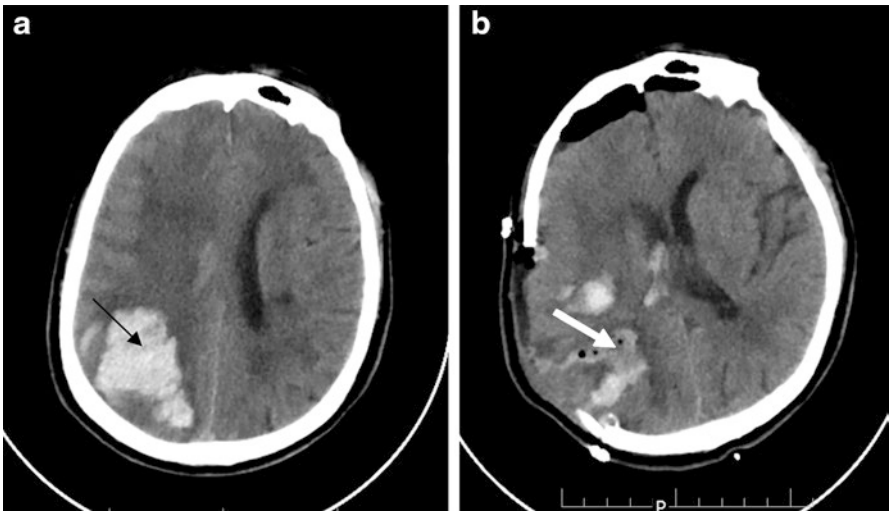

Fig. 1.1 (**a**) CT scan of the brain showing a blood clot (arrow) in the right side of the brain. (**b**) CT scan of the brain of the same patient after a few days of decompressive surgery, showing the absence of bone flap and less amount of clot remaining (white arrow)

1.2 Dementia

With advancing age, due to disturbances of blood supply and neuronal activity, the higher mental functions like memory, intelligence, behaviour, orientation, mood, affect, initiative, attitude, etc. get affected. The daily activities like brushing of teeth, cleaning oneself and dressing up become more difficult. Subsequently, the patient may not be able to perform these activities and may require considerable help from others. This condition is known as dementia.

Alzheimer's disease (AD) is a specific type of dementia, where neurofibrillary tangles are formed in the brain, leading to plaque deposition in neurons, resulting in neuronal dysfunction. Approximately 5–10% of population above 70 years may suffer from AD [5]. Although there is no specific treatment available yet, some medications to halt this degenerative process are available. A lot of research is being done to look at specific proteins in the CSF of these patients with AD in order to find better solution to the problem. Magnetic resonance imaging (MRI) of the brain of these patients may show some diagnostic changes (Fig. 1.2). Presently, the mainstay of managing these patients is by providing specific help and assistance. Most of these are done by the immediate family members at home or in special old-age homes.

Another common group of dementia is vascular dementia resulting usually from uncontrolled or long-standing diabetes and hypertension.

Regular physical and mental activity, proper recreational activities and adequate control of diseases like diabetes and hypertension can prevent vascular dementia to a large extent.

Dementia affects some 24 million people, most of them elderly, worldwide [5]. Up to two thirds of them live in low- and middle-income countries. Awareness of dementia is very low in all world regions. No cure is currently available for dementia [6].

1.3 Parkinson's Disease (PD)

Parkinson's disease is a progressive degenerative disease of the brain occurring usually after the age of 50. It is associated with dysfunction of brain cells producing dopamine. Clinical symptoms can be motor (movement disorder) or non-motor (excessive salivation, constipation, depression, excessive dreaming, etc.). Motor symptoms include tremor, bradykinesia, rigidity and gait problems.

The treatment is essentially medical and rehabilitative. Medicines are mainly aimed at increasing the availability of dopamine at the nerve endings of the brain, regulating co-ordination of motor movements. The role of family or social support is essential in advanced disease. Patients with PD are prone to sustaining injuries due to repeated falls, which may involve the role of other specialists in their treatment.

Severe movement disorders not responding to high dose of medications may benefit from surgery. The surgery for PD may be DBS (deep brain stimulation) or

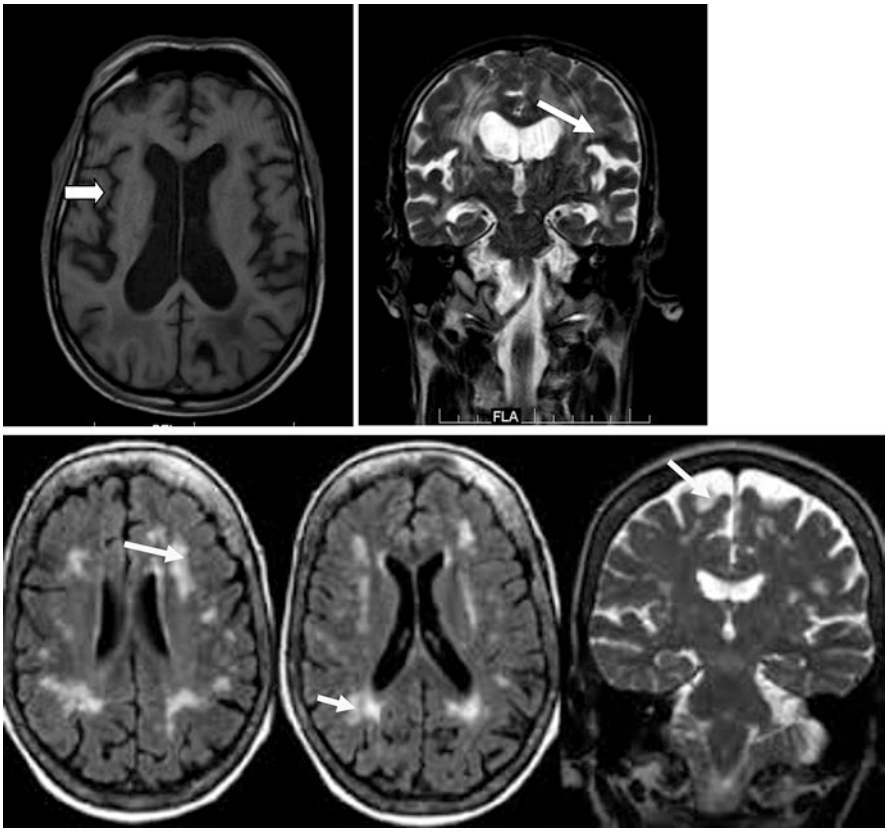

Fig. 1.2 MRI of the brain of patients with dementia showing bilateral ischaemic spots (white arrow) and shrinkage of the brain

lesioning. DBS is otherwise known as pacemaker for the brain. Selected nucleus of the brain, like subthalamic nucleus (STN) or globus pallidus internus (GPI), is stimulated by microelectrode placement using stereotactic methods (Fig. 1.3) [7], connected to internal pulse generator (IPG) placed in a subcutaneous pouch below the collar bone. Lesioning involves permanent destruction of the part of the nucleus using radiofrequency energy delivered through a microelectrode.

1.4 Chronic Subdural Haematoma (CSDH)

The volume of the brain has a tendency to diminish with age resulting in a process called cerebral atrophy [8]. Due to this process, the potential space between the brain and the skull bone increases, thereby putting pressure on the thin-walled veins, bridging between the brain and the dura mater covering the brain which is attached to the inside of the skull bone. Any mild to moderate head injury can result in

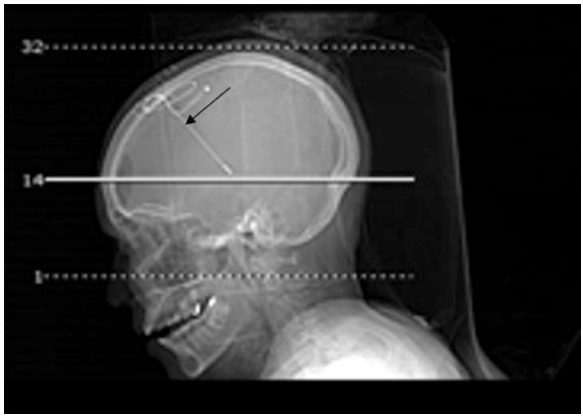

Fig. 1.3 X-ray of the skull (lateral view) showing the microelectrode (arrow)

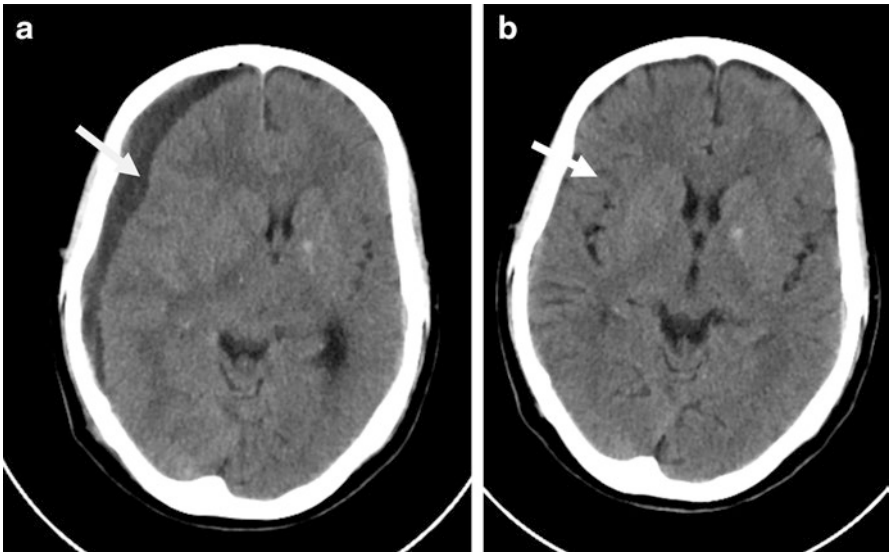

Fig. 1.4 (**a**) CT scan showing CSDH (white arrow) causing mass effect, pressure on the right side of the brain and brain shift. (**b**) CT scan after surgery showing good result with no collection of blood (white arrow)

bleeding from these ruptured veins, thereby causing CSDH formation. This process occurs slowly over the period of days to weeks (usually after three weeks), hence the term chronic. Subsequently, vascular membranes are formed from the fibrin of the blood, which produce more fluid and bleed in to the space causing increase in the amount and produce mass effect on the underlying brain.

Small amounts of CSDH can be absorbed by natural process, but repeated bleeding and use of blood-thinning tablets in these age groups (for brain or heart strokes) can result in large amount of collection of blood products, causing pressure on the brain (Fig. 1.4a) and thereby resulting in headache, vomiting and paralysis.

When significant amount of CSDH is produced, surgical treatment is usually called for. By drilling burr holes into the skull, this liquefied collection can be drained out successfully with a small risk of bleeding, recurrence and infection (Fig. 1.4b). The surgery, if done in time, is curative.

1.5 Normal Pressure Hydrocephalus (NPH)

Discrepancy between cerebrospinal fluid (CSF) production and absorption inside the brain can result in excessive amount of CSF collection in the ventricles when the absorption is less than the production [9]. This condition is called hydrocephalus. In elderly patients, this process develops very slowly, and hence the pressure exerted by the fluid on the brain is usually minimal. Therefore it is called normal pressure hydrocephalus (Fig. 1.5a).

Usually, patients present with gait difficulties, urinary incontinence and dementia. Neurological examination along with CT scan or MRI of the brain usually points to the diagnosis, which is further confirmed by performing a lumbar puncture (LP). After LP, patients with NPH show significant improvement of their symptoms within 24 of the LP. Eventually, patients may require shunting procedure (ventriculoperitoneal shunt) (Fig. 1.5b) using a programmable valve. The opening pressure of the shunt can be controlled from the outside by the use of the programmer to adjust to the required pressure setting, thereby avoiding complications due to over-drainage or under-drainage.

1.6 Hyponatraemia

Due to the use of diuretics in controlling blood pressure, patients in the elderly age group are likely to develop hyponatraemia (low serum sodium), especially when salt intake is restricted. Otherwise also, due to the loss of sodium in excessive diarrhoea and vomiting in this age group, hyponatraemia is encountered in geriatric practice. Patients present with acute confusional states, where the low serum sodium (normal range 135–145 mEq/L) confirms the diagnosis. This is a common cause of falls at home and in hospital in this age group [10]. Correction of hyponatreamia has to be undertaken very carefully and slowly. Hypertonic saline (3% saline) can be used cautiously checking the serum sodium twice daily. Faster corrections of sodium (>12 mEq/day) can result in osmotic demyelination, in which case nerve fibres in the brain, especially brain stem (pontine) and other central areas (extra pontine), can be damaged irreversibly, resulting in severe disability or subsequently death. Correction of hyponatraemia with normal saline (0.9% saline) is safer due to slow correction. Hypertonic saline is indicated in cases of severe sodium depletion causing convulsions, stupor and cerebral oedema.

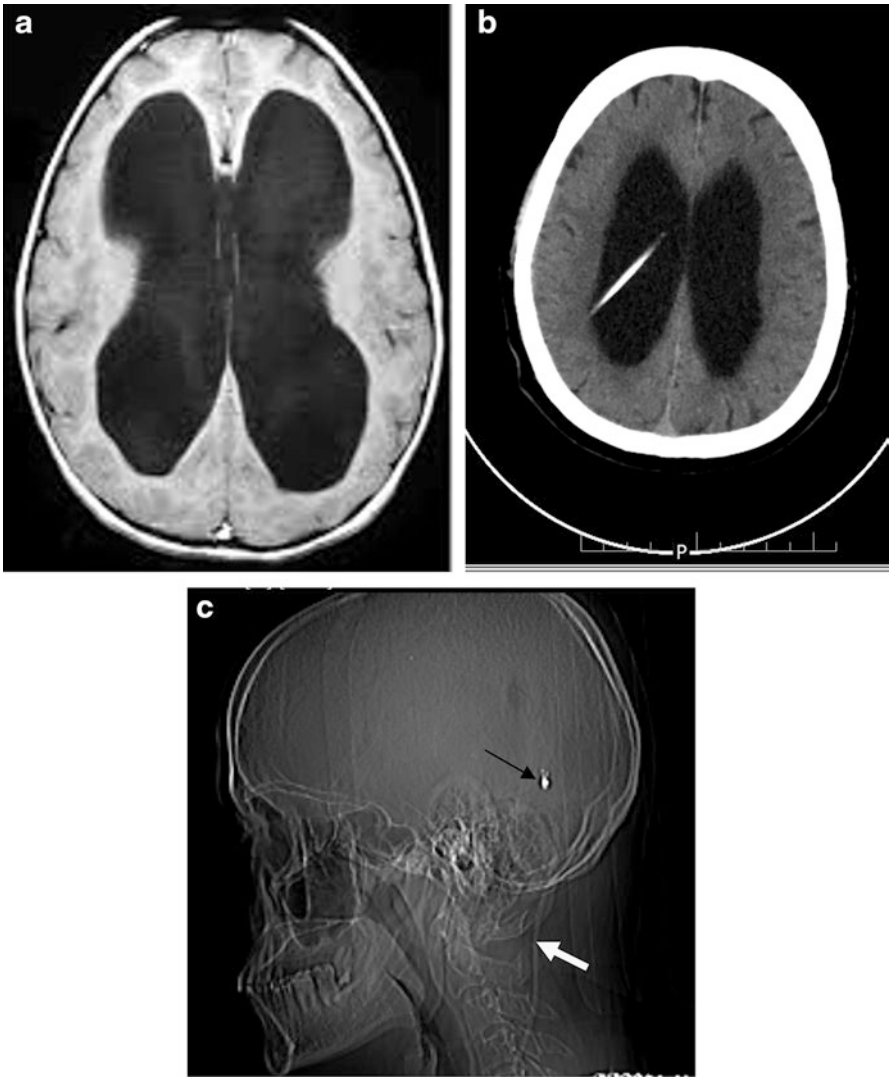

Fig. 1.5 (**a**) MRI brain showing hydrocephalus (left). (**b**) CT scan showing shunt in situ. (**c**) Postoperative X-ray of the skull showing the programmable valve (black arrow) and the connecting tube of the VP shunt (white arrow)

1.7 Spondylosis

Normal wear and tear of the spine is known as spondylosis (Fig. 1.6a, b) also known as degeneration of the spine. The human spine is beautifully designed by nature in the process of evolution to cater to modern living. Basic functions of the spine are stability and mobility. There is a very good balance between both these functions in

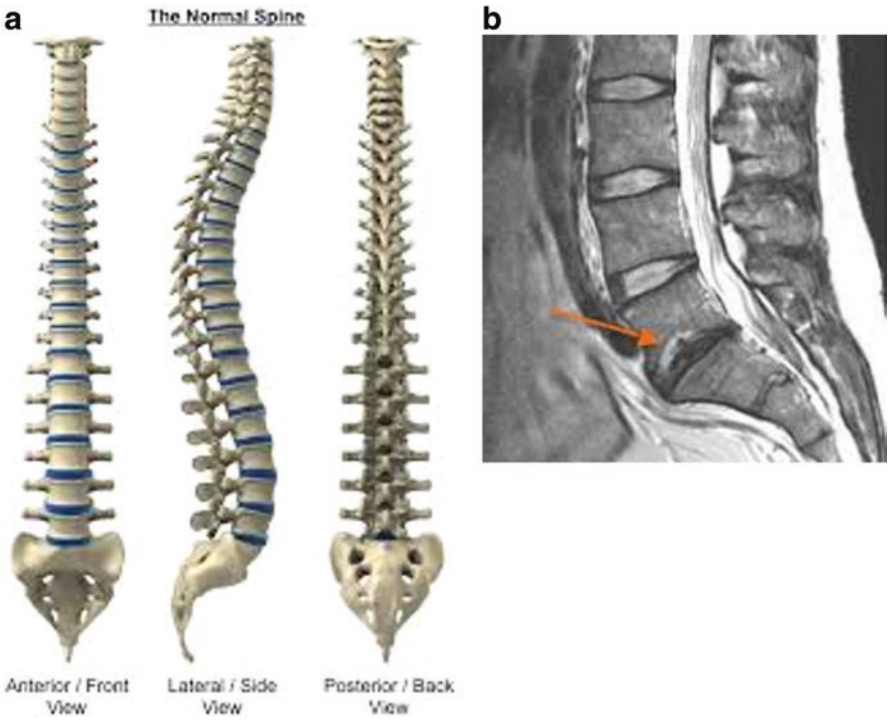

Fig. 1.6 (**a**) Appearance of normal spine (reference from the Internet). (**b**) MRI of lumbar spine with degenerative changes (arrow)

younger age. With advancing age, it gets disturbed. If we are to blame the spine itself for this disturbance, then this is due to stiffness of the joints between the vertebrae, fragility of the ligaments binding them, dehydration of the disks in between the vertebrae and weakness of the bones, etc. These progressive and degenerative processes result in fractures, slipped disks, sciatica (due to nerve root compression by the disks), listhesis (slipping of one vertebra over the other), difficulty in gait due to spinal canal narrowing causing pain and disability of various proportions. Spinal instability is a common cause of spinal degeneration [11].

The initial treatment is medical and physiotherapy. Surgery may be necessary in advanced cases, to relieve pain, weakness and disability.

1.8 Conclusion

Elderly people need special care for their health with prompt attention and quality medical treatment, when necessary. In addition to the abovementioned common geriatric problems, cancers of different organs, both primary and secondary, are more common in this age group. Elderly people sustain frequent domestic falls

resulting in head, spine and other bony injuries as well. Due to the economical constraint of many in this age group, healthcare for elderly is a very important social concern. In addition to medical treatment, geriatric patients need love, affection and care by the family as well as by the society at large. Government as well as NGOs should be involved in thoughtful and meticulous planning of the healthcare of the elderly, because it is only a matter of time that the young become old. By treating the elderly well, mankind preserves the philosophy of 'care for the needy in time of need'.

Acknowledgement The author would like to thank Mr. Shubhro Majumdar for preparing the manuscript.

Declaration All the X-rays, CT scan images and MRI pictures are from the author's own cases (except Fig. 1.6a, which has been taken from the Internet).

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Molecular Marker and Therapeutic Regimen for Neurodegenerative Diseases

2

Sharmistha Dey, Nitish Rai, Shashank Shekhar, Amrendra Pratap Singh, and Vertica Agnihotri

Abstract

The aging brain and nervous system go through changes by natural processes over time. The gradual loss of nerve cells takes place in normal aging process, while in some cases, collapsed old nerve cells lead to lots of accumulation of nerve cell's waste, eventually forming plaques and tangles. The plaques and tangles result in dementia (the memory loss) or movement disorder, which initiate different neurodegenerative diseases in aging. Disease-associated behavioral changes will start and become worse if it could not be detected in the early stage. It can be prevented by mental and physical exercise in normal aging process. Further, neurodegenerative disease in aging could be protected from promoting by early detection with potent molecular markers. The molecule which has direct or indirect role with the pathophysiology of the disease that reflects the insight for early diagnosis can distinguish disease accurately from normal. A molecular marker may simply refer to any biomolecule that can be estimated and utilized as a yardstick of a physiological or pathological state. In this chapter, the molecular markers have been described in context to the neuronal physiology and their potential diagnostic utility in neurodegeneration. This chapter presented the recently exploited biological molecules which have neuropathological role for the development of molecular markers in Alzheimer's disease and Parkinson's disease.

Keywords

Protein marker · Neurodegeneration · Alzheimer's disease · Parkinson's disease · Therapeutics

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2.1 Introduction

Aging, in case of humans, refers to multidimensional processes of physical, psychological, and social change which is characterized by functional decline and disabilities. Aging is due to several complex mechanisms that occur at the molecular and cellular levels. There are various theories that explain the process of aging; however, only few have got experimental support. These are the following:

- *Stochastic theories of aging*: This theory describes aging as a randomly occurring process which accumulates over time. This includes error theory [1], free-radical theory of aging [2], and wear and tear theory [3].
- *Programmed theory*: This theory proposes aging as a programmed and coordinated event rather than a random process. This theory supports cellular senescence theory [4], neuroendocrine theory [5], and immunosenescence theory [6].

According to programmed theory, aging starts at the day we born. When the cells grow and DNA replicates to form new cells, every time a cell divides, telomere present at the end of chromosome gradually becomes shorter. When telomere becomes too short to replicate, after fixed number of cell divisions, cell stops growing and enter into cellular senescence. More accumulation of senescent cells produces reactive oxygen species (ROS) and develops various types of age-associated diseases due to the lack of sufficient antioxidant agent. Overproduction of ROS leads to neurodegeneration and loss of sensory function neuronal cells.

The brain and neuronal tissue are particularly sensitive to ROS. In aging process, some people develop neurodegenerative disease due to the oxidative stress, induced by environmental effect or genetic disorder.

Free radicals are necessary for the living organism for signal transduction, gene transcription, and regulation of vascular muscle cells, platelet aggregation, and hemodynamics. Free radicals, like hydroxyl radical, superoxide anion radical, and reactive oxygen species like hydrogen peroxide, nitric oxide, etc. are produced as by-products during the physiological and biochemical processes. These radicals are removed by many antioxidant reagents like glutathione; vitamin A, C, and E; zinc; etc., with the help of many enzymes like catalase, superoxide dismutase (SOD), glutathione peroxidase, etc.

ROS have a vital role in neurodegenerative diseases. In mitochondria, energy carrier ATP is generated by breakdown of glucose using oxygen through oxidative phosphorylation. The glial cells (neuroglia) present in the brain restrict the entry of various molecules. These cells require more oxygen and glucose for continuous supply of ATP which is needed for controlling all the organs of the human body. The loss of homeostasis between prooxidant and antioxidant overproduces free radicals in the brain. In an aged brain, ROS production increases due to the reduction of antioxidants and low regenerative capacity. As such, the brain contains high amount of unsaturated fatty acid and low amount of antioxidants compared to other tissues. These unsaturated fatty acids consume oxygen and form lipid peroxide. ROS in

glial cells are sensitive to the oxygen-free radicals that damage the neurons. These factors play a key role in developing major neurodegenerative diseases in aging.

Besides being caused by derailed metal metabolism, the oxidative stress-associated neurodegeneration is also predominant in individuals with certain type of mutations as compared to normal individual as shown by genetic evidences.

The rise in the neurodegenerative disease is alarming especially with the projected rise in the elderly population in coming decades. The toll on the patient and caregivers is huge with increasing burden on global economy for disease management. According to the World Alzheimer Report, there were 46.8 million people living with AD and other dementia in 2015 which is predicted to rise to 131.5 million by 2050 worldwide. This translates one in five persons by 2050 [7]. It can be easily stated that early diagnosis and development of novel treatments of neurodegenerative diseases are of utmost importance.

Pathology in neurodegenerative disease does not spread like a fan from one brain area to neighboring areas. Here, in case of neurodegenerative disease, the spread follows disease-specific patterns that look like the architecture of brain connectivity networks. Why pathology spreads along such networks and whether the asset of network connectivity forecasts the severity of neurodegeneration remain blurred.

Although the molecular mechanism pathways channelized by various neurodegenerative disorders are not identical, many processes, like neurite shortening, synaptic loss, and finally neuronal death, are most common features of neurodegenerative disorders. There is no disease-modifying treatment available for neurodegeneration due to lack of understanding of underlying pathophysiology. The regenerative or remodeling approaches for degenerated neurons are only in infancy. This issue is further elevated by unavailability of precise markers which could predict or detect the disease at an early stage.

At present, the gold standard for confirmation of neurodegeneration is the neuropathological screening; however, that is only possible via autopsy of a deceased patient. So, there is a demand for effective diagnostic marker for valid detection of neurodegenerative disease when the scope is still wide open for intervention.

Biomarker is a biologic feature that can be measured and estimated indifferently as a sign of normal biologic processes, pathologic processes, or therapeutically mediated pharmacological responses. Biomarkers take part in various purposes, including disease diagnosis and prognosis, prediction and calculation of treatment response, and safety evaluation. Molecular biomarkers are those markers that can be estimated in biological fluids (plasma, serum, cerebrospinal fluid, urine, and tears) and other samples (like bronchoalveolar cleavage and biopsy) including nucleic acids. The molecular biomarkers are being entirely developed and confirmed to be utilized in drug development and support for the approval of drug products. Biomarker discovery needs targeted identification of a biomarker with real-time quantitative information to indicate which parameters are changing to a statistically pertinent degree in response to disease condition.

In the last few years, there has been a propensity to drive biomarker discovery with "-omics" approaches that thoroughly address a particular biological domain. Whereas this generates huge amounts of data, there is a need for a program to know

Table 2.1 Summary of different of biomarkers [8]

Type of biomarker	Utility
Diagnostic marker	Biological parameter helping in the identification of the disease
Prognostic marker	Biological characteristics that is measured to predict the course of the disease or a response to a therapeutic intervention
Screening marker	Factors helping in early detection of disease
Antecedent markers	Measure the risk of a disorder
Stratification markers	Estimate the probability of drug response or toxicity
Biomarker signatures	Signify the presence of pathophysiological state

disease per se as well, integrating and applying technology to the natural history of a disease in order to understand the pathophysiology over time. Such analyses are massive endeavors, and no single organization, or perhaps even country, can reasonably succeed alone. Therefore for the mutual benefit, national and transnational public/private associations are emerging to drive fundamental molecular understanding of the development and measurement of disease.

Neuroimaging techniques like magnetic resonance imaging (MRI), positron-emission tomography (PET) scan, nuclear magnetic resonance spectroscopy (NMRS), etc. play an important role by not only helping in diagnosis but also measuring the biomarkers such as neuronal metabolites. The imaging techniques coupled with measurement of a biomarker have been found to be quite informative.

The role of microRNAs has also laid down a foundation stone of appreciation, recently. Their altered expression in certain diseases, including neurodegeneration, offers an excellent repertoire of biomarkers (Table 2.1).

Currently, most of the valid biomarkers are obtained from the CSF analysis of the neurodegenerative disease patients, which includes a complex, expensive, and painful extraction procedure for the patients. So, in this context, a simple, most readily accessible and profitable biomarker is needed.

Biomarkers have a huge clinical relevance in which it aids to detect the disease at an early stage when none of the symptoms appear. Thus, considering present situation, molecular biomarkers are highly demanded to spot disease presymptomatically.

Alzheimer's disease (AD) and Parkinson's disease (PD) are most prominent neurodegenerative diseases associated with aging. The other known neurodegenerative diseases are multiple sclerosis, amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease), and Huntington's disease. Mild cognitive impairment (MCI) is a very common early-stage dementia among the elderly.

This chapter will discuss about the proteins which have a critical role in AD and PD phenotype and can be developed as a protein marker for early detection and therapeutic target for AD and PD.

2.2 Alzheimer's Disease

Alzheimer's disease (AD), affecting 35 million people worldwide at the age of above 65 years, is the major cause of dementia. Dementia is the disorder of certain part of the brain that involves thought, memory, and language.

In human brain, all the information is transmitted by one neuron, through its dendrites, to other neurons via electrical and chemical signals. The chemical energy requires oxygen and glucose through blood circulation. When one neuron receives signals from another neuron, it is called synapse. By this way neurons transmit messages from the brain to other muscles and organs. The glial cells called astrocyte protect the neuron from any damages. In aging, due to the overproduction of ROS, glial cells become unable to protect neurons, and the neurons become more vulnerable for damage. Astrocytes help to secrete growth factor for stimulating neurogenesis and form new neurons. Astrocytes react with ROS which generate more in an aged brain and show structural changes which are unable to form new neurons in hippocampus and contribute to age-related decline in neurogenesis.

AD occurs in sporadic as well as familial form. The sporadic form occurs predominantly (>95%), while uncommon, familial form is caused by mutation in three genes related to processing of the amyloid precursor protein (APP) – APP, presenilin 1 (PS1, also known as PSEN1), and PS2 (also known as PSEN2).

Though the exact cause of AD is not absolutely clear, the well-known pathological hallmarks of AD are the extracellular occurrence of plaques of toxic Aβ peptide and intracellular localization of hyperphosphorylated tau forming the neurofibrillary tangles.

The amyloid-beta precursor protein (APP) is a very important protein in neuronal growth and repair. APP is processed by the proteolytic enzymes, α-, β-, and γ-secretase, by two pathways: (1) amyloidogenic pathway and (2) non-amyloidogenic pathway. In amyloidogenic pathway, Aβ protein is generated by sequential action of the β- and γ-secretases outside the membrane and by α-secretase and γ-secretase from a neuroprotective APPα fragment, in non-amyloidogenic pathway. Aβ controls the synaptic activity, and the rate of Aβ production is compensated by regulated removal of Aβ, while in AD, the production and clearance of Aβ peptide become unbalanced. Due to the reduction of clearance activity in some aged brain, the accumulation of Aβ peptide produces senile plaques and induces numerous neurotoxic effects (Fig. 2.1).

In AD, glial cells maintain the amyloid-β (Aβ) peptide levels in the brain. Microglia and astrocytes have a major role in Aβ clearance and degradation. The hydrolysis of Aβ, at different cleavage sites, take place via different methods which include Aβ degradation by proteases in glial cells, like endothelial- converting enzymes and insulin degrading enzyme. Besides these enzymes, other proteases have also been described, suggesting some role in Aβ elimination, such as plasminogen activators, angiotensin-converting enzyme, and matrix metalloproteinases. Extracellular chaperons are released from glial cells to mediate the clearance of Aβ either alone or with receptor/transporter, which make their exit possible through blood circulation. Extracellular chaperons include apolipoproteins, α2macroglobulin,

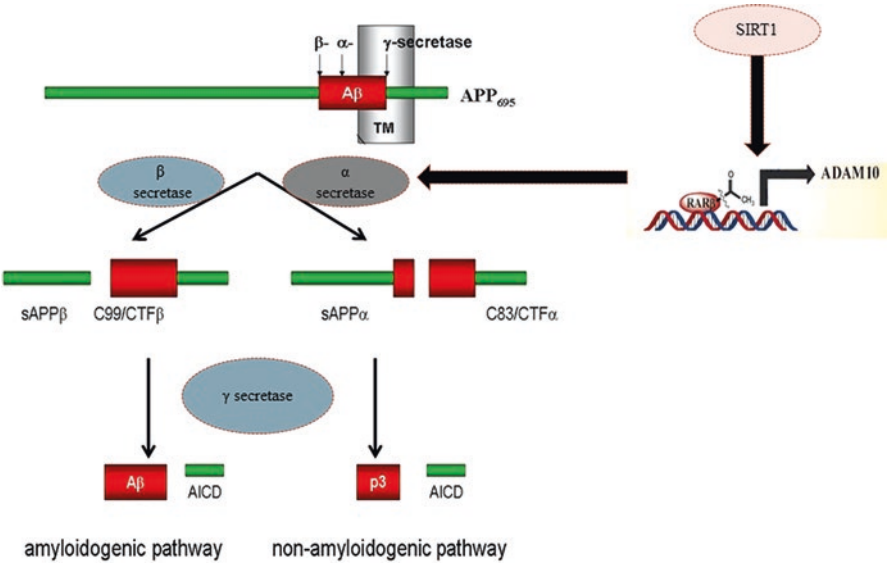

Fig. 2.1 Non-amloidogenic and amyloidogenic pathway of amyloid precursor protein

and α1-antichymotrypsin. Astrocytes and microglia have an essential role in Aβ phagocytosis, in many cases by means of a number of cell surface-expressed receptors.

In AD, amyloid β peptides get deposited by chelating with transition metals (Cu^{2+}, Zn^{2+}, Fe^{3+}) and produce toxic chemical reaction by altering metal oxidation state and toxic hydroxyl (OH) free radicals.

Aβ plaque deposition and neurodegeneration take place in the region that metabolizes glucose-6-phosphate, by aerobic glycolysis, into pyruvate and lactic acid that is important for supplying continuous ATP to the vital process, i.e., brain cell proliferation. This aerobic glycolysis involves NAD^+ depletion and produces NADH. The chemical compound NAD^+ (nicotinamide-adenine dinucleotide) is the key molecule which transfers the information and synchronizes events between the nuclear genome and the mitochondrial genome of a cell.

Tau protein is a normal phosphoprotein which binds to microtubules in the neuronal axons, thereby maintaining the stability of the microtubule. Tau is normally associated with microtubules; however, accumulation of Aβ peptide triggers the changes in tau protein and subsequent formation of neurofibrillary tangle from tau [9]. These filamentous inclusions commonly exist in pyramidal neurons of the AD brain and other neurodegenerative disorders, termed tauopathies [10]. Although Tau is normally soluble, upon phosphorylation by various kinases like cyclin-dependent kinase 5 (CDK5), glycogen synthase kinase 3β (GSK3β), and extracellular signal-related kinase 2 (ERK2), it tends to form insoluble aggregates [11]. Upon phosphorylation, tau loses its affinity from the microtubules and consequently destabilizes them [12] (Fig. 2.2).

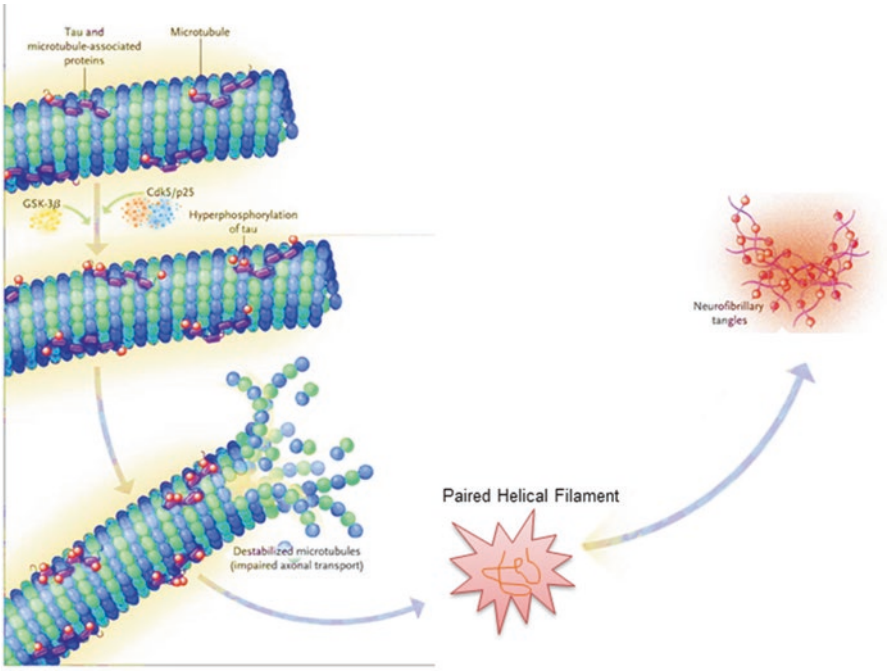

Fig. 2.2 Mechanism of neurofibrillary tangle formation by tau hyperphosphorylation

In previous studies, it has been observed that high mortality rate in AD is associated with high level of Tau. The stability of the microtubule is maintained by the phosphorylated state of Tau by interacting with tubulin. Around 79 Ser and Thr phosphorylation spots are present in Tau, and their phosphorylated states are controlled by Tau phosphatases and kinases [13]. This site-specific hyperphosphorylation of Tau is the result of breakdown of this regulation, which eventually causes development of NFTs and finally causes neuronal cell death [14]. Previous studies show that the formation of NFTs in the brain in the case of AD patients was associated with augmented hyperphosphorylation at p-Tau181 or p-Tau 231 [15]. High p-Tau181 has also been correlated with rapid progression of MCI to AD [16] and swifts cognitive impairment in AD [17].

AD leads to a predominant disturbance in some neuronal populations and brain areas more than the others and results in shrinkage of brain volume and weight. Though AD clearly causes neuronal loss in precise brain regions (e.g., CA1 region of the hippocampus and pyramidal cells in entorhinal cortex), much of the brain volume seems to be lost due to the neurite shortening and shrinkage.

The morphometric measurements in live patients and postmortem tissues have been significantly progressive due to the improvement in radio imaging techniques. For example, progressive decreases in cortical thickness in various brain areas in AD patients can be detected by magnetic resonance imaging (MRI), and it correlates with cognitive decline and predicts conversion from mild cognitive

impairment (MCI) to AD. Accordingly, these MRI data are progressively used in early stage diagnosis of AD and for analyzing clinical trials.

Besides such anatomical changes, the person with the risk of developing AD or patients having alterations in the activity of their neural network can also be exposed by functional MRI (fMRI). These include derailed connectivity and activity in the default mode network, which in normal individual is utmost active during non-active thinking in particular, and reduced hippocampal size and abnormal cortical thinning in AD-vulnerable brain regions correlate with hyperactivation of the hippocampus during the performance of memory tasks [18]. From the biochemical and electrophysiological studies of transgenic mouse models, these findings imply that AD does not simply disturb neural networks and neurons but rather causes abnormal network activity that might vigorously interfere with the complex processes underlying learning, memory, and other cognitive functions. In the early-onset AD, patients are also affected with the increased cases of epileptic seizures. Previous results in transgenic mouse models propose that these difficulties may be the frivolous, representing a boom of more subtle modifications of neural network activity [19, 20].

Though synaptic loss occurs early and correlates with cognitive deficits in patients, but its symptoms develop at very late stage of the AD. The prime hallmarks, Aβ plaques and tau tangles could not be detected at an earlier stage of AD pathology. It is essential to find the solution to prevent or detect early than to reverse dementia disease which is not possible at the late stage. In this situation, recent researches have indicated a possible molecular mechanism involving pathological changes that occur in AD phenotype.

Several efforts have been made to develop a reliable and independent of fluid or tissue diagnostic marker for AD, but still no dependable biomarker could be presented. On the other hand, there is no available biomarker for AD till date which can be unfailingly evaluated in blood samples. Hence, there is an absolute requirement for specific biomarkers which can detect the disease at earlier stage.

Biomarkers reflecting changes in pathophysiological process could be beneficial for understanding of disease mechanisms, to design tools for early diagnosis and prognosis and to analyze drug effects in clinical trials of disease intervention therapies for Alzheimer's disease.

2.3 Markers for Alzheimer's Disease

2.3.1 Aβ42

Aβ plaque deposition is a characterized feature of AD in CSF. Cerebrospinal fluid (CSF) serves as a primary source for the sampling of Aβ [FGA]. CSF exists in close contact with nerve tissue, and therefore an important exchange of substances occurs in the neural environment. So, different groups have studied the alterations in the proteins and substances associated with pathogenesis of AD [-FGA]. Aβ, generated from the large amyloid precursor protein (APP) by secretases, is processed through

amyloidogenic pathway to produce 42-amino-acid peptide [Aβ (1–42)] that can accumulate in the brain under particular conditions (e.g., metals, acidosis). In AD patients, there is a significant reduction in CSF Aβ (1–42) than the controls. The CSF-Aβ42 is quite sensitive (80–90%) to differentiate AD from normal aging and depression. The CSF-Aβ42 is also known to increase in other types of neurodegeneration like Creutzfeldt-Jakob disease, frontal temporal dementia, vascular dementia, and dementia with Lewy bodies; however, in AD, lower levels of Aβ in CSF occur implying amyloid deposition in the cerebrum. This is due to the fact that as Aβ aggregates to form AD plaques in the brain, its concentration in the CSF decreases. The CSF-Aβ42, when used in combination with other AD biomarkers, has remarkably increased sensitivity and specificity for diagnosis. The impaired clearance of Aβ from the brain to the blood/CSF along with the enhanced aggregation and plaque deposition in the brain has been suggested to cause decreased levels of Aβ (1–42) in the CSF. The breakdown of APP is mediated by proteases known as α-, β-, and γ-secretases [21, 22]. The Aβ-amyloid, tau, and phosphorylated (p)-tau are well-known markers to screen MCI and AD, and to anticipate the conversion of preclinical AD and MCI to AD [12, 23].

Although CSF provides a steady pool for biomarker research, lumbar puncture is an invasive diagnostic procedure and seems unsuitable for routine clinical diagnosis. The peripheral biological fluids like blood and saliva provide an excellent option as extraction procedure is less invasive and inexpensive.

The Aβ in the plasma has been also examined as a peripheral biomarker. However, the source of circulating Aβ in plasma is from the brain tissue transported across the blood-brain barrier (BBB) and also from peripheral tissues and organs. This could be problematic since peripheral Aβ evaluation might not show the true dynamics of formation of senile plaque in the brain [FGA].

Gowert et al. [24] reported that Aβ improves platelet activation and ROS generation and concluded that cerebral amyloid angiopathy may involve the role of platelets. AD is characterized by alterations in platelet AβPP since in AD patients, altered proportion of the different forms AβPP was reported compared to control groups [25, 26]. However, it is established that the plasma levels of Aβ (1–40) are not particularly specific for AD and are more dependent on age [27]. Amyloid-beta (Aβ) levels in blood or plasma were found to be differentiated in between AD and control [28].

2.3.2 Total Tau and p-Tau

It has been observed that the total tau in cerebrospinal fluid (CSF-tau) is melodramatically increased in AD patients [29]. It has also been proven that tau binds to microtubules in neuronal axons, thus stabilizing the microtubule accumulation, though the abnormal increase in CSF-tau is not clear yet. While the sensitivity of CSF-tau seems very high for AD, it has lower specificity for other types of dementias [30]. In AD patients, it is well known that the Tau and phosphorylated Tau are elevated in cerebrospinal fluid (CSF) [31–34]. In a recent study, Ming-Jang Chiu

et al. [33] showed the negative association of plasma Tau with visual reproduction, logical memory, and volume of total gray matter in the hippocampus [33]. Elevated plasma Tau has been reported significantly higher in mild cognitive impairment (MCI) and AD patients than the elderly control [33]. The hippocampal atrophy has been correlated with increased phosphorylation at p-Tau_{181} [35]. Recent study reported higher level of both Tau and p-Tau_{181} in serum in the case of AD compared to MCI and control elderly [36].

Farías et al. [37] detected tau protein in platelets with specific antibodies and proposed a new biomarker for AD. Further studies reported that there is a close correlation between the level of tau modification in platelets and the severity of cognitive impairment in AD patients, which have been evaluated as an AD biomarker with specificity of 79.7% and a sensitivity of 75.7% [38].

2.3.3 MicroRNAs

MicroRNAs (miRNA) are small fragments of RNA, about 22 nucleotides long, that control posttranscriptional processes by annealing with target mRNAs at 3′ untranslated region (3′UTR) region, leading to their translational inhibition or sometimes degradation. In animals, the most abundant class of small RNAs belongs to miRNA. Further, miRNAs are usually co-expressed in high abundance with their targets in the nervous system, where they mostly replicate and express in a specific pattern in the brain. They are responsible for regulation of numerous biological processes including neurogenesis and synaptic plasticity, where they drive the cellular processes toward neuronal differentiation.

miRNA expression is influenced by the cell's physiological state; therefore, the circulating miRNA reflects intracellular state in normal and pathological condition. Geekiyanage and Chan [39] reported that expressions of miR-137, miR-181c, miR-9, miR-29a/b are reduced in subgroup of AD patients, upregulating the expression level of serine palmitoyltransferase (SPT). mRNA levels remain unchanged between controls and AD patients; therefore, these are regulated at posttranscriptional level by miR-137/miR-181c (SPT1) and miR-9 and miR-29a/b (SPT2), proposing these miRNAs as promising biomarkers. In addition, it was reported that in sporadic AD subgroup, downregulated miR-9 and miR-29 family members regulate BACE 1, inducing Aβ accumulation [40].

Sheinerman et al. [41] identified various pairs of miRNA (miR-132/miR-491-5p, miR-128/miR-491-5p, miR-134/miR-370, miR-323-3p/miR-491-5p, miR-382/miR-370, and miR-323-3p/miR-370) from the plasma of AD and MCI patients and controls. They proposed that although AD and MCI subgroups could not be distinguished based on these miRNA pairs, they could be characteristics of early pathologic events occurring in neurons.

Leidinger et al. [42] recognized that 12-miRNA signature in blood samples distinguishes AD patients and control with 93% accuracy, 95% specificity, and 92% sensitivity. These miRNAs can also differentiate AD from other CNS disorders.

A previous study reported upregulation of six miRNAs in AD patients compared to control subjects, i.e., miR-342-3p, miR-98-5p, miR-885-5p, miR-let-7d-5p, miR-191-5p, and miR-483-3p. Among these six miRNAs, the miR-342-3p has the highest specificity and sensitivity and might be used as biomarkers in the diagnosis of AD [43].

2.3.4 p97/Melanotransferrin (Mtf)

For the first time, high expression levels of p97 or melanotransferrin (Mtf) were detected in malignant melanoma cells. Mtf are group of iron-binding proteins and possess good sequence homology with serum transferrin and human lactoferrin. Previous studies reported Mtf content as a serum marker for AD patients as it was found elevated in AD patients [44, 45].

Contrary to it, a recent study identified that there was no significant difference in Mtf level between controls and mild or moderate stages of AD patients [46]. Further studies are required in this direction.

2.3.5 Sirtuin

CR is the only effective intervention that causes delayed aging in most organisms and slows down the functional decline and disease onset in lower organism [47] as well as in mammals [48]. Several studies have indicated that the life span can be increased by caloric restriction in diverse species. Thus, an increased effort is put into developing therapeutic agents that can imitate the valuable effect of caloric restriction on longevity without the need for changing dietary intake. Such agents have been termed caloric restriction mimetics (CRMs) [49]. Numerous signaling pathways have been stated to modulate the effects of CR on aging [50]. A series of components involved in these pathways have been confirmed as drug development targets through genetic manipulation studies in different model organisms. One interesting target to appear from such studies is sirtuin, a protein that functions at a regulatory crossroad among nutrient sensing, energy metabolism, and genome stability [51].

Sirtuins are NAD-dependent deacetylases having broad range of metabolic and stress-tolerance properties. SIRT1 is one of the seven mammalian sirtuins, which include a conserved family of NAD^+-dependent deacetylases and ADP. The location of mammalian sirtuins was in different cellular compartments which possess different biochemical activities and molecular masses [52]. SIRT6, SIRT2, and SIRT7 are predominately located in the nucleus, cytoplasm, and nucleolus, whereas SIRT1 is found in both nucleus and cytosol [53]. The location of other sirtuins such as SIRT3, SIRT4, and SIRT5 is reported to be in the mitochondria [53, 54]. In respect to their biochemical properties, SIRT1, SIRT2, SIRT3, and SIRT6 have similar AD-dependent deacetylase activity, while SIRT4 and SIRT6 show ADP-ribosyltransferase activity [53]. NAD^+ is important for uniting the

biochemical and biological functions of sirtuins to the metabolic state of a cell or tissue [55].

Among all forms, SIRT1 is highly described and regarded as controller for delaying the aging process in animal models [56]. SIRT1 has shown neuronal rescue effect against stress in cell cultures [57]. CR which shows a rescue effect on animal models of neurodegenerative diseases such as AD [58] are reported to be driven by SIRT1 [59]. Therapeutic potential of transgenic mouse model upregulating SIRT1 against AD has been shown [60]. The actual pathway of SIRT1 in rescue of AD in animal models is not clear. It has been stated that SIRT1 raised the expression of ADAM10 gene encoding α-secretase which prevents pathogenic Aβ peptide accumulation [61]. The role of sirtuins in the protection of brain deterioration especially in AD has been described. In the experimental model of AD, it is noted that the downregulation of SIRT1 suppresses the expression of α-secretase which further enhances the accumulation of pathogenic Aβ peptide formed by β- and γ-secretase [62]. The serum levels of Aβ (1–40) are upregulated in the AD subgroup than controls and MCI subjects. It can be concluded that as SIRT1 downregulates in AD which driven the expression of Aβ peptide through ADAM10 pathway thus upregulates the level of Aβ peptide [63]. It has also been reported that in transgenic mouse models of AD, amyloid-β plaque levels can be reduced by overexpressing SIRT1 in the brain [64]. SIRT1 activator resveratrol has shown the rescue effect in vitro and in vivo AD rat model and the dropping accumulation of amyloid-β protein [65].

Brain regions that breakdown glucose by aerobic glycolysis experience extreme Aβ plaque deposition and neurodegeneration in AD [66]. Incidentally, aerobic glycolysis may cause increased NADH production and a gradual depletion of NAD^+ reserves within the cells which adversely affect SIRT1 deacetylase activity [67] and thus a shift of APP processing toward the amyloidogenic pathway [68].

The low SIRT1 concentration in autopsy brain tissue of AD patients correlated with the duration of symptoms and tau accumulation in rat provides clinical relevance of the above observations [69]. The age-related serum SIRT1 concentrations decline, and more decline was observed in cases of AD and somehow less marked (though significant) in patients with MCI. Hence, the different SIRT1 levels can be a suggestion for this difference of early detection of AD. The above conclusions put forward a hint that if SIRT1 concentration is considered as the reference, AD and MCI are two conditions which accelerate aging process. Serum SIRT1 does indicate a possible clinical utility for the diagnosis of AD [70].

2.3.6 Sestrin

Accumulation of Aβ amyloid in AD increases the expression of p53 which upregulates phosphorylation rate of tau and eventually causes the neuronal cell death. p53 accumulation induces oxidative stress in response to severe DNA damage [71]. Sestrin is an antioxidant protein, transcriptionally regulated by p53 which is shown to have a neuroprotective role [72]. Sestrin may intervene at multiple stages in AD, from protein accumulation to oxidative stress stimulation [73]. There is only one

Sestrin (Sesn) gene found among invertebrates, whereas vertebrates have three Sesn genes – Sesn 1, Sesn 2, and Sesn 3 [73]. This protein has been emerging as a critical regulator of AMPK-mTOR signaling pathway that extends its role in neurodegenerative disease. Sestrin activation leads to reduction in ROS and increased autophagy and thus may play a therapeutic role in neurodegenerative diseases. Sesn2 has been studied more among all other sestrins.

During aging or environmental stress, our antioxidant system gets weak which does not nullify the excessive oxidative stress resulting in the redox imbalance. During such condition, oxidative stress produces free radicals which get accumulated in the neurons and ultimately causes its death [74, 75]. Autophagy is a tightly regulated process of degradation of intracellular organelles inside the lysosome. The importance of this process lies in the removal of defective organelles like mitochondria and endoplasmic reticulum (ER) which can themselves cause oxidative stress. Autophagic defects can cause oxidative stress and neurodegeneration, and induction of autophagy can be of therapeutic value [76]. Hence, oxidative stress has emerged as a major cause of neurodegenerative diseases in recent times [76]. Such condition demands elucidation and study of new antioxidant genes for the therapeutic purpose. One of the significant genes which play a major role in reducing the level of ROS is Sestrin (Fig. 2.1).

It is a stress response protein which gets upregulated upon variety of insults. Sestrin is known to induce autophagy and prevent oxidative stress. There are three mammalian isoforms of sestrin that are highly conserved. The tumor-suppressor gene p53 activates sesn1 and sesn2 gene [72]. Normally, when p53 gets activated in response to oxidative or other forms of stress, sesn1 and sesn2 genes also get activated subsequently. These genes, in turn, activate AMP-activated protein kinase (AMPK). Its role in various diseased situations has just begun to be recognized. In fact, Sestrin2 was found to be upregulated in CHP134 cell line, exposed to amyloid-beta peptide known to cause AD [77]. Sestrin is a highly conserved gene throughout the animal kingdom [73]. Sestrin is a well-known modulator of AMPK and mTOR, both of which are highly involved in AD [78]. Sestrin promotes activation of AMPK in both mammals and flies. It prevents the accumulation of oxidative stress, caused by N-methyl-D-aspartate (NMDA) receptor activation [79]. Sestrin inhibits ROS accumulation through the maintenance of peroxiredoxin (Prx) activity [80, 81].

According to a recent report, Presenilin (an AD-associated protein) deficiency causes a reduction in sesn2 which renders amino acid sensing of mTORC1 dysfunctional and an attenuated transcription factor EB (TFEB)-mediated coordinated lysosomal expression and regulation (CLEAR) network activity [82]. The sesn activation leads to a reduction in reactive oxygen species (ROS) and increased autophagy and so may play a therapeutic role in neurodegenerative diseases [72, 83, 84]. Altered distribution of sesn2 (colocalized with tau in neurofibrillary lesion) expression was observed in the postmortem brain of 19 AD patients, but the precise clinical role of sesn in AD was not highlighted [85]. Since oxidative stress is important in the progression of AD, it has come up as a basic cause of neurodegeneration, and sesn is an antioxidant protein with a neuroprotective role. The sesn may intervene at multiple stages in AD, from protein accumulation and endoplasmic reticulum (ER) stress to

oxidative stress [72]. The sesn2 was found to induce post-ER stress via PERK and IRE1/XBP1 arms of the unfolded protein response (UPR) leading to mTORC1 inactivation and autophagy induction [86]. Autophagy is a vital phenomenon for relieving the ER stress and clearance of misfolded protein and hence plays an irreplaceable role in AD [87]. It has also been reported that under ER stress condition, sesn2 was specifically upregulated among the other sestrin family members, and loss of sesn2 was not compensated by sesn1. Hence, sesn2 is the only protein in the sesn family that is explicitly associated with ER stress [88].

The serum level of sesn2 was found to be elevated in AD patients in the age group of above 75 years as compared to the same control age group of the study. The level of sesn2 was even higher in AD with disease duration less than 2 years which is important for the detection in the early stage of AD. The ROC curve indicates that sesn2 levels can differentiate the MCI and AD patients from elderly control group with high specificity and sensitivity. A previous study reported that sesn2 mRNA was induced in response to 10 μM Aβ (1–42) in human neuroblastoma CHP 134 cell line [77].

The role of sesn2 in the progression of the disease may open avenues for therapeutic interventions. It may help to establish sesn2 as a potential candidate for a protein marker in the detection of AD. The identification of novel biomarkers would help in the detection of disease possibly before the symptom onset and also for analyzing the effectiveness of any future clinical trials.

2.3.7 Plasma Phospholipids

The phospholipids, mainly phosphotidylcholine (PC) and acylcarnitine (AC), play a major role in maintaining structural and functional integrity of cells. Aβ interacts with phospholipid and disrupts the bilayer integrity. They increased the production of nerve growth factor and thereby regulate the maintenance of neurons, particularly those which are constantly affected by AD within the basal forebrain. Decrease in plasma phospholipids and their different levels between AD and mild cognitive impairment were observed by Wurtman et al. [89]. Plasma phospholipids can serve as biomarker for the early detection of AD. The reduced levels of phospholipids are significantly able to predict development of AD in a normal individual within 2 years [89].

2.3.8 5-Lipoxygenase

AD brain is described by extensive neuroinflammatory processes. The 5-lipoxygenase (5-LOX) is a pro-inflammatory enzyme widely distributed within the central nervous system, and it has been found to be upregulated in AD.

Lipoxygenases are enzymes containing non-heme iron which catalyze the addition of oxygen to arachidonic acid (AA). There are three isoforms of LOX – 5, 12, and 15. 5-LOX adds oxygen on carbon at the fifth position of AA. 5-LOX has been

found to have vital role in AD. In the postmortem brain, the intracellular immunoreactivity of 5-LOX was found to be increasing in the hippocampus of AD patients compared to the normal brain.

A close association of 5-LOX immunoreactivity with Aβ plaques, NEFT, and vasculature was observed by double-labeling analysis. Increased level of 5-LOX mRNA was demonstrated in PBMC in late onset of AD patients.

5-LOX induces Aβ formation via the modulation of the γ-secretase complex and also controls tau metabolism by changing its phosphorylated state at specific epitope by means of cyclin-dependent kinase-5 (CDK5). In vivo study has also found that either inhibition or knockout of 5-LOX considerably reduces γ-secretase expression and Aβ level [90]. Hence, 5-LOX pathway likely plays a critical role in the development of the full pathological phenotype of AD which includes aberrant Aβ production and deposition, as well as altered tau phosphorylation [91]. Therefore, 5-LOX can be a therapeutic as well as preventative target which is able to connect the current AD treatment gap.

Serum 5-LOX was found to be significantly downregulated in case of controls as compared to MCI and AD patients. Significant moderate downhill correlation between HMSE and 5-LOX, and strong downhill correlation between MoCA and 5-LOX was observed [92].

Based on the evidences, 5-LOX can serve as one of the potential serum protein markers for AD. Specific 5-LOX inhibitor can be a significant promising molecule for the therapy of AD. In view of the above information, the proposed study attempts to develop peptide inhibitor against 5-LOX which will be a promising lead molecule for developing therapeutic agent against AD.

2.3.9 ApoE

ApoE is glycoprotein expressed in many organs such as liver and brain with different expression levels. The apoE gene in humans includes several single-nucleotide polymorphisms (SNPs) all over the gene [93]. The most common polymorphic alleles of apoE gene are the following: apoε3 (cys112, arg158), apoε2 (cys112, cys158), and apoε4 (arg112, arg158). One of the genetic risk factors for AD is apoε4 variant of the *ApoE* gene [94, 95]. Since then, many studies established that the genetic risk factor is increased for AD in person with apoε4 [96, 97] than individuals with no ε4 alleles [98, 99]. Furthermore, it is also noted that the ε2 allele of apoE is linked with a lesser risk for AD [100].

2.4 Parkinson's Disease

Parkinson's disease (PD) is the most common age-related movement disorder and second most common neurodegenerative disorder after AD [101]. In PD pathophysiology, there is a loss of dopaminergic neurons and formation of Lewy bodies containing α-synuclein in the region of substantia nigra. Substantia nigra is an area of

the brain that influences motor control by the extrapyramidal pathways. Primary motor symptoms developed in Parkinson's disease include the following:

- Tremor of the legs, hands, jaw, arms, and face
- Bradykinesia (slowness of movement)
- Stiffness or rigidness in the limbs and trunk
- Postural instability or impaired balance and coordination

Before clinical symptoms appear, approximately 60–80% of dopaminergic neurons are lost. The exact cause of PD is still unclear. It is likely due to a complex association of genetic and environmental factors. Age is then most important risk factor for PD. ROS accumulated with age, and it was reported that ROS has special function with loss of dopaminergic neurons. Dopamine is a neurotransmitter and metal chelator, which coordinates with Cu^{2+} and Fe^{3+} to generate free radicals. Antioxidant molecules can prevent ROS-induced protein oxidation, thereby improving mitochondrial function [102]. Significant dopaminergic neuronal loss in PD is due to the loss of glutathione (GSH) and high level of iron and calcium in substantia nigra pars compact (SNpc).

Evidence has emerged that calorie restriction has a significant function in aging which affects the course of PD [103]. Calorie restriction regulates the concentration of NADH/NAD^+. It facilitates active respiration and increases the level of NAD^+ but decreases the concentration of oxygen and reactive oxygen species (ROS) [104].

The familial form of PD exists only in a small proportion of patients (10–15%) which is due to mutation in α-Synuclein and leucine-rich repeat kinase 2 (*LRRK2*) genes (in late-onset cases) and parkin (*PARK2*), ubiquitin carboxy-terminal hydrolase L1 (*UCH-L1*), PTEN-induced putative kinase 1 (*PINK1*), and oncogene DJ-1 (*DJ-1*) (in early-onset cases) [105]. The majority of the patients suffer from a sporadic form and the exact cause is still unclear [106].

The mitochondrial dysfunction, oxidative stress, and protein mistreatment have a vital role in PD pathogenesis, which are induced via non-genetic factor interaction with susceptibility genes [107–109]. Insight into non-genetic causes is needed to be further studied to understand the underlying pathogenesis of the disease and to develop effective therapeutic strategies.

A major pathological feature in Parkinson's disease is misfolding, aggregation, and abnormal accumulation of the protein α-Syn. Lewy's bodies, the round intracellular inclusions that form in degenerating neurons, are composed by polymers of full-length a-synuclein (Fig. 2.3). Point mutations of a-synuclein gene are found to be associated with early-onset familial PD. Mutant a-synuclein proteins show higher tendency to form detergent insoluble filaments as well as a higher toxicity than wild-type peptide in cultured neuronal cells [110].

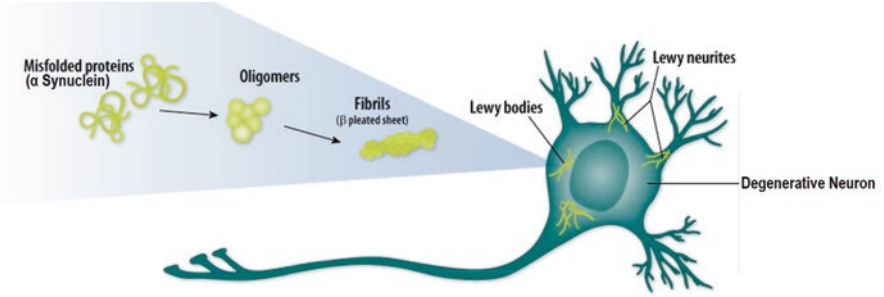

Fig. 2.3 Aggregation of α-synuclein and formation of lewy bodies in Parkinson's disease

2.5 Markers for Parkinson's disease

2.5.1 α-Synuclein

α-Syn protein is secreted into extracellular spaces, so it has been recognized in plasma, saliva, and CSF [111–113]. Secreted fractions of α-Syn protein have also been found to be linked to membrane vesicles of endocytic origin, the exosomes [114–116].

Total α-syn as well as its oligomeric and phosphorylated forms is investigated in PD patients for biomarker development.

Phosphorylated, oligomeric, and total α-syn forms are examined for biomarker development in PD patients.

Phosphorylated form of α-syn protein present in skin nerves has also been reported as sensitive marker for PD after its detection in the skin biopsy of PD patients and compared to controls [117].

α-Syn levels present in CSF have shown to be the most persistent marker. Studies on smaller cohorts did not report much difference in α-syn levels in controls and PD patients [118, 119], whereas larger cohort studies showed contrary results.

Specifically, α-syn protein levels determined through ELISA [120–123], Luminex assays [124–126], or mass spectrometry [125] showed total α-syn protein levels to be significantly lower in PD as compared to control group. α-syn levels in CSF seems to be fairly sensitive in the range of 61–94% and specific from 25% to 64% for distinguishing PD patients and control group [120, 127].

Few studies have reported correlation of total α-syn levels with disease severity [123], but some have reported contradictory results [124]. There was no significant difference observed in monomeric α-syn levels between PD and control group [128, 129], but oligomeric α-syn [119, 123] and phosphorylated form of α-syn [126, 130] were found to be significantly higher in PD [131].

Total plasma α-syn protein levels, observed in PD patients by ELISA, were also found to be elevated [132, 133], whereas western blot reported downregulated levels of plasma α-syn in another study [134]. Few more studies investigated levels of

plasma α-syn by ELISA [119, 135] and mass spectrometry [135] and observed similar results in PD compared with control group.

Plasma total α-syn levels in larger cohorts study were assessed using Luminex assays, where no significant difference was observed between PD patients and control group [130, 136]. On the other hand, another study have found downregulated total α-syn levels in idiopathic PD compared with controls using ELISA, with a likewise trend observed with familial PD [137].

Total α-syn analysis in RBCs in smaller PD cohort study reported upregulated total α-syn in PD patients compared to control group using western blot [138]. Conversely, downregulated total α-syn was observed in PD in comparison with controls in a larger cohort study, using sensitive phospholipid ELISA assay [139]. Furthermore, a larger cohort study reported higher levels of oligomeric α-syn in PD patients than controls but could not be correlated with PD progression [140].

Saliva is easily accessible than other biofluids, salivary α-syn could be helpful in PD detection, but the correlation between salivary α-syn and disease severity remains controversial. When α-syn was studied in an unstimulated saliva by mass spectrometry, western blot, or Luminex assay, no significant differences were observed for the supernatant, cellular component [141], or cellular pellet lysate [142]. Further study done on smaller cohort showed significantly decreased levels of unstimulated saliva (supernatant) α-syn in PD patients compared to controls when assayed by ELISA [143].

2.5.2 Protein Deglycase (DJ-1)

DJ-1, encoded by PARK7 gene, is also analyzed as a candidate biomarker in PD after deletions and point mutations were reported in genetic PD [144]. It is a multifunctional protein that also regulates oxidative stress. DJ-1 isoform levels were analyzed in whole blood from PD patients and control using 2D electrophoresis, mass spectroscopy, and immunoblotting techniques which revealed significant difference between them. Although difference between total DJ-1 levels from PD patients and control subjects was not found significant, some isoforms with 4-hydroxy-2-nonenal modifications in whole blood samples were found to be significantly upregulated and associated to both disease diagnosis and severity [145].

CSF analysis results are not persistent, but one reported downregulation of DJ-1 in PD patients compared to control subjects with a sensitivity of 90% and specificity of 70% [125].

2.5.3 Uric Acid

Uric acid was shown to reduce dopamine oxidation, in the light of accumulating reports that oxidative stress is major factor to the death of dopaminergic neurons in PD. Urate is the principal end product of purine metabolism which circulates at high

concentrations in humans. It has been observed that lower levels of uric acid in PD are associated with higher risk and severity of motor symptoms [146, 147].

2.5.4 ApoA1

Downregulated ApoA1 levels were found to be correlated with early onset PD. Furthermore, smaller increase in plasma ApoA1 level could decrease the risk of developing PD [148]. Since ApoA1 levels are influenced with available PD medication drugs such as statin, therefore development of ApoA1 as a potential marker for PD shows the risks for unbiased biomarker discovery.

Investigators in a cohort study in the United States [149] and Taiwan population [150] have found that the use of ApoA1– elevating statin medications – is linked with lower risk of developing PD.

2.5.5 MicroRNAs

The miRNAs investigation from PD patient's blood reported that miRNA (miR-1, miR-22∗, miR-29a) expression was lower in patients than the controls. The relative expression of another group of miRNA (miR-16-2∗, miR-26-2∗, miR-30a) reported 50% elevation between treated PD patients and control subjects. Furthermore, miR-16-2∗ and miR-26-2∗ levels were upregulated in treated patients compared to non-treated patients [151].

Several circulating miRNAs were reported using the combination of k-TSP analysis and SAM and reported a panel of predictive biomarkers for PD. k-TSP1 (miR-1826/miR-450b-3p), miR-626, and miR-505 produced the highest predictive power of 91% sensitivity and 100% specificity with 100% positive predicted value. However, analysis of the replication set reported 88% negative predicted value [152].

Another study investigated the changes in expression level of serum miRNAs in idiopathic PD and PD with LRRK2 G2019S mutation compared with healthy controls by using RTqPCR and miRNA arrays. Study reported downregulation of four statistically significant miRNAs in either LRRK2 or idiopathic PD (miR-29a, miR-29c, miR-19a, and miR-19b). Subsequently, in validation set, study reported the association of downregulated levels of miR-29a and miR-19b and miR-29c in idiopathic PD [153].

2.5.6 Parkin Gene

The parkin gene is located on chromosome 6q25.2-27. The gene size is approximately over 1.5 Mb with around 12 exons. An early-onset mutation (homozygous exon depletion) in this gene with autosomal recessive inheritance was initially reported in Japanese [153].

At present, there are more than 70 mutations in this gene reported to be associated with the early onset of parkinsonism. These mutations constitute about 50% of familial cases of autosomal recessive juvenile parkinsonism [154].

The clinical sign of disease onset initiates in a vicenarian patient and is conspicuously associated with diurnal fluctuations and dystonia, advancing slowly while escorted by severe levodopa-mediated dyskinesia, without dementia. There is severe neuronal loss in the locus coeruleus and substantia nigra pars compacta which is pathologically confirmed. However, fascinating ubiquitinated Lewy bodies, for degradation process, are inattentive in parkin patient's brains. It signifies that the neurodegeneration pathology varies among several forms of PD. The parkin gene codes for a ubiquitin ligase of 465 amino acids which plays an important role in protein degradation process [155]. It demonstrates that degradation of protein via ubiquitin may play a vital role in the pathology of idiopathic Parkinson's disease. Parkin gene mutations predominantly cause familial early-onset and isolated juvenile Parkinsonism, consequently becoming accurate genetic marker of familial early onset of PD yet.

There are some other genes which are recently identified to have an association with PD, e.g., NR4A2 gene, DJ-1 gene, PINK1 gene, etc. [156–158].

2.6 Conclusion

In the past decade, there are numerous newly identified potential biomarkers which are under investigation for the early diagnosis of respective neurodegenerative disorders with enormous scope for further research. Though the study on development of molecular marker is on a rise, there is currently no potential marker which could detect the disease in a patient-friendly method with considerable sensitivity and specificity. The development (evolution) of novel and specific markers needs more vigorous effort by scientists, and it will help in the disease diagnosis and prognosis and generate new regimens for the therapeutic targets. Therefore, further studies are needed in this area to develop a panel of markers for each neurodegenerative disease which could detect neurodegeneration precisely and accurately. Molecular diagnostic marker would significantly improve the sensitivity accuracy and specificity. With a focused effort, in the future, molecular marker could be a promising tool for developing minimally invasive diagnostic tests for neurodegenerative disorders and therapeutic target to modulate the progression and possibly prevent the disease.

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3 Neurodegeneration During Aging: The Role of Oxidative Stress Through Epigenetic Modifications

Sweta Srivas, Meghraj Singh Baghel, Padmanabh Singh, and Mahendra K. Thakur

Abstract

With global rise in elderly population, there is an increase in the incidence of age-related neurological problems. Hence, achieving healthy aging is a great challenge for scientists, medical practitioners, and social workers. Aging is characterized by enormous remarkable changes in the brain at morphological, cellular, biochemical, and physiological levels leading to cognitive impairment. A key player in this process is reactive oxygen species (ROS) which causes intracellular damage leading to progressive loss of control over biological homeostasis, oxidative stress, and thereby degeneration of different neurons in the brain. Such neurodegeneration is a crucial feature of aging as well as age-related neurological disorders. Therefore, understanding the processes leading to aging and associated neurological problems will help to develop the new therapeutic avenues. The cognitive processes during aging and age-associated neurological disorders are regulated by epigenetic modifications of chromatin. The expression of chromatin-modifying enzymes in turn is regulated by various factors including oxidative stress. As antioxidants prevent oxidative stress, this article reviews the role of ROS in accumulation of intracellular damage along with epigenetic modifications during aging and age-associated neurological disorders and suggests that antioxidants may prove beneficial therapies for these pathologies.

Keywords

Aging · Neurodegeneration · ROS · Oxidative stress · Epigenetic modifications · Antioxidants

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3.1 Introduction

Recent statistics shows that with rise in aged population, there is also increase in the proportion of people suffering from different diseases. Aging as such is associated with different morphological, cellular, biochemical, and physiological changes in the brain. In particular, accumulation of reactive oxygen species (ROS), partially reduced molecular oxygen, causes molecular damages by modulating the function of biomolecules such as protein, DNA, and RNA oxidation or lipid peroxidation [1–3]. The oxidative stress imbalances the normal redox state and decreases the cell repair machinery [4]. Finally, it contributes to the development of neurodegeneration leading to gradual loss of homeostatic mechanisms, cognitive abilities, and memory during aging [5].

Memory is a higher-order brain function which gets affected during aging and age-associated neurological disorders. Several reports have suggested that memory is tightly regulated by expression of synaptic plasticity genes which plays a crucial role in memory formation and stabilization [6]. Research in the past has shown the involvement of epigenetic mechanism in the regulation of synaptic plasticity gene expression at transcriptional level. Epigenetic regulation is defined as changes in gene expression due to covalent modification of DNA and histone without any change in DNA sequence. We have reported upregulation of chromatin-modifying enzymes histone deacetylase (HDAC) 2 and DNA methyltransferase (DNMT) 1 and decrease in synaptic plasticity gene expression during aging and amnesia [7–15]. DNA methylation and histone acetylation also altered at the promoter of synaptic plasticity genes leading to cognitive dysfunction during amnesia [12, 15].

Recent reports have shown that oxidative stress regulates the epigenetic modifications of chromatin and thereby expression and functions of associated genes. As antioxidants detoxify ROS and reduce oxidative stress, they may be used as a therapeutic agent against oxidative stress-mediated epigenetic mechanism involved in memory processes [16]. Therefore, in this article we have reviewed the oxidative stress-mediated epigenetic changes involved in cognitive impairment during aging and associated neurological disorders and their recovery through antioxidant treatment.

3.2 Aging and Oxidative Stress

Aging is a complex programmed phenomenon which influences the physiological and metabolic functions of an organism. It is characterized by enormous changes in the brain at morphological, cellular, biochemical, and physiological levels leading to cognitive impairment. Advanced age affects the cell homeostasis through deregulation of Ca^{2+}, elevation of ROS, and reduction in antioxidant levels, which lead to DNA damage and mitochondrial dysfunction, cell apoptosis, and eventually degeneration of different neurons in the brain. Neurodegeneration is associated with decrease in the number of neuronal cells and subsequently shrinkage and decline in the volume of brain during aging. These changes together contribute to impairment in cognitive function and motor coordination, as observed in age-associated neurological disorders like Alzheimer's disease (AD) and Parkinson's disease (PD) (Fig. 3.1).

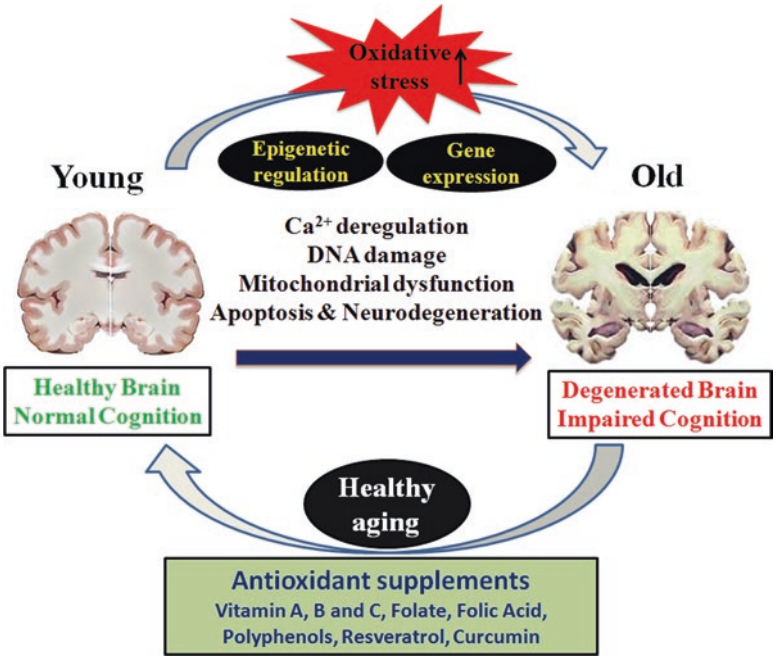

Fig. 3.1 This schematic diagram illustrates the effect of oxidative stress on epigenetic regulation of memory during aging and protective role of antioxidants. The figure depicts a combination of several factors including Ca^{2+} deregulation, elevated ROS level, DNA damage, mitochondrial dysfunction, and apoptosis causing neurodegeneration. The increasing level of oxidative stress and linked factors during aging also affects cognitive processes by modulating the epigenetic machinery and increases the incidence of neurological disorders. Further, antioxidant supplementation modulates these epigenetic changes during aging and could prove beneficial therapeutics for better cognition and healthy aging

3.2.1 Ca^{2+} Regulation, ROS Generation, and DNA Damage

Brain aging is a physiological process that naturally occurs asynchronously in various regions and can be modulated by life style and environmental factors [17]. Progressive normal aging can be converted into pathological, which is represented by neurological disorders with mild cognitive impairment. Disruption in the regulation of intracellular Ca^{2+} levels affects physiological and biochemical processes leading to abnormal homeostasis of the cells [18]. The level of Ca^{2+} determines the transmission of information from presynaptic to postsynaptic neurons across synapses. The level of Ca^{2+} alters during aging and causes disruption in synaptic transmission leading to alteration in cognitive functions [19]. Several reports have shown that Ca^{2+} plays a crucial role in the regulation of ROS production and increased level of intracellular Ca^{2+} activates enzymes which generate free radicals in mitochondria [20, 21]. Moreover, mitochondria are the main source of intracellular ROS generation during oxidation process [22]. It has been elucidated that deregulation of Ca^{2+} signaling, generation of oxidative stress, and mitochondrial dysfunction are

associated with neuronal cell damage during aging and age-associated diseases including AD and PD [23]. Oxidative stress damages several biomolecules like phospholipid, protein, and DNA and also modulates cell signaling pathways and gene expression pattern during brain aging. ROS is one of the markers for neurodegeneration during aging and neurological disorders like AD and PD [24]. ROS is an endogenous inducer of DNA damage, and it makes adduct of 7,8-dihydro-8-oxodeoxyguanosine with DNA, and these adducts accumulate with aging which subsequently reduces the lifespan of an organism [25–28]. Borgesius et al. [29] have shown that mutation in DNA excision repair gene ERCC-1 impairs nucleotide excision repair, inter-strand crosslink repair, and DNA double-strand break repair system and causes age-dependent alteration in neural plasticity leading to neurodegeneration and cognitive impairment. Thus, the age-dependent deregulation of Ca^{2+}, elevation of ROS generation, and damages in genetic material are major factors for neurodegeneration during aging. Age-associated neurodegeneration can be prevented by supplementation of antioxidants, physical exercise, and environment enrichment which helps to regain cognitive ability during aging and age-associated diseases [24, 30].

3.2.2 Mitochondrial Dysfunction and Apoptosis

Mitochondria are major source to produce ROS which targets mitochondrial DNA (mtDNA) [31]. Oxidative lesions of 7,8-dihydro-8-oxodeoxyguanosine are detected more in mitochondria than nuclear DNA, and mtDNA is more susceptible to oxidative damage than nuclear DNA [32, 33]. During aging and age-associated neurodegenerative diseases such as AD and PD, mtDNA accumulate mutations and damages leading to mitochondrial dysfunction [34–39]. Mitochondrial dysfunction is characterized by impaired function, i.e., less oxidation activity, imbalanced mitochondrial fission/fusion events, mitochondrial atrophy, and reduced oxidative phosphorylation, leading to decreased ATP production and reduction in antioxidant activity during aging. These alterations are responsible for disruption of mitochondrial dynamics, integrity, and membrane potential [40].

Disruption of mitochondrial membrane integrity and decreased ATP production are responsible for initiation of release of cytochrome c from mitochondrial membrane to cytosol which activates caspase 3 leading to apoptosis [38]. Moreover, it is well reported that mitochondrial dysfunction leads to apoptosis through release of cytochrome c and activation of caspase 3 in the aging brain [41]. However, this chronic activation of apoptotic markers may cause consistent reduction in neuronal cell number during aging. Several reports have revealed age-related loss of neurons in neocortex and hippocampus which are associated with impaired cognitive functions [42–44].

3.3 Oxidative Stress and Epigenetic Modification

Epigenetic term was introduced in the 1940s by biologist Conrad Hal Waddington and defined as the branch of biology which studies the interaction between gene and their products and the phenotypic change in the organism. Epigenetic modifications mainly through DNA covalent modification and histone post-translational modifications (PTM) play a key role in regulating the gene expression at transcriptional level and their underlying functions.

Methylation of DNA mainly takes place on the cytosine residue which is followed by guanine residue known as CpG islands. It is catalyzed by DNMT which transfers methyl group from S-adenosylmethionine (SAM) to cytosine residue. DNMTs are broadly categorized into *de novo* DNMTs and maintenance DNMTs. The *de novo* DNMTs, DNMT3a and DNMT3b, establish initial methylation patterns on previously unmethylated DNA, and the maintenance DNMT, DNMT1, establishes methylation patterns on hemimethylated replicating DNA.

On the other hand, amino acid at the N-terminal tail of histones undergoes several PTMs, namely, acetylation at lysine, methylation at lysine and arginine, and phosphorylation at serine. They alter the chromatin organization at the local level and regulate the expression of associated genes. These modifications are catalyzed by several chromatin-modifying enzymes which either add or remove small molecule from the histone tail. Among these PTMs, histone acetylation is most studied and catalyzed by histone acetyltransferases (HATs) which add acetyl group from acetyl CoA to lysine residues at N-terminal tail and HDACs which remove acetyl group from lysine residues at N-terminal tail.

Epigenetic modification is regulated by various factors like temperature, light, metal toxicity, anxiety, depression, hormone, drugs, and most importantly oxidative stress [45–52]. Oxidative stress is a common feature during aging and most of the age-related neurological disorders including AD. It also causes alteration in neuronal gene expression, neuronal damage, degeneration, and learning and memory [16]. Recent evidences show that oxidative stress modulates the epigenetic factors and thus associated gene expression and functions.

Oxidative stress induced by H_2O_2 downregulated the expression of DNMT1 and DNMT3a and hypomethylated the promoter region of amyloid precursor protein (APP) and beta-site APP cleaving enzyme (BACE)1 genes leading to their upregulation in SH-SY5Y neuroblastoma cells [53]. DNA cytosine methylation level and expression of DNMT1 decreased in the AD patients as compared to control healthy individuals [54]. Similarly, Chouliaras et al. [55] observed that both DNA methylation and DNA hydroxymethylation level decreased in the hippocampus of AD patients as compared to normal individuals. Oxidative stress is not only observed in neurodegenerative disorder, but it is also a common feature in normal aging. We observed that the expression of DNMT1 was downregulated in the cerebral cortex and hippocampus of old mice as compared to young and adult mice [9]. Similarly,

Oliveira et al. [56] reported that the expression of DNMT3a2 was downregulated in cortex and hippocampus during age-associated decline in memory consolidation. The level of SAM and its precursor molecule folate and vitamin B_{12} decreased in the serum and brain of AD patients [57–59].

Similar to DNA methylation, oxidative stress also modulates histone modifications. HDAC2 expression was upregulated in the hippocampus of old, amnesic, and neurodegenerative mouse model as well as in AD brain [9, 12]. Graff et al. [60] reported that the expression of HDAC2 was upregulated in the hippocampal neuron by oxidative stress when exposed to H_2O_2 or amyloid β_{1-42}, commonly seen in AD. Similarly, the oxidative stress induced by scopolamine upregulated the expression of HDAC2 in the hippocampus of amnesic mice [12]. Though it is not clearly understood how oxidative stress shapes epigenetic landscape, recent reports showed an association between oxidative stress and epigenetic modification during aging and age-associated neurological disorder (Fig. 3.1).

Oxidative stress is in turn regulated by epigenetic mechanisms. Zhao et al. [61] have shown the participation of HDACs in the progress of oxidative stress following stroke by altering the functions of histone or non-histone proteins through deacetylation. Histone phosphorylation also mediated neural necrosis upon oxidative stress in stroke. Also, miRNAs regulate apoptosis and autophagy of neurons, astrocytes, and cerebral vascular endothelial cells after stroke. However, how epigenetic mechanisms regulate oxidative stress during aging is not known.

3.4 Aging and Antioxidants

Antioxidants prevent or delay cellular damage. Antioxidant defense mechanism involves scavenging reactive oxygen/nitrogen species or their precursors, inhibition of ROS formation, binding of metal ions needed for the catalysis of ROS generation, and activation of endogenous antioxidant defenses. The protective efficacy of antioxidants depends on the type of ROS that is generated, the place of generation, and the severity of the damage. With increasing exposure to damaging environmental factors, the endogenous antioxidant defenses are not always completely effective. Therefore, exogenous antioxidants are used to neutralize the cumulative effects of oxidative damage. Antioxidants of widely varying chemical structures have been investigated as potential therapeutic agents for neurodegenerative diseases. Antioxidants have also been shown to modulate the epigenetic modifying enzymes (Table 3.1, Fig. 3.1). These antioxidants are broadly divided into three categories – antioxidant enzymes, vitamins, and phytochemicals.

3.4.1 Antioxidant Enzymes

Antioxidant enzymes (superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase) scavenge ROS and are used as a therapeutic avenue for neurodegenerative diseases and aging. Superoxide, a precursor of free radicals and ROS, regulates major epigenetic processes of DNA methylation, histone methylation, and

Table 3.1 List of antioxidants and their epigenetic targets and effect during aging

Antioxidants	Epigenetic target	Effect	References
Vitamin A	DNA methylation	Causes DNA hypermethylation in dopamine receptor 2 promoter regions and protects dopaminergic neurons	[73]
Vitamin B	DNA methylation	Alters presenilin (PSEN1) expression and neurodegeneration during Alzheimer's disease	[70, 71]
Folate	DNA methylation	Causes global DNA demethylation in the rat brain and thereby affects gene expression during aging	[68, 72]
Folic acid	DNA methylation	Protects against homocysteine-induced neurotoxicity	[72]
Vitamin C	DNA hydroxymethylation, histone methylation	Protects dopaminergic neurons and helps in dopaminergic neurons differentiation	[74]
Polyphenols	Sirtuins (SIRT)	Prevents cognitive decline during aging	[75, 76]
Resveratrol	Sirtuins	Increases cAMP-activated protein kinase phosphorylation	[77]
Icariin	Sirtuins	Protects brain from ischemic injury	[78]
EGb671 (*Ginkgo biloba* extract)	Sirtuins	Protects against β amyloid-induced neurotoxicity	[79]
Curcumin	HDAC isoforms 1, 3, 8, HAT, miRNA-22, miRNA-186a, and miRNA-199a	Inhibits amyloid oligomers and fibrils through binding to amyloid β-plaques and helps in neurogenesis	[80]

histone acetylation [62]. Superoxide-mediated alteration in epigenetic processes might be important for understanding the ROS effects under pathological and physiological conditions like aging. Cu/Zn superoxide dismutase (SOD)1 reduces oxidative damage to DNA and prevents neurodegeneration after brain injury in rodents [63]. Chouliaras et al. [64] have reported that caloric restriction (CR) and upregulation of antioxidants prevents or delays age-related brain pathology. CR also attenuates age-related overexpression of DNMTs. Age-related overexpression of 5-hydroxymethylcytosine in the mouse hippocampus was associated with CR rather than SOD1 overexpression [65]. However, the role of antioxidant enzyme-mediated regulation of epigenetic mechanism during aging is not widely explored.

3.4.2 Antioxidant Vitamins

Antioxidant vitamins play a crucial role in preventing neurodegenerative diseases and aging. Dietary micronutrients including folate, choline, betaine, and other B vitamins have the potential to modulate DNA methylation status [66]. Optimal brain

function including cognition results from highly complex interactions between numerous genetic and environmental factors, including food intake, physical activity, age, and stress which have potential to modulate the DNA methylation status [67]. Folate diet caused global DNA demethylation in the brain of rats and thereby altered the expression of several genes [68]. Therefore, proper maintenance of the epigenomic landscape in normal brain depends on the adequate supply of essential nutrients involved in the metabolism of methyl groups. Kim et al. [69] have also found that folate diet modulates the DNA methylation status during aging. Fuso et al. [70, 71] have shown that vitamin B deficiency (folate, B_{12}, and B_6) leads to an increase of PSEN1 expression probably via impaired DNA methylation in neuroblastoma cells and in the brain of TgCRND8 APP transgenic mice and 129Sv wild-type mice. Further, they suggested that SAM treatment altered DNA methylation pattern and thereby prevented neurodegeneration during AD. Kalani et al. [72] have reported that folic acid supplementation to cystathionine-beta-synthase heterozygote knockout methionine-fed (CBS+/− + Met) mouse brain led to a decrease in the homocysteine level and rescued pathogenic and epigenetic alterations and thereby protected against homocysteine-induced neurotoxicity.

Vitamin A also plays a crucial role in gene methylation. High vitamin A changes in dopamine-related genes expression in all the developmental stages and DNA hypermethylation in dopamine receptor 2 promoter regions [73]. He et al. [74] have shown that vitamin C facilitates dopamine neuron differentiation in fetal midbrain through activation of tet1 and jmjd3 enzymes which further induced 5-hydroxymethylcytosine and reduced H3K27me3 in dopaminergic phenotypes gene promoters.

3.4.3 Antioxidant Phytochemicals

Neuroprotective effects of phytochemicals (carotenoids, flavonoids, allylsulfides, and polyphenols) have been attributed to their antioxidant and anti-inflammatory properties. Dietary sources of flavonoids, the most common group of polyphenolic compounds, can be found in fruits, vegetables, cereals, cocoa, chocolate, and spices. However, the role of antioxidant phytochemicals has been shown to reduce the oxidative damage by altering the epigenetic machinery and thereby improving memory during aging and neurodegenerative diseases. In this regard, many polyphenols display their neuroprotective effects through activation of HDACs. Accumulating evidence suggests that dietary polyphenols (found in chocolate, coffee and cocoa, tea, and red wine) play a significant role in preventing cognitive decline during aging through interaction specifically with the family of SIRTs [75, 76]. It has been shown that resveratrol, a common polyphenol of blueberries, mulberries peanuts, and grapes, activates SIRT1, a member of class III HDAC family, and thereby increases cAMP-activated protein kinase phosphorylation and reduces oxidative damage biomarkers during aging in mice [77]. Similarly, icariin, a flavonol glycoside, showed

protective effect against brain ischemic injury by increasing SIRT1 and PGC-1 alpha expression [78]. EGb671 (*Ginkgo biloba* extract flavonoid fraction) has been shown to protect against β amyloid-induced neurotoxicity through activation of SIRT1. Further, the SIRT1 activation deacetylates Lys 310 subunit of p35 and thereby reduces NF kappa B activity and signaling pathway and MAPK activities [79]. Further, curcumin, a component of the Indian spice *Curcuma longa*, commonly known as turmeric, inhibits amyloid oligomers and fibrils through binding to amyloid β plaques. Curcumin also inhibits HDAC isoforms 1, 3, and 8 and HAT and induces miRNA-22, miRNA-186a, and miRNA-199a in AD model and thereby participates in reprogramming of neural stem cell-directed neurogenesis through its pan-HDAC inhibitory effects [80].

Thus, it is clear that the protection of neurons during aging and neurodegenerative diseases by polyphenols could involve several different mechanisms including epigenetic modifications other than their antioxidant and anti-inflammatory properties.

3.5 Conclusion

The accumulation of ROS through oxidative stress causes neurodegeneration which further leads to altered neuronal functions during aging and age-associated neurological disorders. Here, we reviewed how antioxidants regulate the epigenetic changes such as DNA methylation and histone modifications through oxidative stress modulation during aging and age-associated neurological disorders. In summary, neurodegeneration is a critical feature of aging and age-related neurological disorders. A combination of several linked factors including Ca^{2+} deregulation, elevated ROS level, DNA damage, mitochondrial dysfunction, and apoptosis causes neurodegeneration which further leads to cognitive decline during aging and increases the incidence of neurological disorders. These factors are also responsible for epigenetic modifications by several mechanisms. In turn, these epigenetic changes modulate the expression of memory-related genes and thereby cognitive impairment during aging and age-associated neurological disorders. Further, we discussed that controlling ROS production through antioxidants may prove beneficial to modulate epigenetic changes during aging. In conclusion, we suggest that antioxidants may be used as a therapeutic avenue to prevent cognitive impairment during aging and age-associated neurological disorders.

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Polyglucosan Bodies in Aged Brain and Neurodegeneration: Cause or Consequence?

4

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Abstract

Aging is one of the major risk factors for the onset of a number of idiopathic neurodegenerative disorders. It is not surprising therefore, that the aging process in the brain displays many of the structural and/or physiological changes seen in the brain affected with a neurodegenerative disorder. One of the major hallmarks of such changes includes the formation of proteinaceous and non-proteinaceous (carbohydrate or lipid) inclusions in the neuronal soma and processes. Studies in the recent past have shown a causal correlation between the proteinaceous inclusion and defects in proteolytic processes. However, the physiological basis and significance of the carbohydrate-rich inclusions in the brain have largely been ignored. The carbohydrate inclusions, often referred to as corpora amylacea or the polyglucosan bodies, are aggregates of abnormal forms of glycogen (often lesser branched as compared to normal glycogen), and the inclusions include a small component of proteins as well. Observed both in the neurodegenerative conditions and in the aged brain, whether the carbohydrate inclusions represent causative changes or the end-point changes is yet to be unequivocally resolved. Here, we review our current understanding of the carbohydrate-rich inclusions in the brain and discuss their potential roles in neuronal survival, aging, and death.

Keywords

Lafora disease · Corpora amylacea · Oxidative stress · Neurons · Glia · Alzheimer's disease

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4.1 Introduction

Aging is one of the major risk factors for the onset of a number of idiopathic neurodegenerative disorders. For example, the risk of developing amyotrophic lateral sclerosis (ALS) dramatically increases when a person attains the age of 40 [1, 2] and likewise for Parkinson's disease (PD) and Alzheimer's disease (AD) when one reaches the age of 80 [3]. One of the intriguing correlations between normal aging process and the neurodegenerative process is the presence of structural abnormalities in the brain in both conditions [4–6]. It is widely believed that the age-associated decline in the cognitive functions is likely due to decline in the functionality of the individual neurons rather than the loss of neurons per se [7–9]; therefore the structural abnormalities are likely to contribute the deficit in the neuronal functions [10]. A few of the major cytological changes in the neuronal architecture include the formation of proteinaceous and non-proteinaceous (carbohydrate or lipid) inclusions in the neuronal soma [11] (Table 4.1). However, whether they are the causative changes or end point changes is yet to be unequivocally resolved. Our emerging understanding of the neuronal functions suggests that an imbalance in the homeostatic process could underlie the biochemical and structural changes of the neurons [12]. For example, defects in the protein quality control – the ubiquitin-proteasome system and the autophagy pathway – are thought to result in the deposition of long-lived or abnormally folded proteins in the aged brain [13] as well as in the neurodegenerative disorders [14]. Similarly, the deficit in the lysosomal function is thought to underlie the genesis of lipid-rich pigmented granules, known as lipofuscins [15–17]. Abnormal lysosomes have been also reported in degenerating neurons [18–21]. Supporting this notion, loss of genes coding for the critical regulators of autophagy and/or the proteolysis pathways resulted in shortened life span and neurodegenerative changes in the murine models [22]. Similarly, defects in critical factors involved in the stress response pathways also result in the reduced life span and neurodegeneration [23, 24], thus underscoring the significance of homeostatic processes in neuronal function and survival. One such stress response pathway which is widely implicated in aging and neurodegenerative disorder is the oxidative stress response pathway [25]. Free radical-induced damage and mitochondrial dysfunction are the two key pathways underneath oxidative damage in aging and neurodegenerative diseases like AD, PD, HD, and ALS [26–28] (Table 4.2).

The aged brain and the brain affected with neurodegenerative disorders are characterized by the presence of neuronal inclusions (Fig. 4.1). As mentioned above, a majority of these inclusions are proteinaceous in nature. Some of the well-characterized inclusions include the neurofibrillary tangles [29, 30], amyloid-beta peptide (a-beta) deposits [30], Lewy bodies [31], and the polyglutamine aggregates [32, 33]. In each of these inclusions, the major component of the inclusion is a specific type of protein – either because of an abnormal processing/secondary modification or because of the genetic defect resulting in the altered amino acid composition. For example, in AD, the inclusions are rich in the neurofibrillary tangles or in the a-beta peptides. In PD, the Lewy bodies are characterized by the alpha-synuclein, and in polyglutamine disorders, the inclusions are rich in the

Table 4.1 Types of inclusions reported in aged brain and in degenerating brains (corpora amylacea are covered in Table 4.5)

Inclusion type	In aged brain	References	In brains with neurodegenerative disorder	References
Amyloid plaques (Extracellular Aβ-amyloid peptide)	In parts of limbic system, neocortex, temporal, parietal, and basal ganglia locations	[29, 133–136]	Alzheimer's disease, Down's syndrome	[133, 137]
Neurofibrillary tangles (Intracellular hyperphosphorylated tau protein)	Mostly in the hippocampal and parahippocampal areas and in locus coeruleus	[29, 138]	Alzheimer's disease; progressive supranuclear palsy; Pick's disease; frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17); corticobasal degeneration; frontotemporal degeneration	[139–148]
Lewy bodies (Intracellular inclusions rich in alpha-synuclein protein)	Mostly in amygdala	[138]	Parkinson's disease; dementia with Lewy body; Down's syndrome; Alzheimer's disease; multiple system atrophy; progressive supranuclear palsy	[149–156]
Stress granules (TDP-43 positive inclusions)	Mostly in the medulla, amygdala, and hippocampal areas	[138, 157]	Frontotemporal lobar degeneration with TDP (FTLD-TDP); amyotrophic lateral sclerosis; Huntington's disease; Alzheimer's disease; dementia with Lewy bodies	[158–167]

(continued)

Table 4.1 (continued)

Inclusion type	In aged brain	References	In brains with neurodegenerative disorder	References
Marinesco bodies (Ubiquitinimmunoreactive)	Pigmented neurons, particularly in the substantia nigra	[168]	Alzheimer disease; dementia with Lewy bodies; myotonic dystrophy	[169–171]
Hirano bodies (Intracellular aggregates of actin and actin-associated proteins)	In or adjacent to hippocampal pyramidal cells	[172, 173]	Alzheimer's disease; Parkinson's disease; Pick's disease; amyotrophic lateral sclerosis; ataxic Creutzfeldt-Jakob disease; kuru; scrapie	[173–180]

protein with expanded polyglutamine repeats. These aggregates are also observed in the normally aged brain but are present in lesser extent, and their distribution is also restricted to certain areas of the brain (Table 4.1). These observations have led the researcher to hypothesize that neurodegeneration is an accelerated form of aging and salvage pathways are lost in conditions of neurodegeneration which is functional in a normal aging brain [4]. Among the other forms of inclusions, the lipid-rich lipofuscin granules and the carbohydrate-rich polyglucosan bodies are more common in the aged brain (Fig. 4.2) [4, 34–37]. Studies indicate that lysosomal defects combined with increased oxidative stress could promote the formation of lipofuscin [17, 37]. However, the genesis and the fate of the carbohydrate-rich inclusions, commonly known as the polyglucosan bodies or corpora amylacea (CA), are yet to be fully understood. Since neuronal polyglucosan bodies are ubiquitous in the aged brain and are commonly seen in neurodegenerative conditions, this review will focus on polyglucosan bodies in the neuronal aging and their possible role in neurodegeneration.

4.2 Polyglucosan Bodies: A Historical Perspective

Polyglucosan bodies often referred to as CA in the aged brain were first noted by Jan Evangelista Purkyně (also written as Johann Evangelist Purkinje) in the year 1837 [35]. Rudolf Virchow commonly referred to as the "father of modern pathology" showed in the year 1854 that these bodies reacted with iodine-sulfuric acid and gave violet color [35, 38]. On this basis, Virchow concluded that the CA were celluloselike in nature. The iodine-sulfuric acid reaction was already established for starch in 1814 by Colin and Gaultier de Claubry and independently by Stromeyer

Table 4.2 Changes in cellular physiology associated with aging and neurodegeneration

Physiological process	Aged brain	References	Brain with neurodegenerative disorder	References
Oxidative stress	Positive correlation between age and oxidized protein in specimens from frontal pole and occipital pole	[181–184]	Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, glaucoma, and optic neuritis	[181, 185–201]
	Damage to nuclear DNA and mitochondrial DNA increases with age; however it is tenfold higher in mitochondrial DNA and approximately 15-fold higher in individuals above 70			
	Lipid peroxidation in the cytoplasm of neurons and in the oculomotor nucleus			
ER Stress (Unfolded protein response (UPR) pathway in the ER and calcium homeostasis)	UPR and calcium homeostasis declines with age	[202–208]	Alzheimer's disease, amyotrophic lateral sclerosis, transmissible spongiform encephalopathy, polyglutamine disease, neuronal storage diseases like gangliosidosis, Pelizaeus-Merzbacher disease, Sandhoff's disease, neuronal ceroid lipofuscinosis, Lafora disease	[209–220]
Lysosomal dysfunction (Degradation of proteins transported to lysosomes)	Decline in lysosomal pathway	[221, 222]	Parkinson's disease, Huntington's disease, Alzheimer's disease	[223–227]

(continued)

Table 4.2 (continued)

Physiological process	Aged brain	References	Brain with neurodegenerative disorder	References
Autophagic defects	Decline in autophagic clearance	[228]	Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, Lafora disease, X-linked myopathy, acute central nervous system diseases (stroke, hypoxia/ischemia, brain injury, and experimental brain trauma)	[229–250]
Proteasomal dysfunction	Proteasome activity decreases with age	[251]	Alzheimer's disease, Parkinson's disease, frontotemporal dementia, amyotrophic lateral sclerosis, spinocerebellar ataxia type III, Lafora disease	[252–257]

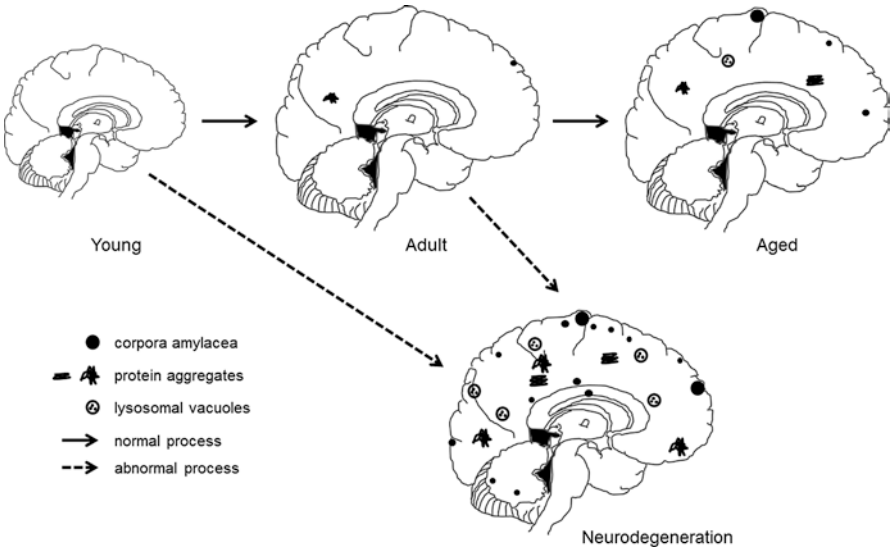

Fig. 4.1 Schematic diagram showing the relative levels and distribution of proteinaceous and non-proteinaceous inclusions in the brain of young, adult, and aged or the neurodegenerative condition, as identified. The depiction is based on the literature survey

[38]. Based on such staining properties, it was G. Busk who first described that CA "were starch and not cellulose, and possessed all the structural, chemical, and optical properties of starch, as it occurs in plants" [38]. The findings of such "plant-specific" starch-like substance in animal tissues stirred the scientific community, as

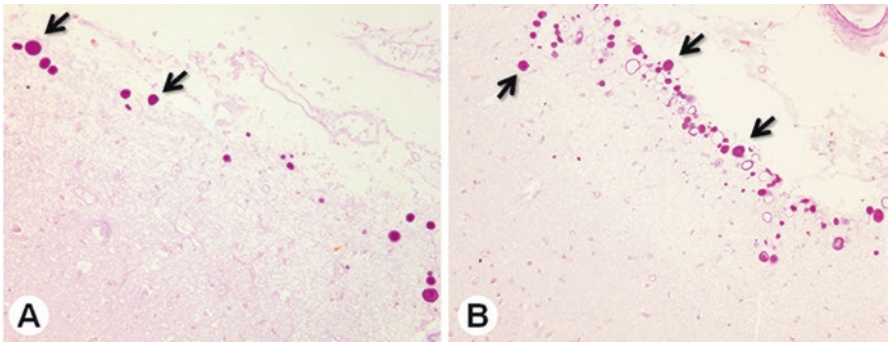

Fig. 4.2 Light micrograph showing the presence of PAS-positive polyglucosan bodies (corpora amylacea) (identified by arrows) in the aged brain (**a**) and in a brain affected with Alzheimer's disease (**b**)

reflected in the words of Ranking and Radcliffe [38]: "The discovery of cellulose and of starch in man is an event of no small moment, for, by demonstrating the presence of vegetable products in the highest animal organism, it does much to break down the remains of that barrier which has been erected between animal and vegetable kingdoms by the dogmatism and prejudices of bygone ages."

The role of polyglucosan bodies in neuropathology was appreciated with the studies on Lafora disease (LD) – a neurodegenerative disorder with progressive myoclonic epilepsy as the primary symptom. In 1911, Lafora and Glueck described "amyloid-like" inclusion in "progressive myoclonic epilepsy" (described first by Unverricht in 1891) [39]. These were later termed as "Lafora bodies." After a year, in 1912, Bielschowsky described the occurrence of intraneuronal inclusions in the brain of the patients with "double athetosis" [40]. It was in 1974 when de Leon proposed the term for these inclusion "Bielschowsky bodies," which were similar to Lafora bodies but differed in their occurrence [41]. It was in 1980 that Robitaille described the chemical nature of the Lafora bodies and of corpora amylacea. He demonstrated both types of the inclusions to be polymers of glucose and gave them a common name, the "polyglucosan bodies" [42]. He also described the occurrence of these inclusions in another disorder, known as adult polyglucosan body disease (APBD) [42]. Since then, Lafora bodies, Bielschowsky bodies, CA, and polyglucosan bodies are categorized under a more generic term called the "polyglucosan bodies." However, the original names are continued to be in use in the literature as they define their occurrence in a specific pathological condition, for example, Lafora bodies in LD, Bielschowsky bodies in Jansky-Bielschowsky disease, CA in the normal aging brain, and the PGBs in the adult polyglucosan body disease [35]. The topological distribution of these inclusions in various pathological conditions differs to an extent. It should, however, be noted that the carbohydrate-rich inclusions represent a "neglected" domain of research. On the contrary, a huge number of studies, in the past two decades, have looked at and dissected the mechanism of disorders involving proteinaceous inclusions.

4.3 Characteristic Features of Polyglucosan Bodies in Nervous System

Corpora Amylacea in Normal Aging Brain CA is observed as a general constituent of the normal aging brain (Fig. 4.1), similar to other characteristic features such as the senile plaques, neurofibrillary tangles in hippocampal neurons, and dystrophic axons in the gracile nucleus [43]. As noted above, CA or the polyglucosan inclusions have attracted much lesser attention as compared to proteinaceous inclusions perhaps because they are generally perceived to be associated only with the senile brain and not with pathological associations. On the contrary, increased density of senile plaques, for example, is associated with neurodegeneration and pathological conditions like AD, which has led to an extensive study into cause and effect of the senile plaques in the brain. However, the origin and fate of the CA in the neurons continue to remain an understudied subject in brain science. CA is known as unbound, filamentous, and spherical or ovoid bodies, ranging from 2 μm to 40 μm in diameter [35, 43, 44]. Sakai et al. [45] were the first one to isolate and describe the chemical composition of CA. They found CA to be majorly a polymer of glucose (87%), with a smaller fraction of protein (4.7%) and phosphate (2.5%) [45]. CA has a dense core and paler periphery [44]. The denser core appears to have randomly oriented branched short linear filaments of glycogen and the periphery to be composed of β-glycogen granules and cell organelles, especially the mitochondria, though the significance of such associations has not be explored [46, 47].

Localization and Distribution of CA in the Human Brain In the aged human brain, CA are reported both in astrocytes and neurons, though they were noted more frequently in the former [42, 44]. Besides, CA are also seen in the extracellular space [48] and in CSF [49], suggesting such bodies could possibly be "secreted" out of the cell. In the neurons, CA are often seen in the axons of the neurons but not in the perikarya (cell body) [35, 50, 51]. While CA have been reported in almost all parts of the aging brain, they have been more frequently observed in the glial feltwork of pia matter; around the blood capillaries, hippocampal regions, and choroid plexus; in the floor of third and fourth ventricle; in the depths of cerebral sulci; and in the white matter [35, 52]. Sakai et al. have drawn a pictorial diagram showing the relative distribution of CA in aging brain [45].

Lafora Bodies Lafora bodies – the other form of polyglucosan inclusions in the brain – were first observed by G.R. Lafora and B. Glueck while performing differential diagnosis for a 17-year-old patient with myoclonus epilepsy [35, 53]. However, they noted that Lafora bodies do not differ much from CA and thus referred to these inclusions with the same name. Later, in 1967, Van Hoof and Hageman-Bal described three different forms of Lafora bodies in cerebral biopsy samples of a 27-year-old LD patient [53, 54]. They being, the "dustlike particles" (referred to as type I), inclusions with intense core and radiating periphery, staining

less densely (type II) and bodies with a "Y" shape crack in the center (type III) [53, 54]. J.B. Cavanagh further suggested that Lafora bodies show a few characteristic features which are not seen in CA and hence supported a distinct name, as Lafora bodies, for these inclusions [35].

Localization and Distribution of Lafora Bodies in the Human Brain Lafora bodies are round or oval in shape, ranging from 10 to 20 microns in diameter, though they show filamentous shape when localized in dendrites [35, 54, 55]. Electron microscopic studies reveal fibrillar structures for Lafora bodies (Fig. 4.3). They are found to be localized in the perikaryon (mostly types II and III) and in the dendrites and neurites of neurons (mostly type I). The anatomical distribution of PAS-positive, "dustlike" (type I) bodies is profuse; however, the type II and III Lafora bodies are most common in the thalamus, locus niger, dentate nucleus, cerebral cortex, basal ganglia, and spinal cord of human brain affected with the LD [54, 56–59].

The Chemical Composition of Lafora Bodies One of the earliest reports about the chemical composition of Lafora bodies is by Yokoi et al. [59]. Lafora bodies were isolated from the cerebral sample of two unrelated patients, and their chemical composition was characterized [59]. They reported that 80–93% of Lafora bodies were polymers of glucose and about 6% was protein. Protein constitutes about 0.6% in amylose and 0.5% in amylopectin, and in Lafora bodies, they are approximately 2% more than in CA [35, 55].

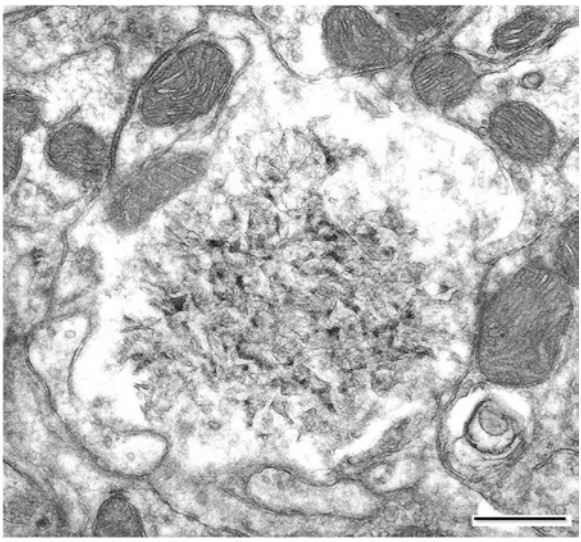

Fig. 4.3 An electron micrograph showing the granular nature of the Lafora bodies in the cerebellar region of Lafora disease mouse model (scale bar = 0.5 μm)

Much of the understandings on the composition and possible genesis of Lafora bodies have come from the studies on the LD animal models. Created by the targeted disruption of the gene involved in the disease, the mouse models displayed most of the symptoms of the LD, including the formation of Lafora bodies in the neuronal and non-neuronal tissues [60] (Fig. 4.3). Confirming the studies from the humans, the Lafora PGBs from the mouse LD models were found to be lesser branched, hyperphosphorylated, and water-insoluble in comparison with the normal glycogen [61, 62]. Specifically, the phosphorylation at C2, C3, and/or C6 carbon of glucose was approximately eightfold higher in tissues of the LD mouse models [63]. Besides the abnormal glycogen, the Lafora bodies were also shown to recruit specific proteins – both in the patients and in animal models of LD (see Table 4.3).

Bielschowsky Bodies Bielschowsky bodies were first described in the late-infantile neuronal ceroid lipofuscinoses (NCL) by Jan Jansky and Max Bielschowsky [64]. Late-infantile NCL is also commonly referred to as "Jansky-Bielschowsky disease," thus honoring the discoverers. These bodies are an uncommon form of PGBs and are also observed in status marmoratus of basal ganglia [65, 66]. Bielschowsky bodies are similar to Lafora bodies and are found in the perikarya of neurons, dendrites, and axons. However, these are typically restricted to pallidum region of the brain [65–67], while Lafora bodies are more spread out in their distribution. A small number of Bielschowsky bodies are also reported in the substantia nigra, putamen, and inner globus pallidus and in brain stem regions [66, 68]. Bielschowsky bodies observed in perikarya were similar to Lafora bodies with dark-staining central core, while the one seen in the neuropil resembles CA [35, 41]. Bielschowsky bodies are noted for their variable size, ranging from 30 to 40 μm in size, and are round to oval in shape and often seen elongated in dendrites and neurites [35]. Ultrastructurally they were composed of ovoid or cylindrical fibrillary aggregates and were electron-dense in the core [35, 69]. Probst et al. identified ribbonlike flattened structure in Bielschowsky bodies which were earlier described as common fasciolar substructures in CA and Lafora bodies [35, 69].

4.4 Hypothesis on the Origin and Function of Corpora Amylacea in the Brain

As mentioned earlier, CA are glycoproteinaceous inclusions observed in the aging brain. The small fraction of these bodies (4%) accounts for proteins, which represent an extraordinary array of proteins of neuronal, oligodendrocyte, and glial origin [34, 35, 52, 70] (see Table 4.3). The origin of these bodies is in part disputed because of such wide variety of proteins and their diverse origin. The following are some of the suggestions with regard to the origin of CA: they are mere artifact of postmortem brain [34, 35, 71], accumulation of protein product derived from degenerating neurons and oligodendrocytes [72, 73], composed of protein secreted from lymphatic system [34, 35, 71], result of abnormal glycogen metabolism [45], and possibly of fungal origin, since fungal proteins are co-localized with these bodies which

Table 4.3 Protein components localized with polyglucosan bodies in aged brain on the basis of immunocytochemical studies

Proteins	Normal aging	Neurodegenerative disease	Reference
Fungal protein (anti-*C. glabrata*)	-	AD,PD,ALS	[70]
Mylen basic protein (anti-MBP)	+	AD	[72]
Proteolipid protein (anti-PLP)	+	AD	[72]
Galactocerebroside (anti-GalC)	+	AD	[72]
Ferritin (anti-fer)	+	AD (S)	[72]
Myelin/oligodendrocyte glycoprotein (anti-MOG)	+	AD (W)	[72]
Heat shock protein (hsp) 27, 28, 70, and 60	Hsp60: N/A	Hsp60: ALS, MS, DE	[100, 101, 258, 259]
	Hsp27: +	Hsp27: CPS, AHS	
	Hsp28 and 70: +	Hsp28 and 70: AD	
		Hsp70: LD	
Keratin sulfate proteoglycan	+	NR	[260]
Tau	+	VaD	[261, 262]
Mitochondrial constituents	+	AD, I	[84, 85]
Advanced glycation end product	+	NR	[83]
Heme oxygenase-1	+	AD (++)	[78, 263]
Complement pathway proteins (Anti-c3D)	-	AD, MS, Pick's disease	[86]
S100 proteins	+	NR	[264]
Reelin	+	AD(S)	[99]
Transglutaminase 1(TG) and TG catalyzed cross-links	+	AD, PD (++)	[262]
β-Actin and β-tubulin	+	AD,PD	[262]
Parkin	+	AD,PD	[262]
Alpha-synuclein	+	AD,PD	[262]
Ubiquitin	+	AD,LD	[258, 259, 262, 265, 266]
Thrombospondin 1	+	VaD	[267]
ADAMTS 13	+	VaD	[267]
NeuN	+	PD	[74, 76]
Adenosine-2A receptor antisense (nucleic acid)	+	-	[268]
Bcl-2	+	I	[85]
c-Jun	+	I	[85]
Nestin	+	PD	[76]
Extracytoplasmic APP	+	AD	[48]
Hsp32	+	-	[83]
Neuronatin	NR	LD	[265]
20 S proteasome	NR	LD	[258]
Malin	NR	LD	[258]
Neurofilaments	NR	LD	[269]

(continued)

Table 4.3 (continued)

Proteins	Normal aging	Neurodegenerative disease	Reference
Desmin	NR	LD	[269]
Concanavalin A	NR	LD	[269]

Abbreviations: *DE* disseminated encephalomyelitis, *CPS* complex partial seizures, *AHS* Ammon's horn sclerosis, *VaD* vascular dementia, *I* ischemia, *AD* Alzheimer's disease, *MS* multiple sclerosis, *PD* Parkinson's disease, *LD* Lafora disease, *ADAMTS 13* a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13, *Bcl-2* B-cell lymphoma 2
(+) present, (++) high reactivity, (–) absent, *NR* not reported

are probably found due to fungal infection [70]. In the following section, we will restrict our discussion to the neuronal and astrocytic origin of these bodies.

Neuronal Origin of Corpora Amylacea The possible neuronal origin of CA was proposed due to its localization with neuron-specific proteins such as NeuN [74], carnosinase (neuronal GABA releasing enzyme) [75], nestin [76], and others (listed in Table 4.3). Similarly, a good number of proteins with glial origin have also been reported to co-localize with CA [77]. Based on these observations, Cavanagh opined that if the neuronal origin CA has to become part of the main peripheral mass of CA found in the subpial regions, then it has to be transferred from neurons to glial cells [72, 78]. In support of this hypothesis, it has been further suggested that such bulk transfer of material from neurons to astrocytes is possible since neuronal protrusions connecting with the oligodendrocytes and Schwann cells are well documented [35, 79–81]. Powell et al. [82] observed polyglucosan body in the intra-axonal protrusion of Schwann cells in the diabetic rat, suggesting this to be the natural route for the expulsion of these bodies from axons. However not much advancement has been made in the recent years to test this hypothesis, and thus it still remains a well-supported hypothesis.

Selmaj and colleagues [73] microdissected CA using laser technology from the postmortem multiple sclerosis brain lesions. Using modern and advanced molecular biology techniques, they identified 24 proteins which were suspected to be of neuronal origin. The majority of these proteins were of cytoskeletal in origin, and thus they proposed that CA is remnants of degenerating and aggregated neurons.

Glial Origin of Corpora Amylacea In comparison with evidence for the neuronal origin of CA, an equally compelling evidence/suggestions support the glial origin of CA. Similar to neuronal hypothesis, CA has been observed in the glial cells, and CA contain a good number of proteins of glial origin, for example, GFAP [77], stress-related proteins, and AGEP (advanced glycan end products) [83]. Another compelling evidence that Cavanagh suggested is the close resemblance of CA with gomori-positive granules found in periventricular astroglia [35]. Gomori-positive bodies are analogous to the CA in many aspects; for example, their number also increases with age, immunocytochemically both are reactive for

mitochondrial-derived proteins and DNA, and they both increase in number in conditions of stress [84, 85]. Thus, these are compelling and persuasive evidence to suggest the glial origin for CA. Recently, Song et al. [78] demonstrated that over-expressing heme oxygenase-1(HO-1) in astrocytes culture leads to vacuolization and accumulation of PAS+ granules. Further, using a transgenic mice GFAP:HMOX-1(heme oxygenase-1expression driven by GFAP promoter), they show that numerous CA were positive for HO-1, ubiquitin, and MnSOD (mitochondrial superoxide dismutase). Thus, the authors concluded that HO-1 causes mitochondrial damage and CA biogenesis in astrocyte culture and in aging brain [78]. This is the first ever attempt to show a direct relation between a protein and its role in the biogenesis of CA.

Function of Corpora Amylacea in Aging Brain Similar to their origin, the biological function or significance of CA in aging brain is not well understood. Some consider CA to be the cause of aging-associated neurodegeneration, and they are also observed in various neurodegenerative disorders, while others propose CA to be "waste containers" where all the deleterious wastes are sequestered to protect the surrounding from their effect. For example, Singharo et al. [86] found CA present in AD to be immunopositive for complement pathway proteins and regulators – a form of innate immune response. Thus, CA might be neuroprotective, by sequestering and preventing the immunogenic protein recognition from lymphocyte and microglia [86]. On the basis of their location, Cavanagh further suggests that these bodies are unlikely to disturb neuronal system; he proposes that CA are observed in the subpial and subependymal regions, and some of these bodies by means of regular tissue movement and vascular pulsation might reach the subarachnoid space. However, very few reports confirm their presence in cerebrospinal fluid (CSF) [49], but an enormous number of CA are observed lying close to the CSF pathway [35]. Cavanagh further suggests that in ageing brain, when the number of CA increases, their expulsion from the brain is not required as they are sequestered in places like subpial and subependymal, where they are unlikely to be harmful to the nervous system [35].

On the basis of chemical nature of CA, which is glycogen, it is suggested that these bodies might trap/sequester products from the oxidative damage to mitochondria and other potentially damaging proteins derived from the aging process, which are nondegradable in nature (AGEP) [35]. Such scaffolding function for glycogen has been recently been proposed in various reports. For example, Puri et al. [87] have shown glycogen accumulation near aggresome under proteasomal stress, suggesting them to sequester damaged proteins. Groen et al. [88, 89] proposed scaffolding function of glycogen by using in vitro setup and showed that they are required for microtubule assembly. Since glycogen synthesis in our nervous system is an attribute of astrocytes, and since these bodies are immunopositive for proteins of neurons/oligodendrocytes origin, it is suggested that these bodies also collect material from extracellular space [48]. Recently, Rai et al. [306] propose another

equally compelling hypothesis that under stress neurons resort to make glycogen and sequester the damaged proteins in them, thus suggesting protective role of glycogen in neuronal survival under stress.

4.5 Polyglucosan Bodies in Nonhuman Brains

PGBs are observed in nonhuman vertebrate brains as well. However due to the scarcity of reports, whether or not these structures have any pathological importance is not known. In this section of the chapter, we will try to compile and present all the available information on animals in which substantial reports are available. These include cattle, dogs, cats, rat, and mouse, and they are detailed in Table 4.4. One of the earliest reports of Lafora body-like inclusions in aged dogs was by Suzuki et al. [90]. The occurrence of these Lafora-like bodies was described in 91 dogs from 1 month to 19 years old and in the gracile nucleus and conus medullaris and their frequency increased with the age [90]. Intriguingly, these bodies were localized in the axon but never in the perikarya of the neurons [91]. It was in 2010 that Márquez et al. described the occurrence of Lafora body-like structures in two canines with a neurological disorder [92]. They found that radial PGBs resembling Lafora bodies were found in the brain stem and diencephalon and were also located in the perikarya of the neurons. Ultrastructural studies showed that these PGBs were similar to the one found in LD patients [92]. These inclusions were found to be immunopositive for the monoclonal antibody raised against human PGBs, thus suggesting the similarity between them [93, 94]. Yamanami and colleagues [94] compared the intraneuronal Lafora bodies in two Lafora patients with the Lafora body-like inclusions in 18 dogs and found that these canine inclusions were histologically, immunohistochemically, and ultrastructurally similar to that of humans. Atoji et al.

Table 4.4 Polyglucosan bodies in nonhuman brains

	References	
Species	Normal aging	Disease
Mice	[270, 271]	NA
Fox (LB)	[272, 273]	NA
Dog (PGB and LB)	[90, 93, 95, 273, 274]	[92, 94]
Rat (PGB)	[275]	NA
Cat (LB)	[93, 273, 276, 277]	[278]
Cow (PGB and LB)	[279]	[280]
Pig	[35]	NA
sheep	[35]	NA
Horse	[35]	NA
Rhesus monkey (PGB)	[281]	NA
Baboon (LB)	[273]	NA

Abbreviations: *LB* Lafora bodies, *PGB* polyglucosan bodies, *NR* not reported

[95] investigated these Lafora body-like inclusions using lectin histochemistry and found these PGBs to be composed of mannose and glucose residue and suggested components to be derived from mitochondria, rough endoplasmic reticulum, Golgi apparatus, and hypolemmal cisternae. Subsequently, some of these breeds of dogs were shown to have mutations in the *NHLRC1* gene - the gene defective in Lafora disease in humans [96].

4.6 Corpora Amylacea in Neurodegenerative Brains

The frequency of CA increases in pathological conditions like AD, temporal lobe epilepsy, PD, and multiple sclerosis [34, 52, 97]. However, other than the massive accumulation of CA in such conditions, there are a few compositional differences in CA as well (Table 4.3). For example, proteins related to an anion exchanger gene family, which included proteins facilitating the movement of ions like chloride and bicarbonate across the membrane and membrane repair proteins, are found to show more reactivity in CA of AD [98]. Another group of proteins that are reported to show strong reactivity in CA from multiple sclerosis is protein related to complement pathway. It was noted that the complement pathway is activated in a neurodegenerative diseases like AD, Pick's disease, and MS and thus CA were examined for the immunoreactivity for the complement pathway proteins. In contrast, the CA in the normal brain were either negative or less active as compared to the one found in these pathological condition [86]. Similarly, Reelin, an important modulator of cytoskeleton elements (actin and microtubule), was found to show more reactivity with CA in AD brains as compared to non-demented control brains [99]. Botez and colleagues [85] have reported increased CA in an individual with the history of repetitive hypoxic episodes as compared to control in Ammon's horn and dentate gyrus [85]. The CA in control as well as the one with repetitive hypoxia episodes was negative for Bax and stained positive for Bcl and c-jun/AP1 proteins [85]. These proteins are involved in cerebral ischemia pathogenesis and thus were tested in this report [85]. Erdmar et al. [100] reported an increase in the reactivity of heat shock protein 27 (Hsp 27) significantly in temporal lobe specimens from patients with complex partial seizures. Hsps are produced in the cells in response to stress conditions (heats, hypoxia, and heavy metal) and pathophysiological conditions like inflammation and ischemia and in growth and development [100, 101]. Thus, CA has been reported to be positive for these proteins as it has been suggestive to be formed in response to stress [102]. A large number of neurodegenerative disorders are associated with the formation of CA in the brain, and they have been detailed in Table 4.5.

Table 4.5 Polyglucosan bodies in neurodegenerative disorders

Disease	Symptoms	Corpora amylacea	References
Alzheimer's disease	Progressive dementia	Similar regions as in normal aged brain but in greater density	[52, 98, 282, 283]
Mesial temporal lobe epilepsy	Epilepsy	Hippocampus	[284–289]
Parkinson's disease	Bradykinesia, rest tremor, rigidity, and postural instability	Substantia nigra and ependyma of the lateral and fourth ventricle	[76, 262, 290]
Huntington's disease	Progressive motor, cognitive, and psychiatric symptoms	Synaptic processes in striatum	[291, 292]
Pick's diseases	Semantic dementia with breakdown in the conceptual database which underlies language production and comprehension. Changes in social behavior and personality predominate	Hippocampus	[86, 293, 294]
Multiple and hippocampal sclerosis	Multiple sclerosis is an inflammatory autoimmune demyelinating disease	Margins of blood vessels	[73, 295]
Focal cortical dysplasia	Epilepsy	Neocortex	[296, 297]

4.7 Neurodegenerative Disorders with Polyglucosan Bodies Resulting from Genetic Defects in the Glycogen Metabolism

Unlike the CA, the genesis of which is still unknown, a few neurodegenerative disorders associating with the PGBs in the neurons are caused by genetic defect altering the glycogen metabolism. This group of disorders, though small in number, offers some insight into the formation of PGBs in the neurons. A few of the well-known examples are discussed below:

Lafora Disease LD is an autosomal recessive disease and one of the most severe forms of the neurodegenerative disease with a defining symptom of progressive myoclonus epilepsy. The age of onset is 10–12 years, and the patient dies within 10 years of the onset, mostly due to respiratory failure. As the age progresses, LD patients exhibit cognitive decline, ataxia, and increasing episodes of myoclonic seizures [103–107]. The hallmark of this disease is the abnormal accumulation of glycogen as Lafora bodies, and these inclusions grow in size and number with age [108]. The causative gene underlying this disease was identified to be *EPM2A*, which encodes laforin, a dual specificity phosphatase, and *NHLRC1* which encodes malin, an E3 ubiquitin ligase [109, 110].

Laforin, a protein phosphatase has a carbohydrate-binding domain at the amino terminal through which it binds to carbohydrate moieties including PGBs [111,

112]. Laforin was shown to function as a glycogen sensor in the cell [113]. Thus, the reduced glycogen reserve is thought to release laforin from the glycogen, leading to its malin-dependent degradation and to increased glucose uptake [113, 114]. Laforin is also proposed to remove phosphate group from glycogen which is incorporated during synthesis as an error by glycogen synthase, and thus glycogen in Lafora bodies is more phosphorylated as compared to normal glycogen [115]. Malin, an E3 ubiquitin ligase, has been shown to regulate the protein level of laforin [116]. Both the proteins have been shown to regulate glycogen levels by regulating the level of proteins involved in glycogen metabolism, for example, PTG [117], debranching enzyme [118], neuronatin [119], and muscle isoform of glycogen synthase [120]. However, reports regarding the glycogen synthase and the debranching enzyme are yet to be unequivocally established as there are reports which suggest no change in activity or levels of these enzymes in LD [121]. As suggested above, the enhanced glucose uptake in LD model is proposed to be another reason behind increased glycogen levels, on the basis of increased glucose transporter levels on the plasma membrane [113]. Thus, the genesis of Lafora bodies in LD can be attributed to the loss of direct regulatory roles of genes involved in the glycogen metabolism.

Adult Polyglucosan Body Disease The adult polyglucosan body disease (APBD) is caused due to homozygous or compound heterozygous mutations in the glycogen branching enzyme 1 (GBE1) [122]. The disease, with age at onset 40 years, progresses slowly, affecting the lower motor and upper motor neuron functions. The symptoms include cognitive decline, peripheral neuropathy, and neurogenic bladder (OMIM#263570). The hallmark of APBD is the accumulation of large PGBs throughout the nervous system – both in the neurons and in the astrocytes. GBE1 (glycogen branching enzyme 1) is involved in creating branches during the synthesis of glycogen. Due to the branched nature of glycogen, it is soluble in the cell. Thus, due to the deficiency of GBE1, less branched and insoluble form of glycogen is formed, which is termed as polyglucosan.

Type IV Glycogenosis or Andersen's Disease Type IV glycogenosis or Andersen's disease, as otherwise it is known, is a rare inherited disorder and is found usually in infants and young children. Polyglucosan-like structures accumulate in many tissues, including the brain, in this condition [35, 123]. The symptoms of this disease are variable, and usually, liver is affected, and death occurs due to liver failure [124, 125]. Involvement of neurological symptoms is uncommon, and if at all it occurs, then it is usually seen in older children [35]. Type IV glycogenosis is caused due to the absence of glycogen branching enzyme. The ADPB is an allelic variant of type IV glycogenosis. PGB has been observed in the spinal cord at 4 months of age. The number of PGB also increases at places (subpial, subependymal, clustering around blood vessels) where they are usually found in elderly subjects [35]. In addition to the usual places, PGBs are also observed in the innermost and outer layer of cortex, in the molecular layer of the cerebellum, in basal ganglia, and in dentate nuclei [35]. As compared to CA, the phosphate content of PGB in type IV glycogenosis is lower, similar to Lafora bodies.

4.8 Concluding Remarks

Neurons are a unique type of cells in that they normally do not store glycogen like the other cell types do [126]. The energy demands of the neurons are normally met by the glucose provided by the supporting cell types, such as the astrocytes [127, 128]. Despite this apparent dependence for the glucose metabolism, the neurons, however, are known to possess required machinery for the glycogen synthesis. For example, glycogen synthase, glycogen phosphorylase, and branching enzyme among others are known to be synthesized and functional in the brain tissue (Table 4.6). Thus, the occurrence of polyglucosan-rich inclusions in the aged brain or in the neurodegenerative disease conditions might likely represent an active process. Given that healthy neurons do not store glycogen, and yet they are endowed with the glycogen synthetic machinery, it points to the possibility that the glycogen buildup is a transient response to a neuronal stress. A support for the hypothesis comes from studies wherein neurons exposed to physiological stress, such as hypoxia or endoplasmic reticulum (ER) stress, are known to have higher levels of glycogen [129, 130, 306]. This would either mean that the glycogen might protect the neurons under stress or that the activated glycogen synthetic machinery is involved in the protective response and thus the glycogen buildup is a by-product. Since aged neurons are known to be under increased stress, the polyglucosan inclusions could possibly represent a failed attempt to cope up with such physiological stress, and that PGBs are neuroprotective. A support for hypothesis comes from the studies wherein the knockdown of glycogen synthase in the neurons leads to decrease in the life span of the fly model under hypoxic stress [130]. An alternate and equally compelling hypothesis is that the PGBs are neurotoxic. Here, the glycogen buildup, possibly resulting from an abnormal physiological response, results in the death of the neurons. Indeed, forced expression of the glycogen synthase in the neurons of murine and fly models leads to an enhancement in the aging process of the brain and neuronal death [131, 132]. Thus, the PGBs seen in the aged brain possibly represent the aging process itself, and they might contribute to the neurodegeneration. A third possibility could be that the neuronal glycogen synthesis is transient stress response mechanism, possibly to protect neurons under transient physiological stress – such as hypoxia and ER stress. However, the prolonged synthesis of glycogen and accumulation could be toxic and detrimental to neurons. The next decade might answer many of these questions and bring the focus back on glycogen in neurodegeneration and neuronal survival.

Table 4.6 Key glycogen metabolic enzymes in observed in neurons

Enzymes	References
Hexokinase	[298, 299]
Glycogen synthase	[300]
Glycogen branching enzyme	[301]
Glycogen phosphorylase	[302, 303]
Glycogen debranching enzyme	[304, 305]

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Signaling of Nociceptors and Pain Perception: Impact of Age

5

Manjula Vinayak and Ajeet Kumar Singh

Abstract

Nociceptor is receptor of noxious stimulus, i.e., a stimulus that potentially leads to tissue damage. Signaling of nociceptors leads to pain perception. Thus, pain (hyperalgesia) is a signal of tissue damage. Acute hyperalgesia is sudden pain and is an essential constituent of protective system, whereas long persisting chronic hyperalgesia with no obvious use has generated great concern for physicians. Persistent chronic hyperalgesia is generally associated with long illness like diabetes, osteoarthritis, and cancer. Research in pain therapy has improved the quality of life to some extent; however, it is still a great challenge to overcome chronic hyperalgesia.

The challenge to treat chronic pain is even more difficult for elderly individuals as the elderly people over 65 constitute the fastest-growing group of people in today's society due to decline in fertility and improvement in longevity. Although the normal aging process does not lead to chronic pain, these painful conditions are more frequent in elderly people than the younger ones. Therefore, the subfield of geriatric pain has grown dramatically over the last two decades. The literature supports that pain in older and younger adults differs in clinically and theoretically significant ways. Several unique characteristics of geriatric pain are proposed due to the possibility of nonuniform age-related variations in physiology and prolonged recovery from tissue and nerve injury. The discrepant findings of age-related increase/decrease or stability in pain require a concrete theoretical framework based on the molecular mechanism of hyperalgesia. Another challenge for the society is to tackle the psychosocial factors important in adjustment to chronic pain in older people. The present chapter reviews the signaling of nociceptors, various types of hyperalgesia with insight in the

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molecular mechanism, age-related patterns in pain, and challenges in assessment strategies to evaluate pain; and establishment of clinically relevant animal models as well as sociopsychological aspect of pain in old people. The chapter also presents avenues for future research.

Keywords
Nociceptive signaling · Inflammation · Ion channels · Hyperalgesia · Chronic pain · Neuropathy

5.1 Introduction

Pain is the terrible fear of mankind and it is more terrible than death itself. Pain relief is the most important task of a physician. Various theories and treatments are associated with acute and chronic pain; still chronic pain most of the time remains untreated throughout the world. Research on pain has gained importance in the last two decades and has become the most burning field of research of the twenty-first century. Challenge of pain treatment mostly lies on complexity of pain physiology. Although pain is not categorized as a disease, persistent pain is difficult to bear, and people must get total or partial relief from it. The experience of pain is a multidimensional process that may include various sensory aspects and emotional components, all of which are involved in activation of different brain areas and neuronal ensembles.

Therefore, insight into molecular mechanism of the complex process of pain perception is demand of the day. The in-depth information will identify potential therapeutic targets for the treatment of pain.

5.1.1 Pain Perception

Pain perception or feeling of pain includes the process of reception of pain stimulus by nociceptors and nociceptive signaling to the spinal cord and ultimately to the brain, where pain is identified.

5.1.1.1 Nociceptive Signaling

Nociceptors are peripheral sensory (afferent) neurons which are stimulated by intense thermal, mechanical, or chemical stimuli and initiate the process of pain perception or nociception [1]. Unlike general concept of carrying information in one direction, the nociceptors exhibit bidirectional signaling while transmitting noxious stimuli from the periphery to the spinal cord. Nociceptors show pseudounipolar morphology, wherein the axon originating from cell body is bifurcated and distributed toward central and peripheral regions. The cell body is located in dorsal

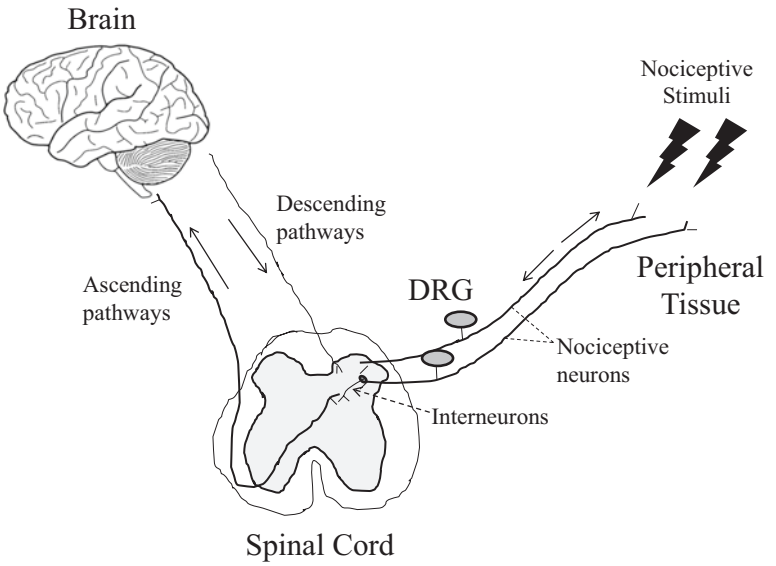

Fig. 5.1 Bidirectional signaling of nociceptors
Nociceptors convey information from the peripheral tissues to interneurons in the dorsal horn of the spinal cord. This noxious information is transmitted from the dorsal horn to the thalamus, cerebral cortex, and higher centers of the brain. The central terminals of nociceptive neurons also transmit information to polysynaptic interneurons. Descending pathways provide feedback signals at the dorsal horn of the spinal cord. Nociceptors may also transmit signals back to the peripheral nerve endings, which results in neurogenic inflammation

root ganglia (DRG) or the trigeminal ganglion. The axonal branch innervates the spinal cord or the target organ, respectively (Fig. 5.1).

The two branches cannot be distinguished biochemically as most of the proteins synthesized by the DRG or trigeminal ganglion cell-like TRPV1, CGRP, and substance P are distributed to both central and peripheral terminals [2–4]. In case of neurogenic inflammation, nociceptive nerve terminals secrete substance P (SP) and CGRP through antidromic activation [5]. This special character distinguishes nociceptors from the prototypic neuron having biochemically distinct dendrite and axon. Because of the unique property, the nociceptor can receive and send messages from either end. Antidromic stimulation of the afferent nerve fibers results in vasodilation, increased vascular permeability, and edema. Apart from central route, the nerve impulses travel to the collateral branches as well as to unstimulated nerve endings to cause release of neuropeptides (axon reflex). As a result the effect is observed in the surrounding area, apart from the point of initial stimulus leading to primary hyperalgesia at the point of injury as well as secondary hyperalgesia in the surrounding area.

There are two major classes of nociceptors based on types of axonal fibers [6]. The Aδ fiber axons are myelinated and fast-conducting (~20 m/s), and C fiber axons are light or non-myelinated and slow-conducting (~2 m/s) action potential toward the CNS. The fast-conducting Aδ fibers mediate initial sharp pain, whereas the C fibers are responsible for slow-reaching prolonged pain with lesser intensity. As a result, two phases of pain are distinguished in case of acute damage. Massive or prolonged input to C fibers may build up in the spinal dorsal horn with increased sensitivity to pain.

In mammals, nociceptors are found throughout the body either externally or internally. Examples of external are the skin, cornea, and mucosa, whereas internal nociceptors are in a variety of organs such as the muscle, joint, bladder, gut, and digestive tract. Based on the variety of stimuli to which nociceptors respond, they may be classified as thermal, mechanical, chemical, sleeping/silent, and polymodal. Silent or sleeping nociceptors do not respond at all to chemical, thermal, or mechanical stimuli unless injury actually has occurred. They respond only on the onset of inflammation to the surrounding tissue. Polymodal nociceptors respond to a variety of stimuli.

5.1.1.2 Supraspinal Processing of Pain

Until recently, little was understood about the cortical mechanisms that underlie the perception of pain. Although electrophysiological studies have demonstrated that some neurons in the cortex respond to noxious stimuli, its correlation with pain was not clear. The critical information about the cortical processing of pain-related information provided with powerful imaging methods [7–9] has revealed that pain is not processed in a single area of the brain.

Central terminals of activated C and Aδ nociceptors in the dorsal horn of the spinal cord release a variety of neurotransmitters. Further interaction with NMDA glutamate receptors located in the postsynaptic neuron triggers calcium-dependent signaling pathways. The cascade via downstream signaling pathways like MAPK, PKC, PKA, PI3K, and Src leads to excitation of neurons to propagate message to the brain, which is responsible for pain perception. Projection neurons within laminas I and V of the dorsal horn carry pain messages to the thalamus and brain stem. The thalamus is capable of sensing the intensity and location of pain, whereas the brain stem is relevant to poorly localized pain. Normally inhibitory interneurons secrete GABA and/or Gly which decreases the excitability of the next neurons. These interneurons modulate (inhibit) pain transmission. The inhibition may be lost in case of injury, resulting in hyperalgesia. Further, microglial cells are activated due to release of ATP and chemokines by peripheral afferent nerves which results in the release of brain-derived neurotrophic factor (BDNF) promoting enhanced pain or hyperalgesia. Release of cytokines like IL-1β, IL-6, and TNFα by activated microglia may lead to central sensitization.

5.1.1.3 Role of Inflammatory Mediators and Ion Channels

Tissue damage leads to secretion of inflammatory mediators by migrated cells (basophils, platelets, macrophages, neutrophils) and resident cells (mast cells,

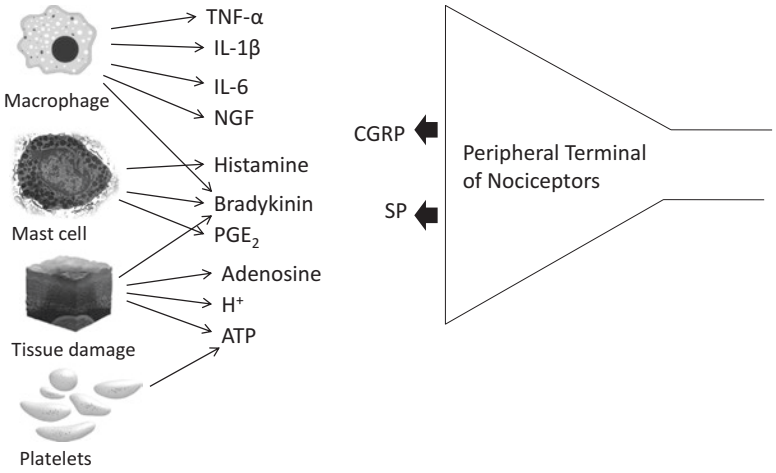

Fig. 5.2 Inflammatory mediators of pain
Peripheral tissue injury leads to the release of inflammatory mediators by resident cell, activated nociceptors, or infiltrated immune cells like neutrophils, mast cells, macrophages, basophils, and platelets. The inflammatory soup includes histamine, serotonin ATP, glutamate, calcitonin-gene-related peptide (CGRP), substance P, bradykinin, prostaglandins, leukotrienes, nerve growth factor (NGF), proinflammatory cytokines (TNF-α, IL-6, IL-1β), and protons. These factors act on the nociceptors by binding to cell surface receptors or by retrograde transport to DRG and spinal cord

endothelial cells, keratinocytes, and fibroblasts). Inflammatory soup includes serotonin, histamine, glutamate, ATP, adenosine, SP, CGRP, bradykinin, eicosanoids (PG, TX, LT), NGF, endocannabinoids, TNF-α, IL-1β, extracellular proteases, protons, etc. which initiate a cascade of molecular events to initiate sensitization of nociceptors and generation of hyperalgesia [10, 11] (Fig. 5.2).

The mechanism of generation of hyperalgesia by inflammatory soup involves ion channels as the key components. Intervention of the channels individually by mutation or molecular blockers has revealed their role in different types of pain sensation [12]. TRP channels function as receptors for plant-derived irritants, including capsaicin (TRPV1), menthol (TRPM8), and the pungent ingredients isothiocyanates and thiosulfinates of mustard and garlic (TRPA1), respectively [13–17]. Molecular insight highlights the contribution of various voltage-gated channels in pain transmission, for example, acid-sensitive ion channels (ASIC), voltage-gated sodium channels, voltage-gated potassium channels, two-pore potassium channels (K2P), voltage-gated calcium channels, TRPM8, and KCNK channel family, KCNK2 (TREK-1) and KCNK4 (TRAAK). Various receptors are responsible for integration of stimuli in order to differentiate the nature of injury (Table 5.1). Such studies have made tremendous improvement in pain therapy.

Noxious stimulation leads to neuronal plasticity which may decrease the body's own pain inhibitory systems, resulting in increased pain. Neuronal plasticity in response to injury, inflammation, and disease may exaggerate pain by increasing

Table 5.1 Nociceptor diversity shown by unique repertoires of ion channels specialized to detect one or more stimulus modalities

Nociceptor subsets	Expressed ion channels
Thermal	
i. Heat	TRPV1 (transient receptor potential cation channel subfamily V member 1)
	Non-TRPV1 heat-sensitive channel
ii. Cold	$Na_V1.8$ (sodium voltage-gated channel 1.8)
	KCNK4 (potassium channel subfamily K member 4)
	TRPM8 (transient receptor potential cation channel subfamily M member 8)
	KCNK2 (potassium channel subfamily K member 2)
	Non-TRPM8 cold-sensitive channel
Mechanical	TRPA1(transient receptor potential ankyrin 1)
	KCNK2 (potassium channel subfamily K member 2)
	KCNK4 (potassium channel subfamily K member 4)
Chemical	TRPV1 (transient receptor potential cation channel subfamily V member 1)
Polymodal	
i. Peptidergic	KCNK4 (potassium channel subfamily K member 4)
	TRPV1 (transient receptor potential cation channel subfamily V member 1)
	TRPA1(transient receptor potential ankyrin 1)
	KCNK2 (potassium channel subfamily K member 2)
	Non-TRPV1 heat-sensitive channel
	Mechano-transduction channel
ii. Non-peptidergic	KCNK4 (potassium channel subfamily K member 4)
	KCNK2 (potassium channel subfamily K member 2)
	Mechano-transduction channel

excitatory or decreasing inhibitory mechanisms. Plasticity may cause short-term changes which last for minutes to hours or long-term changes which may be permanent.

5.1.1.4 Acute and Chronic Hyperalgesia

Hyperalgesia is defined as augmented response to mild painful stimuli resulting in increased pain sensation. Primary hyperalgesia develops at the site of injury due to sensitization of afferent peripheral nerves or nociceptors, known as peripheral sensitization. Intense stimuli initiated due to tissue injury induce acute pain at the site of damage. The acute or inflammatory hyperalgesia detects environmental stimuli (thermal, mechanical, or chemical) and subsides once the cause of injury is withdrawn. Whereas, secondary hyperalgesia develops in the undamaged tissue surrounding the injury making it hypersensitive (increased pain sensitivity outside of the area of injury or inflammation). It is due to impulses which travel to the collateral branches as well as to unstimulated nerve endings. Secondary hyperalgesia develops after a wide range of cutaneous injuries including burns, mechanical

trauma, and freeze injuries and persists long after injury. This is known as persistent or chronic pain. Chronic pain is due to central neuron sensitization where continuous nociceptor input is required from the site of primary hyperalgesia for its maintenance. Chronic hyperalgesia associated with the diseases like diabetes and arthritis develops due to nerve damage which results in persistent inflammation leading to increased spontaneous firing and release of neurotransmitters. It is known as neuropathic hyperalgesia. Chronic hyperalgesia is not restricted to only peripheral region. Nerve injury involves changes in the properties of not only nociceptors but also of the circuits engage in the spinal cord and the central nervous system [1]. In the setting of persistent injury, tremendous plasticity is exhibited by the pain transmission pathway of both the peripheral and central nervous system which enhances pain signals causing hyperalgesia.

5.1.2 Effect of Age on Pain (Nociceptor Sensitization)

Pain is not a direct by-product of aging but rather is a result of pathological conditions that have developed during aging process and are prevalent in elderly population [18]. Increase in neuropathic pain with increasing age is a common feature. There are similarities between the pathophysiological changes during the emergence of neuropathic pain and age-related changes in nociceptors which may be responsible for the development of chronic pain with aging.

There are various types of ailments associated with people with advancing age like high blood pressure, cardiac problem, neurodegenerative diseases, muscular weakness, etc. It is possible that the same factors may operate in a somewhat different manner in multidimensional nature of pain across age groups. Some of the unique characteristics of geriatric pain are correlated with predictors and mediators of pain and their differential adjustment with age. For instance, higher blood pressure has been associated with decreased pain sensitivity in younger but not older people. Female gender has been associated with more intense postoperative pain in younger but not older patients.

5.1.2.1 Somatosensory Changes in Nociceptors

Clinical studies support that neurochemical and neuroanatomical changes taking place in mid-life alter the response to tonic and chronic painful stimuli later in life. Endogenous inhibitory systems degenerate, and cell death increases in advancing age which has been correlated with increased susceptibility to neuropathies. Consistent with other sensory modalities, numerous age-related anatomical, chemical, and functional changes occur in the somatosensory system of both human and animal models [19]. Reduction in the number of peripheral afferents and demyelination of fibers along with increased inflammation are common features of age-associated changes which are similar to the pathological changes that occur following nerve or tissue injury in animals [20]. The number and size of sensory neurons in dorsal root ganglia (DRG) increase throughout early adulthood till midlife of rat and then decrease thereafter [21].

Table 5.2 Effect of aging on pain sensitivity in animals

Animal	Type of pain stimulus	Method	Increase/ decrease sensitivity with age	References
Rat	Thermal and electric shock sensitivity	Paw lick and tail flick	Decrease	[23]
Mouse	Thermal	Hargreaves test	Decrease	[24]
	Mechanical	von Frey test	No change	
Mouse	Thermal	Hargreaves test	Decrease	[25]
Rat	Thermal	Hot plate test	Increase	[26]
Rat	Thermal	Paw immersion test	Increase	[27]
	Mechanical	Paw pressure test		
Rat	Thermal	Licking and guarding	Increase	[28]
Rat	Thermal and mechanical	Neuronal activity and substance P-like immunoreactivity (SP-LI) of the gracile nucleus (GN)	Increase	[29]

Table 5.3 Effect of aging on pain sensitivity during inflammatory hyperalgesia in animals models

Animal	Pain model	Type of pain stimulus	Method	Increase/decrease sensitivity with age	References
Rat	CFA	Thermal	Hot plate test, spinal dynorphin expression	Increase	[30]
Rat	Formalin	–	Paw licking and flinching response	First increase and then decrease	[31]
Rat	Formalin	–	c-Fos expression	Increase	[32]
Rat	Formalin	Thermal	Operant escape testing	Increase	[33]

Further, the cellular and molecular mechanisms produce ROS during the development of chronic pain which is also associated with aging [22], thus providing the rationale for synergistic relations between the process of aging and the pathological condition of chronic pain (Tables 5.2, 5.3, and 5.4).

Effect of age on pain sensation varies in population due to difference in age-related changes in pain signaling. Pain sensations are processed by multiple nervous system components that do not age uniformly [39, 40]. The impact of age on pain sensitivity ranges from increased sensitivity to decreased sensitivity to no change. With advancing age, sensitivity for hearing, taste, smell, vision, and touch decreases due to diminished number of specialized peripheral receptors combined with a deterioration of supporting tissues. Consistent with other sensory modalities, reduction of myelinated and unmyelinated fibers and damage in peripheral nerves lead to diminished excitability of sensory neurons which are responsible for behavioral changes with advancing age of man [41–43]. Alternatively, increased excitability within intact pain pathways has been suggested to increase the magnitude of

Table 5.4 Effect of aging on pain sensitivity during neuropathic hyperalgesia in animal models

Animal	Pain model	Type of pain stimulus	Method	Increase/decrease sensitivity with age	References
Rat	Sciatic nerve ligation (SNL)	Thermal	Hargreaves test	Increase	[34]
Rat	CCI and PSNL	Thermal	Hot plate test	Increase	[35]
		Mechanical	von Frey method		
Rat	L5/L6 spinal nerve ligation	Mechanical	von Frey method	Decrease	[36]
		Cold	Cold plate	Decrease	
Rat	CCI	Thermal	Paw immersion test	Decrease	[27]
		Mechanical	Paw pressure test		
Rat	Partial denervation of tail	Thermal	Tail flick test	No difference	[37]
		Mechanical	von Frey method	Increase	
Rat	CCI	Thermal	Hargreaves test	No difference	[38]
		Mechanical	von Frey method	No difference	

suprathreshold pain sensations in old people. Pain sensitivity in humans includes an increased threshold and decreased tolerance with advancing age leading to age-associated opposite influences on the pain. In spite of the controversial results of preclinical studies, age-related increases in nociceptive sensitivity have been demonstrated under naïve, inflammatory, and neuropathic conditions. One of the potential explanations for these changes is believed to be associated with degeneration of endogenous inhibitory systems, increasing cell death and loss of "buffering" mechanism provided by microglia, rendering compensatory homeostatic mechanisms ineffective, and thus leading to a permissive environment for the increased susceptibility to neuropathies and development of pain [44].

A combination of behavioral, electrophysiological, and molecular approaches revealed that nociceptor sensitization to mechanical stimulation depends on age and the chronicity of injury. C fibers of inflamed aged mice showed reduced or lack of sensitization during acute and chronic inflammation, although these mice exhibited continued behavioral sensitization. The findings provide insight to the processes that may be responsible for differences in pain sensation between young and aged populations [45]. Persistent alterations in immune cells, in particular mast cells and microglia, give rise to chronic pain. Mast cells and microglia communicate with pain neurons, both in the periphery and at the spinal and supraspinal levels, and promote persistent neuroinflammation [46].

5.1.2.2 Chronic Pain in Old Age

Advancing age has been consistently associated with increased risk for neuropathic pain. Pain prevails in old age due to pathological conditions that have developed during aging process [18]. Most common pathologies that cause pain in the older age include diabetic neuropathy, osteoarthritis, postherpetic neuralgia, and lower back pain [47, 48].

5.1.2.2.1 Diabetic Neuropathy

Diabetic neuropathy is a nerve-damaging disorder resulted from diabetic microvascular injury involving small blood vessels that supply nerves (*vasa nervorum*). It constitutes one of the major causes of pain in the older population. The role of diabetic neuropathy in occurrence of pain is reflected by 8.6% increase in the prevalence of pain in diabetic people as compared to overall population of adults [49] which increases with age and with the duration of diabetes [50]. In older adults with diabetes, peripheral neuropathies lead to their adverse effects on stability, sensorimotor function, gait, and day-to-day activities.

The abnormalities that could contribute to neuropathy include oxidative stress generated due to high glucose metabolism and activation of protein kinase C. Progression of these abnormalities might be facilitated by several biological changes during the aging process which involves an increase in the production of advanced glycosylation end products (AGEs), nerve vascular alterations, and impaired resistance to oxidative stress. Furthermore, aging and type 2 diabetes are associated with increased levels of inflammatory markers, such as cytokines IL-6 and TNF-α, leading to nociceptor sensitization and hyperalgesia [51, 52].

5.1.2.2.2 Osteoarthritis

Osteoarthritis (OA) is the most common cause of chronic disability in older adults [53]. Prevalence of osteoarthritis in elderly population ranges from 33% to 50% in different studies [54, 55]. Classically, osteoarthritis has been described as a consequence of wear and tear of the cartilage during aging and subsequent involvement of other structures in the joints. Whereas, current concept for the relationship between aging and OA emphasizes that the aging increases the susceptibility to OA but is not sufficient to cause it. A significant involvement of tissue inflammation has been shown in the arthritic pain [54, 56]. Chronic damage to the joint resulting in inflammation leads to activation of nociceptors. Oxidative stress might be a link between aging and arthritic pain. There are several evidences for oxidative damage in articular cartilage with aging, as well as with OA [57]. Therefore, age-related increase in ROS levels could play an important role in OA [58]. Various inflammatory mediators such as IL-1, IL-6, TNF-α, and other cytokines released during OA may stimulate the further production of ROS. Ineffectiveness of classical analgesics (COX-2 inhibitors, oral NSAIDS, opioids) suggests the presence of neuropathic component in arthritic pain [54, 59–61]. Interestingly, neuropathic pain is also intrinsically linked with oxidative stress [62]. Therefore, the use of antioxidants may prove to be useful in the treatment of arthritic pain in conjunction with classical medications.

5.1.2.2.3 Postherpetic Neuralgia

Postherpetic neuralgia (PHN) is a painful condition which is neuropathic in nature and develops due to reactivation of dormant *Varicella zoster* virus in sensory ganglia. PHN is associated with 20% of patients over the age of 50, which rises up to 35% in patients over the age of 80. The severity of pain also increases with advancing age [63, 64]. High risk of PHN in elderly people is generally associated with their decreased cell-mediated immunity. Although pathophysiology of PHN is not fully understood, it has been shown clearly that it includes neuronal injury at peripheral and central component of nociceptive transmission [65]. Molecular alterations include increased expression of voltage-gated sodium and potassium channels as well as TRPV1 channel [66, 67]. Loss of GABAergic inhibition may intensify the symptoms [67]. There are two clinical patterns of PHN, i.e., irritable nociceptor and deafferentation. Irritable nociceptor is due to hyperactivity of C fibers resulting in allodynia (pain response to normally non-painful stimuli), whereas deafferentation results due to loss of C fibers and rewiring of peripheral neurons causes cross talk between touch stimuli and pain transmitting tracts in the spinal cord, producing allodynia [68].

5.1.2.2.4 Lower Back Pain

Lower back pain (LBP) is a serious debilitating condition which affects almost 30% of elderly population [49]. A clear correlation has been found between back pain with age [69, 70]. The degenerative and inflammatory changes in the facet joints of vertebrae result in the enlargement of these joints. Lower back pain is most often the result of compression of the nerve roots due to radiculopathies (compression or irritation of a nerve). Therefore, LBP-induced pain is supposed to be neuropathic in nature. Few investigators have reported inflammatory cytokines (e.g., TNF-α, IL-6) in degenerated facet joints [71]. As such, it is plausible that aging-associated systemic inflammation may play a role in the development of disk degeneration and subsequently LBP. Therefore, combinatorial approach to target neuropathic and inflammatory components of LBP may be beneficial.

5.1.2.3 Pain and Sociopsychological Factors in Old Age

Persistent pain in old people may limit the physical activities of old people as the activities induce pain. They are also afraid of reinjury or falling [72, 73]. The limiting activity may lead to a cycle of restriction, decreased social participation, and greater disability. Comorbid depression and other psychological factors can worsen pain intensity and cause difficulty controlling pain [74]. Therefore, psychological support should be recommended as an integral component of pain management. Changes such as lapses in memory and concentration may be especially uncomfortable for older adults because of worries about the potential to develop dementia [75]. Older persons generally hesitate to take analgesic medications as they are concerned about the side effects. They also seem to be more worried about becoming addicted to analgesics than are younger people [76].

5.1.2.3.1 Pain Modulation by Emotion

Pain sensitivity is regulated by emotions like fear, anxiety, and stress. Stress may induce or suppress the pain. Stress-induced hyperalgesia and stress-induced analgesia depend on nature and duration of stress as well as on the intensity of stressor. [77]. Similarly pain may be enhanced by anxiety [78]. Pain sensitivity is found to positively correlate with basal anxiety levels in case of some pain patients [79]. Chronic stress induces hyperalgesia. Repeated exposure to a cold environment, a nonnoxious stressful situation, causes mechanical hyperalgesia. Similarly, chronic restraint stress leads to thermal hyperalgesia as observed in the tail flick assay [80].

5.1.2.4 Treatment

Even though there is a wide range of available medicines for pain, the management of pain is sometimes inadequate leading to inappropriate pain control and patient suffering. Non-opioid like NSAIDs and opioid analgesics like morphine are the most common drugs used to manage different types of pain. NSAIDs are used to relieve minor aches and pains which include naproxen, ibuprofen, aspirin, diclofenac, and celecoxib. However, long-term use of these medicines may cause the adverse effects like risk of blood clots, elevated blood pressure, platelet dysfunction, peptic ulcer, nephropathy and renal failure, inhibition of labor, cardiac failure, and sudden cardiac death. Opioids are used in chronic hyperalgesic treatment which has raised safety issues due to increased risk of their side effects. The major concern of opioid treatment includes addiction, tolerance, and neuropsychological effects. The symptoms are nausea, vomiting, constipation, itching, dizziness, sweating, sedation, lethargy, CNS adverse events, and overdose leading to death. Recently antioxidants have shown promising effect in elimination of pain. Polyphenols of dietary source like vegetables, fruits, and drinks (wine and tea) are being tested for their analgesic action [10, 11, 81].

Pain in the elderly population is associated with functional impairment, decreased appetite, impaired sleep, depression, and social isolation. Therefore, a multidisciplinary approach is recommended to investigate all possible options for optimal management: pharmacotherapy, psychological support, and physical rehabilitation. The psychological aspect of pain management such as relaxation, prayer, acupuncture, and physical rehabilitation for adaptation to loss of physical, psychological, or social skills results in the delayed progress in chronic pain conditions and impacts the patient adherence for pain medications [82].

5.1.2.4.1 Challenges in Evaluation of the Effects of Age on Pain Sensitivity

The effects of age on pain sensitivity include increases, decreases, or no change in cutaneous sensitivity with advancing age [83]. Majority of the animal studies examining on age-related changes in pain sensitivity (thermal and/or mechanical sensitivity) rely on reflex-based behavioral measures. Unfortunately these methods do not reveal the same effects on pain sensitivity in humans. Recent studies include reflex-based response along with cortical behavior; however, results are contradictory.

Aging adversely affects the clinical diagnosis of diabetic neuropathy in elderly patients. Most of the diagnostic procedures like nerve conduction studies, determination of vibratory perception threshold, and autonomic function tests are affected as reference values are markedly influenced by age. As a result the pain experienced by aged patients is often unresolved despite pharmacological treatment [84]. Pain remains under-assessed, underdiagnosed, and undertreated in older persons across all healthcare settings [85]. Furthermore, the relationship between neuropathy and *diabetes mellitus* is more difficult to ascertain in elderly patients due to age-related changes in the peripheral as well as autonomic nervous system and the associated diseases. For this reason, careful evaluations with age-adjusted references are very important. For effective diagnosis and treatment of pain in older patients, a proper history and physical examination is essential, whereas pain assessment scales such as the verbal descriptor scales (VDS), the numerical rating scales (NRS), and the visual analogue scales (VAS) generally prove beneficial [86].

Selection of a clinical condition which may serve as the model for preclinical studies is another challenge for studying age-dependent changes. Basic research conducted on pain mechanisms in aged animal models is insufficient, and the reports on pain responses in aged animals have shown conflicting results [33, 45]. Pain in advancing age is generally associated with arthritis, nerve injury, postoperative procedure, stroke, etc. In many instances more than one of these conditions exists, which add to the challenge creating corresponding preclinical model.

5.2 Future Prospects

The translational research in the field of pain and aging needs to focus on establishment of clinically relevant animal models and assessment strategies to evaluate the causal relationships between age-related biological and behavioral changes in pain sensitivity. More recent studies have grown in methodological and statistical quality to analyze pain in a geriatric population [87]. However, additional research is needed to clearly define the nature of behavioral, physiological, biochemical, and molecular changes that occur with aging in normal and pathological conditions. Nociceptors are logical targets for the development of novel therapeutic interventions. Regarding treatment of pain, many receptors and ion channels are recently identified specifically in nociceptors, making these proteins as future targets for pain elimination. Further, novel pain-relieving agents, without serious side effects of NSAIDs or opioids, are needed to be established. Recent studies have demonstrated antioxidants as promising pain-relieving agents. However, more in-depth studies are needed for establishment and clinical use of these agents.

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Association Between Ageing and REM Sleep Loss: Noradrenaline Acting as a Mediator

6

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Abstract

Rapid eye movement sleep (REMS) constitutes a distinct and unique stage within sleep, which is essential for the maintenance of normal physiological processes. It is maximum in the babies, reduces with increased age, and is expressed least in the old age. REMS loss is associated with various pathophysiological disorders; expressions of several of the symptoms are common with those associated with ageing. As many of those common symptoms are induced by elevated levels of noradrenaline in the brain, we propose that the ageing-associated symptoms could be due to REMS loss and consequent increase in noradrenaline in the brain.

Keywords

Brain ageing · Locus coeruleus · Monoamines · Neurodegenerative disorders · REMS deprivation · REM-OFF NA-ergic neurons

Abbreviations

AD	Alzheimer's disease
aDMRs	Ageing-associated differentially methylated regions
GABA	Gamma-aminobutyric acid

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LC	Locus coeruleus
NA	Noradrenaline
NREMS	Non-REMS
PD	Parkinson's disease
REMS	Rapid eye movement sleep
REMSD	REMS deprivation
ROS	Reactive oxygen species
SD	Sleep deprivation

6.1 Introduction

Rest and activity are instinct, reversible physiological states expressed by all living beings; the former has further evolved into sleep particularly in species higher in evolutionary order. The sleep state is a pillar of good health when the body physiology attempts to re-boot itself by replenishing the exhausted component(s). Sleep serves numerous purposes in the short and long term causing acute to chronic conditions including energy conservation [1], tissue renewal [2, 3], memory consolidation [4, 5], brain excitability [6], and maintaining synaptic homeostasis [7]. Based on characteristic electrophysiological signals, sleep has been broadly classified into rapid eye movement sleep (REMS) and non-REMS (NREMS). Normally the former is embedded deep within NREMS as if being protected as a prized possession. Interestingly, one spends the least time in REMS, which is expressed maximum in the babies and reduces with ageing [8]; however, it is never absent in life.

Ageing is an inevitable process, which in a sense is a necessary evil. It is defined as "an intrinsic, progressive and generalized process of physical deterioration that continues over time beginning about the age of reproductive maturity" [9]. Deteriorative physiological changes; increased mortality and reaction time; decreased sensitivity, reactivity, and motor performance; changes in cellular and biochemical composition; reduced adaptability to environmental changes; increased susceptibility; and incidence of some diseases are few of the characteristic features of ageing [10, 11]. Several theories/hypotheses/molecular models have been proposed to understand ageing which includes damage by reactive oxygen species (ROS), telomere shortening, genomic instability, cell death, cross-linking hypothesis, evolutionary senescence theory, etc. [12]. However, our understanding of their mechanism of action is far from satisfactory.

In spite of variations in ageing-associated symptoms, some of the symptoms, e.g., sleep disturbances, are common to ageing population. Most of the elderly people complain of deteriorated sleep quality to various degrees as expressed by reduction in sleep duration, poor efficiency, increased latency, excessive daytime sleepiness, increased sleep fragmentation, and other sleep-related problems [13–16]. Ageing impacts sleep duration, timing, quality, and quantity. The ability of the brain to initiate and maintain this essential phenomenon, sleep, reduces with age (Fig. 6.1). Whether increased sleep problems/issues in aged population can

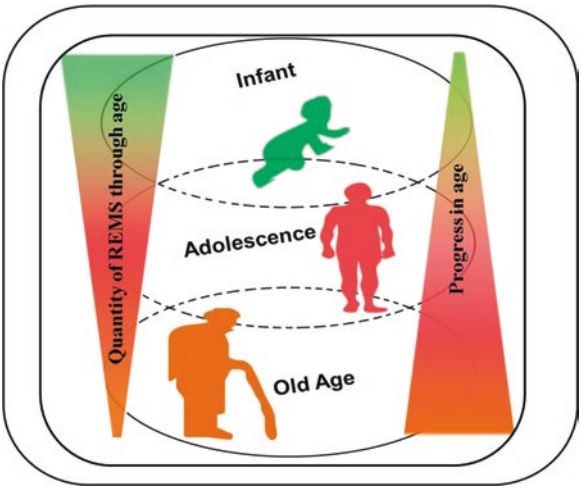

Fig. 6.1 Persistent presence of REMS through the life of an individual reflects its importance in survival and for leading a normal life. This figure shows that REMS is maximum during early life (birth to infant) and decreases through adolescence to old age; however, interestingly, it is never absent in life.

serve as a marker of poor physical health condition associated with ageing is not very apparent.

As like ageing, sleep is a natural instinct behavior, and the two may have a close link to maintain normal physiological processes. Despite recent developments in medicine, molecular biology, biotechnology, and genetics, ambiguity exists on several questions, including those related to ageing and sleep disorders. One of the important questions is can healthy sleep habit help ameliorate ageing-related disturbance in physiological processes and improve quality of life? If yes, what are the means to achieve the same and what could be the possible mechanism? In this chapter, we would explore if and how ageing-associated pathophysiological changes or dysfunctions could be linked to disturbances in sleep, REMS in particular. Further, we would correlate how changes in REMS loss associated with at least one factor, noradrenaline (NA) in particular, could be responsible for ageing-associated various dysfunctions leading to increased neurodegeneration and susceptibility to pathophysiological disorders (Fig. 6.2).

6.2 Ageing and Sleep Disturbances

Both qualitative and quantitative changes in sleep patterns have been linked with ageing. This is reflected by the fact that sleep duration gradually reduces from infancy to childhood to adulthood and progressing towards old age, importantly without compromising the quality of life [13]. However, this is debatable since several studies report more sleep fragmentation than reduced sleep duration in elderly people [16, 17]. The number of naps increases from one per day to more than four on an average [18–20]. It has been reported that the total quantity of sleep reduces linearly with age with a loss of approx. 10 min per decade [8]. Sleep disturbances are multifactorial; for example, some sleep disorders may be associated with

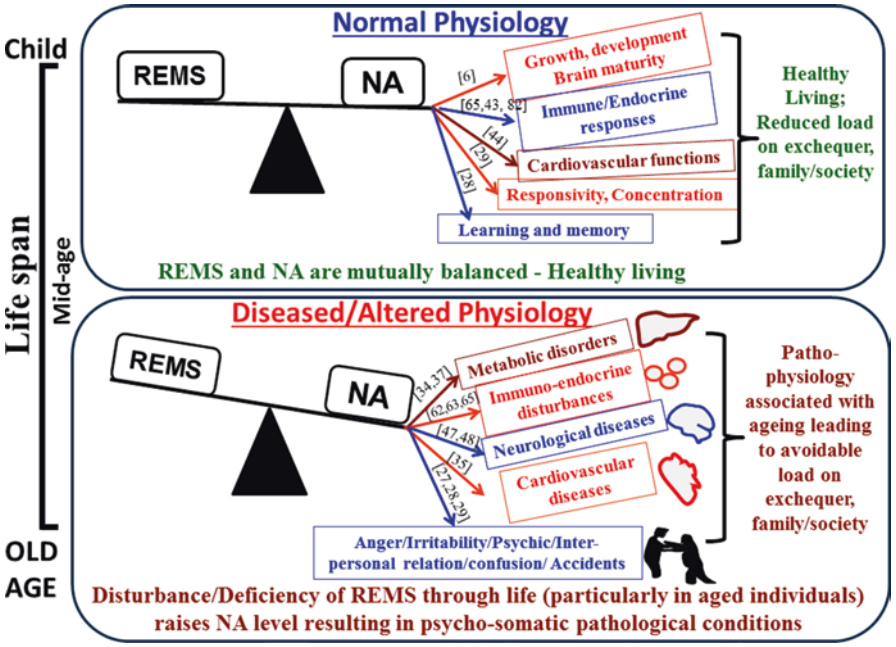

Fig. 6.2 Diagrammatic representation of the role of REMS in maintaining the level of noradrenaline (NA) in the brain, which then modulates most physiological processes of an individual. Upon ageing either the level of NA or REMS is affected, which then directly or indirectly modulates other physiological processes to maintain them to an optimum level. However, due to changes in one or more socio-psycho-somato-pathological conditions/factors, REMS and/or NA is affected, resulting in disorder(s) and/or expression of symptoms including those associated to ageing.

ageing and some circumstantially associated with a disease or disturbance in circadian rhythm, while some may be secondary sleep disturbances induced by treatment [21]. On one hand, sleep disturbance is possibly the most common disorder associated with ageing with or without any associated age-related dysfunction or pathophysiological changes. On the other hand, the increased frequency of cumulative health problems may also contribute to compromised sleep quality in elderly people. Due to the tight association between sleep disorders and many of the multifactorial health problems challenging the elderly people, it is often difficult to distinguish whether pathophysiological changes or dysfunctions through ageing are associated with sleep disturbances [22].

6.2.1 REMS and Ageing

Sleep is divided into NREMS and REMS. As normally REMS appears only after a period of NREMS, a total loss of NREMS prevents REMS as well. Therefore, most experimental studies have been conducted after loss of either total sleep (which includes loss of NREMS as well as REMS) or after loss of REMS. The percentage

of REMS and its latency decreases with age [8], and the prevalence of REMS shifts toward the earlier part of the night [23] with age. REMS was therefore found to increase during the first quarter of the sleep episode, and any further augmentation in REMS during the total sleep period was reduced in old age [24]. Along with REMS, density of rapid eye movements is also affected with ageing. Comparison of the eye movements between young and old age subjects depicted decreased incidence of rapid eye movements in elderly people [25]. It has been reported in a study examining sleep pattern in young (19–28 years old) and older (60–82 years old) subjects that young adults arose preferentially from REMS stage, while older adults arose mostly from NREMS stage [26]. Since REMS is essential for learning and memory consolidation, its gradual loss in aged people may be at least partially responsible for compromised neurocognitive function.

6.2.2 Common Symptoms Associated with Both Ageing and REMS Loss

REMS plays an essential role in several physiological processes. It has been proposed that REMS maintains brain excitability and thus maintains the "**housekeeping functions of the brain**" [6]. Many of the REMS loss-associated symptoms have been reported upon ageing, for example, reduction in excitability [27], memory loss [28], loss of concentration [29], and neurodegeneration [30], while REMS is reduced in ageing-associated diseases, e.g. Alzheimer's Disease (AD) [31], Parkinson's Disease (PD) [32], etc.

6.3 Ageing and Pathophysiological Changes

Ageing is a multifactorial process associated with accumulation of molecular and cellular dysfunction over time leading to physical, social, mental, and cognitive decline. Ageing often leads to emergence of complex disorders, which may lead to conditions not falling under the classically defined diseases and are grouped under geriatric diseases; some of the common ones are detailed below.

6.3.1 Metabolic Disorders

Ageing is one of the important correlates affecting metabolism in the body. The decrease in metabolic rate of an organism is directly proportional to the age [33]. Also, there are changes in body composition, weight, and body mass index with age [34]. The prevalence of many predisposing conditions like insulin resistance, stress, hypertension, obesity, and inflammation increases with ageing which further increases the propensity of cardiovascular and other metabolic diseases [35]. Damage caused by the excess generation of ROS is one of the most prevalent causes of metabolic disorders in old age [36]. Total sleep deprivation (SD), which

includes REMS deprivation (REMSD) as well, has been linked with alteration in metabolic profiling, cardiovascular deficiencies, and neural, respiratory, as well as digestive disturbances, due to fluctuations in different autocrine, paracrine, endocrine, as well as inflammatory molecules in the body. Poor sleep is an important factor for expressing metabolic syndrome and serves as a risk factor for obesity and diabetes [37]. It has been shown that orexin, leptin [38], ghrelin [39], increased body mass index [40], corticosterone, and insulin secretion [41, 42] have also been impaired in sleep-deprived persons. Gradual sleep loss in this day-to-day fast lifestyle enhances the onset of geriatric symptoms early and also makes a person prone to disease manifestation via immunoendocrine disturbances and alteration in secretions of hypothalamic-pituitary-adrenal axis [43]. During SD, there is respiratory acidosis, fluctuation in respiratory exchange ratio and aerobic oxidation, and depletion in glycogen storage [44]. Short sleep duration and associated risk factor have close links for the development and increased risk of future metabolic syndrome [45]. Obstructive sleep apnea patients, who often suffer from reduced REMS, often show increased insulin resistance and glucose intolerance. The severity of obstructive sleep apnea is directly proportional to the progression of metabolic dysfunction [46].

6.3.2 Neurodegenerative Disorders

Ageing affects several molecular, cellular, and physiological processes. Age-related change in these processes predisposes the organism to neurodegeneration and associated pathologies. However, the neurodegenerative disorders develop due to accumulation of factors in the body resulting from consistent insult due to exposure to extrinsic and intrinsic variables. The progression of diseases occurs from presymptomatic stage to mild to severe abnormal state. The AD is the most prevalent neurological disease associated with ageing followed by PD [47]. Thus, ageing is the greatest risk factor for these diseases, and the prevalence of these diseases increases in an exponential manner post 60 years [48]. As ageing progresses, the neurons of the substantia nigra degenerate due to exposure of various stressors. This is also associated with loss of mitochondrial dysfunction and decline in proteasome activity which results in a reduced ability of the neurons to respond to physiological conditions [49]. Sleep disturbances, particularly reduced REMS [32], has been reported in PD patients. In addition, PD patients show increased latency to sleep, fragmented sleep, and daytime sleepiness which are usually associated with REMS behavior disorders [50]. Patients with PD show decreased level of NA and some loss of NA-ergic neurons in the locus coeruleus (LC) [51, 52].

In AD, structural, functional, and cognitive impairment occurs due to tauopathy. There is a metabolic decline in preclinical AD due to amyloid and hyperphosphorylated tau deposition [53] [54]. A gut neurohormone amylin, which has close similarities with β-amyloid (in terms of β-sheet structure as well as receptor function), has been approved for the treatment of AD. This depicts that there is similarity in treatment approach for metabolic and neurodegenerative disorders due to the

complexity and interconnected web of network in molecular pathways in both conditions. Like other neurodegenerative diseases, AD patients also suffer from sleep disturbances. Polysomnography indicated the loss of REMS and increased REMS latency in AD patients [55–57]. REMS behavior disorder, like REMS without atonia, is a distinguishing feature in AD [31]. Although the brain of AD patients shows a significant loss of cholinergic population, loss of LC neurons has also been reported with progression of AD [58–60]. The surviving NA-ergic neurons are reported to be highly active possibly for maintenance of high NA level in the brain in ageing and AD [61].

6.3.3 Immunological Diseases

Ageing is often associated with dysregulation of the immune system, leading to decline in T-cell number and function [62]. There are changes in the innate and adaptive immune systems, increased susceptibility to infection, autoimmunity, malignancy, and impaired wound healing [63]. These are often associated with a decrease in B- and T-cell production in primary and secondary lymphoid organs along with diminished function of mature lymphocytes [64]. Also, sleep is compromised in most infections and diseased conditions [65–70]. The amount of time spent in NREMS increases, while that in REMS is reduced in cases of several infections [71]. REMSD has been reported to alter levels (or release) of several hormones, metabolites [72], interleukins [68, 73–75], enzymes and their activities [76], neuronal structural proteins, and apoptosis [77, 78] in the brain. Older people show a decreased response to immune challenges as compared to younger individuals. In nonobese mouse model of diabetes, it has been shown that 96 h of SD significantly decreased the number of lymphocyte in peripheral blood [79]. Thus, sleep loss (total or REMS loss) might trigger autoimmune disorders and weakening of host defense against diseases. SD also causes upregulation of different inflammatory cytokines like IL-1 and TNF-α which have been shown to be associated with various types of sleep disorders [80, 81].

We have seen above that total and/or REMS loss affects the immune responses; however, the mechanism of action is not known. The loss of sleep (a behavior) is likely to modulate (induce or reduce) a factor, which then would affect the immune responses. As we have seen that at least REMS loss elevates NA level and that affects many of the sleep/REMS loss-associated changes, we propose that altered NA level could be responsible for at least some of the sleep loss-associated changes in immune responses. This view may be supported by the fact that NA is essential for the maintenance of normal level of antibody production in vivo and thus augments the $CD4^+$ T- and B-cell activity [82] and modulation of the immune responses [82].

6.4 Molecular Changes in the Ageing Brain

Gene expression patterns vary through the life of an organism. Ageing is genetically regulated, and variations in the expression of genes can either extend or shorten longevity. As described in introduction, several hypotheses/theories have been proposed in order to understand the deteriorative changes associated with ageing. However, still the molecular aspects of ageing are not completely known. Oxidative stress associated with ageing can cause DNA damage which leads to changes in gene expression and so on [12]. Ageing is associated with significant changes in brain architecture (molecules to neuronal connections), loss of neurons and glia [83, 84], myelination [85], and synaptic plasticity [86], and changes in gene expression in the brain [87], sleep disturbances, and many pathophysiological conditions [48]. Interestingly, many of these changes are common to be associated with sleep disturbances or experimental sleep loss.

6.4.1 Changes in Gene Expression

Molecular characterization of age-related changes in hypothalamus and cortex shows different patterns of gene expression. High expression of mitochondrial respiratory enzymes in the hypothalamus and not in cortex suggests increased production of hypothalamic ROS in aged mice [88]. Gene expression of gamma-aminobutyric acid (GABA) receptor α-1 subunit, essential in signal transduction of GABA, decreases only in aged cortex and not in hypothalamus. This difference in the gene expression between hypothalamus and cortex could be due to the differences in the rate at which different brain tissues age. This may be due to tissue-specific metabolic as well as environmental variations. Therefore, some molecular mechanisms would be specific, and others would be common in different brain tissues during the ageing process. In the same study, the gene expressions of Na^+/K^+-ATPase and synaptotagmin-I have been shown to decrease in both aged cortex and hypothalamus. The expression of the enzyme tyrosine hydroxylase also varies differentially in a region-specific manner [89].

Several genes involved in neuronal growth are downregulated with ageing. Significant changes in gene expression of proteins associated with synaptic plasticity, mitochondrial function, and vesicle transport occur with ageing [90]. Age-related changes in gene expression in the brain have also been associated with cancer and other age-related disorders [91]. Gender-based differences also exist in the ageing brain. Different categories of genes across all brain regions were primarily affected more in males than in females [92]. These gender differences could be due to differences in the lifestyle, hormonal levels, and environmental factors interacting with the genome. *Although several studies have identified variations in gene expression of enzymes/factors involved in REMS regulation* [93–96] [97], *their correlation with ageing is still not completely known and needs to be established.*

6.4.2 Age-Related Epigenetic Changes

Epigenetic variabilities such as DNA methylation, histone modifications, and microRNA regulation are common mechanism related with pathophysiological changes. Epigenetic changes are associated with ageing process, and they affect the life span of an organism [98]. Persistent epigenetic alterations occur in the cells that keep changing their state through development and differentiation and also during ageing. Therefore, epigenetic changes in those genes which are related to ageing affect the life expectancy. Epigenetic modifications may compromise transcription accuracy leading to unfavorable effects on life span in ageing cells [99]. Age- and tissue-dependent DNA hypo- and hypermethylation have been reported in several studies [100–102]. DNA methylations are counterbalanced by demethylases along with the involvement of several other proteins in the pathways required for carrying out oxidation, hydroxylation, and repair. Therefore, it is quite possible that such complex phenomena of methylation and demethylation involving several proteins may become inefficient, with the age of the organism [103, 104].

DNA methylation of gene promoter significantly increases in human cerebral cortex which negatively affects expression of genes involved in synaptic signaling and related functions in old age brains [105–107]. Histone acetylation decreases, while phosphorylation increases with ageing [108]. The histone modifying-enzymes sirtuins 1–7, especially sirtuin 1, tend to be downregulated with ageing, and activation of sirtuins through calorie-restriction extends the life span [109]. The ageing-associated differentially methylated regions (aDMRs) and promoters of age-associated genes have a common polycomb repressive complex 2 sequence which represents an epigenomic mark. These polycomb sequences are associated with aDMRs hypermethylation with ageing, while the hypomethylated aDMRs are associated with enhancer sequences [110]. Mammalian ageing is associated with several epigenetic modifications particularly in the promoters of cell cycle regulatory genes which also include the senescence-associated heterochromatin loci [111]. In the prefrontal cortex, there is loss of histone acetylation at the proximal promoters of genes essential for GABA-ergic neurotransmission, 5-hydroxytryptamine (5-HT)-ergic signaling, and mitochondrial functions [112]. In the cerebral cortex and hippocampus of ageing rodents, chromatin opening/accessibility is not facilitated due to the loss of favorable epigenetic marks such as histone acetylation, while the repressive chromatin marks, including di- or trimethyl histone H3K9, are upregulated [113]. Also, the hippocampus of aged mice depicts decrease in acetylated H4K12 at regions of actively expressed genes [114].

Altogether, such changes in the ageing brain chromatin might impair neuronal gene expression [90, 115], thus causing a decline in the signaling capacity of nerve cells, deficits in axon myelination, and so forth ultimately leading to neurodegeneration [116, 117]. Several isolated and independent studies indicate the crucial role of epigenetic modifications in the development of age-associated diseases such as AD, PD, dementia, and cardiovascular disorders [118]. Earlier we have discussed in detail the role of these modifications in the regulation of NA level in the brain, REMS, and associated neurodegenerative disorders [119]. *However, the role of*

epigenetic changes in the regulation of age-associated REMS loss and NA-associated modulation needs systematic study.

6.4.3 REMS Loss-Associated Molecular and Epigenetic Changes in the Brain

Although some isolated attempts have been made, detailed study on the molecular and epigenetic changes after REMS loss is still missing; therefore, thorough and systematic investigations in these directions are needed. Nevertheless, we summarize here the findings available in the literature on the changes in neural gene expression after total SD. Using microarray, 14 mRNAs have been identified which were modulated by sleep and wake in specific brain regions of rats [120]. More recently transcriptome analysis of brain samples after REMSD revealed alterations in the expression of several genes involved in chromatin assembly, methylation, learning, memory, neuronal plasticity, as well as genes related with synaptic transmission and neuronal excitability [121]. These and related molecular studies strongly suggest that sleep disturbance affects basic cellular machineries, neural plasticity, metabolism, and neurotransmission. However, characterization of molecular correlates of REMS loss and wakefulness is still a far cry and needs to be done using modern technology.

6.5 Age-Related Changes in NA Levels

It has been reported that the levels of monoamines including NA, dopamine (DA), and 5-hydroxytryptamine (5-HT) decreased in different brain regions in aged rats as compared to young rats [122]. However, the levels increased after the rats were treated with antioxidant, lipoic acid, which reduced the oxidative stress. In the elderly, decrease in DA and 5-HT levels was observed in hippocampus, amygdala, and striatum, the areas linked with age-related neurodegeneration, while NA and 5-HT decreased in the brainstem, the region related with REMS regulation [123]. The NA level was found to reduce by about 50% in the hypothalamus in aged Sprague-Dawley rats [124].

The peripheral sympathetic nervous system activity increases in the elderly people in a region-specific manner [125]. The forebrain NA-ergic projections cause an increase in peripheral sympathetic activity [126, 127]. Elevated NA turnover has been reported in the elderly men as compared to young men [125]. Increase in plasma NA in older people [128–130] and aged Sprague-Dawley rats [131] has been reported, which could at least partly be due to reduced NA reuptake [125, 132]. Although the plasma NA content increases, reduced neuronal [133] and cytoplasmic NA contents [134, 135] have been observed along with a reduced, unchanged, or slightly elevated tissue [136] NA levels. Although sporadic and isolated studies reported reduced NA-ergic activity, majority of the studies showed overall elevated NA-ergic activity in the brain as well as in the periphery of aged humans and

animals. Differences in results in some studies could be due to differences in conditions of the experiments and other non-specific factors. Notwithstanding, it is probably safe to interpret that at least in those conditions where there was elevated NA-ergic activity in aged subjects, it could be related to reduced REMS.

6.5.1 REMS Loss-Associated Effects Could be Induced by Elevated NA in the Brain

REMS loss-associated dysfunctions are likely to be induced by affecting the REMS regulatory mechanism(s). In brief, REMS is controlled by the reciprocal interaction between the REM-ON (cholinergic) and REM-OFF (NA-ergic) neurons located in the brainstem; neurons from other brain regions may affect REMS by modulating these neurons [137]. The NA-ergic neurons in the LC are REM-OFF type; they are continuously active during all stages except during REMS, when they cease activity [138]; however they continue activity during REMSD [139]. Because of such nature of the NA-ergic REM-OFF neurons, it was proposed that NA level would rise in the brain upon REMSD [139], which has been confirmed recently (Mehta et al., 2017). Additionally, increased tyrosine hydroxylase [140] and dopamine β-hydroxylase and decreased monoamine oxidase A [141] and corresponding changes in their gene expressions [142, 143] also support effective elevated levels of NA in the brain upon REMSD.

6.6 Conclusion and Working Hypothesis/Model

Several studies have reported that ageing is associated with sleep/REMS disturbance as well as host of physiological dysfunctions, which may or may not be associated with significant pathological conditions. Many of the altered physiological conditions are common to ageing as well as sleep/REMS loss. We argue that both the ageing and sleep loss, REMS loss in particular, are likely to affect one or more common factor(s), which in turn affects the former and induces associated changes directly or indirectly. We have argued that the level of NA changes upon REMS loss as well as with ageing. Also, many of the common altered physiological conditions (irrespective of expression of full-blown pathology or not) are associated with REMS loss as well as ageing and are induced by NA. Therefore, *we propose that alterations in the level of NA could be central for ageing-related sleep disturbance and pathological conditions. Hence, maintenance of NA level in the body including in the brain could ameliorate the ageing-associated symptoms, although ageing* per se *is inevitable and may not be avoided.*

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Conflict of Interest Authors declare no conflict of interest.

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Age-Related Changes in the Human Retina: A Role for Oxidative Stress

7

Tapas Chandra Nag

Abstract

The human retina undergoes subtle, age-related changes with aging. The changes are obvious in most layers and especially in photoreceptor cells. Examinations of postmortem donor retinas (35–94 years) by light and electron microscopy revealed significant structural alterations of the components of photoreceptor outer and inner segments. Immunohistochemical localization with biomarkers of oxidative stress showed an age-dependent intensification of oxidative stress; both lipid peroxidation and protein nitration occurred predominantly in aging photoreceptors, with the former restricted to photoreceptor outer segments and the latter is predominant in their inner segments. Besides, lipid peroxidation is a problem for Müller cells of the aged retina. Antioxidant support by way of upregulation of antioxidant enzymes is not robust enough to counteract the oxidative stress. The mitochondrial superoxide dismutase-2 shows a clear upregulation; other enzymes, such as glutathione peroxidase-1 and glutathione S-transferase, show a decrease in expression with aging and this may be responsible, in part, for the age-related alterations as well as loss of neurons from the aging human retina. Mechanisms for increased antioxidant support, via both exogenous and endogenous routes, seem an important area of future investigation.

Keywords

Aging · Oxidative stress · Cell loss · Antioxidant enzymes · Photoreceptors

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7.1 Introduction

The retina is a delicate neural tissue that serves important tasks like recognition of objects, clear perception of their shape, and size and color discrimination. It possesses a diverse kind of neurons, each performing a dedicated specialized task in the interpretation of the visual world. The number of neurons is clearly high to perform these acts, and the highest density of certain neurons, for example, the cone photoreceptor and bipolar and ganglion cells, are characteristically concentrated in a circumscribed retinal region, the fovea, which works to achieve the highest visual resolution.

The general histological organization of the human retina is illustrated in Fig. 7.1. The macula is characterized by the preponderance of the cells in the ganglion cell layer and inner nuclear layer and a thin outer nuclear layer (Fig. 7.1a). Outside of the macula, the thickness of the ganglion cell layer and inner nuclear layer gradually decreases (Fig. 7.1 b, c), while that of the outer nuclear layer increases in the far periphery due to abundance of rods (Fig. 7.1c). Cone photoreceptor cells are abundant in the macular region, the greatest being in the center of the fovea.

During the course of normal aging, the retina undergoes significant morphological and neurochemical alterations [49, 61, 63, 65, 66, 76, 86, 97]. Quantitative analysis revealed a gradual loss in the abundance of several classes of retinal neurons, particularly the rod photoreceptor cells [40, 49] and bipolar [1] and ganglion cells [40, 45]. Factors contributory to those age-related alterations in the retina seem multifactorial, though these are at present essentially undefined.

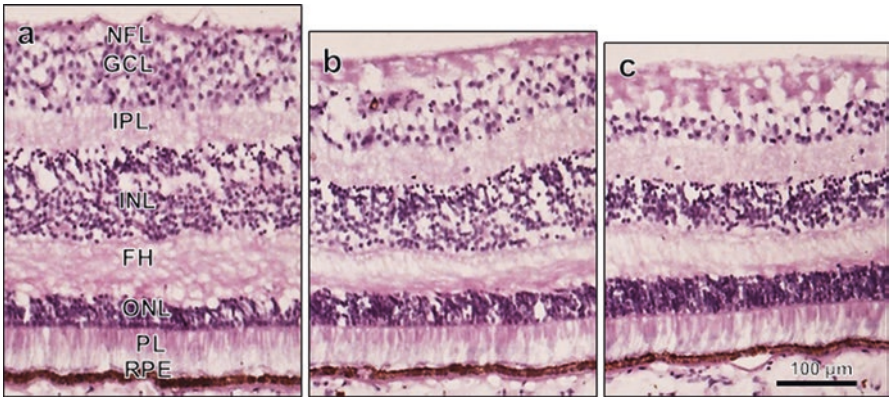

Fig. 7.1 Histological organization of human retina (72-year-old donor) in the macula (**a**), near peripheral (**b**) and far peripheral region (**c**), stained with hematoxylin and eosin. Note that the thickness of the ganglion cell layer (GCL) and inner nuclear layer (INL) gradually deceases away from the macula, whereas the outer nuclear layer (ONL) is the thickest in the far periphery (**c**). Other layers are indicated. *NFL* nerve fiber layer, *IPL* inner plexiform layer, *FH* fiber layer of Henle (present in macula; A), *PL* photoreceptor layer, *RPE* retinal pigment epithelium. Scale bar (shown in **c**) applies to all other figures

It is reasonable to assume that the human retina, like many other tissues, is prone to be attacked with reactive oxygen species and free radicals that are generated in metabolic processes, since the tissue is highly metabolically active and thus has greater oxygen demand (in fact the highest of all body tissues). Also, owing to its continual exposure to visible light and high lipid content in photoreceptor outer segments, the human retina is susceptible to be impaired by oxidative stress [7, 47]. Oxidative stress ensues when there is a gap between the high generation of free radicals and limited defense mechanisms via the endogenous levels of cellular antioxidants. However, relatively little is known on oxidative stress in the aging human retina. It is rich with a diverse kind of vitamins as antioxidants (vitamin C and E), antioxidant enzymes [glutathione peroxidase, glutathione S-transferase, and superoxide dismutase], and a major cellular antioxidant, glutathione. These are essentially involved in counteracting with the production of free radicals and reactive oxygen species, as happens in many tissues [27, 44, 59, 100, 104]. More interestingly, the human retina is endowed with a rich amount of certain carotenoids (lutein and zeaxanthin) in the macular and peripheral regions [90, 91], which can slow down the inception of the age-related maculopathy [57, 85, 90], the pathological damage that occurs with aging of the macula. Furthermore, these naturally occurring compounds are proven to protect the retina from light-induced oxidative damage [8], which a major problem for the light-sensitive photoreceptor cells. Thus, inevitably, if the levels of carotenoids and antioxidant enzymes fall short of, excessive generation of free radicals can have a devastating oxidative effect on cellular proteins, lipids, and nucleic acids, leading to cell damage and ultimate death.

In this paper, an attempt is made to elucidate the various age-related morphological alterations of the retinal neurons, especially the photoreceptor cells and gradual decline in their density, showing evidence that oxidative stress, which intensifies with aging, could be one of the leading factors involved in those events.

7.2 Aging Changes in the Human Retina

7.2.1 The Retinal Pigment Epithelium

The retinal pigment epithelium, a single layer of cuboidal to low columnar epithelial cells, suffers the most in aging, perhaps due oxidative stress. These cells are rich in lysosomes, smooth endoplasmic reticulum, and melanosomes ([105]; Fig. 7.2a). Their thin apical cytoplasmic processes interdigitate with the photoreceptor outer segments lying in the outer (scleral) one-third of the photoreceptor layer [80]. The latter are composed of numerous plasma membrane discs that are periodically shed and phagocytosed by the retinal pigment epithelium [10, 94, 107]. Defects in the clearance of shed outer segment discs, due to a loss of lysosomal cathepsin-D activity via iron accumulation [17], are a feature of aging retinal pigment epithelium. It is known that iron causes oxidative stress in many tissues, including the retina. There is a report indicating signs of degeneration of mitochondria of the retinal pigment epithelium with aging [38]. With aging, the

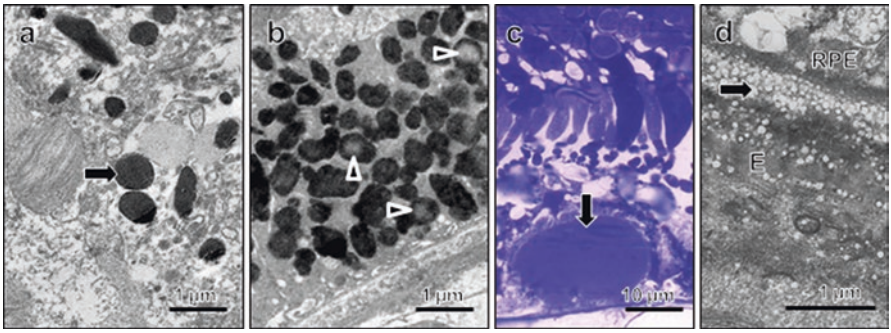

Fig. 7.2 Transmission electron micrographs showing changes at the retinal pigment epithelium-Bruch's membrane interface. (**a**) Melanin granules (arrow) of young RPE. (**b**) Lipofuscin (arrowheads) accumulation in aged RPE. (**c**) Drusen (arrow) lying beneath the pigment epithelial plasma membrane. (**d**) Accumulation of lipid spheres (arrow) in Bruch's membrane. (**e**) Elastic lamina of Bruch's membrane

retinal pigment epithelium develops autofluorescent lipofuscin granules, which are more in older retinal samples during eighth and ninth decades than in lower decades ([28, 35–37, 42, 48, 65, 82]; Fig. 7.2b). These granules are lysosomal derivatives that contain indigestible proteins and lipids of photoreceptor outer segments and a photoreactive compound, called A2E. Their gradual accumulation in retinal pigment epithelial cells is a key step that activates generation of free radicals [11, 92, 104], causing photoreceptor damage and ultimately their death. Also, with aging, there is formation of drusen (Fig. 7.2c) and basal laminar deposit underneath the basal lamina of the retinal pigment epithelium and basal linear deposit between the retinal pigment epithelium and inner collagenous layer of Bruch's membrane [19, 34, 53, 58, 82, 101, 102]. These substances result from partial removal of defective retinal pigment epithelial components and later act as debris [34]. They limit the accessibility of nutrients and oxygen from the choriocapillaris to the underlying photoreceptor cells. Additionally, lipid-rich, thick deposits are seen between the retinal pigment epithelium and Bruch's membrane (Fig. 7.2d) in aging eyes. A role for local inflammation in drusen biogenesis in aging eyes and AMD has been proposed [3]. On chemical analysis, abundant esterified cholesterol has been detected in age-related maculopathy [22, 106]. Besides, aging retinal pigment epithelial cells contain large number of melanolipofuscin and melanolysosomes that leads to gradual atrophy of this layer [11, 12, 16, 25, 37, 65, 83].

7.2.2 Photoreceptor Cells

In normal photoreceptor cells, the plasma membrane discs of the outer segments are intact and aligned properly, i.e., perpendicular to the photoreceptor axis. In aging, the photoreceptor outer segment discs are found to be randomly oriented, fragmented, or disorganized into membranous whorls [61]. Fragmented photoreceptor

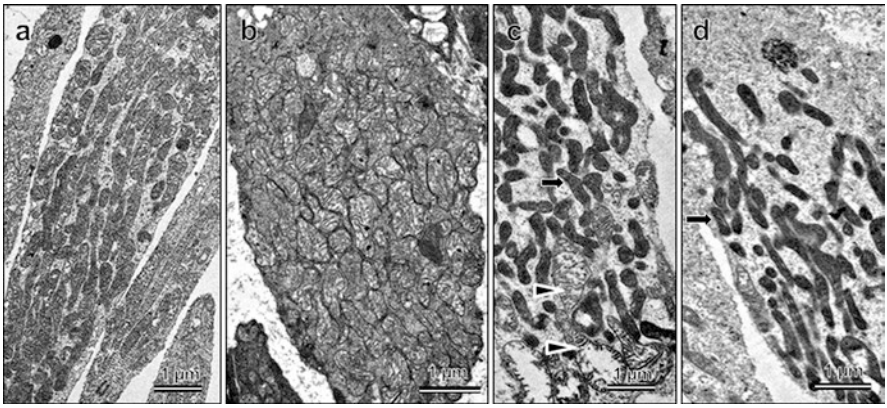

Fig. 7.3 Transmission electron micrographs showing mitochondrial alterations in photoreceptor inner segments with aging. (**a**) Normal well-aligned mitochondria in a macular cone (56-year-old). (**b**) Unusual aggregation of mitochondria of a mid-peripheral cone (74-year-old donor). (**c**, **d**) Mitochondria change to irregular profiles with electron-dense matrix (arrows; 85- and 87-year-old donors, respectively). Few swollen mitochondria are also indicated (**c**, arrowheads)

outer segment discs are normally seen in vitamin A-deficiency [29, 55] and light-induced retinopathy [50, 51, 56, 72] in experimental rodents and chicks that are perhaps caused by oxidative stress.

The photoreceptor inner segments show an ellipsoid, a myoid portion, a nucleus, and the inner fiber that terminates into a synaptic terminal [13, 46]. Most mitochondria of photoreceptor cells are located in the ellipsoid. They appear elongate with well-aligned cristae and homogeneous, less dense matrix (Fig. 7.3a). With aging, cone mitochondria show partial loss of cristae and densification of matrix (Fig. 7.3c, d; [63, 66]). These changes are prevalent in the macular cone inner segments. Also, an unusual aggregation of mitochondria in a tightly packed manner in the cone inner segments is seen in mid-peripheral cone cells (Fig. 7.3b). Their occurrence in the human retina with aging is likely due to increased mitochondrial vulnerability via oxidative stress and light or toxic substances that interfere with mitochondrial biology. Like the retinal pigment epithelium, lipofuscin granules occur in photoreceptor inner segments in aging [48, 63]. From chemical viewpoint, there are alterations in the immunohistochemical expressions of the enzymes of oxidative phosphorylation in aging photoreceptor cells [6]: it has been shown that complex I (subunit 15 kDa, NDUFB4) shows a reduced expression in the macular as well as peripheral cone cells in advanced ages (eight decades onward), while patchy distribution of complex IV (subunits I and II) immunoreactivity in cone inner segments is noted over the retina with aging [66]. Complexes II, III, and V (ATPase 6 and alpha subunits) do not show any obvious alterations in their expression over the decades. Such mitochondrial changes (structural and biochemical) in the aged photoreceptor cells are likely to cause energy depletion, ultimately interfering with cone viability in the human retina with aging.

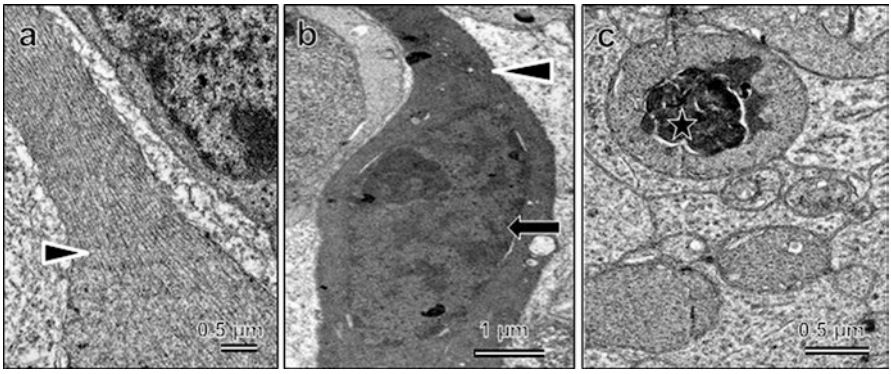

Fig. 7.4 Transmission electron micrographs showing changes in photoreceptor cells with aging. (**a**) A normal cone axon with longitudinally aligned microtubules (arrowhead, 56-year-old). (**b**) Shrinkage of the axon (arrowhead) and nucleus (arrow) of a cone. (**c**) A degenerative fiber of Henle (star). Other fibers (FH) appear normal with cytoskeleton

The photoreceptor inner segments possess numerous microtubules that run parallel to the alignment of ellipsoids, inner fibers, and fibers of Henle (Fig. 7.4a). The latter, anatomically the photoreceptor axons in the macular region, are extremely long fibers (about 530 µm) that course obliquely toward the foveal slope [13, 23, 30], being clearly visible in the parafoveal and perifoveal regions. In advanced state of aging, there is evidence of selective darkening of parafoveal fibers (Fig. 7.4b, c). Also, the microtubules of photoreceptor inner segments undergo clumping and disorganization, resulting in the formation of membrane-bound, filamentous bodies with dense materials [63]. The significance of their occurrence in the macular cone photoreceptor cells with advanced aging is not clear. It is speculated that in AMD, there may be disturbances in the macular cone cytoskeleton and associated proteins, which can trigger photoreceptor cell death [31]. The present study emphasizes that this is indeed possible in the aging human retina. Significant morphological alterations of photoreceptor axons, often in the form of bent or tortuous projections, and neurite extensions from axons, were reported in the aging human retina [76].

7.2.3 Photoreceptor Synaptic Changes

The synaptic terminals of the photoreceptor cells are arranged in one to two rows in the outer plexiform layer of the retina. They contain synaptic ribbons, small, neurotransmitter-loaded synaptic vesicles and few mitochondria [93]. In young donor retinas, synaptic terminals show presence of few, straight synaptic ribbons (about 500 nm long) that remain associated with horizontal cell processes and bipolar cell dendrites. In aging retina, the synaptic terminals of photoreceptor cells show prominent degenerative changes (Fig. 7.5a). The synaptic ribbons, which are dynamic membranous organelles, show altered structures under physiological stress [62, 65]. They appear tiny or partly disorganized (< 300 nm in length; mean normal;

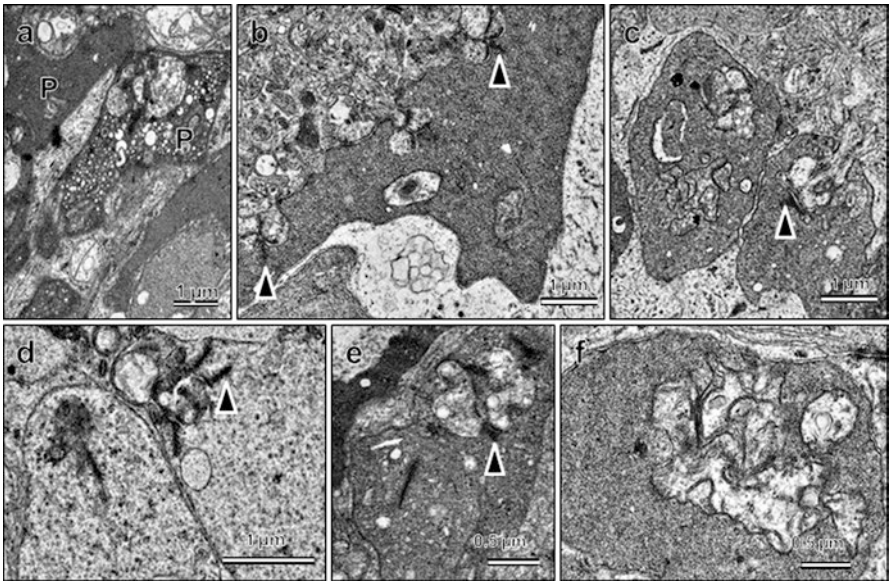

Fig. 7.5 Transmission electron micrographs showing photoreceptor synaptic changes in the aging human retina. (**a**) Cone terminals (pedicles, P), showing degenerated cytoplasm. (**b**–**e**) Photoreceptor terminals with inconspicuous, short synaptic ribbons (arrowheads). (**f**) A cone pedicle with abnormal, swollen mitochondria. From 75- (**a**), 81- (**b**, **c**), 85- (**d**, **e**), and 89-year-old (**f**) donor retinas

Fig. 7.5b–e) and often remain floated in photoreceptor terminals without being associated with postsynaptic horizontal and bipolar cells. The mitochondria of the photoreceptor terminals also show a swollen appearance (Fig. 7.5f). Besides these events, two reports have indicated the rod spherules to withdraw from their normal position in the outer plexiform layer and invade the outer nuclear layer. Also, at the same time, the dendrites of the rod bipolar cells (postsynaptic to photoreceptor cells) extend into the outer nuclear layer to establish connection with the retracted rod terminals [32, 95]. There are a number of known proteins (RIBEYE, bassoon, and piccolo) that appear to regulate the organization of the ribbon synapses in photoreceptor synaptic terminals [24, 84]; it is possible that some of them may undergo oxidative damage, rendering synaptic ribbons to be altered into bent or tiny structures.

7.2.4 Age-Related Loss of Photoreceptor Cells

The outer nuclear layer, wherein the nuclei of rod and cone cells are arranged into several rows, shows a gradual thinning with aging. Quantification of the number of nuclei per unit length of the outer nuclear layer revealed a gradual loss of photoreceptor nuclei with aging, both from the macular and peripheral part of the retina [32, 40,

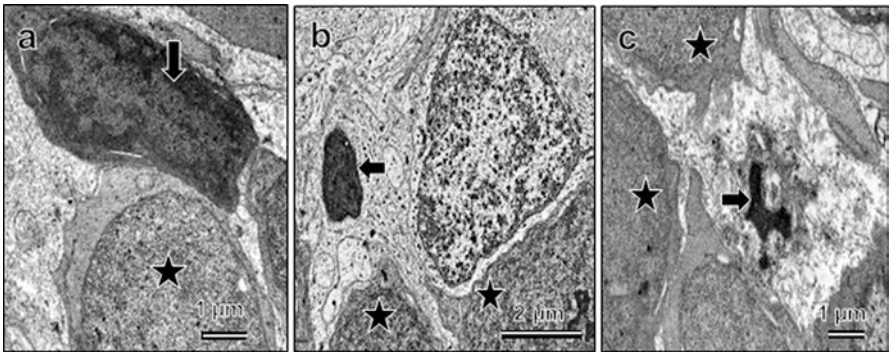

Fig. 7.6 Transmission electron micrographs showing darkening of photoreceptor nuclei (arrows, **a**, **b**) and fragmentation (arrow, **c**) in the outer nuclear layer, indicating their possible loss by apoptosis, other nuclei appear normal (stars; 85-, 87- and 89-year-old donors, respectively)

41, 49]. These observations clearly reflect photoreceptor cell loss in aging, assuming that retinal shrinkage with aging is insignificant. These studies reported on the significant loss of rods, but not cones, from the peripheral retina [40] and macula with aging [20, 49]. However, the mechanisms of rod and cone cell death (apoptosis versus necrosis) remain unknown for the aging human retina. In the aging human retina, the outer nuclear layer shows instances of the presence of shrunken, condensed, pyknotic, and fragmented nuclear profiles (Fig. 7.6a–c). From their deep location in the outer nuclear layer (i.e., toward the vitreal aspect), it is likely that those nuclei belong to rod cells. Clear, empty spaces are often noticeable in the outer nuclear layer of the aged retina, thereby reinforcing nuclear loss. It is possible that the rod cells undergo apoptosis during normal aging; this aspect, however, remains to be seen for cone cells that die sporadically and at a relatively late age [86].

7.2.5 Age-Related Changes in the Inner Retinal Layers

The inner retina contains two nuclear layers, the inner nuclear layer and ganglion cell layer and one synaptic layer and inner plexiform layer. The inner nuclear layer consists of horizontal, bipolar, and amacrine neurons and one type of glial cells, Müller cells. Reports on how the inner nuclear layer cells alter with aging, especially after photoreceptor cell death, are limited. In advanced ages, lipofuscin granules were found in bipolar cells of temporal peripheral retina [65]. Although rare, the significance of their occurrence in the peripheral retinal cells is not clear. A decrease in the density of rod bipolar cells (identified by immunoreactivity to protein kinase C-alpha, a marker for them) was also reported for the aging human retina [1].

Müller cells, however, show some conspicuous changes in aging human retina. The junctional complexes (desmosomes), which hold the adjacent end-feet, show

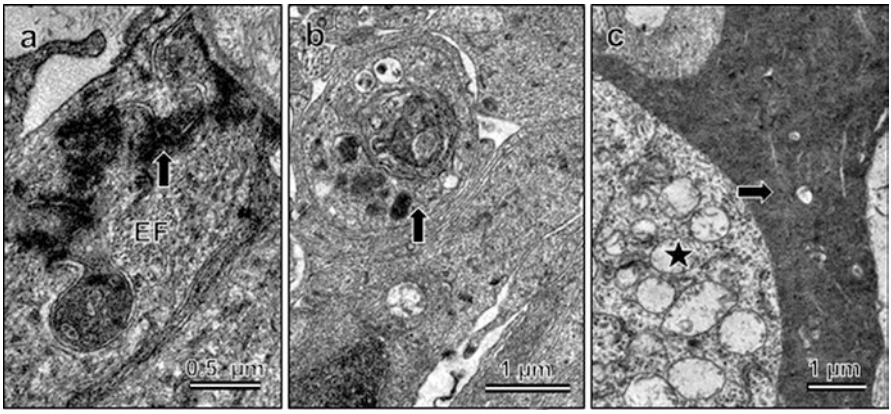

Fig. 7.7 Transmission electron micrographs showing of Müller cell alterations in aging retina. (**a**) Degenerative and misaligned desmosomes in end-feet (EF, arrow). (**b**) Lysosomal granules (arrows) in a vitreal process. (**c**) The ensheathment of a neuronal cell body (star) by thick hypertrophied process (arrow). From 76-, 85-, and 89-year-old donors, respectively)

altered appearance with dissolution of anchoring materials (Fig. 7.7a). A varied degree of Müller cell gliosis occurs in the inner retina with aging during eighth and ninth decades. Their inner processes, which are relatively thin in the foveal region [69], appear thick and replete with intermediate filaments (Fig. 7.7b) and smooth endoplasmic reticulum. Later, with advanced aging, these processes become highly electron-dense and intimately ensheath the neuronal cell bodies (Fig. 7.7c), especially those located in the inner nuclear layer, where gliosis is prominent. These processes often contain debris, lipidic substances, and lysosomes of various stages (Fig. 7.7b) and seemingly play a dominant role in the phagocytosis of dead retinal neurons. Bringmann et al. [14] reported an age-related decrease in potassium currents in Müller cells of the human retina.

Relatively little information is available for the aging human retinal ganglion cells. A study reported their loss from the macula with aging [18], although relatively at a much lower rate than that happens outside of the macula [45]. Balaszi et al. [5] reported significant decrease in the number of ganglion cells and their axons with aging. In relatively lower-age donor retinas, the axons show numerous neurotubules in a homogeneous axoplasm (Fig. 7.8a), whereas dark, shrunken ganglion cell axons are often seen in the advance-aged nerve fiber layer (Fig. 7.8b). Optical coherence tomography detected thinning of the nerve fiber layer with aging [70]. Lipofuscin granules prominently occur in ganglion cell somata with aging [63]. These cells are, however, much vulnerable to oxidative stress than any other inner retinal neurons, as is the case in pressure-induced glaucoma.

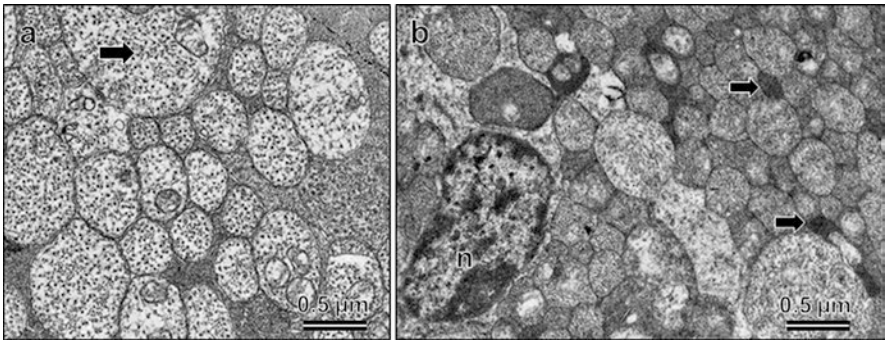

Fig. 7.8 Transverse sections of ganglion cell axons of nerve fiber layer. Relatively healthy axons (arrow, **a**) are seen in a lower-age donor retina (74-year-old), compared to few dark, shrunken axons in old donor retina (arrows, **b**; 86-year-old). The nucleus (n) of an astrocyte is present in the vicinity

7.3 Localization of Biomarkers of Oxidative Stress in Human Retina

7.3.1 4-Hydroxy-2-Nonenal (HNE)

To understand possible oxidative changes in the aging human retina, few established immunohistochemical markers, indicating sites of cellular oxidative stress, were employed. In the donor retinas of lower age group (40–60 years), the immunoreactivity to HNE (a marker of lipid peroxidation) is initially observed in few peripheral end-feet of Müller cells and cells of the inner nuclear layer (Fig. 7.9a): with aging, the immunoreactivity becomes widespread in many cells of the inner nuclear layer (age > 70 years; Fig. 7.9b; [64]). At the same time, the immunoreactivity appears in the fibers of Henle (Fig. 7.9b) and cone outer segments, more prominently in those located in the parafovea (Fig. 7.9b, c), bur less in the perifovea (Fig. 7.9d). In the retinal periphery, only limited cone or rod cells show immunoreactivity in their outer segments (Fig. 7.9e), which is perhaps due to abundant carotenoids (lutein and zeaxanthin) detected in those structures at this retinal region [77]. With regard to the immunopositive cells of the inner nuclear layer, quantification has revealed an increase in their percentage in the parafoveal and peripheral retina with advanced aging, when compared with that in young age group [64]. Double localization study of HNE with glutamine synthetase (a marker for Müller cells) by confocal laser scanning microscopy has revealed that many HNE-positive cells of the inner nuclear layer and outer fibers (in the fiber layer of Henle) belonged to Müller cells [64]. The outer fibers, along with the perikarya and end-feet of Müller cells and cone outer segments, denote the sites of lipid peroxidation in aging human retina.

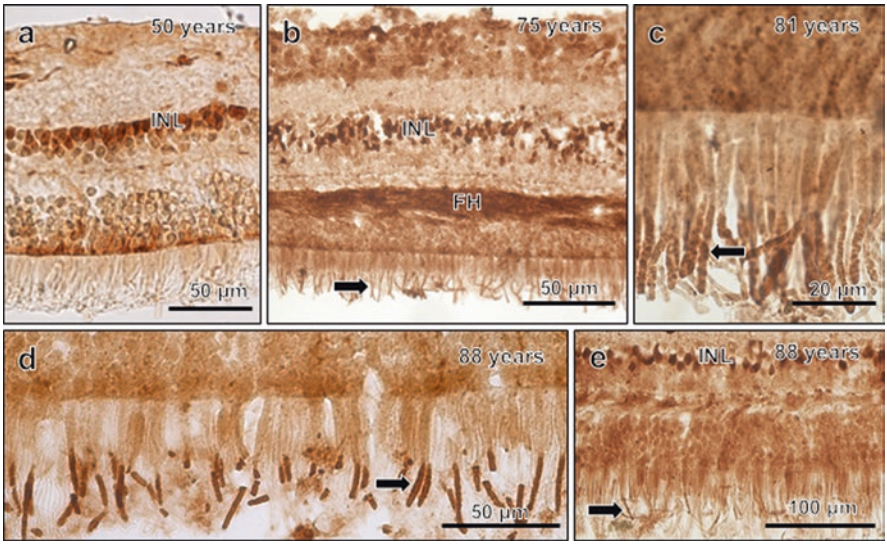

Fig. 7.9 HNE immunoreactivity in the human retina at different ages. Immunoreactivity is initially present in the inner nuclear layer (INL) only (**a**), and then with aging it appears additionally in photoreceptor (cone) outer segments (**b**–**e**; arrows). The fibers of Henle also show immunoreactivity (**b**). **a** and **e** are from mid-peripheral retina; other figures are from parafoveal (**b**, **c**) and perifoveal parts (**d**). Donor ages appear on the top right-hand corners of the figures

7.3.2 Nitrotyrosine

Immunoreactivity to nitrotyrosine antibody indicates cellular sites of protein tyrosine nitration. Unlike HNE, which is localized to photoreceptor outer segments, immunoreactivity to nitrotyrosine is restricted to the photoreceptor inner segments (Fig. 7.10a–d), which often extends into their synaptic terminals. Both macular (Fig. 7.10a, b) and peripheral parts (Fig. 7.10c, d) of the human retina show appearance of the nitrotyrosine immunoreactivity with aging [66]. Besides, few radial processes of Müller cells in the inner nuclear layer and nerve fiber layer show prominent immunoreactivity. As with HNE, nitrotyrosine immunoreactivity appears to accumulate in the photoreceptor cells with progression of aging (e.g., Fig. 7.10d, [68]).

7.4 Immunohistochemical Localization of Antioxidant Enzymes in the Human Retina

Among the antioxidant enzymes operative to counteract oxidative stress in the human retina, mitochondrial superoxide dismutase-2 (SOD-2) shows an age-related increase in the photoreceptor cells throughout the retina [66]. Additionally, the aged retina shows an increase in SOD-2 immunoreactivity in the inner nuclear layer, but

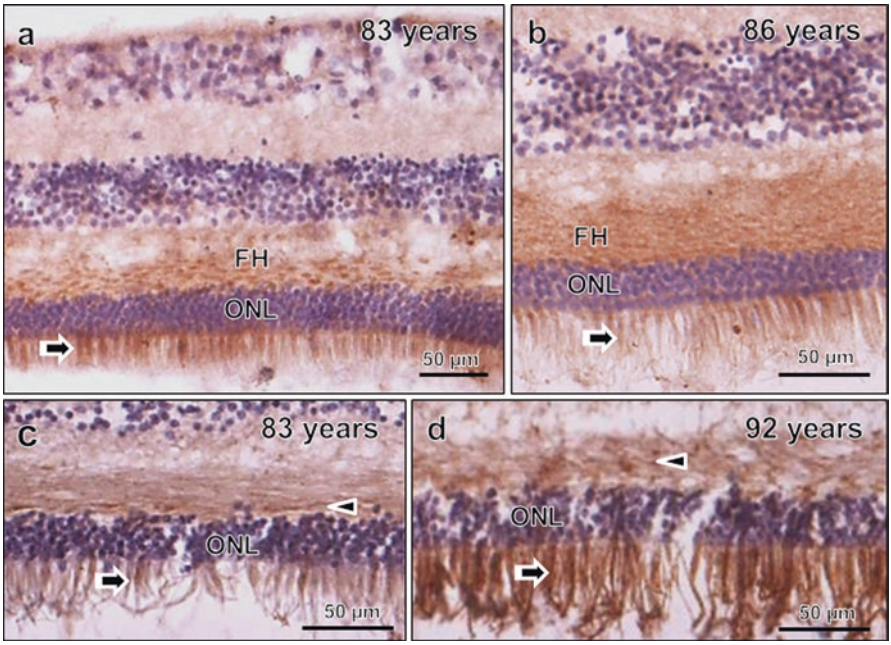

Fig. 7.10 Nitrotyrosine localization in the human retina. Photoreceptor inner segments show immunoreactivity (arrows; **a**–**d**), as are the fibers of Henle (FH) in the parafovea (**a**, **b**). Fibers at the level of the outer plexiform layer are also immunopositive in mid-peripheral retina (arrowheads; **c**, **d**). ONL, outer nuclear layer. Donor ages appear on the top right-hand corners of the figures

more prominently in ganglion cells and the nerve fiber layer of the macular region. Other antioxidant enzymes, such as glutathione peroxidase-1 and glutathione S-transferase-π 1, show a low to weak level of expressions in the photoreceptor cells (inner segments), fibers of Henle, and outer and inner plexiform layers (Fig. 7.11, glutathione peroxidase-1).

7.5 Oxidative Stress in the Aging Human Retina

To understand how the human retina suffers from oxidative damage, immunohistochemical localization of biomarkers of oxidative stress and antioxidant enzymes was analyzed in human retina at different ages. The aldehyde HNE, a marker of lipid peroxidation, is a potent cytotoxic agent generated during lipid peroxidation in cells [33]. Nitrotyrosine, a marker of protein nitration, appears during protein tyrosine nitration by reactive nitrogen species such as peroxynitrite [74]. The products keep on gradually accumulating in a cell undergoing oxidative stress and ultimately cause tissue damage when their level is exceedingly high. However, the accumulation of HNE is reported to induce the upregulation of several antioxidant enzymes,

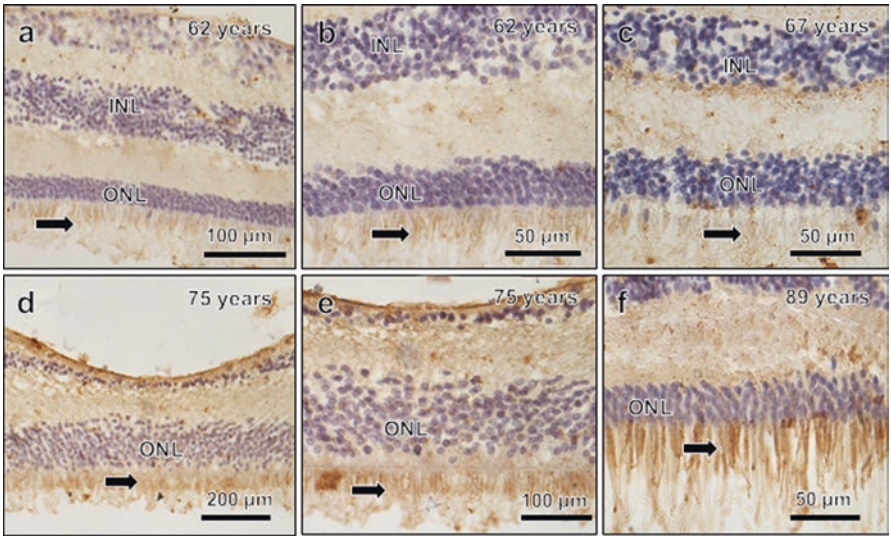

Fig. 7.11 (**a**–**f**) Glutathione peroxidase-1 immunoreactivity in the human retina. Photoreceptor inner segments show weak to moderate immunoreactivity (arrows). *GCL* ganglion cell layer, *INL* inner nuclear layer, *ONL* outer nuclear layer. Donor ages appear on the top right-hand corners of the figures

especially glutathione S-transferase [4], and exerts neuroprotection. In the retina, Malone and Hernandez [60] reported HNE-induced antioxidant responses in astrocytes of the optic disc.

Experimental studies in rodents have reported that the oxidative stress in the retina may stem from different physical factors (e. g., exposure to visible light) or biochemical status of the tissue (antioxidant deficiency or age-related gradual iron overload). These stressors may contribute to lipid peroxidation of photoreceptor cells [26, 81, 103]. Circumstantial evidence also suggested that retinal damage upon prolonged exposure to light can be slowed down and even ameliorated by certain groups of synthetic antioxidants tested to this end. Several studies [52, 96, 99] also reported a variety of retinal proteins that can undergo modification upon accumulation of HNE in a cell. Because prominent immunoreactivity to HNE was localized in Müller cell end-feet of the aged retina [64], it is inevitable that this aldehyde can modify the specific proteins present in those glial compartments (e.g., inward-rectifying potassium channels and aquaporins).

Earlier, oxidative stress has been implicated in several retinal diseases, such as age-related macular degeneration, Eales disease, retinitis pigmentosa, glaucoma, and diabetic retinopathy [7, 54, 87]. The present study indicates a possible role for oxidative stress in retinal changes that occur with progressive aging. However, the precise reasons for oxidative stress in aging human retina are not clear. Possible factors that can be ascribed to this process are sunlight, smoking, nutritional status, lack of carotenoids in diets, and a decrease in the levels of endogenous antioxidants and antioxidant enzymes (rendering decreased antioxidant defense mechanisms)

with normal aging. In the aging retina, the production of reactive oxygen species could be even more than that in other tissues, and they can cause the oxidation of lipids, proteins, and nucleic acids in the absence of the substantial level of retinal endogenous antioxidants (e. g., carotenoids, vitamin E) and antioxidant enzymes (glutathione peroxidase, glutathione S-transferases, catalase, and SOD-2). A previous study emphasized that oxidative stress in the aging human retina could involve Müller cells [64], a major glial cell type of the retina. These cells support many vital physiological functions of the retinal neurons [15]. They are quite resilient to physiological changes in certain processes in which the other cells of the retina show the early signs of damage. Owing to age-related oxidative stress in Müller cells, as shown by Nag et al. [64], the maintenance of normal physiological processes of the retinal neurons could be hampered. These put the retinal neurons in extra risk of developing susceptibility to oxidative stress with aging. For example, Müller cells uptake glutamate, a major neurotransmitter that is released at the synaptic cleft by neurons after synaptic neurotransmission, by glutamate-aspartate transporter-1. Glutamate is then converted into harmless glutamine by glutamine synthetase present in those glial cells [15]. A decreased glutamate uptake due to low expression of glutamate-aspartate transporter-1 [78] has been suggested to be an unfavorable situation that can lead to a reduction of glutathione synthesis in Müller cells [79]. This is likely to enhance oxidative stress in the retina, since reduced glutathione is the substrate for many antioxidant enzymes inside cells. Thus, one reason for oxidative stress in Müller cells could be related to the endogenous levels of glutathione with aging, and remains for future study. It is known that these cells synthesize and release glutathione for neuronal use [73, 75].

On the other hand, several other reports have indicated a protective role for HNE in oxidative stress [98]. The accumulation of HNE induces the upregulation of glutathione S-transferases [4, 39], which are mainly involved in cellular detoxification. Thus, it is important to know how redox status is affected by oxidative stress in the retina and the precise endogenous antioxidant defense mechanism against oxidative stress because of aging.

7.6 Oxidative Stress Responses in the Aging Human Retina

The intracellular environment is enriched with a variety of endogenous antioxidant molecules (e.g., reduced glutathione), antioxidant enzymes, and vitamins to protect the cells from oxidative damage caused by free radicals and reactive oxygen species generated during metabolic reactions (Handelman and Dratz [43]. A pioneering study on biochemical estimation of the levels of major antioxidant enzymes by De la Paz et al. [27] reported that the activity of catalase, superoxide dismutase, and glutathione peroxidase remains stable in the aged human macula up to 86 years, the last age studied by the group. Spatiotemporal expressions of the markers of antioxidant enzymes in the aging human retina show that only SOD-2 shows a clear age-related increase in the human retina [66]. The present observations also support that the pattern of immunoreactivity to glutathione S-transferase and glutathione

peroxidase-1 in the human retina remains almost unaltered up to the eighth decade, after which the immunoreactivity appears low in advanced-aged retina, indicating their minimal role in the detoxification of free radicals, reactive oxygen species and lipid peroxides [67]. In most tissues, glutathione S-transferase is predominantly involved in counteracting toxic metabolites generated during lipid peroxidation [88, 89]. In the human retina, three classes of glutathione S-transferase, namely, α, μ, and π, have been characterized [2, 88]. One isoform, GST π-1, has been shown to bind to the carotenoid zeaxanthin present in the fibers of Henle in the macula for possible protection against oxidative stress [9].

7.7 Does Oxidative Stress Affect Vision in the Elderly Humans?

Aging is a risk factor for various ocular changes that often culminate in disease manifestations. In a somewhat mysterious way, it initiates the pathogenesis of certain diseases, like AMD, wherein the retinal pigment epithelium-choriocapillaris interface is primarily affected. The disease secondarily affects the photoreceptor cells, when they do not receive adequate nutrient and oxygen supply from the choriocapillaris due to local structural defects that result in faulty/decreased delivery mechanisms. Thus, photoreceptor cells, especially the rods, die in this process [20, 21]. It is interesting to note that in aging, photoreceptor cells also die from the macula and periphery of the retina [20, 21, 40]. Also, there is a loss of rod bipolar cells [1] and ganglion cells and their axons from the macula and periphery with aging [5, 18, 45, 70]. Although not proven, all these changes should limit visual perception, especially acuity and sensitivity to dark adaptation in the elderly individuals. It should also be realized that the photoreceptor cells and other retinal neurons that are altered in a subtle manner with normal aging (summarized in Table 7.1) and yet do not die may be dysfunctional or sub-optimal in their functions, which could also exert an impact on vision in the elderly.

7.8 Future Research

Several lines of evidence have now established that the retinal vulnerability to alterations in aging as well as in certain diseases (e.g., AMD) is centered in the outer retina, especially at the level of retinal pigment epithelium-Bruch's membrane interface. It is known that the gradual accumulation of autofluorescent lipofuscin granules in aging and diseases is a devastating condition that provokes oxidative stress in the retinal pigment epithelium. Another cellular event that prominently occurs in aging retinal pigment epithelium is a decreased rate of phagocytosis of remnants of photoreceptor outer segment discs (shed during circadian light-dark cycle) by the retinal pigment epithelium due to decreased activity of the lysosomal enzyme, cathepsin-D [17]. Oxidative mechanisms are considered to be active in these detrimental events for the outer retina and remain for an active area of investigation.

Table 7.1 Details of human retinal changes in aging

Layers/cell types	Histological/ultrastructural features	References
Retinal pigment epithelium	Formation of drusen and basal laminar deposit underneath the basal lamina	Farkas et al. [34], Loeffler and Lee [58], van der Schaft et al. [99, 102], Kliffen et al. [53], Curcio and Millican [19], Sarks et al. [80], Del Priore et al. [25], Bonilha [12], and Nag and Wadhwa [65]
	Accumulation of lipofuscin	Feeney [35], Weiter et al. [105], Sarks et al. [80], Dorey et al. [28], and Nag and Wadhwa [65]
	Formation of melanolipofuscin and melanolysosomes	Feeney-Burns et al. [36, 37]
	Loss of melanin	Weiter et al. [105], and Sarna et al. [83]
Rods	Abnormalities in outer segment disc orientation	Nag and Wadhwa [65]
	Convolutions in rod outer segments	Marshall et al. [61]
	Loss in the peripheral retina	Gao and Hollyfield [40], and Eliasieh et al. [32]
	Loss in the macula	Curcio et al. [20], and Jackson et al. [49]
Cones	Swollen, dumbbell-shaped mitochondria of inner segments; loss of cone mitochondria	Nag and Wadhwa [66]
	Lipofuscin-like refractile globules in inner segments	Tucker [97], Iwasaki and Inomata [48], and Nag et al. [63]
	Distal axonal swelling, tortuous axons	Pow and Sullivan [76], Shelley et al. [84], and Nag et al. [68]
	Shortening of synaptic ribbons	Nag and Wadhwa [62]
Outer nuclear layer	Gradual thinning with age	Gartner and Henkind [41], Gao and Hollyfield [40], Jackson et al. [49], and Eliasieh et al. [32]
Bipolar cells	Lipofuscin granules	Nag and Wadhwa [65]
	Loss of rod bipolar cells in the macula	Aggarwal et al. [1]
	Dendritic extension of rod bipolar cells into outer nuclear layer	Eliasieh et al. [32], and Sullivan et al. [95]
Müller cells	Hypertrophy, proliferation of smooth endoplasmic reticulum	Nag and Wadhwa [65]
Ganglion cells	Loss in the macula	Balaszi et al. [5], Gao and Hollyfield [40], and Curcio and Drucker [18]
	Lipofuscin granules in soma	Nag et al. [63]
Nerve fiber layer	Thinning with age	Neuville et al. [70]

These changes in the retinal pigment epithelium ultimately create an unfavorable environment for the photoreceptor cells, leading to their degeneration and gradual loss with aging and in AMD. There is also a need to examine in details on how the cells of the inner retina react to the effects of oxidative stress, especially the bipolar and ganglion cells that also die in the course of aging [1, 5, 45, 70]. Lipid peroxidation occurs in Müller cells (somata) and their processes [64], whereas protein tyrosine nitration is prominent in the nerve fiber layer [64]. Both processes are likely to influence critically for the well-being of inner retinal neurons. The retinal antioxidants molecules (e. g., carotenoids and reduced glutathione) play a critical role in the defense mechanisms against oxidative stress. Carotenoids (lutein and zeaxanthin), which are abundant in the human macula and retinal periphery, are reported to scavenge efficiently free radicals generated in metabolic processes. Reports on whether there is depletion of carotenoid levels in the aged human retina, and also of reduced glutathione are at present limited ([71]; carotenoids) and remain for future studies.

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Aging: Influence on Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD)

8

Niraj Kumar Srivastava, Ramakant Yadav, and Deepak Sharma

Abstract

Muscular dystrophies are genetic diseases of muscles. These diseases are characterized by progressive muscle wasting and weakness of variable distribution and severity. Selective involvement, significant wasting, and weakness of muscles are significant characteristics of muscular dystrophies. The most important types of X-linked dystrophy are Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD). DMD is the most rapidly progressive and lethal form of dystrophy, but BMD is a milder form. Both the types of dystrophy are caused by a mutation in a specific gene (dystrophin gene) within the X chromosome (gene map locus 12q21, Xp21.2) that affords commands for the formation of the dystrophin protein, and this protein is an important structural component of muscle cell. Aging is an important parameter for patients with DMD and BMD. This influences the clinical symptoms, disease progression, serum enzyme abnormalities, morphological changes in muscle, and medications for DMD and BMD patients. Aging factor is responsible for pathogenesis in DMD and BMD. With increased knowledge about the understanding of DMD and BMD, still there is an uncertainty of the exact mechanism of progressive muscle degeneration in these disorders. On the basis of numerous reports, oxidative stress may be one of the causes for the muscle degeneration in DMD and BMD, which increases with age. The present chapter described the effect of aging on all the parameters of disease in DMD and BMD.

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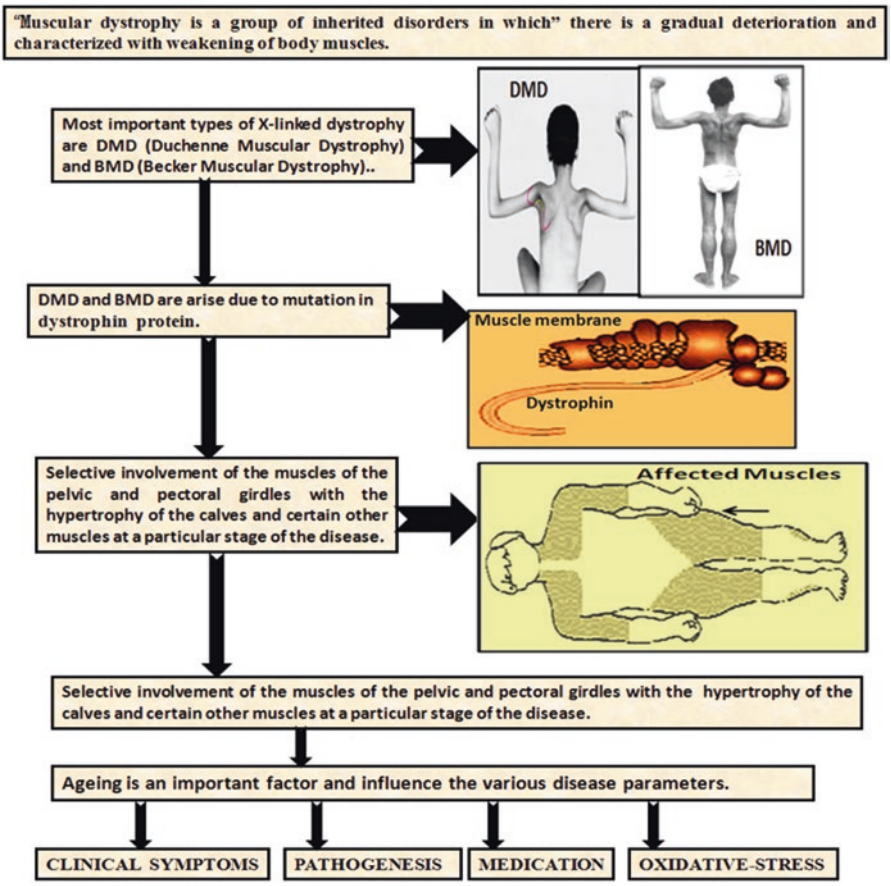
Graphical Abstract



Keywords

Muscular dystrophy · DMD · BMD · Aging · Genetic diseases · Hereditary diseases · Dystrophinopathies · Myopathies · Neuromuscular diseases

Abbreviations

BMD	Becker muscular dystrophy
CK	creatine kinase
CMD	congenital muscular dystrophy
DM	myotonic dystrophy
DMD	Duchenne muscular dystrophy

EDMD	Emery-Dreifus muscular dystrophy
EMG	electromyography
FSHD	facioscapulohumeral muscular dystrophy
LGMD-2B	limb-girdle muscular dystrophy-2B

8.1 Introduction

Muscular dystrophy is one of the numerous hereditary muscular diseases in which a person's muscles progressively and permanently deteriorate, causing weakness and eventually complete disability. Selective involvement, significant wasting, and weakness of muscles are significant characteristics of muscular dystrophies [1]. This is in distinction with other variety of myopathies, where the weakness is dispersing and moderately more than wasting, and muscle enlargement is exceptional. Numerous types of muscular dystrophies have been illustrated in the literature, and typical categorization of these depends upon the age, advancement, location of involvement, and inheritance pattern. Most of these disorders have now been well-recognized and completely identified with the mutated genes and their protein products. Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD), Emery-Dreifus muscular dystrophy (EDMD), facioscapulohumeral dystrophy (FSHD), limb-girdle muscular dystrophy (LGMD), myotonic dystrophy (DM), and congenital muscular dystrophy (CMD) are predominate types of muscular dystrophy [1–5].

8.2 DMD and BMD

Two major forms of X-linked dystrophy are DMD and BMD. DMD is recognized as a lethal and rapidly progressive form of dystrophy, while BMD is a milder form [1].

8.2.1 Incidence

The majority of assessment and surveys showed that DMD has an incidence of 1 in 3500 survive male births and a prevalence of about $50–70 \times 10^{-6}$ male population. Outcome of the majority of assessment and consistent estimate of the BMD vary from 18 to 30 per 100,000 survive born males and of its prevalence in the population from 1.9 to 4.8 per 100,000. One-third of cases are new mutants, another one-third have a preceding family history, and the rest of one-third are born to unsuspecting and frequently mutant carriers in all the cases of DMD and BMD. The mutational rate is $7–10 \times 10^{-5}$ per gene per generation in dystrophinopathies. The cumulative observation of BMD birth incidence (at least 1 in 18, 450 survive male births) is about one-third that of DMD (1 in 5618 survive male births) [1–5]. In India, hospital-based reports represented that in all patients of muscular dystrophy, 30% patients were suffering with DMD and 0.6% with BMD [6].

8.2.2 Cause

DMD and BMD are observed due to mutation in a specific gene within the X chromosome (gene map locus 12q21, Xp21.2) that affords directions for the construction of the dystrophin protein, a significant structural constituent of muscle cell. Noticeable deficiency or absence of dystrophin is acquired in the case of DMD, whereas minor deficiency of dystrophin is observed in BMD patients. Dystrophin is absolutely nonfunctional in DMD and moderately functional in BMD patients [1–5].

8.2.3 Clinical Symptoms and Signs

DMD happened predominantly in male and characterized by:

(a) Onset of symptoms usually before the age of 4 years and rarely as late as the seventh year of age.
(b) Selective involvement of the muscles of the pelvic and pectoral girdles in a symmetrical manner.
(c) Observation of hypertrophy of the calves and certain other muscles at a particular stage of the disease.
(d) Relentlessly progressive weakness in every case, leading to inability to walk within 10 years of the onset and later to contracture and thoracic deformity.
(e) Invariable cardiac involvement.
(f) Frequent intellectual impairment with variation.
(g) Occurrence of death by the second or third decade due to respiratory or less frequently, cardiac failure, often associated with inanition and respiratory infection.
(h) High level of serum CK in early stages of the disease.
(i) Certain histological features characteristic in muscles [4, 5, 7].

Both patients with DMD and BMD are showing similarity in several clinical symptoms. Severity of these symptoms is less in BMD as compared to DMD. Incident of cardiac failure or respiratory arrest is more recurrent in DMD as compared to patients with BMD. Clinical symptoms between the ages of 5 and 15 years are observed in most patients with BMD, and in few cases the onset symptoms are missing until the third or the fourth decade of life. There is an identical involvement of selective muscles in patients with DMD and BMD. There are selective bilateral and symmetrical wasting and weakness of the costal origin of pectoralis major, latissimus dorsi, brachioradialis, hip flexors and extensors, and medial vastus of quadriceps. Afterward the supinator, biceps, triceps, serratus anterior, and neck flexors turn weak [2, 4, 5, 8].

Gower's maneuver (Fig. 8.1) or sign is very crucial and helpful for making the first step of the diagnosis in both DMD and BMD [1, 8]. "Pradhan sign" (Fig. 8.2) or "valley sign" [9] is another important clinical sign for patients with DMD and BMD. The value of this sign is that it is visible even in those DMD patients who do

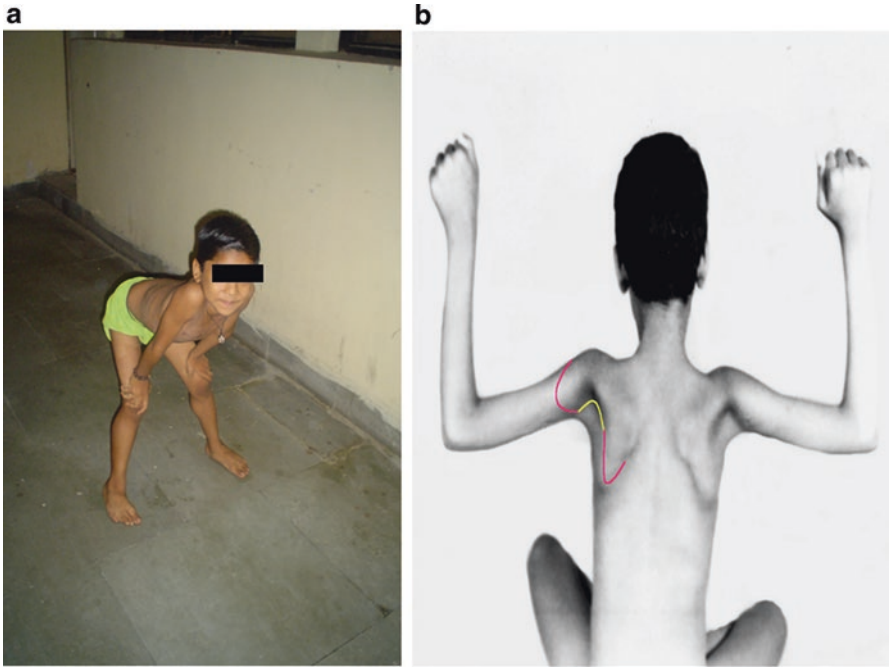


Fig. 8.1 (**a**) Gower's maneuver or sign in DMD patient, (**b**) "valley sign" in DMD – a valley (yellow) between the two mounts (red)

not demonstrate convincing diagnostic calf enlargement or at the late stage of the disease when the Gower's sign cannot be tested and the calves are much reduced. This is the most remarkable and specific sign for DMD with 90% sensitivity at the ages of 8–11 years [9, 10].

8.2.4 Diagnosis

Patients with DMD and BMD are examined by the clinicians on the basis of clinical symptoms, signs, and family history. They performed or advised to perform the various laboratory tests to confirm the diagnosis on the basis of these clinical examinations. The diagnostic confirmation of DMD and BMD is a combined effort of clinical symptoms, EMG (electromyography), blood enzyme estimation, and molecular genetic analysis [11, 12]. Multiplex PCR is a commonly used procedure for DMD diagnosis, which intentions about 18–32 exons of the dystrophin gene to recognize entire exons deletions. This approach of PCR is frequently qualitative or semiquantitative and serves for the exons in the hot spot region [13, 14]. Southern blot analysis and dosimetric PCR-based methods or techniques, for example, multiplex ligation-dependent probe amplification (MLPA), are also used for the detection of duplication [15–18]. The ultimate diagnosis is made by histopathological- and

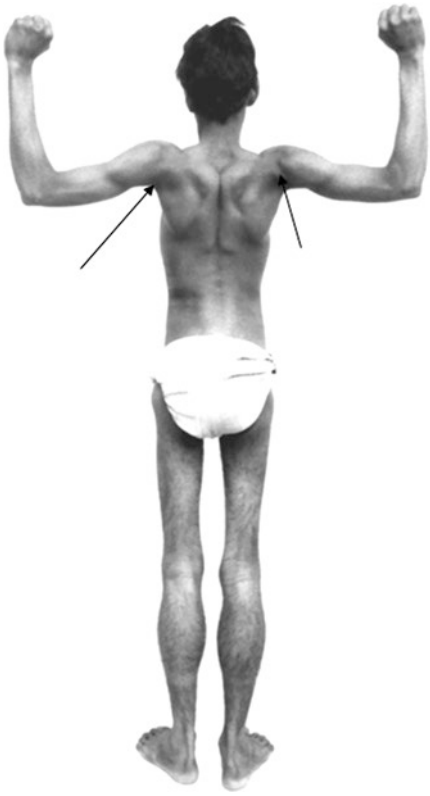


Fig. 8.2 "Valley sign" in BMD patient (sign is showing by arrows)

immunohistochemical-based lab studies for patients with DMD and BMD in the clinical arrangement [11]. The sketch of the intact methods of the diagnosis in a sequential manner is represented by Fig. 8.3. Nuclear magnetic resonance (NMR) spectroscopy-based metabolomics analysis is also one of the possible approaches for the diagnostic confirmation of DMD and BMD [11–19].

8.2.4.1 EMG (Electromyography)

The electrical activity of muscle is studying by electromyography. Electromyography (EMG) is an important clinical approach and is used as a precious support to the neurological diagnosis. When clinical assessment is complicated or ambiguous, EMG examination is helpful to support the diagnosis. The established role of clinical neurophysiology in the management of principal disorders of muscle has been in diagnosis, but the beginning of quantitative techniques and advanced electromyography (EMG) machines, which allocate quick acquisition and analysis of data, opens the way for consecutive studies to review disease progression and response to treatment [20]

Pathophysiological process of affecting muscle has been evaluated by neurophysiology-based EMG technique. This technique has diagnostic importance

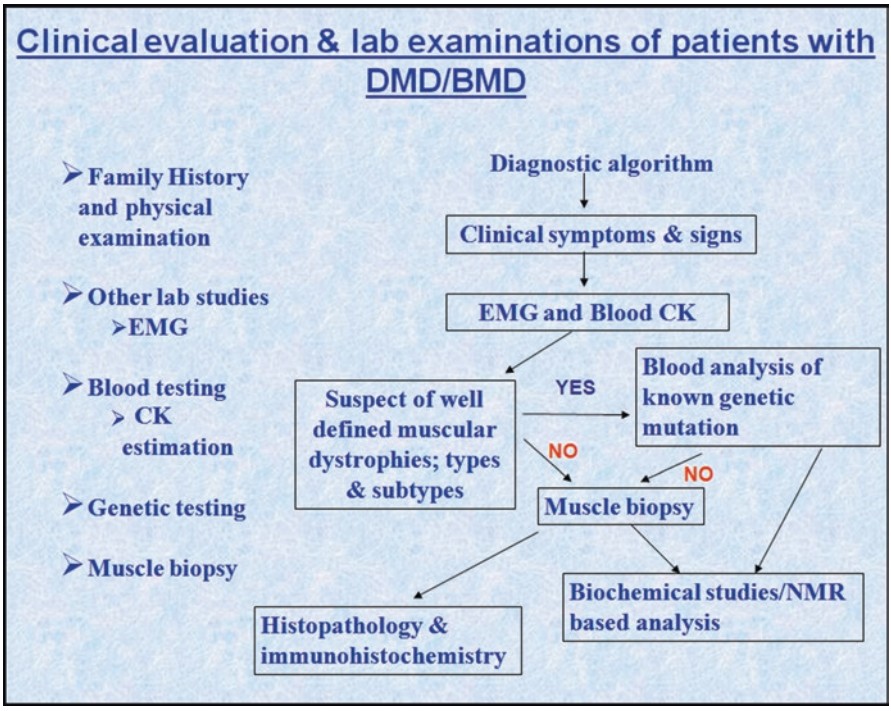


Fig. 8.3 Diagram represented the confirmation of the diagnosis of patients with DMD and BMD

and also investigated by numerous sites of muscle. EMG myopathic pattern is represented by Fig. 8.4 [11, 20].

8.2.4.2 Blood Enzyme Estimation

Observation of the high level of CK (creatine kinase), diphosphofructose aldolase, pyruvate kinase, aspartate aminotransferase, glucosephosphate isomerase, lactate dehydrogenase, carbonic anhydrase III, and enolase is found in the serum of patients with DMD and BMD. In all of these enzymes, most marked and consistent elevation in serum is CK. The skeletal muscle, heart, and brain (but not in the erythrocytes, liver, or other organs) are particular organs which contain the significant amount of CK. Due to this important fact, evaluation of CK in serum has been paid to the diagnostic aid in clinical medicine for patients with DMD and BMD [21]. Observation of 10–20 times higher CK was in serum of patients with DMD [8]. CK enzyme is a protein and produced by two subunits. These two subunits are M (prevalent in muscle tissue) and B (prevalent in brain tissue). Production of three isoenzymes is observed due to different association of these two subunits. Three isoenzymes of CK are CK_1, CK_2, and CK_3. CK_1 is composed of BB, CK_2 is composed of MB, and CK_3 is composed of MM subunits. There is a predominant occurrence of enzyme CK_1 in brain tissue, CK_2 mostly in heart muscle and CK_3 in skeletal muscle tissue. Mitochondrial CK enzyme has also been identified as additional CK isoenzymes [22–24].

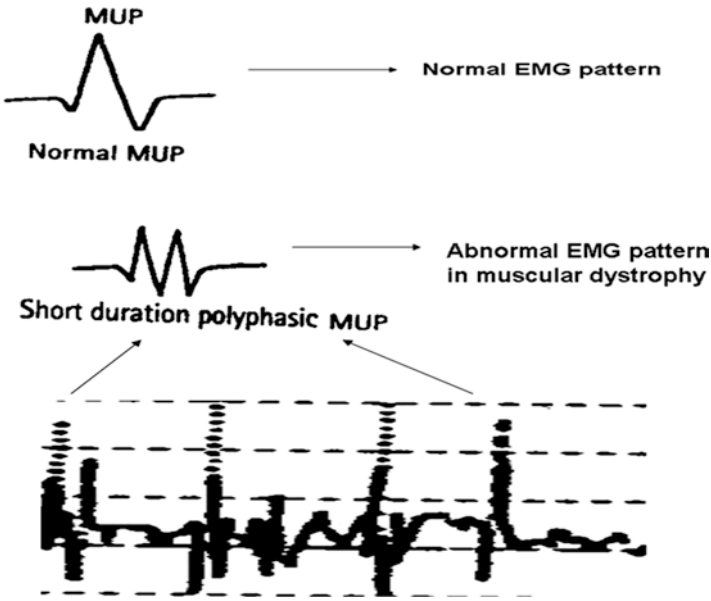


Fig. 8.4 Electromyography measurement showed the myopathic pattern in the DMD patients

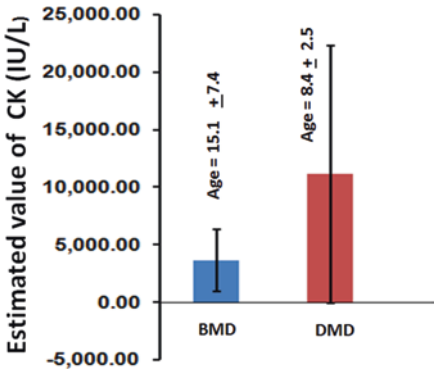


Fig. 8.5 Bar diagram represented the CK value (IU/L) in patients with BMD and DMD (with respective to mean age)

Serum CK levels in both DMD and BMD patients as compared to control or normal subjects are represented by Fig. 8.5. This figure showed the huge variation in the level of serum CK in patients with DMD and BMD. A number of factors are associated with this variation, and these factors are age, lifestyle, physical activity, and severity of the disease. Level of serum CK is reached to the peak in between 3 and 5 years of age and then reduces with increasing age in DMD. The rate of muscle decay may be responsible for the characteristic alterations of serum CK. This is very interesting to know that children with DMD had higher CK levels when they were younger and had more muscle function. What is the reason behind this? This happened due to rapid muscle degeneration at the earlier stages and more muscle bulk

available to release CK into the circulation at this time. There was a 19-fold enhancement found in the mean CK level in BMD patients as compared to control subjects, and pattern of elevation is similar to DMD with less severity [25–27].

8.2.4.3 Gene Mutation Analysis

Diagnostic determination of the patients with DMD and BMD is carried out by molecular or gene mutation-based analyses. Outcome of the genetic testing is most important clinical information, which is required for genetic counseling, prenatal diagnosis, and consideration for future mutation-specific therapies [1–5]. Dystrophin mutations are confirmed by multiplex PCR, multiplex ligation-dependent probe amplification, single-condition amplification/internal primer, and multiplex amplifiable probe hybridization. Least expensive and an easily available technique is multiplex PCR. The whole mutated gene is not characterized, and only deletions are identified by this technique. Deletions, duplications, and covering of all exons are identified by multiplex ligation-dependent probe amplification and amplifiable probe hybridization. Detection of deletions and data sequencing is carried out by single-condition amplification/internal primer [15–18]. No further testing is required after identification and full characterization of a dystrophin mutation by one or more of these techniques.

There is a need of dystrophin gene sequencing to detect the point mutations or small deletions/insertions in the case of negative outcome of deletion/duplication testing. Complete characterization of the mutation (deletion endpoints or exact position of any point mutation) is needed to allow the correlation of the predicted effect of the mutation on the reading frame of the gene. This determined the phenotypic variability seen in dystrophinopathy and also helpful to decide the mutation-specific treatments [4, 5, 14–18]. Multiplex PCR result for gene mutation in DMD/BMD patients is represented by Fig. 8.6.

8.2.4.4 Histopathological- and Immunohistochemical-Based Examination

Diagnostic confirmation of DMD and BMD is based on the histopathology and immunohistochemistry in the case of failure to provide the diagnosis by genetic testing. Staining of muscle tissue sections with hematoxylin and eosin demonstrated the dystrophic features in patients with DMD and BMD. Distortion of fascicular architecture with marked variation of fiber size in muscle tissue sections of DMD patients was observed [28]. Numerous minute atrophic fibers, bulky hyalinized hypertrophied fibers, necrotic fibers, and intrafascicular fibrosis were observed in the muscle biopsy sections of DMD patients. Incidents of nuclei internalization and myophagocytosis were also seen in the section of DMD muscles. There was an observation of degenerated fibers with marked newly produced fibers in sections of muscle biopsies of patients with BMD as well as in patients with DMD with the staining of hematoxylin and eosin [29].

Immunohistochemical-based dystrophin staining is showing the diagnostic confirmation of patients with DMD and BMD. There is an observation of complete

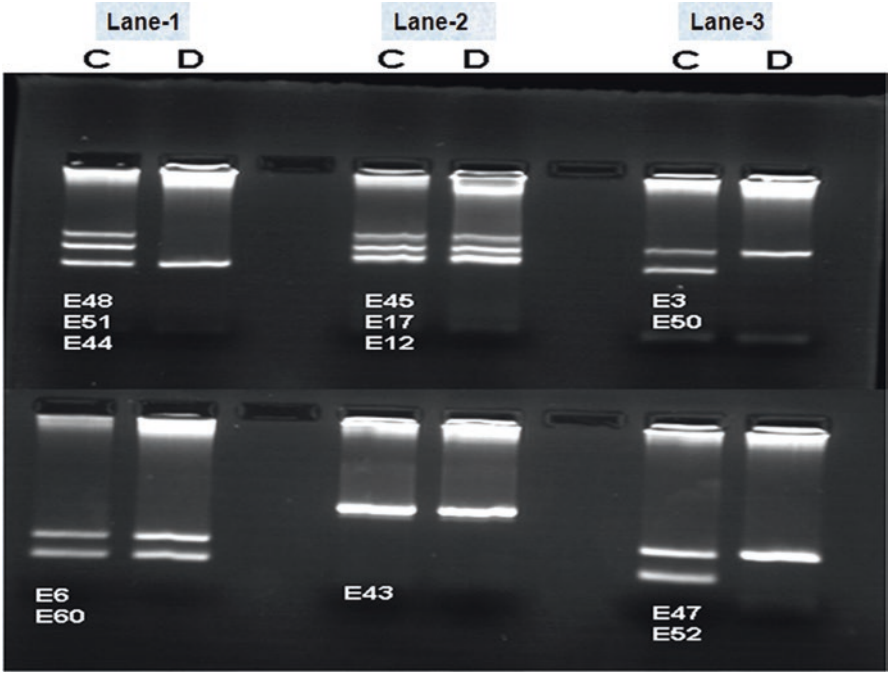


Fig. 8.6 Electrophoretic pattern in Lane-1 showed the deletion in exons 48 and 51, Lane-2, showed no deletion in exons, and Lane-3 showed the deletion in exon 50 and 52 (*C* control, *D* disease)

absence of dystrophin protein in the muscle biopsy of DMD cases and discontinuous dystrophin staining pattern with reduced amount in cases of BMD (Fig. 8.7) [28].

8.2.4.5 Metabolomics-Based Possible Diagnostic Approach

Differentiation of DMD patients from healthy subjects was determined by measurement of serum lipids through ^{1}H-NMR spectroscopy. High levels of phospholipids and elevated phospholipids to cholesterol ratio were observed in serum of patients with DMD as compared to healthy subjects. Outcome of this study may be used for diagnostic purposes even in negative gene deletion cases of DMD (Fig. 8.8) [11]. Branched chain amino acids, acetate, glutamine, and tyrosine are significant parameters in serum for the differentiation of patients with DMD and BMD as compared to normal subjects. The above description showed that NMR spectroscopy-based metabolomics have a potential to perform the diagnosis of patients with DMD and BMD [19].

8.2.5 Aging

Aging is the progression of becoming older. There is a characteristic of change in human being over time by aging. These changes are physical, psychological, and social [29]. Activities and quality of life are affected by aging. Disease-related

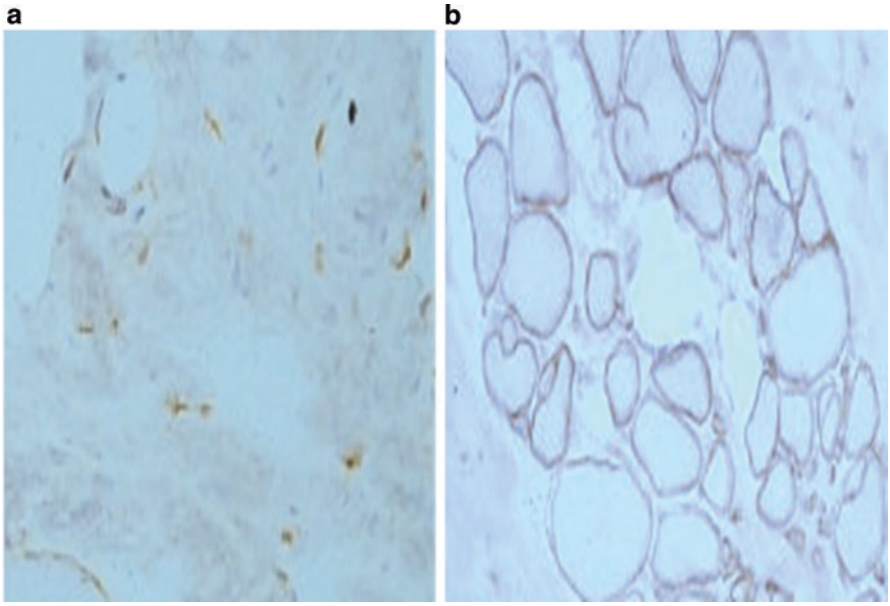


Fig. 8.7 Dystrophin staining performed in muscle biopsy: (**a**) in DMD muscle, dystrophin is completely absent; (**b**) in BMD muscle, dystrophin is reduced and discontinuous

structural and functional alteration is also affected by the aging process and the rate of aging. A functional and structural change in aging muscle is completely explained and a comprehensive reduction in the muscle protein synthesis with aging [30].

Aging is a noteworthy parameter for patients with DMD and BMD. This influences the clinical symptoms, disease progression, serum enzyme abnormalities, morphological changes in muscle, and medications for DMD and BMD patients. Aging aspect is responsible for pathogenesis in every stages of patient's life in DMD and BMD.

8.2.5.1 Clinical Symptoms and Disease Progression

Height and weight are normal at birth in patients with DMD. Rate of successive growth is slow, and falling in the growth curve below the normal centiles is observed in the first year of life of patients with DMD. Short stature at a preclinical stage in these patients is a common finding. Developmental delays, difficulty in running or climbing stairs, frequent falls, and the enlargements of the calf muscles are early symptoms reported by parents of patients. Appearance of Gower's sign and the gait becomes lordotic and waddling at the age of 3–6 years. Enlargement of the calf, gluteal, lateral vastus, deltoid, and infraspinatus muscles are presented at the age of 5 or 6 years. Weakness is more proximal than distal, and the muscles of lower extremity and torso appear to be more affected than those of the upper extremities at this moment. The sparing of the upper extremities, however, is more apparent than real, because the patient's stance and locomotion reveal the weakness of the

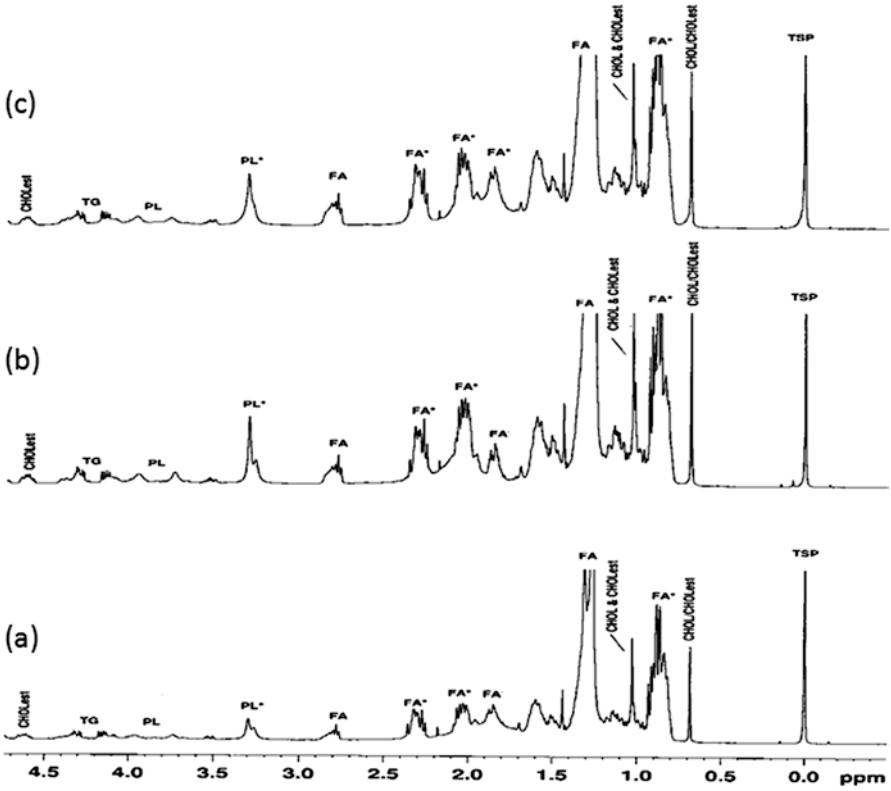


Fig. 8.8 1H-NMR (proton nuclear magnetic resonance) spectra are showing the lipid components, i.e., TG (triglycerides), PL (phospholipids), CHOL (cholesterol), CHOLest (cholesterol esters), and FA (fatty acids) in (**a**) control subjects, (**b**) gene deletion positive case of DMD, and (**c**) gene deletion negative case of DMD

torso and lower extremities, whereas manual muscle testing, which is difficult to perform in young children, is required to demonstrate weakness in the upper extremities [31, 32].

Gradual and linear decrease is observed in the strength of limb and torso muscles in patient with DMD. Distal muscles remain stronger than proximal ones at the age of 6–11 years in DMD patients. The neck flexors are more involved than extensors, biceps, and triceps more than deltoid, wrist extensors more than flexors, quadriceps more than hamstrings, and the tibialis anterior and peronei more than gastrocnemius, soleus, and tibialis posterior. Except for the sternocleidomastoids, muscles innervated by the cranial nerves, levator ani, and the external anal sphincter muscle are spared [33–39].

Weak muscles represented the gradual decrease and finishing of the tendon reflexes in DMD patients. Disappearance of reflexes is observed in the biceps, triceps, and knee in about 50% of the patients before the age of 10. At the advanced stage of disease, the brachioradial reflex persists longer, but the ankle reflex can be elicited in one-third. Unequal weakness of agonists and antagonists joint

contractures emerge with the disease progression. Significant contractures are developed in 70% of the patients between 6 and 10 years of age in the iliotibial bands, hip flexors, and heel cords [39–43].

There is no fine correlation observed for selected muscles in between the loss of strength and the loss of ability to perform specific functional tasks in DMD patients. There is an unexpected decline observed for the ability to climb stairs, rise from the supine position, climb stairs with rails, and walk a short distance at the age of 7–11 years. When DMD patients are evaluated by the Vignos scale (1, normal function, unable to ambulate), most spend a long time in functional grade 2 and then traverse the remaining stages rapidly, over a course of 2 or 3 years. In a large group of DMD patients, 94% under age of 8 years could climb stairs with mild difficulty, and 14% were still able to do so after the age of 10 years [34, 40, 41, 43].

All limbs and torso muscles are decreased in size in DMD patients with the loss of ambulation in the second decade of life. Patient can perform only limited activities involving the forearm and hand muscles due to increasing weakness of the upper extremities. Weakness of paraspinal muscle produces the progressive kyphoscoliosis. There is a considerable weakness of respiratory muscles that showed the abnormal low maximal inspiratory and expiratory pressures with decrease of vital and total lung capacities, which begins at the age of 8 or 9 years and decreases consistently. There is an observation of declining the forced vital capacity annually by about 4% of the predicted value. There is an occurrence of respiratory failure with carbon dioxide retention and anoxemia due to respiratory infections in DMD patients. There is also an observation of pure respiratory failure without infection and signals of an irreversible terminal event in DMD patients. The age at respiratory failure correlates positively with the degree of thoracic scoliosis. About 40% of the patients die of respiratory failure, with or without infection, and 40% of cardiac failure. The age of death of DMD patients ranged from 10 to 29 years with a mean of 18.3 ± 3.6 years [44–47].

The mean age of onset symptoms in BMD is about 12 years (range 1–70 years). DMD and BMD patients are not differentiated clinically before the age of 8 years. Symptoms appeared in 50% of BMD patients at the age of 10 years and about 90% by the age of 20 years. However, few patients are free of muscle symptoms until their 50 years of age. There was an observation of lower limb weakness at the mean age of 11 years and upper limb weakness at the mean age of 31 years in BMD patients. Observation of ambulatory loss in BMD patients varied from 10 to 78 years of age. The mean age at loss of ambulation is in the fourth decade. Patients with mild BMD remain ambulatory beyond the age of 40 years. The mean age of survival are lying in between 23 and 89 years [48–59].

8.2.5.2 Pattern of Muscle Power in DMD and BMD

A rapid deteriorated form of muscular dystrophy is recognized as a DMD. Pattern of linear decrease in muscle power with age is observed in DMD patients. Muscle power is also positively correlated with age in DMD patients. There is a decrease of average muscle strength with every year increment in age of patients. The pattern of decrease in average muscle strength is 3.9% of the muscle power of a normal

individual. Lower extremities are weaker than the upper extremities with the retention of the half of the muscle strength in DMD patients at the age of 12 years. Power of proximal muscle is weaker than distal muscle. So, the dominancy in the weakness of the elbow and wrist extensors is more than that of the flexors in DMD patients. Hip and knee extensors are parts of the lower extremities, and these parts are weaker than the hip and knee flexors. In the upper extremities, elbow and wrist flexion contracture is easily found due to difference in the strengths of agonist and antagonist muscles of a joint, which is responsible for shortening of the muscles of stronger side and developing of joint contracture. Hip flexor (lower extremities) contracture is commonly found with the development of severe flexion contracture in the knee joints of DMD patients in the early stage of the disease. The ultimate results of these events are incapability of walking of DMD patients. Clinicians advised to use computer games as finger exercises or a leisure activity for managing the strength of finger flexors in patients with DMD (at the age of twenty) [60, 61].

The muscle power strength of DMD or BMD patients is ranked by Brooke and Vignos scales. The Brooke and Vignos scales in patients with DMD and BMD were successfully applied in one of the multicenter study. The outcome of such studies represented the classification of patients with DMD as severely progressive group, whereas BMD was classified as slowly progressive group. Brooke and Vignos scales are easy to explain with the requirement of the little time to finish the tests. None of the inconvenience is created by using these tests for patients with DMD and BMD. The Brooke scale is acceptable to grade the arm function of the DMD, and each grade of the Brooke scale is distributed with the acceptable percentage (ranging from 7.1% to 33.3%). Difficulties arise to discriminate the various levels of severity for BMD patients with slow rate of the disease progression [60, 62].

8.2.5.3 Cardiac Involvement in DMD and BMD

Cardiac abnormalities are life-threatening troubles for patients with DMD and BMD. Frequent problems for patients with BMD and DMD are cardiomyopathy and cardiac conduction deficit. Frequency of these incidences is more in DMD as compared to BMD patients. The incidence of cardiac abnormalities is up to 90% at 18 years and nearly 100% at 30 years of age in DMD patients. Cardiac rate, rhythm, and conduction are the pattern of cardiac abnormalities in patients with DMD. There was an observation of the average pulse rate of 100 beats per minute in DMD children after the age of 5 years as compared to the corresponding value in age-matched controls which was only 77 beats per minute. Abnormalities in the cardiac conduction system appeared in 50% of the patients with DMD. Intra-arterial conduction defects are common in DMD patients, but infranodal conduction defects appear in 10%, and more than one type of defect occurs in one-third of patients [63–69].

BMD patients suffered repeatedly of myocardial abnormalities. The outcome of ECG was similar to those noted in DMD patients. Bushby and Gardner Medwin observed ECG abnormalities in 41% of whole BMD patients in a retrospective survey of 52 patients. Appearance of cardiomyopathy was not related to the course or severity of the myopathy, and severe cardiac abnormalities were occurring in some patients with only mild muscle weakness. ECG (electrocardiography) showed

cardiac abnormalities in 47% of carriers (85 in DMD/44 BMD; 129) aged between 18 and 60 years. Abnormal ECG finding was observed in 38% of DMD and 34% of BMD carriers. Left ventricular dilation was observed in 19% and 16% of DMD and BMD cases, respectively. There was 8% of DMD carriers, represented the dilated cardiomyopathy. No cardiac abnormalities were observed in 38% of the carriers of DMD/BMD [70–73].

8.2.5.4 Central Nervous System Involvement in DMD and BMD

Little, Meryon, Duchenne, and Erb had documented the mental retardation in patients with DMD. The mental deformity is present at an early age (3–5 years of age), is nonprogressive, cannot be accredited to the anomalous motor growth, does not correlate with phase of the disease, and affects verbal more than realistic intelligence. According to a report or study, mental retardation is observed in a small percentage of the patients. In 38 patients with DMD, the mean intelligence quotient (IQ) was 83. The observed values ranged 46–134 and showed a bell-shaped distribution curve [40, 41, 70–72].

Average IQs have been represented by most patients with BMD. Appearance of reduced IQ is finely correlated with early phase of the disease (5–6 years of age) rather than with rapid progression of the disease. Loss of expression of the Dp 140 dystrophin isoform observed due to mutation also represented the cognitive impairment in a few DMD patients [40, 41].

8.2.5.5 Biochemical Abnormalities in Body Fluids in Patients with DMD and BMD

Serum CK (creatine kinase), diphosphofructose aldolase, pyruvate kinase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucosephosphate isomerase, lactate dehydrogenase (LDH), carbonic anhydrase III, and enolase are elevated in patients with DMD and BMD. Hypersecretion of enzymes is a highly marked point in the early stages of the patients with DMD and BMD. Marked decrease in the secretion of enzymes is observed with the degeneration of functioning muscle mass in these patients. Serum CK elevation is the most marked and consistent, and CK assay is most useful of the serum enzyme tests for the diagnosis of patients with DMD and BMD because considerable amounts of CK are present only in the skeletal muscle, heart, and brain (but not in erythrocytes, liver, or other organs) [74, 75].

Elevation of serum CK is 10–100 times more than normal at birth in DMD. Similarly, serum CK is elevated in DMD infants without showing clinical manifestation of disease. There is an increase of up to two orders of magnitude of the serum CK activity in DMD in the first 3 years of life and reached up to peaks around the age of 3 years. After the age of 3 years of the DMD patient, serum CK decreases exponentially by about 20% per year. There is an observation of 25–200-fold elevation of the serum CK levels in the first 10 years of life of typical cases of BMD. Marked elevation of serum CK is observed in the preclinical stages and decreases as disease progresses or with increase of age. Mean value of serum CK elevation (mean age of 20) was 35-fold for 52 patients with BMD. The serum CK

elevation was 3- to 20-fold in mild form of BMD with exertional muscle cramps and mild weakness, or mild weakness, or mild weakness and early myocardial disease [1–6, 40, 41].

A repetitive myoglobinemia was observed in DMD and BMD patients. Myoglobinemia frequency was highest in patients with DMD and BMD. No specific relationship between the CK and myoglobin levels was observed, but patients with the highest CK levels also acquired the highest myoglobin levels. Level of myoglobinemia was reduced in DMD patients with age and disease progression. Observation of a marked decrease in myoglobinemia was seen in DMD patients at the age of 12 or more than 12 or with the loss of ambulation. Level of myoglobin and CK was fluctuating in serum of DMD patients with ambulation. Physical activity of patients determined the alteration in the level of myoglobin and CK in serum. Increase or decrease of physical activity was responsible for increase or decrease in the level of CK and myoglobin in serum of patients. There was a prompt and marked increase of serum myoglobin on mild exercise in ambulatory DMD patients (up to 10–11 years of age) followed within 4 h by a marked increase of serum CK (e.g., from 6000 to 25,000 U/L). Mild increase of serum myoglobin was observed in normal subjects due to exhausting exercise followed by a smaller increase of CK 10–18 h after exercise (e.g., from 70 to 230 U/L). One conclusion was drawn on the basis of all these findings that mild exercise was responsible for muscle fiber injury in DMD patients [76, 77].

Observation of reduced creatine and amplified creatinine excretion in urine was seen in DMD patients. Initiation of creatine synthesis takes place in the kidney with the production of guanidoacetic acid from glycine and arginine and is finished in the liver with the methylation of guanidoacetic acid, which produces creatine. Later, creatine is transported via blood to muscle and holds 95% of the body pool in a concentration of about 4 g/kg. Eighty percent of total body creatine is present as phosphocreatine, and the rest of 20% is present as free creatine. Energy stored in the form of phosphocreatine is responsible for rephosphorylate ADP to ATP in the reaction catalyzed by CK in a normal adult, the total body creatine pool is 100 to 120 g approximately 1% of which (or 40 mg/kg of muscle) is formed daily, and the same amount is dehydrated nonenzymatically to creatinine, which is excreted. Functional muscle mass is therefore proportionate to the total creatinine excretion in DMD. There was a reduction of 41–87% of creatinine excretion in DMD patients, and the excretion in a given patient declines progressively with progression of the disease and age [78–84]. DMD patients excreted about 22 mg of creatine per kilogram per day at the age of 6–11 years, but normal boys of the same age excreted 11 mg of creatine per kilogram per day [40, 41].

Elevation in the urinary excretions per gram of creatinine of taurine, 3-methylhistidine, dimethylarginine, polyamines (putrescine, spermidine, and spermine), and carnosine (beta-alanyl-histidine) was observed in patients with DMD. Elevated level of excretion of 3-methylhistidine and dimethylarginine is an index of increased actin and myosin catabolism in muscle with disease progression and age. Marker of disease activity in patients with DMD is an augmented polyamines excretion [85–88].

8.2.5.6 Morphological Abnormalities in Patients with DMD and BMD

Pathological alterations are similar in muscle of patients with DMD and BMD. These alterations are necrosis, regeneration, endomysial fibrosis, branching or splitting of fibers, and abnormal variation in fiber size. The factors of differentiation between DMD and BMD are age, stage, and severity of the disease. Reduction in the clinical severity of the dystrophinopathy is responsible for downward trend in the frequency of necrotic, hypercontracted, and regenerating fibers [40, 41].

Necrotic Fibers Muscle tissue of DMD and BMD represented the specific differentiation on the basis of the presence of necrotic fibers. The necrotic fibers have the following distinguishing features in muscle of DMD: (1) In trichromatically stained fresh-frozen sections, they have a green-blue color (normal color, deep blue) and a glassy or homogeneous cytoplasm; the intermyofibrillar membranous network is absent, attenuated, or clumped; (2) the histochemical reactions for oxidative enzymes and glycogen are markedly reduced or lost; (3) the myofibrillar ATPase reaction is also reduced, but may persist even after reactivity for oxidative enzymes, and glycogen has disappeared; (4) the necrotic fibers may or may not be invaded by mononuclear cells; and (5) those portions of the necrotic fibers not replaced by invading cells invariably react for the C5b-9 complement membrane attack complex (MAC) and usually contain granular calcium deposits. Fibers in early age and stages of necrosis immunoreactions for hematopoietic prostaglandin D synthase showed that prostaglandin D2 is produced by these fibers in DMD and not observed in BMD.

This has not been established till date in DMD or BMD that the earliest age when necrotic fibers appear in groups. Single necrotic fibers appear in the neonatal period and appear in the groups at the age of 3 years. Longitudinally oriented sections of paraffin or resin showed that the necrosis was segmental and the necrotic fiber segments extending over a distance of 0.2–2.5 mm. Both necrotic and non-necrotic segments of a longitudinal traverses showed the normal appearance of hypercontracted non-necrotic part or signs of regeneration. A transverse section of muscle showed the central fiber necrosis with partly or completely filling of macrophages and normal appearance of fiber in the peripheral parts [89–94] (Fig. 8.9).

Regenerating Fibers Muscle of DMD and BMD showed the regenerating fibers with a consistent finding. These have (1) large vesicular nuclei with prominent nucleoli, basophilic cytoplasm, and poorly developed cross-striations, (2) an uneven distribution or sometimes subsarcolemmal increases of oxidative enzyme activity, (3) small lakes of glycogen, (4) moderate reactivity for lysosomal enzymes (e.g., acid phosphatase or acid cathepsin), and (5) a type 2C staining pattern in the myofibrillar ATPase reaction. Regenerating fibers of muscle myofibrils are immunoreactive for the fetal isoform of myosin heavy chain and express utrophin under the sarcolemma [92–99] (Figs. 8.10 and 8.11).

Regenerating elements can occur next to necrotic fiber remnants within the confines of an area previously occupied by the non-necrotic fiber. More often, in both DMD and BMD, the regenerating fibers occur in clusters of varying size. This may be related to the grouping of the necrotic fibers they replace, to incomplete lateral fusion of myotubes arising within the basal lamina cylinder of a necrotic fiber, or

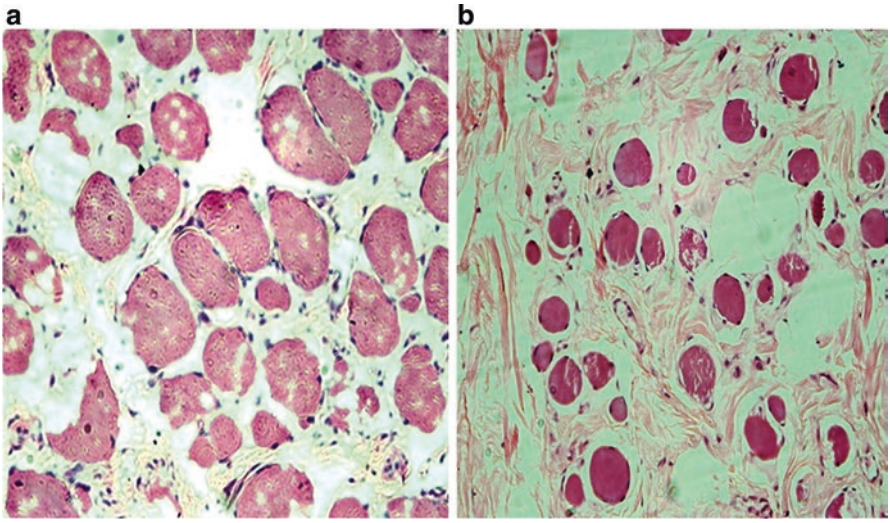


Fig. 8.9 H & E stain of (**a**) DMD patient showed marked variation in fiber size, splitting of fibers, internalization of nuclei, small atrophic fibers, and intrafascicular fibrosis; (**b**) DMD patient showed large amount of degenerated fibers, variable fiber size, and connective tissue

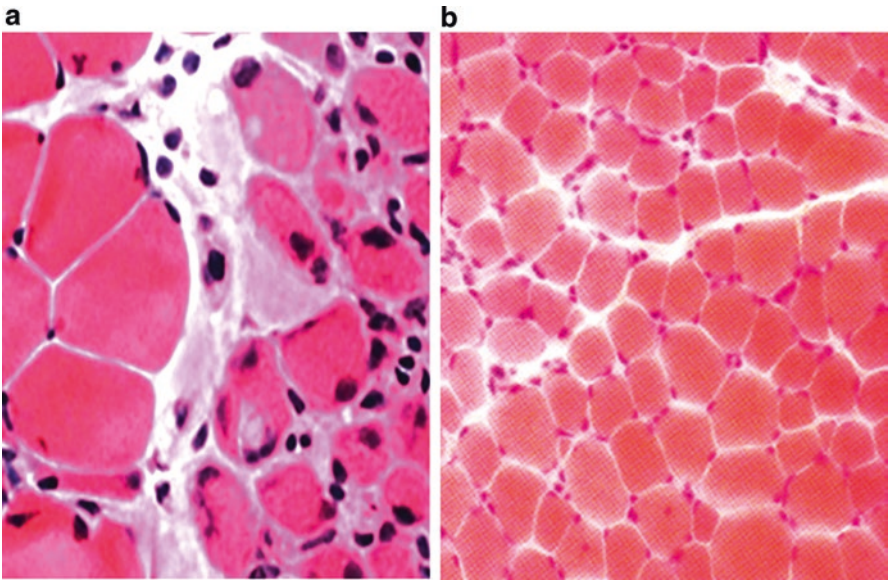


Fig. 8.10 H & E stain of (**a**) BMD patient showed marked degenerated and regenerated muscle fibers; (**b**) BMD patient showed marked necrotic fibers and variable fiber size

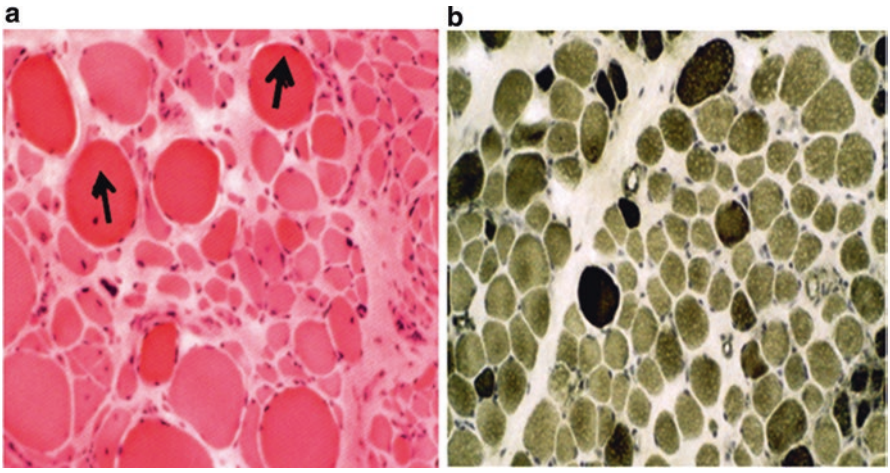


Fig. 8.11 (**a**) H & E stain of BMD patient showed the clusters of small fibers and also the darkly stained hypercontracted fibers; (**b**) ATPase at pH 9.4 in DMD case showing variation in size of both fiber types and a marked predominance of the pale type 1 fibers. Several of the dark-type fibers are 2C fibers and were also dark when stained for ATPase with acid preincubation at pH 4.6 and pH 4.3

both. It is also possible that myogenic cells leave disrupted muscle fibers or migrate from undamaged fibers in response to a signal from damaged fibers and attempt to form myotubes in the endomysium [100–104].

With the maturation of regenerating fibers and their increase in girth, showing a more even distribution of oxidative enzyme activity and glycogen, their nuclei also become smaller and less vesicular. They are continuing to express the fetal isoform of heavy chain, remain type 2C fibers in the ATPase reaction, and retain faint acid phosphatase positivity until they are nearly normal in size. Proportion of acid phosphatase-positive type 2C fibers are expressing fetal myosin isoforms in a given specimen, which is a sensitive indicator of the total number of immature fibers and regenerating activity. The time required for a regenerating fiber to become fully mature, for the fetal myosin isoform to disappear, and for the ATPase reactivity to reflect innervation has not been established in DMD or BMD [40, 41, 103, 104].

Grouping of Necrotic and Regenerating Fibers The reason for the grouping of necrotic fibers in DMD, and sometimes in BMD, is not fully understood. A possible explanation is as follows: fiber necrosis is segmental, extending up to several millimeters in length. The muscle fibers branch and anastomose because of splitting of existing fibers or because of incomplete lateral fusion of regenerating myotubes. On average, 2–3% of the fibers are subdividing in a given plane of sectioning; these are six branching per centimeter fiber length, and as many as six parallel branches of a single fiber can exist in a given transverse plane of sectioning. Thus, a group of necrotic fibers may simply arise from segmental necrosis of all branches of a given fiber. Regeneration occurring within a group of necrotic fibers would then result in

an even larger group of regenerating fibers because of incomplete lateral fusion of the regenerating myotubes. This view is consistent with the observations that (1) necrotic fibers are not grouped at birth and (2) in a given biopsy the average number of fibers in a regenerating group is high as or higher than the average number of fibers in a necrotic group [40, 41, 103, 104] (Figs. 8.9 and 8.10).

Distribution of Histochemical Fiber Types A random distribution of histochemical fiber types is observed in the neonatal period. In older patients, this distribution is slightly disturbed, with 6–15 fibers of a given type appearing adjacent to each other. This finding is readily explained by the presence of branching fibers (i.e., by the same factors that contribute to grouping of the necrotic fibers) and by the assumption that branching fibers are innervated by a single endplate [1–6, 40, 41].

Type 1 fiber preponderance has been described in DMD, but the proportion of type 1 fibers may vary from muscle to muscle and with age. The mean percentages of type 1 fibers in biceps brachii, gastrocnemius, and quadriceps muscles were 41%, 48–56%, and 45–76%, respectively. Although the number of type 2 fibers is reduced, the mean diameter of type 2 fibers is markedly larger than that of type 1 fibers. In DMD, type 2B fibers decrease in number after the age of 2 years and disappear entirely after the age of 5 years. A plausible explanation for the early destruction of type 2B fibers is that their sarcolemma is subject to the highest mechanical stress due to rapid, strong contractions, dictated by rapidly firing, physically active motor neurons. Selective depletion of type 2B fibers is not a feature of BMD [105, 106].

The proportion of type 2C fibers is higher than normal because both regenerating and recently regenerated fibers are type 2C. The number of type 2C fibers increases with age in DMD [40, 41] (Fig. 8.11).

Fiber Size Abnormal variation in fiber size and an increase of the mean fiber diameter has been observed in some muscles of 14- to 21-week old male fetuses at risk of developing DMD. Variation in fiber size is slightly increased in the neonatal period, probably because of the presence of scattered large fibers. Between the ages of 1 and 5 years, the mean muscle fiber diameter is greater in DMD than in corresponding control samples. After 5 years of age, the variation in fiber size continues to increase, but the mean fiber diameter returns to normal because of an increased replacement of normal and large fibers by abnormally small fibers. Small clusters of highly atrophic fibers are conspicuous in some specimens. Some of these atrophic fibers are immature by histochemical criteria and could be regenerating fibers that did not become innervated. Interestingly, in gastrocnemius muscle fiber size is increased at all ages [107–109].

The hypertrophied fibers in DMD are of either histochemical type, although in some specimens more type 2 than type 1 fibers are hypertrophied. Some large fibers are also hypercontracted, but not all hypercontracted fibers are large, and not all large fibers are hypercontracted [40, 41, 107].

Central Nuclei Abundance of more **c**entral nuclei in the muscle fibers of DMD and BMD are responsible for moderate or severe disability. Quantitative observation of central nuclei is 2–4% in the muscle fibers of DMD and 7–25% of muscle fibers in BMD.

Hypercontracted Fibers Hyaline, hyperreactive, opaque, or large dark fibers (but not all hypercontracted fibers are large) are the properties of hypercontracted fibers. A defect in plasma membrane is responsible for the influx of calcium-rich extracellular fluid, which is further responsible for the development of hypercontraction. Instantly due to such defects, the sarcomeres are pulled apart in a wedge-shaped zone, which possess the resemblances with the delta-shaped lesions and also observed in some fibers in DMD. The significance of hypercontracted fibers and delta lesions in DMD has been disputed. Studies showed the mean incidence of hypercontracted fibers in DMD has ranged from 4.3% to 8.3%, but this is less than 0.4% in normal subjects [110–112] (Fig. 8.11).

Other Pathologic Changes in Muscle Disease progression in both DMD and BMD is responsible for the enhancement of endomysial and perimysial fibrosis. Normal connective tissue elements are observed in the neonatal period of DMD. Endomysial and perimysial fibrosis is a prominent feature at the 4 years of age of DMD. Type 3 collagen is predominantly present in the fibrous connective tissue, and the disorganization is taking place in the architecture of the fascicles. Fatty and fibrous connective tissue replaces the disappearing fibers. Tissue fibrosis is promoted by transforming growth factor-β1 (TGF-β1) and its binding protein, which is highly expressed in muscle fibers of DMD and to a lesser extent in BMD. The interstitial fibrosis in DMD and BMD is possibly promoted by TGF-β1 with its binding protein [113, 114].

8.2.5.7 Medication [Corticosteroids (Prednisone and Deflazacort)] in DMD and BMD

None of the medication is capable for permanent inhibition of the rate of degeneration in DMD. By knowing this disgusting fact, the physician, physical therapist, social worker, and organization are still working for the development of the quality of living for patients. There is a requirement of an efficient curative mediator for DMD patients. Currently available solitary medication is glucocorticoids. These are slowing the decline in muscle strength and function in DMD, which in turn decreases the danger of scoliosis and steady the pulmonary function. This is also responsible for the limited degree of improvement in cardiac function [115].

Development in muscle potency was observed in a preliminary randomized controlled trials with prednisone treatment (0·75 mg/kg per day) for up to 6 months in patients. Another form of glucocorticoid is deflazacort, available in numerous countries, has a comparable efficacy at per day dose of 0·9 mg/kg, and has a vaguely diverse chronic threat outline [1–5, 115].

Most accurate time to initiate glucocorticoid therapy in an ambulatory boy with DMD is not established in the literature. Accurate timing of beginning of glucocorticoid therapy depends upon the personage resolution, supported on functional situation and in addition taking into account the age and pre-existing threat characteristic for adverse side effects. There is no compulsion of glucocorticoid dealing for a child who is still gaining motor skills or is below 2 years of age. Glucocorticoid medication is started at age of 4–8 years in DMD patients after the recognition of stability

phase by clinicians with the major concerns of pre-existing threat features for side effects to stay until the decline phase. Medication of glucocorticoid is also recommended by the clinicians in patients with DMD in the complete decline phase or in marginal condition of ambulation with limited benefit [1–5, 40, 41, 115].

The sequential evaluation and parental reports are supported to make a proper decision for initiating the glucocorticoids treatment. An additional attention is required for beginning the glucocorticoid medication in a first or second consultation. Evaluation of motor function (making progress, area of stability phase, and decline) in DMD patients is based on the clinical history with the extra care of child aged less than 6 years. Avoiding the potential side effects of the long-term use of glucocorticoids is required for taking the specific precautions and management by their primary-care physician and specialty health-care panel [115].

Medication of glucocorticoids is continuing in ambulatory phase of patients. But several clinicians also continued this medication after loss of ambulation for preserving the upper limb strength, reducing progression of scoliosis, and delaying the loss in respiratory and cardiac function. Beginning of glucocorticoids in non-ambulatory patients is more proportional as compared to categorical. This is not well-established and well-recognized facts for the effectiveness of glucocorticoid treatment in avoiding scoliosis or in stabilizing cardiac or respiratory function in this situation [1–5, 39–41, 115].

Spectacular and continue improvement in potency with therapeutic utilization of prednisone was observed in BMD patients [116]. BMD patient (26 years) administrated 30 mg prednisolone and showed the decrease level of CK with improvement in the cardiomegaly at the stage of severe heart failure. Regular administration of 7.5 mg prednisolone in this patient was advantageous for returning to normal life with the freedom of the heart malfunction. Corticosteroids are effective for skeletal muscle improvement and rarely effective for heart failure in DMD [117].

8.2.5.8 Oxidative Stress in DMD and BMD

Oxidative stress is one of the causes for muscle degeneration in patients with DMD and BMD. Levels of antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase) were elevated in muscle specimens of mdx (prenecrotic), mdx (4–5 mo), and dy/dy mouse model of muscular dystrophy [118]. Higher levels of these antioxidants enzymes and lipid peroxidation were found in patients with DMD and BMD [118].

Due to mutated dystrophin protein, the plasma membrane becomes fragile with reduced stiffness and increased leakiness with exposing dystrophin-deficient muscle to hypoosmotic conditions. All these characteristics are linked with an enhancement of intracellular calcium, reactive oxygen species, and activation of a protease cascade. Significant loss of myofibers is largely mediated by a necrotic cell death process and also linked with progressive replacement of the myofibers by fibrosis. Production of reactive oxygen species is the cause of oxidative stress [119]. Oxidative stress is responsible for weakening of integrity and abnormal behavior of muscle membrane. Abnormal membranes are more susceptible to oxidative stress-induced lipid peroxidation. Lipid peroxidation is responsible for the damaging of

lipid bilayer structure of membrane and finally the cell death occurs. In this way, muscle cell degenerated, and this event increases with progression of disease and age of the patients [40, 41, 118].

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Mitophagy, Diseases, and Aging

9

Sujit K. Bhutia, Prajna P. Naik, Debasna P. Panigrahi, Chandra S. Bhol, and Kewal K. Mahapatra

Abstract

Mitochondrial dysfunction contributes to age-associated disease phenotypes and aging. With age, mitochondria show change in morphology, mutation and change in mtDNA, increase in oxidative stress, epigenetic change in mitochondrial proteins, and defect in mitochondrial quality control leading to accumulation of dysfunctional mitochondria. Mitophagy, a specified form of autophagy, regulates the turnover of damaged and dysfunctional mitochondria to govern energy homeostasis. The age-dependent impairment of mitophagy inhibits removal of superfluous or dysfunctional mitochondria as well as weakens the biogenesis of mitochondria resulting in the aggregation of reactive mitochondrial mass and consequently leads to the deterioration of cellular function. Novel therapeutic strategies have been articulated for maintaining healthy mitophagy level which could delay aging and extend health span. This chapter provides an updated mechanistic overview of mitophagy pathways and discusses the effect of mitophagy in aging.

Keywords

Mitochondria · Mitophagy · Aging · Senescence · mtDNA · ROS

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9.1 Introduction

Aging is a biological phenomenon characterized by progressive deterioration of the physio-metabolic state of an organism with time. In particular, aging is the inability of an individual to get fit with the environmental needs with respect to increased age. It is associated with the loss of physiological integrity and disease susceptibility by accumulation of damages inside a cell which ultimately leads to diminish the function [1–3]. During aging, the cells undergo irreversible cell cycle arrest and distinct phenotypic alterations termed as senescence which are found to be accumulated at sub-cellular level to accelerate the aging process [4, 5]. Several theories assume that aging is the sum of accumulative damages at the cellular level rather than at the organism level, i.e., it starts at the molecular level and has the potential to exploit it from cellular to tissue level and then finally leads to organ level damage [6]. Bernard Strehler, an American gerontologist in 1977, set four postulates to define aging, i.e., aging is universal, intrinsic, progressive, and deleterious [7]. Later in 2013, Lopez-Otin set nine hallmarks for aging which are genomic instability, telomere attrition, loss of proteostasis, deregulated nutrient sensing, altered intercellular communication, cellular senescence, stem cell exhaustion, epigenetic alterations, and mitochondrial dysfunction [1].

Although the process of aging is known and discussed for decades, the advances in molecular and cell biology show new directions to understand the basic mechanism of aging. Autophagy, a cytoprotective mechanism, has been identified as a critical regulator for the process of aging [8]. In this setting, mitophagy, a specialized autophagy, is implicated in many neurological diseases and degenerative aging. In this connection, maintenance of mitochondrial quality control and homeostasis is of utmost importance in cellular physiology to regulate aging through mitochondrial autophagy. Deficiency in mitophagy results in the substantial increase in reactive oxygen species (ROS) production which subsequently leads to aging [9] which is well supported by "free radical theory of aging" given by Denham Harman in the 1950s [10, 11]. Several reports have been stated on different models including *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster*, and mice which show decreased life span in mutants with reduced expression of autophagy genes and vice versa [12]. The decrease in mitochondrial quality is associated with defective metabolism of the living system causing many age-related disorders; therefore, focus was instigated to understand and discuss the effect of mitophagy in aging.

9.2 Causes of Aging

9.2.1 Mitochondrial DNA Mutation

Mitochondria are dynamic cell organelles which produce ATP through oxidative phosphorylation and play a key role in cellular metabolic processes. Alterations in mitochondria and mitochondrial DNA (mtDNA) have been detected in somatic tissues and are highly associated in the multifactorial aging process in both humans

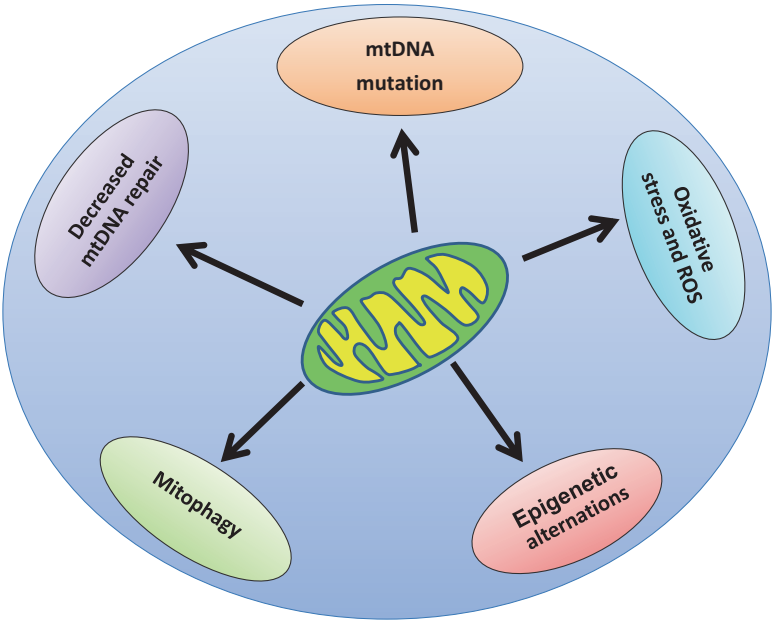

Fig. 9.1 Role of mitochondria in aging. Accumulation of somatic mutation in the mtDNA provokes the respiratory chain dysfunctions which in turn enhances the production of DNA-damaging oxygen free radicals ultimately contributing to progeroid phenotypes [13]. However, some recent evidence suggests that increased mtDNA mutations associated with aging originate due to clonal expansion of mtDNA replication errors made by the mtDNA polymerase during development [14]. The production of ATP through aerobic metabolism and generation of ROS may result in tissue dysfunction and onset of aging supported by free radical theory of aging [17, 18]. Sometimes, epigenetic alternation in mitochondria changes the expression of mitochondrial genes and enzymes which ultimately altered mitochondrial dysfunction and metabolism and may result in tissue dysfunction and onset of aging [21–26]

and animals. According to the mitochondrial theory of aging, accumulation of somatic mutation in the mtDNA provokes the respiratory chain dysfunction which in turn enhances the production of DNA-damaging oxygen free radicals which ultimately contributes to progeroid phenotypes [13] (Fig. 9.1). Various types of age-related mtDNA mutations have been reported including large-scale deletions, tandem duplications, and point mutations. However, in contrast to the original theory, recent evidence suggests that increased mtDNA mutations associated with aging originate due to clonal expansion of mtDNA replication errors made by the mtDNA polymerase during development [14]. Moreover, various environmental factors (e.g., UV radiation, air pollutants), lifestyle (e.g., alcohol drinking, cigarette smoking), and xenobiotics (e.g., drugs) are reported to generate ROS and free radicals which may induce the aging process in the human tissues by accumulation of mtDNA mutations. Surprisingly, it is important to note that the sum total mtDNA mutations in human tissues involved in aging rarely exceed 1%. Now question arises, how such a low fraction of mtDNA mutation can have significant impact on

aging. The possible answer to this question may be either due to the lack of detection methods or due to the uneven distribution of the mutated mtDNA in the target cells and tissues [15, 16].

9.2.2 Oxidative Stress and ROS

Functional mitochondria utilize most of the oxygen for the production of ATP through aerobic metabolism and generate ROS including hydrogen peroxide, superoxide anions, and hydroxyl radicals to maintain the redox balance [17]. According to the "free radical theory of aging," mitochondria are the ultimate target of free radical attack which contribute to the aging process and are later supported by the "mitochondrial theory of aging," suggesting accumulation of damage to mitochondria, mitochondrial DNA (mtDNA), and RNA which leads to aging in both humans and animals [18] (Fig. 9.1). Respiratory enzyme complex I, i.e., NADH dehydrogenase, and proton-motive Q cycle operating in complex III are the major sites for the generation of ROS in the respiratory chain. Mitochondria are the both important sources and target of ROS which damages the mitochondrial proteins leading to the dysfunction and plays a key role in aging process and neurodegenerative diseases. The rate of production of hydrogen peroxide and superoxide anions in mitochondria is increased with age which results in decreased mitochondria number, increased mitochondrial structural abnormalities, reduced in mitochondrial enzyme activities, and decreased respiratory control [19]. But it has been reported that ROS at low concentration can function as signaling molecules and are essential for a number of biological processes, such as defense against invasion of microorganisms and cell growth. Moreover, it has been shown that life span of an organism is inversely correlated to the rate of oxygen metabolism and directly to the antioxidant capacity. The increased production of hydrogen peroxide was observed in *D. melanogaster* under oxidative stress which caused oxidative damage to mtDNA and membrane lipids of mitochondria [20].

9.2.3 Epigenetic Regulation

Epigenetic modifications are changes in the expression of critical genes associated with various physiopathological processes without affecting the DNA sequence. Several epigenetic mechanisms including DNA methylation and posttranslational modification of histones and microRNAs (miRNAs) have been reported to regulate various cellular processes [8, 13]. It is highly essential for the normal development and maintenance of tissue-specific gene expression and its disruption leads to improper activation or inhibition of various signaling pathways resulting in altered gene function and disease condition. Recent advances have shown that epigenetic changes have a huge influence on the aging process and have been regarded as a hallmark of aging [21]. The relationship between epigenetics and aging was first noticed almost about 40 years ago in humpbacked salmon which showed a decrease

of genomic DNA methylation with increasing age [22]. Similarly, Ashapkin et al. have reported the loss of methylated cytosines from DNA in mammals with progression of age [23]. Although the epigenetic mechanism in the nuclear DNA is well studied and correlated with aging, it is a new and incompletely described phenomenon in mtDNA. The first evidence about an association between mitochondrial epigenetics and aging was observed in 1983, when it was reported that methylation of mtDNA decreases with the increasing age of the cultured fibroblasts [24]. Epigenetic alternation changes the expression of mitochondrial genes which ultimately alteres mitochondrial metabolism and results in the formation of "senile phenotype." Mitochondrial epigenetic signature can be influenced by various ubiquitous factors including airborne pollutants, metal-rich particulates, drugs, and diet and correlates to peculiar phenotypes, including aging and several diseases like Down's syndrome, amyotrophic lateral sclerosis, Alzheimer's and Parkinson's diseases, and cancer. Some of the mitochondrial enzymes such as manganese superoxide dismutase (MnSOD) and NADH:ubiquinone oxidoreductase (respiratory complex I) are found to be acetylated and have impacts on mammalian aging and longevity [25]. In mammals, sirtuin family members (SIRT3, SIRT4, and SIRT5) are found to be localized exclusively within the mitochondria and act as metabolic sensors and NAD-dependent lysine deacetylation linked to aging [26]. Some of the miRNAs such as miR-335 and miR-34a regulate the genes of antioxidant enzymes such as superoxide dismutase 2 (SOD2) and thioredoxin reductase 2 (TRDX2) and thereby protect the mitochondrion from free radicals [27]. Similarly, miRNA-15b acts as a negative regulator of stress-induced SIRT4 and thereby regulates mitochondrial ROS production and generation of senescence-associated secretory phenotype [28] (Fig. 9.1).

9.3 Mitophagy

Mitophagy is the selective degradation process by which damaged or dysfunctional mitochondria are captured by nascent phagophore membranes and are degraded in lysosomes for maintaining the mitochondrial integrity and cellular homeostasis [2, 3, 29]. Mitophagy initiation is highly related to the mechanism regulating morphology of mitochondrial dynamics (Fig. 9.2). The key event of the quality control process by mitophagy is the distinction between damaged mitochondria and healthy mitochondria. The sensing process of the mitochondria is still not discovered, but it suggests the PTEN-induced putative kinase 1 (PINK1) molecule as a molecular sensor for depolarized mitochondria [30]. PINK1 is a gene product of PARK6 having serine/threonine kinase activity, firstly identified as mutated in Parkinson's disease. Under homeostatic conditions, PINK1 is imported into the mitochondrial inner membrane due to potential difference across the membrane leading to its proteolysis by the presenilin-associated rhomboid-like protease (PARL) and other proteasomes. In case of dysfunctional/depolarized mitochondria or accumulation of unfolded proteins, PINK1 is not processed, and the full-length PINK1 (64 kDa) is accumulated on the mitochondrial outer membrane of dysfunctional mitochondria

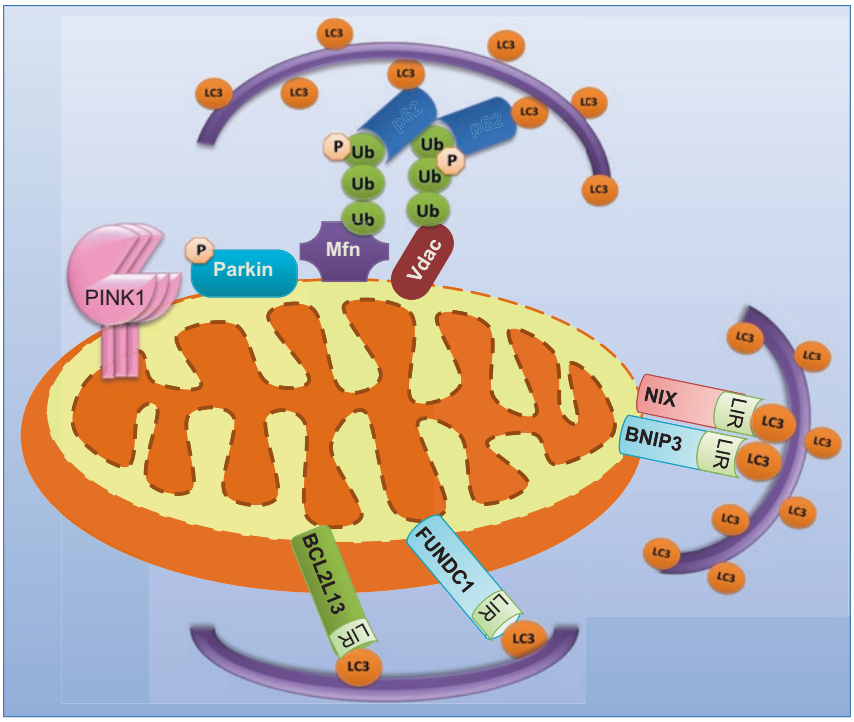

Fig. 9.2 Mechanism of mitophagy. Upon membrane depolarization, PINK1 is stabilized and accumulates at the outer mitochondrial membrane, where it phosphorylates the substrates like MFN2, VDAC1, and ubiquitin, which promotes interaction with p62 that in turn facilitates interaction with LC3 to induce mitophagy [29]. The other mitochondrial proteins like NIX, BNIP3, BCL2L13, and FUNDC1 directly interact with LC3 via LIR motif at nascent phagophore to target damaged mitochondria for degradation by autophagy [37, 38]

with the kinase domain facing toward cytosol. The accumulated PINK1 phosphorylates ubiquitin on serine 65, which is highly essential for Parkin recruitment to mitochondria [31, 32]. PINK1 then phosphorylates Parkin on serine 65 in its ubiquitin-like domain and, together with ubiquitin-S65 binding, fully activates Parkin's E3 ubiquitin ligase activity [33]. There are several mitochondrial proteins such as VDAC1, MIRO1, and MFN-2 which are identified as substrates for Parkin at the outer mitochondrial membrane to commit mitochondria for autophagic degradation [34]. Most of the Parkin substrates specify that the mitochondrial proteins are remarkably altered by Parkin activity. Phosphorylation of MFN2 by PINK1 is required for selective recruitment Parkin to depolarize mitochondria. When the substrates such as VDAC1 and MFN2 are ubiquitinated by Parkin, they create a docking site for the LC3-interacting proteins p62/SQSTM1 and NBR-1 [29], allowing selective Parkin-dependent degradation of mitochondria within autophagolysosome. The Bcl-2 family anti-apoptotic proteins such as Bcl-xL and Mcl-1 directly interact with Parkin and impede the interaction of PINK1 with Parkin to prevent the Parkin-dependent

mitophagy. On the contrary, the pro-apoptotic BH3 proteins PUMA, NOXA, Bim, and Bad promote the recruitment of Parkin to damaged mitochondria by interfering the interaction of anti-apoptotic protein with Parkin to initiate mitophagy [35].

A number of mitochondrial membrane proteins such as BNIP3, NIX, VDAC1, FUNDC1, BCL2L13, MUL1, and prohibitin 2 can interact with the autophagy marker protein LC3 through their LC3-interacting region (LIR) to induce mitophagy. It was reported that BNIP3 and Nix are the key molecules for the mitophagy in response to the hypoxia. NIX primarily regulates basal level of mitophagy in physiological conditions by directly interacting with LC3 or LC3 homologue GABARAP, which is essential for reticulocyte maturation. Defective NIX causes defect in erythroid development, but the mechanism is not clearly defined [36]. Likely, BNIP3, a homologue of NIX, activates excessive mitophagy via its LIR motif to modulate mitochondrial integrity in the skeletal muscle and liver [37]. Another mitophagy receptor FUNDC1 is located at the outer mitochondrial membrane and controlled by the phosphorylation of ULK1 at serine 17 position. FUNDC1 can directly interact with LC3 at the isolation membrane through the conserved LIR motif. Likely, BCL2L13, a member of Bcl-2 pro-apoptotic protein, regulates mitophagy through phosphorylation at Ser 272 residue which is highly essential for the receptor activity of BCL2L13 [38]. Under stress and serum starvation, the transcription factor FoxO1 and FoxO3 induce another novel mitophagy receptor MUL1 which promote mitophagy in skeletal muscles by ubiquitination of MFN2 [39]. Recently, it has been observed that inner mitochondrial membrane protein, prohibitin 2, is associated in targeting mitochondria for autophagic degradation. The LIR domain of prohibitin 2 binds to the autophagosomal membrane-associated protein LC3 which led to Parkin-dependent mitophagy in response to mitochondrial depolarization and proteasome-dependent outer membrane degradation in mammalian cells [40].

9.4 Mitophagy and Aging

One of the principal characteristics of aging includes excessive dysfunctional mitochondrial content and impaired mitochondrial function, highlighting the need of mitochondrial elimination through autophagy [2, 29]. The growing population of cells with dysfunctional mitochondria causes significant harm by exporting reactive molecules that damage other cellular contents. There occurs an age-dependent impairment of mitophagy which not only inhibits both removal of superfluous or dysfunctional mitochondria but also weakens the biogenesis of mitochondria resulting in the aggregation of reactive mitochondrial mass and consequently leads to the deterioration of cellular function. Yet, the precise cellular and molecular basis of mitochondrial homeostasis with respect to aging remains elusive. A diverse group of organisms ranging from yeast to mammals show dysfunctional mitochondria accumulation and defective mitophagy during aging. Interestingly, in yeast mtDNA-deficient strains, mitophagy is responsible for extending the replicative life span (RLS), and the mitophagy-associated proteins AUP1 and UTH1 greatly influence aging in yeast [41, 42] . Moreover, fission, fusion, and mitophagy along with

mitochondrial segregation during cell division are also found to decline with increasing age [43]. In a fungal model of aging, *Podospora anserina*, mitochondrial matrix AAA^+ LON protease regulates mitochondrial levels of PINK1, and its deficiency is strongly coupled with impairment of mitochondrial function and aging [44]. It showed the process of spatiotemporal regulation of autophagy in association with age and found that there is indeed an age-dependent decline in autophagy in the intestine, muscle, pharynx, and neurons at different time points during the adult life of *C. elegans* [45]. Moreover, it indicated that with respect to age, the autophagy recycling process becomes incomplete by stopping somewhere after the autophagosomes are formed. Various reports advocate that mitophagy protects organisms from mitochondrial dysfunction during aging and promotes healthy aging. Shaik et al. recently reported that partial suppression of electron transport chain regulatory protein frataxin of mitochondrial membrane extends the life span of *Caenorhabditis elegans* through the activation of a pdr-1-/Parkin-, PINK1-/PINK-, and dct-1-/BNIP3-dependent mitophagy [46]. Moreover, it is reported that mitophagy restricts the process of mitochondrial biogenesis to normalize mitochondrial content and promote healthy longevity [47]. IGF/IGF-1 mutants of long-lived *C. elegans* show increased induction of Parkin-/BNIP3-regulated mitophagy which is responsible for its life span extension and delayed aging. The PINK/Parkin homologues of *D. melanogaster* established a strong link between mitophagy proteins and aging. Interestingly, Parkin expression is shown to reduce misfolded ΔOTC (deletion mutant of ornithine transcarbamylase) accumulation indicating the role of Parkin as downstream of mitochondrial DNA mutation leads to clear proteotoxic stress during aging. The overexpression of Parkin diminishes the ubiquitin/protein aggregate load in *Drosophila* muscle and delayed aging and fostered longevity by mitigating the deleterious consequences of mitochondria DNA mutation and reactive protein aggregates [48]. Moreover, the PINK1 deletion mutant flies have an overall shorter life span with respect to their wild-type controls indicating the significance of mitophagy in regulating mitochondrial quality control and aging [49]. In mammals, for the execution of mitophagy, the PINK1-Parkin axis requires SIRT1, the NAD-dependent deacetylase, a molecule associated with aging. SIRT1 inhibition decreases the PGC-1α activation followed to abrupt PINK1-/Parkin-mediated mitophagy and thereby promotes aging [50]. In mice models, transgenic (Tg) mice with cardiac-specific expression of the HSP27 gene had higher levels of PINK1 and Parkin as compared to old WT hearts suggesting that occurrence of mitophagy alleviated cardiac aging [51]. An increase in mitophagy activity in polymerase G (PolG) mutated mouse is associated with increase in mtDNA damage. Furthermore, a mt-Keima mouse model developed by Sun et al. demonstrated that there occurs a 70% decline in the mitophagy in the dentate gyrus region responsible for memory and learning in the age group between 3- and 21-month-old mice suggesting that mitophagy decreases with age [8]. Further, increased mitochondrial damage and decreased capacity to mitigate the damage via mitophagy contribute to mitochondrial-driven age-related pathologies [14, 52] (Fig. 9.3).

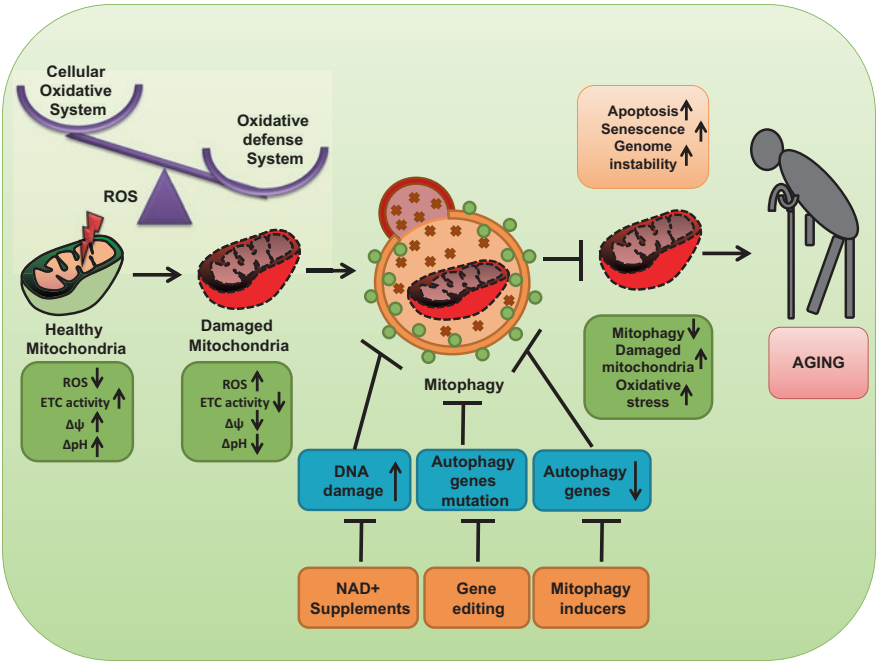

Fig. 9.3 Mitophagic regulation of aging. Inactivation of antioxidant defense system increases the population of dysfunctional mitochondria. This increase in the reactive mitochondria contents urges its elimination through the selective mitochondrial autophagy (mitophagy) [2, 29]. However, there occurs a decline in the efficient mitophagy with increasing age, and the reactive mitochondrial population deteriorates the cellular function leading to the aging-associated disorders [41, 42, 44–50]. In simple words, decline in autophagy with respect to time leads to the senescence [53–60] and aging process [44–50]. However, reversal of mitophagy inhibition by NAD^+ supplements, gene editing, or mitophagy inducers would eliminate the super-reactive mitochondrial content, delay aging, reduce the aging-associated disorder, and enhance the healthy life span of organisms [8, 74–77]

9.5 Mitophagy and Senescence

Cellular senescence may get activated following any alteration in the mitochondrial homeostasis or mitochondrial dynamics [1, 4]. Mitophagic activity was shown to be decreased during senescence [53]. Impairment of autophagy may occur due to the interaction of cytoplasmic p53 with Parkin which inhibits translocation to dysfunctional mitochondria and stimulates senescence [54]. Moreover, the inhibition of mitochondrial fission process by downregulation of fission 1 (FIS1) protein is also shown to promote cellular senescence. FIS1 downregulation leads to the reduced recruitment of DRP1 and restoration of membrane-associated ring finger C3HC4 5 (MARCH5) leading to least DRP1 activity, mitophagy inhibition, and senescence [55]. Cigarette smoke extract (CSE)-induced cellular senescence in human bronchial epithelial cells (HBEC) via mitochondrial fragmentation is through the

mitochondrial ROS production [56]. Moreover, cigarette smoke (CS)-induced impaired mitophagy and perinuclear accumulation of damaged mitochondria are associated with cellular senescence in both human lung fibroblasts and small airway epithelial cells [54]. Interestingly, it is observed that reduced PARK2 expression levels in primary human bronchial epithelial cells (HBEC) impede mitophagy and promote senescence as a part of the pathogenesis of chronic obstructive pulmonary disease (COPD) [57]. Overexpression of FIS1 leads to increased mitochondrial fission and reduces the senescence-associated phenotypic changes suggesting the role of mitophagy in inducing cellular senescence [58]. Moreover, decreased expression of DRP1 and FIS1 is shown to mediate the mitochondrial elongation in senescent cells and augment resistance to oxidative stress through the PINK1 pathway [59]. Furthermore, the inhibition of MARCH5, a mitochondrial E3 ubiquitin ligase, is demonstrated to induce cellular senescence through DRP1 and MFN [60]. Again, the depletion of FIS1 and OPA1 promotes massive mitochondrial fragmentation to reverse the cellular senescence [60]. It can be concluded that sustained mitochondrial elongation and reduced mitochondrial fission lead to reduced mitophagy and the emergence of senescence-associated phenotypic changes (Fig. 9.3).

9.6 Reversal of Aging Through Therapeutic Intervention of Mitophagy

Mitochondrial dysfunction and compromised mitophagy play an important role in aging in different species including human being, and intervention of mitophagy with pharmacological and genetic approaches may have therapeutic benefit for aging [3, 12]. Caloric restriction (CR) has been considered as the first and foremost parameter to prolong life span and delay aging [61]. For example, CR is found to enhance renal mitophagy and diminished the expression of aging markers including p16, senescence-associated galactosidase, and oxidative stress in the kidneys of rat [62]. Moreover, it has been shown that regular exercise improved mitophagy and lysosomal proteolysis to regulate cognitive function in aged hippocampus in rats [63]. Likely, mitophagy plays an important role in restoring preconditioning in aging hearts. For instance, TEMPOL, an intracellular antioxidant, rescued the isoflurane preconditioning through mitophagy induction in the cardiocytes of old rats [64]. Direct activation of mitophagy machinery has also been shown to delay aging. PINK1, Parkin, and sirtuin modulator have shown a viable strategy to activate mitophagy [65–68]. It is reported that sirtuin-activating compounds including resveratrol and metformin delay aging through mitophagy [69, 70]. It showed that metformin suppresses stress-induced senescence, decreases function of mitochondrial complex I [71], prevents mTORC1 signaling by triggering AMPK [72], and inhibits pro-inflammatory NF-κB signaling [73] to enhance life span and health in different organisms including human being. Further, sirtuin activation through NAD+ precursor including NR and NMN supplement stimulates mitophagy to improve both life span and health life in mice and other organisms [74].

Mitophagy induced by bioactive natural compound from antibiotics and plant secondary metabolites has been identified to regulate aging. Actinonin, a natural-occurring antibacterial agent, was shown to activate mitophagy in neural stem cells in mt-Keima mice although detailed mechanism of the role of mitophagy needs to be investigated [8]. A natural polyamine, spermidine, has been documented as a potent inducer of mitophagy and provides protection in several tissues including the heart, brain, and muscle and extends life span in different organisms [75]. Similarly, urolithin A from fruits especially pomegranates was shown to induce mitophagy both in vitro and in vivo. It is found to prevent accumulation of dysfunction in the mitochondria and prolong life span in *C. elegans* and stimulated muscle function in rats [76]. Tomatidine, a metabolite from leaves and stem of tomato, was demonstrated to activate mitophagy and mitochondrial biogenesis through PINK1/DCT-1 pathway which improved the life span and health span in *C. elegans* [77].

9.7 Conclusion and Future Prospective

It has been recently recognized that mitochondrial dysfunction is well accepted as a driving force for tissue and organism aging and age-related diseases. During aging, mitophagy is inhibited by reduction in expression of autophagic proteins, mutation in mitophagic regulators, as well as increase in mitochondrial DNA damage. Moreover, depletion of metabolites including NAD+ is also associated in mitophagy deficiency and promotes aging. Thus attempts to reverse the decline in mitochondria function to improve mitochondrial quality control might be an effective way to control aging. Several recent approaches including pharmacological activation of mitophagy with small molecules, development of methods to restore NAD^+ levels, and modulation of PINK1, Parkin, and USP30 have been undertaken for developing a wide range of mitochondrial therapies for aging. In many studies, the connection between mitophagy and aging was correlative, and detailed causative mechanisms are to be investigated. Further, the current studies often neglect to decipher the existence of the role of mitochondrial quality control in aging in human. Does aging-related mitochondrial dysfunction cause senescence in human? What is the role of mitophagy in cancer patients especially at old age? The roles and molecular mechanisms of mitophagy in maintenance of stem cell pool are to be investigated. Moreover, it will be important to inspect the time and site of action information and pinpoint exactly how autophagy fails to complete its cycle during aging. In summary, future study on mitophagy may expand our understanding on mechanism of aging and other diseases and definitely provide novel therapeutic strategies to delay biological aging.

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Genetics, Ageing and Human Health

10

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Abstract

Ageing is a gradual impairment of physiological processes, leading to compromised cellular functions enhancing susceptibility to death. Ageing also becomes the primary cause of major human pathological disorders, comprising neurodegenerative diseases, cardiovascular disorders, cancer, and many more. In the developed as well as the developing world, ageing represents the biggest cause of illness and mortality. Ageing can be controlled to some extent by targeting genetic corridors and biochemical pathways implicated with human health. The idea of targeting ageing by reversing the pathogenesis of diseases seems to be promising but poses its own challenges. This chapter elucidates the genetic disorders affecting the aged people highlighting the challenges related to their healthcare system.

Keywords

Neurodegenerative · Methylation · Pathological · Genetic disorders

10.1 Introduction

Ageing is an innate biological phenomenon of getting old with the passage of time. This represents one of the greatest challenges to modern society. In *Homo sapiens* ageing highlights the accumulation of changes over a period of time [1] including

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physical, psychological, and social ones. Ageing is the biggest risk factor for some of the most devastating and distressing diseases like neurodegenerative, cardiovascular, inflammatory, and metabolic ones.

At the cell level, ageing causes a drift in DNA methylation. During ageing in various tissues, DNA methylation declines. Culture of mammalian fibroblasts to senescence results in loss of DNA methylation [2]. Due to passive demethylation during ageing, a decrease in global DNA methylation takes place.

Due to gradual depletion in DNMT1 efficacy or inaccurate focusing of enzyme by various cofactors [3]. DNA hypomethylation, unusual hypermethylation of promoter, and unassertive DNMT overexpression are epigenetic alterations that are known in cancer [4]. Therefore the epigenetic alterations throughout ageing are expected to confer tumorigenic transformation. Besides epigenetic alterations, Lopez-Otin et al. [5] describe some hallmarks, which leads to ageing. They identified different cellular and molecular symbols that are generally considered to contribute to ageing and together decide the resultant phenotype (Figs. 10.1 and 10.2). Each cause of ageing shown in Fig. 10.1 meets the three fundamental criteria: (i) its

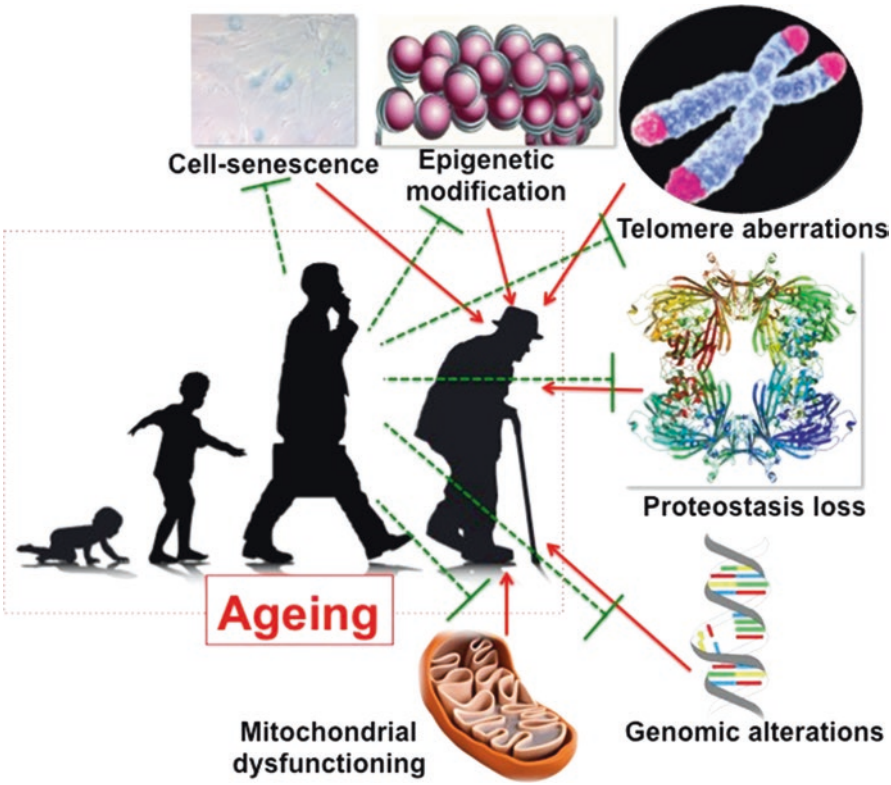

Fig. 10.1 A schematic presentation describes the causes of ageing (red solid arrows) and strategies to combat (green blunt end dotted arrows) ageing. (Part of figure is taken from www.szpin.ca)

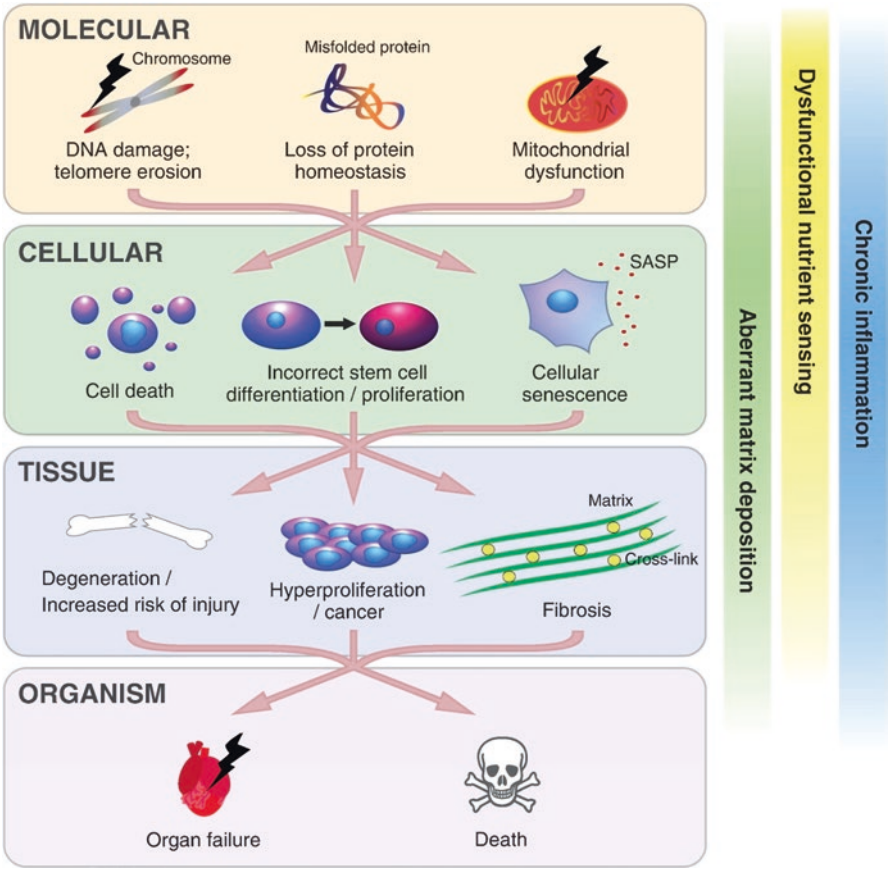

Fig. 10.2 Mechanisms of human ageing. Ageing affects physiological processes taking place at different levels (molecular events such as mitochondrial dysfunction to genomic lesions, cellular measures such as senescence, or over-proliferation). Dysfunctional cells lead to tissue dysfunction, ending in organ failure and finally death. Interactions between each level serve to intensify pathology at all levels. Black lightning symbolizes organ damage or failure; SASP refers to the senescence-associated secretory phenotype. Figure reprinted with permission from Mallikarjun V, Swift J (2016) EBioMedicine, 14, 24–31 [50]

manifestation should occur during natural ageing, (ii) its experimental augmentation should hasten ageing, and (iii) its improvement should delay the normal ageing progression and hence increase healthful lifespan.

In the developed world, ageing represents the biggest causes of illness and mortality [6]. The study of ageing has resulted in the treatment of a number of diseases and has yielded an important application of knowledge and a significant improvement in patient quality of life. An aged individual may bear any number of comorbidities, which can complicate required therapeutic interventions exponentially. The idea of targeting ageing itself by reversing the pathogenesis of several diseases is an appealing task. As per the investigation through data mining, genes are regulated

differentially at senescence and are conferred with atherosclerosis development and vascular calcification. Mainly the genes IL8, IL1β, ICAM1, ESM1, TNFAP3, and CCL2 having the roles in inflammation; ADM, VEGFβ, VEGF, and MMP14 in tissue remodeling; and BMP2, MGP, DCN, OPG, and SPP1 in vascular calcification have been examined [7].

10.2 Ageing as a Clinical Indication

D.E.B Powell proposed that the apparently independent disease of ageing is considered separately in nature for possible treatments [8]. Gerontology reveals that many age-related diseases share common causes and may be reversed or prevented through common cures. Ageing occurs by the developmental processes for successful reproductive maturation. It occurs even after reproductive maturity is reached [9]. The molecular and physiological defects can be distinguished in many forms at molecular, cellular, and tissue levels in biology. Old senescent cells possess dysfunctional mitochondria, aggregated proteins, epigenetic lesions, and eroded telomeres.

This can lead to senescence and subsequent depletion of active stem cell populations, impairing regenerative capacity and further promoting tissue ageing. "Aged" extracellular environment can be characterized by age-related immuno-degeneration, disrupted circadian rhythms, impaired nutrient sensing, and improper autocrine and paracrine signaling processes. These processes combine to promote organ failure leading to an increase in morbidity at an exponential rate and finally mortality [10]. Thus ageing comprises the highest risk factors for a number of human diseases that include neurodegeneration, cancer, diabetes, and metabolic syndrome. Besides these, other diseases are also known (mentioned in Table 10.1).

10.3 Alzheimer's Disease

Alzheimer's disease is the most prevalent dementia amidst aged people. Dementia can be described as a disorder of the brain that seriously affects a person's capability for doing daily activities. It causes brain damage by the death of brain cells affecting brain function and intellectual ability. Alzheimer's starts silently; initially it involves those brain parts which control memory, language, and thought. People suffering from Alzheimer's face trouble in reminding the incidents that occur recently and the identity of known people. Mild cognitive impairment results in memory problems in affected individuals. The chances of having Alzheimer's are more in patients suffering from mild cognitive impairment [11]. Alzheimer's begins mostly after the age of 60, and the risk increases with age. The risk is also higher if the parent or grandparent had the disease in family history.

In case of Alzheimer's disease, there is β-amyloid deposition in brain parenchyma in the form of congophilic plaques. β-amyloid is known to be a 39–42 amino acid peptide derived from several transcripts of β-amyloid precursor protein encoded

Table 10.1 Some other genetic diseases associated with ageing

S. no.	Genetic disorder	Gene involved and location	Protein	Inheritance	Symptoms	Reference	OMIM
1.	Premature ageing syndrome, Okamoto type	SGCG gene	35kD dystrophin-associated glycoprotein	Autosomal recessive	Osteosarcoma, cataracts, diabetes mellitus, osteoporosis, and erythroid macrocytosis	Okamoto et al. (1997), PubMed: 9056555	601,811
2.	Premature ageing syndrome, penttinen type (PENTT)	Heterozygous mutation in the PDGFRB gene, gene location: 5q32	Platelet-derived growth factor receptor beta	Autosomal dominant	Underdeveloped cheekbones, lipoatrophy, dermal and epidermal atrophy, hypertrophic lesions that resemble scars, thin hair, and marked acroosteolysis	Johnston et al. (2015), PubMed: 26279204	601,812
3.	Branchiooculofacial syndrome (BOFS)	Heterozygous deletion/insertion mutation in TFAP2A gene, gene location: 6p24.3	Transcription factor AP-2-alpha	Autosomal dominant	Imperforate nasolacrimal duct, branchial clefts with characteristic facies, premature ageing, growth retardation, lip pseudocleft-hemangiomatous branchial cyst syndrome, and hemangiomatous branchial clefts-lip pseudocleft syndrome	Milunsky et al. (2008), PubMed: 18423521	113,620
4.	Mandibuloacral dysplasia	LMNA gene, gene location: 1q22	Lamin A/C	Autosomal recessive	Skeletal abnormalities with progressive osteolysis of the distal phalanges and clavicles, pigmentary skin changes, growth retardation, and craniofacial anomalies with mandibular hypoplasia	Simha and Garg, 2002, PubMed: 11836320,Garavelli et al., 2009, PubMed: 19764019	248,370
5.	Platelet-derived growth factor receptor beta (PDGFRB)	PDGFRB gene, gene location: 5q32	Platelet-derived growth factor receptor beta	Autosomal dominant	Brain calcification, scleroderma, myofibromatosis, and Primary familial Myeloproliferative disorder with eosinophilia	Nicolas et al. (2013), PubMed: 23255827	173,410

(continued)

Table 10.1 (continued)

S. no.	Genetic disorder	Gene involved and location	Protein	Inheritance	Symptoms	Reference	OMIM
6.	Microphthalmia/ microcephaly/ ectrodactyly of lower limbs	SNX3, gene location: 6q21	Sorting nexin-3	…..	Palate, ectrodactyly of the feet, mental retardation, prognathism, borderline microcephaly, microphthalmia, cleft lip, and premature ageing of the skin	Viljoen and Smart (1993), PubMed: 8287191, Suthers and Morris (1996), PubMed: 8867664	601,349
7.	Ataxia-telangiectasia-like disorder 2 (ATLD2)	PCNA, gene location: 20p12.3	Proliferating cell nuclear antigen	Autosomal recessive	Neurodegenerative phenotype characterized by sensorineural hearing loss, developmental delay, short stature, ataxia, photosensitivity, cutaneous, and ocular telangiectasia	Baple et al. (2014), PubMed: 24911150	615,919
8.	Williams-Beuren syndrome (WBS)	WBSCR22, gene location: 7q11.23	Probable 18S rRNA (guanine-N(7))-methyltransferase	Autosomal dominant	Supra valvular aortic stenosis (SVAS), dental anomalies, mental retardation, peripheral pulmonary artery stenosis, and distinctive facial features	Jones (1990), PubMed: 2118785, Osborne et al. (2001), PubMed: 11685205	194,050
9.	Werner syndrome	RECQL2, gene location: 8p12	WRN/DNA helicase	Autosomal recessive	Scleroderma-like skin changes, specifically in the extremities, subcutaneous calcification, premature arteriosclerosis, cataract, diabetes mellitus, and prematurely aged facies	McKusick et al. (1963), PubMed:14042963	277,700
10.	Bloom syndrome	RECQL3, gene location: 15q26	DNA helicase RecQ protein-like 3	Autosomal recessive	Sun sensitivity, proportionate pre- and postnatal growth deficiency, hyper- and hypopigmented skin, and chromosomal instability	Vijayalaxmi et al. (1983), PubMed: 6879180, Seal et al. (1991), PubMed: 1742335	210,900

by exon 18 on chromosome 21 [12]. Alzheimer's disease is characterized by the abrasion of tissues and various inflammatory proteins. This observation has led to hypothesize that the inflammation of brain results in neuronal injury in Alzheimer's. As per various epidemiological studies in nine different countries, it has been suggested that the anti-inflammatory drugs used to treat arthritis reduce the occurrence of Alzheimer's disease as well [13].

10.3.1 Genetic Basis of Alzheimer's Disease

Induction of the repressor element 1-silencing transcription factor (REST) or neuron-restrictive silencer factor (NRSF) is a ubiquitous characteristic feature in normal ageing in hippocampal and human cortical neurons. REST declines in Alzheimer's disease and mild cognitive impairment. With ageing, REST is induced by cell nonautonomous Wnt signaling as normal phenomenon. In Alzheimer's disease, dementia with Lewy bodies and frontotemporal dementia strike the onset of loss of REST from nucleus and are visible in pathological misfolded proteins and autophagosomes [14].

10.3.2 Early-Onset Alzheimer's Disease

Early-onset Alzheimer's disease occurs in people of 30–60 years of age and accounts for $\leq$5% of the individuals suffering with this disease. It can be due to inherited changes in one among the three genes. It results in the early-onset familial Alzheimer's disease (FAD) [15].

A child develops 50% probability of mutation for early onset of FAD if his biological mother or father carries a genetic mutation. Upon the inheritance of mutation, a child cultivates very strong possibility of having an early onset of FAD. Early-onset FAD occurs due to the single-gene mutations on chromosomes 1, 14, and 21. These mutations results in faulty protein formation. Mutation on chromosome 21 leads to abnormal amyloid precursor protein (APP) formation. Chromosome 14 mutation result in malfunction of presenilin 1, and chromosome 1 mutation leads to abnormal presenilin 2. These mutations cause the breakdown of APP leading to the generation of harmful forms of amyloid plaques.

10.3.3 Late-Onset Alzheimer's Disease

A number of individuals with Alzheimer's encounter late onset of the disease. Here the signs occur in the middle of 60 years of age and later. The scientific reasons of late-onset Alzheimer's are not known, but they comprise genetic aspects along with the environmental factors and lifestyle that cause risk for the onset of disease.

The role of a single gene involved in the late-onset form of Alzheimer's is still a mystery. But apolipoprotein E (APOE), a gene genetic risk factor on chromosome

19, might enhance the chance of late onset of the disease. APOE arrives in various alleles or forms. To treat the prevailing disorder, various actions have been employed. The first one is the partial inhibition of either of the two proteases, γ- and β-secretase, partially generated through amyloid precursor protein. For β-secretase, the effective small-molecule inhibitors are screened, and their medicinal chemistry is studied in order to find the large active site for the fitting of these molecules. For γ-secretase, potent membrane-permeable inhibitors have been synthesized and tested in humans. But the literature suggests that a number of these compounds influence the signaling by Notch proteins and other receptors on cell surface [16].

Another strategy is to enhance the clearance of Aβ from the cerebral cortex or to avert the oligomerization of Aβ. This can be pictured by using the passive or active Aβ immunization, where antibodies to Aβ cease the peptide cerebral levels by propagating clearance of microglial cells [17, 18]. It can also be achieved by replacing the peptide to the systemic circulation from the brain [19].

Thirdly, an anti-inflammatory phenomenon lies on the fact that inflammatory response at cellular level in the cerebral cortex is evoked by the successive Aβ accumulation [20]. Based on these findings, clinical trials of such compounds are to be planned.

The fourth approach comprises the cholesterol homeostasis modulation. Long-term use of cholesterol-decreasing drugs like statins – HMG-CoA reductase inhibitors – lowers incidence of Alzheimer's disease [21, 22].

The fifth strategy includes the observation that Aβ aggregation depends on Zn^{+2} and Cu^{+2} [23]. It suggests that chelation of these bivalent ions in vivo may prevent Aβ deposition [24].

The sixth strategy is based on the prevention of the neurodegenerative and synaptotoxic effects that are assumed to be triggered by Aβ accumulation. It includes some antioxidant and neuroprotective compounds. But success of this approach is still far to reach to humans.

Thus, the correct treatment of Alzheimer's disease has not yet been discovered, and a lot of research is being carried out to find cure for this devastating disorder of intellect.

10.4 Parkinson Disease (PD)

Parkinson disease is the second highest occurring neurodegenerative and ageing-related disorder, approximately affecting about 1% of the population of ≥50 years of age [25]. James Parkinson (in 1817) gave the first description of PD. The main clinical manifestations of PD comprise muscular rigidity, postural instability, resting tremor, dementia, postural abnormalities, dystonic cramps, and bradykinesia.

Pathologically PD is represented by degeneration of selective neurons of *substantia nigra*, locus ceruleus, and peripheral autonomous nervous system, cerebral cortex, and hypothalamus. Specific types of eosinophilic inclusion bodies named Lewy bodies are present in the affected area of the brain. The exact mechanism of PD is not known, but presently different types of genetic factors have been

investigated in the etiology of this disease. Generally, inheritors of PD are considered on the comparative and complex interactions of genetic and environmental factors. In 1880 Gowers found that about 15% of the PD sufferers are having affected relatives. In multicase families, present familial studies highlight the observation of an autosomal dominant pattern of inheritance in PD patients and have suggested that genetic elements play a significant etiologic role. In some cases PD has also been diagnosed in people who are having no family history; this condition is sporadic, and causes remain unclear.

10.4.1 Genetic Heterogeneity of PD.

About 15% of Parkinson patients bear a family history of the disease concerned. Familial incidents of autosomal dominant PD can be initiated by genetic alterations in different gene loci including PARK1 and PARK4 of *SNCA* (OMIM: 163890) present on chromosome 4q22, PARK5 (191342), due to mutation in the *UCHL1* gene present on chromosome 4p13; PARK8 (607060), mutation in the *LRRK2* gene (609007) on chromosome 12q12; PARK11 (607688), mutation in the *GIGYF2* gene (612003) on chromosome 2q37; PARK13 (610297), caused by mutation in the *HTRA2* gene (606441) on chromosome 2p13; PARK17 (614203), mutation in the *VPS35* gene (601501) on chromosome 16q11; and PARK18 (614251), which happens due to mutation in the *EIF4G1* gene (600495) present on chromosome 3q27.

Similarly, a number of loci have been identified that are responsible for early onset of autosomal recessive PD: PARK2 (PARK2; 602,544, 600,116), caused by mutation in parkin-encoding gene present on chromosome 6q26; PARK6 (605909), due to mutation in the *PINK1* gene (608309) on chromosome 1p36; PARK7 (606324), caused by mutation in the *DJ1* gene (PARK7; 602,533) on chromosome 1p36; PARK14 (612953), caused by mutation in the *PLA2G6* gene (603604) on chromosome 22q13; PARK15 (260300), caused by mutation in the *FBXO7* gene (605648) on chromosome 22q12–q13; PARK19A (615528) and PARK19B (see 615,528), caused by mutation in the *DNAJC6* gene (608375) on chromosome 1p32; and PARK20 (615530), caused by mutation in the *SYNJ1* gene (604297) on chromosome 21q22.

Mutations in one or all of the above genes appear to be sporadic and are not inherited. Similarly variations in some genes, like GBA and UCHL1, do not result in PD but increases the risk of this condition in some families. Alterations in some other unidentified genes also contribute to the risk of developing PD.

In some cases the development of late-onset type of PD has been linked with mutations or polymorphisms in certain genes, including *GBA* (606463), *MC1R* (155555), *ADH1C* (103730), *MAPT* (157140), and genes at the HLA locus (142860). Individually, each of the concern risk factors faces low penetrance on the development of disease but shows significant cumulative effect when present together (Hamza et al., 2010). Receptiveness to PD is governed by expanded trinucleotide repeats in various genes that cause other neurologic disorders marked by

spinocerebellar ataxia including the *TBP* (600075), *ATXN2* (601517), *ATXN3* (607047), and *ATXN8OS* (603680) genes.

The exact genetic cause of PD is still not clear. A number of PD symptoms appear when neurons in the substantia nigra expire/are impaired. Neurons produce dopamines that transmit signals in the brain for smooth physical movements. Upon the damage of dopamine-producing neurons, the ability of the brain to communicate with muscles weakens. As a result the brain is unable to control the movement of muscle.

The gene mutations also degrade some nonfunctional proteins in dopamine-producing nerve cells. When the undegraded proteins gather in neurons, it leads to the death of these cells. Mutations also affect the mitochondrial function. As a result the faulty mitochondria give rise to free radicals that damage nerve cells. Free radicals may gather and cause impairment of dopamine-producing neurons.

Another significant feature of PD is the appearance of Lewy bodies in dead or dying dopamine-producing neurons. Whether Lewy bodies are involved in killing neurons or not is still a mystery.

10.5 Osteoarthritis

The frequency of osteoarthritis intensifies with age. It is reported that 30–50% of adults aged ≥65 years faces this condition [26, 27]. Osteoarthritis is an age-related degeneration of the surface of articular cartilage, i.e., the soft white tissue covering the bones. The occurrence of osteophytes in radiographic analysis of multiple joints like the spine, hands, knees, and hips discloses osteoarthritis within a single joint in about 80 percent of older individuals [28]. Recent research has shown an age-related decrease in the levels of the high-mobility group box protein 2 (HMGB2) that is observed in the superficial zone of the cartilage which may lead to an increase in the death of chondrocyte. This may be due to the fact that in human there is a loss of HMGB2 expression and murine cartilage with age, leading to the most severe onset of osteoarthritis [29].

A great change in the anabolic and catabolic activity is seen in osteoarthritis. Cell senescence due to ageing in the cartilage results in the decline in chondrocytes ability to respond to growth factors. This changes the balance of catabolic and anabolic activity as seen in osteoarthritis. There is substantial decline in the chondrocyte response to IGF-I which is the key matrix stimulating growth factor in the cartilage with ageing [30, 31]. IGF-I is an important autocrine survival factor in the cartilage [32]. The expression and amount of another factor OP-1 which exists in the cartilage decrease upon ageing [33] which leads to osteoarthritis.

10.5.1 Genetic Basis

It is observed that various types of osteoarthritis depend upon genetic component to a large extent. The genetic basis of osteoarthritis can be due to the alterations in

multiple genes and do not proceed as per Mendelian inheritance pattern. Some reports of unrelated families showed co-inheritance with generalized osteoarthritis with specific alleles of the gene for type II procollagen (COL2A1) on chromosome 12 [34]. Upon familial disease survey, it was found that out of seven families, two bear mutation, both having evidence of associated chondrodysplasia [35]. Linkage between COL2A1 and the occurrence of osteoarthritis has been observed in several families [36]. The early onset of calcium pyrophosphate deposition disease and severe degenerative osteoarthritis is reported to possess a genetic linkage between the disease in some individuals and chromosome 8q that suggests a defective gene located at this position may be responsible for the same [37].

Rheumatoid arthritis is an autoimmune disease of adults. With advancing age, the immune system undergoes a number of changes. Among them is the age-dependent decrease in the functioning of the thymus gland. T-cell homeostasis gets increased in patients suffering with RA upon ageing. The expansion of naive and memory T cells is less varied, possibly due to thymic insufficiency, and it is influenced upon autoreactive cells. Pre-senescent T cells are resistant to apoptosis and expand to large clonal populations. These cells are in regulatory control of nonconventional co-stimulatory molecules, display potent effector functions, and appear to be critical in the synovial and extra-articular appearance of RA [38].

10.6 Huntington Disease

Huntington disease is a neurodegenerative disorder that progresses with age. It is autosomal dominant having a unique phenotype characterized by dystonia; chorea; incoordination; cognitive decline, i.e., loss of thinking ability; uncontrolled movements; and difficulties in behavior. There is a significant loss of neural cells and atrophy of caudate and putamen.

Adult onset is the most occurrent form of Huntington disorder. It is usually seen upon the onset of 30–40 years of age. Some early signs and symptoms include trouble in learning new information, irritability, small involuntary movements, depression, poor coordination, and difficulty in decision making. A number of individuals suffering from Huntington disease develop chorea that involves involuntary jerking movements. These movements are more pronounced on progresses of disease. Affected individuals face trouble in walking, speaking, and swallowing. People suffering with this disorder also experience changes in personality and a significant decline in thinking and reasoning abilities. After the signs and symptoms begin, the affected patients live about 15–20 years in the adult-onset form of Huntington disease.

The average age for the onset for HD is 35–44 years [39]. Diseased individuals usually face neurologic manifestations (≈ two thirds), and some face psychiatric changes. Early diagnostic stage includes difficulty in mental planning, slight alterations in movement of the eye, minor involuntary movements, coordination, and often a depressed or irritable mood. Affected individuals can do their ordinary activities and are able to continue work [39].

About 25% of individuals affected with HD faces delayed onset, nearly after age of 50 or 70 years. These individuals encounter chorea, gait or walking disturbances, and dysphagia. The next stage is characterized by chorea, voluntary activity becomes increasingly difficult, and also dysarthria worsens. Further stage of HD comprises severe motor disability and the complete dependency of individual, mute, and incontinent. After onset of HD, the median survival time of an individual is 15–18 years, and so the age at death lies between 54 and 55 years [40].

The genetic cause of HD is basically HTT gene mutation. This HTT gene gives command for huntingtin protein. Though the function of huntingtin protein is not known, it seems to play a significant role in neurons. DNA segment CAG, i.e., a trinucleotide, gets repeated in HTT mutation. This segment comprises a series of three DNA building blocks, namely, adenine, cytosine, and guanine, that gets repeated a number of times in a row about 10–35 times within the gene. People having 36–39 CAG repeats may or may not develop the symptoms of HD, while people with ≥40 repeats are prone to this disorder.

Also as the size of the CAG segment increases, it results in the formation of huntingtin protein abnormally. The normal function is disrupted when the elongated protein is transferred to smaller and toxic fragments resulting in the binding and accumulation of toxic fragments in neurons. The ceasing of neuronal functioning and neuronal death in some areas of the brain highlights the symptoms of HD.

Huntington disease is an autosomal dominant inherited disease. One copy of the altered gene is sufficient to cause the disorder, in each cell. Progeny inherits the mutated gene from the affected parent. It is exceptional that an individual with HD bears normal parents because generally the affected individuals have diseased or carrier parents.

10.7 Cardiovascular Disease

Ageing represents declining of cardioprotective systems and increasing disease processes that develops the chances of development of heart failure to a great extent. Out of 50% of all cardiac arrest diagnoses, 90% of cardiac failure deaths occur in people over the age of 70. Ageing on its own does not lead to cardiac failure; instead it lowers the ability to bear the disease. In most of the developed countries, maximum population is older, and so ageing is a risk factor for all cardiovascular diseases [41]. Heart failure is one of the most prevalent cardiovascular disorders of ageing.

In spite of major advancement in curing heart failure over the last 30 years, the prognosis is still not advanced, with an average survival rate in older adults [42]. The heart undergoes a number of functional changes with age, and its ability to respond to workload decreases. Its reserve capacity also ceases upon ageing. Maximal heart rate, end-systolic volume, end-diastolic volume, prolonged systolic contraction, contractility, prolonged diastolic relaxation, sympathetic signaling, etc. are influenced upon ageing. Some of the cardiac disorders that occur with ageing

are myocardial infarction, myocardial ischemia, and idiopathic ventricular fibrillation. Idiopathic ventricular fibrillation is one of the deadly cardiovascular diseases.

10.7.1 Genetic Basis of Idiopathic Ventricular Fibrillation (IVF)

The most significant reason of cardiac death is ventricular fibrillation. Peripheral pulses and blood pressure are lost when there is no cardiac output, and the patient becomes unconscious. Arrhythmia causes death if not immediately controlled. Only 3% of IVF patients survive out-of-cardiac arrest [43]. Malfunction of ion channels causes IVF due to mutations in SCN5A gene that lie in the cardiac sodium channel. A splice donor mutation, missense mutation, and a frameshift mutation in the coding region of SCN5A have been identified in IVF patient history. Missense mutation in sodium channels results in its inactivation more rapidly than normal, and frameshift mutation makes the sodium channels nonfunctional. The results show that the risk of developing IVF is due to the mutations in cardiac ion channel genes [44].

10.8 Adult Polyglucosan Body Disease

Adult polyglucosan body (APBD) neuropathy is a progressive disorder that slowly affects the central and peripheral nervous systems. After the age of 40, patients suffer with a varied combination of pyramidal tetraparesis, cognitive impairment, neurogenic bladder, and peripheral neuropathy. Also cerebellar dysfunction and extrapyramidal signs are seen in the affected individuals. The pathology of this disorder is aggregation of oval, intracellular polyglucosan bodies in the nervous system, which lies in neuronal and astrocytic processes [45].

10.8.1 Clinical Feature

The clinical manifestations include marked sensory loss in the legs, gradual upper and lower motor neuron deficits, and neurogenic bladder in 50% dementia. Corpora amylacea or Lafora bodies which are restricted to neurons' processes and astrocytes are abundantly discovered during autopsy of such individuals. Biopsy of some patients shows large bodies within axons of sural nerves. Polyglucosan bodies also occur in type IV glycogenosis along with Lafora disease, in the "normal" course of ageing [46].

10.8.2 Molecular Genetics

A study in seven Jewish patients identified homozygosity with APBD for a missense mutation in the glycogen-branching enzyme (GBE) gene [45]. Related family members having heterozygous condition for mutation face an incomplete

biochemical defect. It leads mutant allele with simple autosomal recessive transmission. This mutation was seen in compound heterozygous state in a 20-year-old person having normal function of the liver. It is thus a nonprogressive form of type IV GSD. It is marked that APBD is a variant of GSD type IV.

In a non-Ashkenazi patient with APBD, mutation in GBE1 gene for compound heterozygosity had been identified with GSD type IV [47]. The patient faces disturbance in walking, urinary urge indulgence, and hearing loss. Biopsy of sural nerve suggested the presence of leukocyte and polyglucosan bodies, and GBE activity was 20% normal. Each of the 50% of clinically unaffected daughters of the diseased mother acts as carrier of one of the mutations and exhibits intermediate levels of GBE activity, i.e., 80% of normal. These findings lead to the conclusions that APBD and GSD IV are allelic disorders.

In Ashkenazi Jewish descent, 46 APBD patients were examined having mutations in the GBE1 gene [48]. In 22 patients, Y329S was known to be most prevalent mutation in the homozygous state and in the heterozygous state in 13 patients. Three related people were homozygous for E242Q mutation. Akman et al. [49] demonstrated that three individuals were compound heterozygous for Y329S and L224P substitution. Ashkenazi patients having APBD were compound heterozygotes for two mutations, viz., c.986A > C(p.Y329S) and IVS15 + 5289_5297delGTGTGGTGGinsTGTTTTTTACATGACAGGT, both being founder mutations. Not a single patient was homozygous for this new mutation. Sixteen heterozygous patients were found to be carriers of a complex deep intronic deletion/insertion mutation in intron 15 (607839.0020) that resulted in a truncated protein. It was observed that intronic mutation was missing during the whole genomic sequencing.

Thus APBD is caused by mutations in *GBE1* gene. The *GBE1* gene plays an important role for making the glycogen-branching enzyme. Glycogen-branching enzyme takes part in the formation of glycogen responsible for stored energy in the body. *GBE1* gene mutations cause deficiency of glycogen-branching enzyme, resulting in abnormal glycogen molecule production. The abnormal glycogen molecules are known as polyglucosan bodies. They accumulate within cells and cause damage. Nerve cells are susceptible to the aggregation of polyglucosan bodies in people with APBD, leading to impaired neuronal function.

In some cases mutation in the *GBE1* gene do not result in a shortage of glycogen-branching enzyme in APBD individuals. The cause of disease in such individuals is unclear. APBD is an autosomal recessive pattern that is inheritable. Here both copies of the gene in each cell are having mutations. An individual's parent having an autosomal recessive condition carry one copy of the mutated gene but do not exhibit any signs and symptoms of this condition.

In conclusion, genetic discrepancies have been shown to play an important role in the progression of human ageing and lifespan. Different studies carried out on human populations show that particular genetic variations in humans are linked to different human diseases. Here, we have described diseases associated with human ageing (Table 10.1). Identification of genetic variations related to ageing will uncover more candidate genes and underlying pathways. This may offer a chance to detect premature ageing and disabilities involved. Ageing should not be viewed as

disease instead a way of ending genetic programming for sustenance and survival. There is a strong genetic component to diseases common during old age. However, the environment and lifestyle make a major difference. Thus, many age-related diseases may be circumvented if a well-disciplined life is lived and extreme behavior is avoided.

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Genetic Syndromes and Aging

11

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Abstract

Involvement of a genetic component in the phenomenon of aging has been evidenced through tinkering with the gene(s) or genomic elements and manipulating life span of experimental organisms, which understandably is further influenced by a multitude of other nongenetic factors. Genomic studies related to aging till date on experimental model organisms have suggested the involvement of several pathways and candidates in the process. Studies in different syndromes or diseases associated with premature aging, involving either single or a set of genes, have added to the notion that life span has a genetic component and certain genetic diseases enhance the process of aging. Understanding the mechanism of these genetic components and the pathways suggests that the intervention at such levels would check the process of premature aging and enhance healthy life span.

Keywords

Aging syndromes · Hutchinson-Gilford syndrome · Cockayne syndrome · Down syndrome · Werner syndrome · Ataxia telangiectasia · Bloom syndrome · Fanconi anemia · Xeroderma pigmentosum · Rothmund-Thomson syndrome

11.1 Background

Human aging is a complex and inevitable process we go through at roughly similar rates, suggesting that aging is a prudently time-controlled developmental program of interaction between an individual's genetic makeup and their environment. The

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apparent symptoms of aging invariably are characterized by the development of wrinkles, graying and loss of hair, osteoarthritis, osteoporosis, progressive loss of fertility, loss of muscle mass and mobility, decreased cognitive ability, hearing loss, and succumbing to age-related metabolic diseases, such as cancer, diabetes, hypertension, atherosclerosis, and several neurological disorders [1, 2]. The key molecular hallmarks of aging phenotype include telomere attrition, genomic instability, loss of proteostasis, epigenetic alterations, mitochondrial dysfunction, deregulated nutrient sensing, stem cell exhaustion, cellular senescence, and altered intercellular communication [1]. Model organisms, such as nematodes (*Caenorhabditis elegans*), yeast (*Saccharomyces cerevisiae*), fruit flies (*Drosophila melanogaster*), and mice (*Mus musculus*), mimicking in vitro genetic studies, have helped identify many aging-related genes and conserved pathways in evolution. These are factors like sirtuins; specific mitochondrial genes, *p53*, *FOXO* genes, and microRNAs; and cellular pathways, TOR, JNK signaling, insulin, and IGF-1 signaling [3–5]. Increase in the life span by several folds has been reported by the genetic manipulation of laboratory model organisms for some of these, such as insulin growth factor, AMPK signaling, and the presence of longer telomeres [1, 6–8]. Added to this, most of the environmental effects on an individual's germline cells, resulting in the epigenetic changes during a lifetime, which is erased and re-established in each generation through a developmental reprogramming, contribute to the inheritable process of aging [9]. Compared to the model organisms, humans, however, have significant differences. Besides the human-specific aging characteristics, differences in inflammatory responses, and the involvement of more number of genes than in lower organisms [10, 11], the dissimilarity emerges in the longer life span when compared to the model laboratory organisms. Further, the genes and their mutant forms implicated in model organisms at times do not show their involvement in aging in humans. For example, mutations in homologs of the nematode insulin-signaling pathway known to increase the life span in nematodes have been identified in obese and diabetic humans [12]. Similarly, one should take care of other allelic variations present in the genome when interpreting the outcome of genetic manipulation and treatments in inbred mice. For example, C57BL/6J, most commonly used inbred strain of mice, carries a deletion in nicotinamide nucleotide transhydrogenase (*Nnt*) gene which is necessary for glucose tolerance, suggestive of using C57BL/6J with caution for aging studies given the close association of aging and metabolism [13–16]. Nevertheless, longevity induced by genetic manipulations on lower organisms has provided evidence in support for the concept that genes regulate life span and orchestrate cellular and metabolic pathways to mediate this phenotype. Mutations and polymorphisms of various nutrient sensors and their downstream effectors have been associated with the longevity in humans as well as model organisms [1, 6, 17, 18].

One of the approaches adopted to study the role of the genetic component in aging and its heritability in humans has been to compare identical with fraternal twins. Interestingly, the heterozygotic and homozygotic twins in various populations have reported heritability of longer life spans by >20% with more or less additive effect when compared to the life span of parents [19, 20] (Table 11.1). Further support to these conclusions has emerged from a study comprising of 20,502 individuals of Scandinavian twins, where if a female lived till 92 years, the probability of a fraternal twin was 1.57 times and that of an identical

Table 11.1 Heritability of aging (adapted and modified from [20]

Sample type	h^2	References
1766 aged subjects and their 7103 offsprings born between 1822 and 1915	10%	[26]
French Canadian isolate; offspring of parents who married between 1820 and 1869	~0	[27]
Genealogies of 6 large New England families born between 1650 and 1874	16–32%	[28]
2872 same-sex twin pairs (Denmark) born between 1870 and 1900	26% men; 23% women	[19]
600 same-sex twin pairs (Denmark) born between 1870 and 1880	33.33%	[29]
31,608 same-sex twin pairs (Denmark, Sweden, Finland)	57% men; 51% women	[30]
10,505 same-sex twin pairs (Sweden) born between 1886 and 1925	10–35%	[31]
1655 individuals of the old order Amish (OOA) population of Lancaster County	25%	[20]
20,502 individual (Danish, Finnish, and Swedish) twins born between 1870 and 1910	Male MZ twins 39%; male DZ twins 21%	[21]

MZ monozygotic, *DZ* dizygotic

twin 2.5 times more to reach that age in comparison to other females. For males, a fraternal twin was 1.76 times and an identical twin 4.83 times likely to reach the age of 92 years [21]. The offspring of the long-lived parents had a low risk for cardiovascular disease, cancer, and diabetes when compared to the offspring of the parents who died young [22], apparently supporting the concept of inheritance of longevity. Added to these traditional approaches, even in the genome era, polymorphisms, such as SNP, rs2149954, located on chromosome 5q33.3 associated with lower blood pressure (12,704 cases vs. 75,374 controls <65 years; p-value = 1.7×10^{-8}) [23], and SNP, rs2075650, located in TOMM40 at chromosome 19q13.32 close to the apolipoprotein E (APOE) gene [24, 25], have been associated with survival up to >90 years of age.

This chapter deals with progeroid syndromes (PS) characterized by signs of premature aging. Since the year 1886, when the first clinical aging (Hutchinson-Gilford) syndrome or progeria was identified, >75 syndromes have been recognized with the symptoms of premature aging [32]. Progeria (PS) invariably is classified into two categories: (1) segmental PS, affecting multiple organs and tissues, and (2) unimodal PS, which affects a single organ or tissue. Segmental PSs include Hutchinson-Gilford progeria syndrome (HGPS), Cockayne syndrome (CS), Down syndrome (DS), Werner syndrome (WS), Ataxia-telangiectasia (AT), Bloom syndrome (BS), Fanconi anemia (FA), and Xeroderma pigmentosum (XP), whereas familial Alzheimer's, Parkinson's, and attenuated polyposis are included in unimodal PS [32, 33].

Detractors argue that PS could not be used as a model to understand aging as they do not reflect the process of healthy aging, which is genetically complex and may not necessarily explain the involvement of a minor set of genes implicated in these syndromes. However, these shortcomings of PS as models of healthy aging also are their strengths. Involvement of single genes in these defects facilitates biochemical characterization of the etiologies which accelerate attainment of aging characters in progeria

syndromes, a correlation challenging to establish in normal processes of aging due to genetic complexity [32, 34, 35]. Thus, attaining accelerated aging characteristics in PS helps in the identification of specific pathways and underlying mechanisms that fail and in all probabilities operate at a slower pace through the later stages of the normal processes of aging. Documentation of such defective genes in monogenic progeria-related syndromes with accelerated aging allows the discovery of alleles of these genes that may either postpone or discreetly to accelerate the onset of typical aging characteristics [36]. Table 11.2 provides a brief description of PSs, considered as the human models of accelerated aging, which are dealt with in this chapter in detail.

Table 11.2 Examples of progeroid syndromes

Syndrome	Frequency	Gene	Inheritance pattern	Median age
Hutchinson-Gilford syndrome	1:4,000,000	Heterozygous dominant mutations in the *LMNA* gene	Nonheritable	~13.5 years
Cockayne syndrome (CS)	1:500,000	Mutations in *CSA* and *CSB* genes	Heritable; autosomal recessive	Type I ~16 years; type II ~5 to 7 years; type III ~30 years
Down syndrome (DS)	1:200,000	Partial or full trisomy at chromosome 21	Nonhereditary; except a few cases of rare Robertsonian translocation	~60 years
Werner syndrome (WS)	1:200,000; in Japan 1:20,000 to 1:40,000	Mutations of the *WRN* gene	Heritable; autosomal recessive	~54 years
Ataxia-telangiectasia (AT)	1:40,000 to 1:200,000	Mutations in the *ATM* gene	Heritable; autosomal recessive pattern (carriers have high a risk of cancer)	~16 to 25 years
Bloom syndrome (BS)	Very rare; few hundred cases are reported	Mutations of the *BLM* gene	Heritable; autosomal recessive	~26 years
Fanconi anemia (FA)	1:2,00,000 for affected and 1:300 for carriers	Homozygous or compound heterozygous mutations *FANCA* gene	Autosomal recessive	~25 years
Xeroderma pigmentosum (XP)	1:1,000,000 but have a high frequency in Japan	Mutations in DNA repair genes	Autosomal recessive	Depends on the management of syndrome
Rothmund-Thomson syndrome (RTS)	Not available	Mutations in *RECQL4* gene	Autosomal recessive	Normal in the absence of malignancy

11.2 Premature Aging and Genetic Syndromes

11.2.1 Hutchinson-Gilford Progeria Syndrome (HGPS)

Hutchinson-Gilford **progeria** syndrome is the most widely studied progeroid syndrome, which is extremely rare. The de novo dominant heterozygous mutation either within exon 11 or at the cryptic splice site of exon 11 (G608G) in lamin A (*LMNA*) gene, located at 1q22 position [2, 37], has been implicated as the causal factor of the syndrome. The presence of a heterozygous mutation in exon 11 of *LMNA* gene leads to a deletion of 50 amino acids spanning cleavage site for CAAX prenyl protease 1 homolog (ZMPSTE24) accountable for the cleavage of prelamin A to produce lamin A. Lamin A protein, as a part of the nuclear inner membrane, is significant in maintaining the integrity of the nuclear envelope, performing a part in chromatin arrangement and epigenetic regulation [38]. The dysregulation of lamin A, therefore, leads to an accumulation of progerin protein, a toxic form of lamin A, disrupting the integrity of the nuclear envelope [39, 40]. The accumulation of progerin in HGPS cells leads to an irregular nuclear morphology, genetic instability, and P53-dependent premature senescence. Unprocessed lamin A and the progerin are also known to interfere with the functions of essential DNA replication factors causing replication stress, resulting in the chronic DNA damage during S phase and delayed replication fork progress with an outcome of premature senescence in HGPS [40]. Excessive accumulation of prelamin A due to trisomy of chromosome 1, resulting in its overexpression has also been proposed to induce mild-HGPS-like defects [41].

Individuals with Hutchinson-Gilford syndrome display an accelerated loss of hair (alopecia), decrease in subcutaneous fat (lipodystrophy), wrinkling of the skin, osteoporosis, and severe atherosclerosis mimicking the normal aging process. Cardiovascular failure of Hutchinson-Gilford syndrome patients, due to increased vascular stiffening followed by hypertension, angina, cardiomegaly, and congestive heart failure, is responsible for almost all mortality [37, 42, 43]. There are variations in this syndrome where growth retardation and other features not associated with aging are observed; and some features of aging, such as deterioration of the nervous system-related functions and increased susceptibility to cancer, are not observed [43]. In recent studies, brain cells have been shown to express low level of lamin A, explaining the absence of deterioration of CNS in progeria patients [44]. Further studies using ZMPSTE24-deficient mosaic mice have shown that the accumulation of prelamin A does not increase the susceptibility of mosaic mice toward carcinogens to develop a tumor; instead, it prevents cancer invasion. Incidentally, the presence of progerin RNA and protein accumulation in healthy individuals during normal aging process confirms its role in aging, drawing parallels between normal and pathological conditions [45]. This gradual increase during physiological aging could be due to several reasons, including *LMNA* mutations, epigenetic regulation, or abnormal farnesylation [46]. Age-induced pluripotent stem cells (iPSC) used as a new cellular model for studying premature aging have recently shown the involvement of significant constituents of the nuclear envelope, including lamin A [46].

Another most predominant physiological anomaly associated with progeria is elevated hyaluronic acid in urine [47]. Hyaluronic acid levels usually increase in urine with aging [48], though at levels lower than that observed in progeria. Hyaluronic acid is required for the maintenance of skeletal, muscular, cutaneous, and vascular systems of the body. It is also thought to block angiogenesis (vascularization). Hence, a possible fault in hyaluronic acid metabolism may disrupt several developmental pathways.

11.2.2 Cockayne Syndrome (CS)

Cockayne syndrome, a heritable autosomal recessive disease, is also characterized by a variety of features resembling accelerated aging. These include several neurological manifestations, cognitive deficits, pigmentary retinopathy, cataracts, sensory neural deafness, loss of subcutaneous fat, progressive hearing loss, cognitive decline, nephronic reduction, atherosclerosis, arteriolosclerosis, chronic hypertension, diabetes, and ambulatory and feeding difficulties, besides characteristic dwarfism, microcephaly, photosensitivity, etc. [46, 49–52].

Based on the age of onset of symptoms and severity of the disease, CS is divided into three types: type I, type II, and type III [53, 54]. CS type II is the most severe form and shows growth defect in infants, such as congenital cataracts or other structural anomalies of the eye with little postnatal neurological development and an increase in height or head circumference [53]. Type I is a classic CS with the onset of growth and developmental abnormalities in the first two years, whereas type III (mild) CS patients show symptoms in the later stages of life [53]. The primary cause of death in CS is respiratory failure, but the average age may vary with the type of CS. Mean life expectancies of type I and III individuals are 16 and 30 years, whereas the average survival of type II CS patients is 5 to 6 years [50, 51, 53, 54].

CS requires a mutation in both the alleles of either Cockayne syndrome A (*CSA*, also known as *ERCC8*) or Cockayne syndrome B (*CSB*, alias *ERCC6*) genes [49, 54, 55]. *CSA*, located at 5q21, is mostly associated with type I and III CS and is responsible for 20% to 25% of CS cases [49, 53, 54]. The majority of CS cases (75–80%), especially type II, are caused by mutant CSB (located at 10q11–21) [49, 53, 54]. Apart from these two, a smaller number of CS cases may arise from mutations in one of the three Xeroderma pigmentosum (XP) genes [*XPB* (*ERCC3*), *XPD* (*ERCC2*), or *XPG* (*ERCC5*)] in individuals who have clinical features of both disorders, CS and XP [56]. Mutations in *CSA* and *CSB* genes affect transcription-coupled repair (TCR), a subpathway of the nucleotide excision repair (NER), dedicated to the removal of transcription-blocking lesions from the genome [57]. CS individuals lack this exceedingly efficient repair of the transcribed strand of active genes and thus have difficulty in recommencing transcription following DNA damage. Persistent damage in the transcribed strand may seize RNA polymerase, and the related transcription deficits may explain many CS abnormalities. Moreover, both CSA and CSB are known to localize to

mitochondria, where they protect against oxidative stress-induced mtDNA damage by interacting with base excision repair (BER)-associated mitochondrial 8-oxoguanine glycosylase-1 [58, 59]. Increased oxidative stress-induced mtDNA damage and mutations in mitochondrial DNA are well-established markers of healthy aging [1, 60]. The presence of CSB in mitochondria also increases the mitochondrial transcript and protein levels by interacting with the core transcription machinery [61]. Another hypothesis proposes that blocking of cellular transcriptional process induces programmed cell death (apoptosis). This cell loss is sufficient to elicit signs of aging and other characteristics of CS [62]. Accumulation of endogenous oxidative DNA damage in neurons is known to inhibit transcription or induce apoptosis, thus explaining the profound neurodegeneration associated with this disease [62, 63]. In addition to its DNA- and nucleosome-stimulated ATP hydrolysis activities, CSB is also known to alter the conformation of the DNA duplex. Several in vitro assays have revealed that CSB actively wraps and unwraps DNA around itself, which may account for CSB function in altering DNA-protein interaction and transcription in cells [64]. Thus, the involvement of the specific genes in this syndrome through more than one pathway could influence the process of progeria, the premature aging.

11.2.3 Down Syndrome (DS)

DS is a chromosomal disorder, and the affected individuals have a partial or full copy of extra chromosome 21, referred to as trisomy 21 [65]. Almost all DS adults develop Alzheimer's disease in their early 50s, due to the triplication of amyloid precursor protein (APP), which is earlier than the healthy aging individuals [66]. Apart from early-age cognitive deficits and intellectual disability, hearing loss is prevalent in DS adults, and the pattern is found to be compatible with the precocious aging of the hearing system [67]. One in 800 newborns is affected by Down syndrome, and the frequency of having a child with DS, researchers believed in the past, increases with advancing age of the mother [68]. The reason for this is not fully understood; probably the extra gene dosage of some of the genes present on chromosome 21 is suspected to be involved. More than half of children born with DS have congenital heart defects. Down syndrome patients have delayed development of speech and language and early onset of osteoarthritis and osteoporosis, in addition to the hearing loss, than the same age group of healthy persons [69]. DS also affects behavioral development, such as attention problem, stubbornness, and obsessive-compulsive disorder. DS individuals have an increased risk for cancer, almost 10–20 times higher for leukemia, particularly for childhood leukemia than the average population [70–72]. Other than these features, certain autoimmune conditions, such as diabetes mellitus, thyroid gland-related disorder, and celiac disease (gluten enteropathy), are more prevalent in DS children [73, 74]. Over the past few decades, due to increased management of the disease and awareness, the average age of death in DS cases has significantly increased from 40 to 60 years [75, 76].

Trisomy associated with DS is of three types: (1) 90–95% of DS patients have three copies of chromosome 21 in all the cells of body; thus the person with DS has 47 chromosomes instead of 46 [77]; (2) in ~5% of patients, a part of chromosome 21 translocates to another acrocentric chromosome, mostly chromosome 14 [78]; (3) in rare cases, when nondisjunction of chromosome 21 takes place in one of the initial cell divisions in the zygote, mosaicism occurs in DS creating a mosaic pattern of cells, some with 46 and others with 47 chromosomes [79]. The trisomy of chromosome 21 is mostly maternal in origin with nondisjunction at meiosis I [80–82]. Sequencing of chromosome 21 in early 2000 allowed the identification and prediction of genes that could be important for DS [83]. Genes linked with mitochondrial energy metabolism, reactive oxygen species (ROS) metabolism, and structural and functional genes of the central nervous system are found to be present on chromosome 21 [83, 84]. Several studies have been published linking mitochondria, ROS, and APP protein to DS and Alzheimer's disease [85–87]. Disproportionate oxidative metabolism could lead to increased cellular damage. This scenario is unswerving with increased lipofuscin and oxidative DNA damage observed in DS and the projected association between accumulation of oxidative damage and aging characteristics [88]. The contribution, however, of any gene on chromosome 21 to any premature aging characteristics associated with Down syndrome remains unclear.

11.2.4 Werner Syndrome (WS)

Werner syndrome (WS), also known as "progeria of adults," is characterized by multiple features of aging, beginning in early adulthood. Affected cases appear relatively healthy until adolescence and show change toward premature aging after that. Individuals with WS usually develop normal until the first 10 years, followed by the lack of growth spurt, loss and graying of hair, hoarseness, flat feet, and scleroderma-like skin changes, followed by bilateral ocular cataracts, type 2 diabetes mellitus, hypogonadism, skin ulcers up to the 20s, and osteoporosis in the 30s [89]. WS is inherited in a monogenic autosomal recessive manner, where 25% progeny of an affected individual has a chance of being affected and 50% could be a carrier and asymptomatic for the syndrome. WS patients have 2- to 60-fold increased risk of cancer, including thyroid, melanoma, meningioma, bone tumors, and leukemia, as well as some sporadic tumors than what is expected for the age-matched controls [90, 91]. Cancer and increased myocardial infarction are the primary cause of death in WS patients [89]. The average age of death in WS has increased in the last decade to 54 years due to improved diagnosis and management of the syndrome [89, 92, 93]. Other than these signs, individuals with WS do not suffer from mental retardation or dementia, hypertension, skeletal anomalies, osteoarthritis, and developmental defects [89, 93, 94].

The WS was first identified in 1904 by Otto Werner, but the gene responsible for WS was discovered in 1996 through a positional cloning approach [95]. The mutations of *WRN* gene, present approximately 90% of WS cases, results in the

production of the truncated WRN protein responsible for all the phenotypes of WS. WRN is a RecQ-helicase-like protein of 1432 aa and possesses 3′–5′ helicase with 3′–5′ exonuclease activity [96, 97]. WRN is a DNA structure-specific helicase and is involved in DNA repair, recombination, and telomere maintenance [98]. Cells of WS patients have hyper-recombination potential, which leads to increased chromosomal aberrations and global genomic instability with the deletion of a large number of genes [99, 100]. Importantly, fibroblasts derived from WS patients show premature aging, such as replicative senescence and telomere shortening with unusual extended S phase in comparison to normal cells [101]. Overall, the Werner syndrome phenotype points to an association between the collection of genetic changes, cellular senescence, and aging. In the recent past, WS has also been related with an increased epigenetic age (DNA methylation age), calculated as units of years based on the weighted average across multiple C_pG sites [102]. The presence of hyper-recombination and multiple chromosomal aberrations in WS cells suggests that aging and increased cancer susceptibility could stem from the failure to suppress illegitimate recombination events and global genomic instability.

11.2.5 Ataxia-Telangiectasia (AT)

Ataxia-Telangiectasia or Louis-Barr syndrome is an autosomal recessive syndrome, in which patients suffer from accelerated aging as well, and characterized by progressive cerebellar ataxia, telangiectasia (dilation of blood vessels), immune defects, and increased frequencies of malignancy [103, 104]. Features of this disease, surface during infancy, and progress during childhood. In AT, the cerebellum is the most affected area of the brain which associates with the muscular control of the body [105, 106] and develops into general motor dysfunction, eventually confining most patients to the wheelchair around the end of their first decade. Abnormal swallowing and limited facial expression are common to AT patients. Dysfunctional swallowing not only causes the nutritional problem but also has an association with pulmonary aspiration-mediated infections of lower respiratory tract [107, 108]. Immunodeficiency is another hallmark of AT where B- and T-cell counts are severely affected [109]. A wide range of cancer predisposition to Hodgkin's lymphoma, non-Hodgkin's lymphoma, invasive breast cancer, gastrointestinal mucinous adenocarcinoma, and a variety of leukemia, including lymphocytic leukemia, has been observed in the AT patients [110–112]. *ATM*, the gene implicated in the disease, is extensively studied for its role as a chief mobilizer of the DNA damage response pathway for double-strand break (DSBs).

The primary cause of the disease is the mutation in both the alleles of an autosomal gene, *ATM*, which encodes a multifunctional protein kinase ATM [113, 114]. The majority of AT patients have a truncated form of ATM protein as a result of either homozygous or compound heterozygous null mutations in the *ATM* gene [115–117]. A few substitution mutations in the kinase domain of ATM are also found [115]. Cells which lack ATM function are severely sensitive to ionizing radiation, thereby having chromosomal instability (with augmented telomere

shortening) and premature replicative senescence. There are several reports which have demonstrated the role of ATM kinase in regulating cell cycle progression. In response to DNA damage, ATM kinase phosphorylates several protein targets, notably the tumor suppressor protein P53 that in turn either delays the cell cycle progression or initiates programmed cell death. Consequently, loss of ATM function results in accumulation of damaged DNA and/or increased chromosomal breaks, after the replication of the damaged DNA in surviving cells. These outcomes endanger the ataxia telangiectasia individuals with increased tumorigenesis. Those with one mutated ATM allele (~1% of the population) are suggested to have a slightly elevated cancer risk, although without the apparent phenotypes of ataxia-telangiectasia.

11.2.6 Bloom Syndrome (BS)

Bloom syndrome (BS) is a rare autosomal recessive disorder with chromosomal instability [118]. The syndrome is characterized by small but proportional body size [119]. Mean birth weight and mean birth length in BS are >2 SD below the normal, suggesting that the growth retardation in BS is prenatal [120]. Body mass index at birth and during postnatal development is also well below average, but the deficit decreases into adulthood [121, 122]. At birth, the skin of individuals with BS syndrome may appear normal, but gradually in the first or second year of life and response to sun exposure, children develop an erythematous rash on the nose and cheeks and around the mouth [122, 123]. Children usually complain of severe gastroesophageal reflux and diarrhea. The increased gastroesophageal reflux could lead to increased frequency of pneumonia in BS due to increased exposure to gastric contents following aspiration [124]. Various other clinical features of BS include a mild immunodeficiency, azoospermia or severe oligospermia in males, premature cessation of menstruation in females, and minor anatomic defects. Normal to compromised intellectual abilities have been reported in different BS individuals [123]. The cells in BS cases show an increase in chromosomal aberrations and characteristic quadri-radial (Qr) chromosome formation, the latter as an indicator of unresolved recombination between homologous chromosomes [125–128]. Spontaneous occurrence of cancer is the leading cause of death associated with BS. Three main characteristics of cancer have been suggested in BS: (1) early development of cancer for each tumor type, (2) development of multiple independent primary cancers in a single individual, and (3) a broad range of the types and sites of cancer [123, 124]. The standardized incidence ratios in BS show that persons with BS are 99 times (l83–117) more likely to be diagnosed with any cancer relative to the general population [123]. Additionally, BS individuals also suffer from adult-onset type of diabetes, associated with impaired glucose tolerance and insulin resistance [129].

BS is caused by homozygous or compound heterozygous mutations in the BLM gene localized to chromosome 15q26.1, which result in either BLM inactivation due to premature protein translation termination or abrogation of its helicase activity

due to missense mutations [130]. BLM hydrolyzes ATP to unwind DNA by traversing ssDNA in a 3′–5′ direction [131] thus playing a critical role in HR, in DNA replication, and stabilization and repair of damaged replication forks. BLM is also crucial for the proper segregation of sister chromatid in mitosis and telomere maintenance [123]. Unlike WS, individuals who suffer from BS do not develop the features associated with aging, such as gray hair, cataracts, osteoporosis, skin changes, arteriosclerosis, and atherosclerosis prematurely. Surviving persons with BS are relatively young with an average age at death of <30 years, and the oldest Bloom syndrome reported in the registry is 53 years old.

Another gene located in 15q26 position was found to be mutated in a BS case reported from India [132, 133]. This gene codes for an isozyme, pyruvate kinase (PK) M2, which explains the different pleiotropic features of the syndrome, not rationally understood by mutations in the BLM gene alone [134, 135]. Further, the absence of established mutations in the BLM gene in another BS case from India (unpublished results) suggested the role of PKM2 in the syndrome and its role in cellular growth and development, hence the process of aging [134].

11.2.7 Fanconi Anemia (FA)

Fanconi anemia (FA) is a rare autosomal genetic disorder characterized by progressive bone marrow failure (BMF), endocrine dysfunction, cancer, and other clinical features commonly associated with healthy aging [136–138]. FA patients show early onset of several age-associated diseases such as myelodysplastic syndromes (10 years vs. 70 years), acute myeloid leukemia (30 years vs. 70 years), and squamous cell carcinoma (40 years vs. 75 years) [139–142]. FA is proposed to be a segmental disorder [139, 143]. Unlike other genomic instability syndromes, FA is characterized by a prooxidative condition, and cultured cells are highly sensitive, show weak growth at normal oxygen levels, and are impaired for ROS-induced DNA damage repair [144–146]. The presence of high levels of 8-oxo-deoxyguanosine (8-oxo-dG) in cells of FA patients than the age-matched healthy controls further suggests the prooxidant state [143, 147]. FA cells have more mutation frequency than the healthy cells in culture and have mitochondrial dysfunction along with high levels of hydroxyl radicals in leukocytes [148–150]. FA patients also accumulate high levels of inflammatory cytokines, TNFα, IL6, and IL1β at an early age, suggesting that FA should be characterized as a premature inflammation disorder [151–154]. Increased ROS, along with inflammatory cytokines, also leads to significant alterations in hematopoietic stem cells, which include activation of differentiation checkpoints and exhaustion of stem cells in bone marrow [155, 156]. Although minimal differences in the size of telomere of leukocytes of FA patients have been seen, the laboratory models used for the study of the functions of FA proteins have suggested a very significant role in the recruitment of shelterin proteins and telomere length maintenance [157–160].

The first case report of FA was published in 1927 by a Swiss pediatrician, Guido Fanconi. Although a number of clinical heterogeneity has been reported, common

clinical manifestations include pre- and postnatal growth retardation, malformation of the skeleton, and hearing loss; hypogonadism and reduced fertility; cutaneous abnormalities and bone marrow failure; and susceptibility to cancer, predominantly acute myeloid leukemia [161, 162]. Several genetic strategies resulted in identifying 19 different complementation groups (Table 11.3). The primary function of FA proteins is to activate or drive DNA repair and homologous recombination [163]. Genetic studies have revealed that mutations in the FANCA, FANCC, and FANCG genes are most common and account for ~85% of FA cases [164, 165]. FANCC

Table 11.3 FA complementation groups and associated genes

Complementation group	Chromosome location	Gene involved	Mutation type
Group A	16q24	*FANCA*	Homozygous or compound heterozygous
Group B	Xp22	*FANCB*	Frameshift and deletion mutation
Group C	9q22	*FANCC*	Homozygous, compound heterozygous, insertion, deletions
Group D1	13q12	*FANCD1/ BRCA2*	Homozygous or compound heterozygous
Group D2	3p25	*FANCD2*	Homozygous or compound heterozygous
Group E	6p21	*FANCE*	Homozygous
Group F	11p15	*FANCF*	Homozygous or compound heterozygous
Group G	9p13	*FANCG/ XRCC9*	Homozygous or compound heterozygous
Group I	15q26	*FANCI*	Homozygous or compound heterozygous
Group J	17q22	*FANCJ/ BRIP1*	Homozygous or compound heterozygous
Group L	2p16	*FANCL/ PHF9*	Homozygous or compound heterozygous
Group N	16p12	*FANCN/ PALB2*	Compound heterozygous
Group O	17q22	*FANCO/ RAD51C*	Homozygous
Group P	16p13	*FANCP/SLX4*	Homozygous or compound heterozygous
Group Q	16p13	*FANCQ/ ERCC4*	Compound heterozygous
Group R	15q15	*FANCR/ RAD51*	Heterozygous
Group T	1q31	*FANCT/ UBE2T*	Compound heterozygous
Group U	7q36	*FANCU/ XRCC2*	Homozygous
Group V	1p36	*FANCV/ MAD2L2*	Homozygous

mutations account for ~14% of FA cases [165]. FANCD2 encodes a protein that plays a central role in the FA pathway of DNA repair [166]. The proposed model for DNA repair by FA proteins involves a nuclear FA core complex made up of eight proteins, FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, and FANCM. This core complex receives the upstream DNA damage response signal and then activates the FANCT enzyme, which mono-ubiquitinates the FANCD2/FANCD1 complex.

This modified complex translocates to the DNA damage site and recruits the downstream effector proteins BRCA1, BRCA2, BRIP1, PALB2, RAD51C, SLX4, ERCC4, and RAD51 to DNA damage site [164]. This model also helps to explain the hypersensitivity of cells from FA patients to the clastogenic and cytostatic effects of DNA cross-linking agents such as diepoxybutane and mitomycin C [164].

11.2.8 Xeroderma Pigmentosum (XP)

XP is a rare autosomal recessive disease, where patients exposed to sunlight exhibit strong freckle-like pigmentation and lesions in the exposed areas. The disease is characterized by defects in nucleotide excision repair (NER), which normally is responsible for the removal of DNA lesions induced by the UV component of sunlight. Some XP patients also show defects in the replication of UV-damaged DNA. Several skin aging symptoms may appear as precancerous skin lesions, such as actinic keratosis; and most unprotected XP patients have been reported to develop skin cancer (non-melanoma and melanoma) before the age of 10 [167]. XP patients have 10,000-fold higher chances of developing non-melanoma skin tumors when compared to the normal population and a 2000-fold increase in the incidence of melanoma before the age of 20 [167]. Using different cell fusion experiments, seven different complementation groups (XPA to XPG), corresponding to seven different genes, were identified [167]. Other than skin-related abnormalities, 20~30% of XP patients also develop progressive neuronal impairment, growth retardation, and microcephaly. These developmental problems cause premature aging [167] characterized by loss of hearing, difficulty in swallowing, and mental retardation.

Interestingly, not all the mutations associated with XP complementation group genes lead to neurological disorders associated with severe XPs [167]. These observations explain that defects in NER pathways could explain the increased tumorigenesis observed in the skin of XP patients. Oxidative stress-induced DNA damage has been proposed as the cause of severe phenotypes associated with XP, which is further supported by the involvement of XPC proteins in oxidative stress-induced DNA damage repair [167]. XP, as one of the first syndromes, was found to show aberrant DNA repair processes, since these cells were not able to show unscheduled DNA synthesis after UV exposure [168]. However, these findings are still not enough to explain the severe neurological condition and accelerated aging of XP patients.

11.2.9 Rothmund-Thomson Syndrome (RTS)

Rothmund-Thomson syndrome (RTS) is a rare autosomal recessive disease with complex characters and severity. Most of the patients have skin rashes known as poikiloderma, atrophy, and telangiectases [169]. RTS patients also have several features of accelerated aging, such as sparse and thin hairs of scalp, eyebrow, and eyelashes; irregular pigmentation of the skin; osteoporosis with increased frequency of fractures; and increased risk of cancer especially squamous and basal cell carcinomas [170–172]. For a maximum number of RTS cases, homozygous or compound heterozygous mutations in *RECQL4* gene are found to be responsible, and for the rest still, the gene(s) need to be identified [173, 174]. *RECQL4* functions in DNA replication, homologous recombination, DNA damage repair, and maintenance of telomeres, thus mutations affecting its activity, could lead to accelerated aging phenotypes, such as genomic instability, cellular senescence, mitochondrial dysfunction, and telomere attrition. RECQL4 participates in multiple DNA repair pathways including, HR, NHEJ, NER, and BER by directly interacting with RAD51, Ku70/Ku80, and XPA [175–178]. Apart from defective DNA repair-induced genomic instability, abnormal replication also induces genomic instability in *RECQL4* mutant cells. The presence of cellular senescence with activated P38 pathway in primary dermal fibroblasts isolated from RTS patients confirms the stress-induced premature senescence in RTS [179]. Recently, RECQL4 has been found to be localized in mitochondria, where it plays an important role in maintaining the integrity of mtDNA [180, 181]. RECQL4 has also been found to be interacting with TRF2 of shelterin complex of telomere and can unwind the D-loops indicating its role in telomere maintenance [182, 183]. RTS patients without mutations in *RECQL4* also show some of the accelerated aging phenotypes of chromosomal instability, early onset of cancer [169, 171]; thus identifying the mutated genes of these patients would be more helpful for preventing age-related diseases.

11.3 Recapitulation of Features of Aging in Syndromes and Aging Theories

Many biological theories have been proposed to explain the million dollar question of "Why do we age?"; however, none of them provide complete explanations (Table 11.4). Out of all aging theories, the free radical theory of aging, the mitochondrial theory of aging, and the telomere theory of aging are the most commonly discussed ones. However, the accumulation of senescent cells leading to the deterioration of the system has always been the central point of discussion. Natural aging has been thought to be the result of the accumulation of senescent cells in the organs to the extent of interference in normal function [184]. Cellular senescence is the irreversible loss of replicative potential of cells caused by various extrinsic and intrinsic factors. As discussed above, many genes have been identified in humans to accelerate the process of senescence and aging, although only some of the standard aging features are recapitulated with the individual gene. Senescent cells contribute

Table 11.4 Theories of aging

Category/name of the theory		Proposal
Programmed longevity theories	Endocrine theory	Loss of coordinated action of the neuroendocrine system to maintain the functional homeostasis of the body system as well as response to environmental stimuli
	Programmed senescence theory	Aging is a result of replicative senescence induced by continuous shortening of telomeres
	Immunological theory	Decreased effectiveness of the immune system with age results in increased incidences of infectious disease as well as the inability to recognize self-antigens driving autoimmune pathologies
Damage and error theories	Wear and tear theory	Continuous and repeated use leads to dysfunction of organ or tissues due to accumulation damage from diseases, toxins, and food
	Rate of living theory	Every individual has a fixed amount of metabolic potential; thus, the higher the metabolic activity, the shorter the life span
	Cross-linking theory	Aging occurs due to increased inter- and intramolecular cross-links which are not repairable by cellular repair enzymes leading to systemic failure
	Free radical theory	Accumulation of by-products of oxidative metabolism, free radicals, and superoxides subsequently damages the macromolecules and induces the systemic dysfunction
	Mitochondrial theory	Increased damage to mtDNA leads to functional decline of mitochondria and accumulation of mutations driving the process of aging
Gene regulation theory		Aging is driven by the change in expression of genes associated with development, differentiation, homeostasis, and senescence

to aging by secreting pro-inflammatory cytokines, chemokines, and proteases known as senescence-associated secretory phenotypes (SASPs) [185]. These SASPs have a role in reinforcing the senescence in nearby cells, promoting the tumorigenesis as well as chronic inflammatory response associated with aging [186, 187]. Increased expression of SASPs in progeroid syndromes is a very well-established phenomenon and has also been associated with the progression of syndromes [188–191].

Studies have shown that knockout of key inflammatory transcription factors such as NFκB not only reduces the age-associated chronic inflammatory response and tumorigenesis but also improves the life span [192]. Similarly, accumulation of progeria-associated prelamin A (progerin) activates NFκB by ATM-dependent signaling pathway and increases the secretion of age-associated pro-inflammatory cytokines in two different mouse models of accelerated aging [193]. Interestingly, the abilities of NFκB to activate inflammatory response and other epigenetic regulators make it as a significant roadblock for the reprogramming of naturally aged as well as prematurely aged somatic cells [194, 195].

In order to propose a unifying mechanism for the process of aging, a comprehensive understanding of aging-associated phenotypes and regulatory signaling pathways is essential. Accumulation of nuclear and mitochondrial DNA damage, one of the key features of healthy aging, is common to all progeroid syndromes and one of the critical stimulants of senescence in cells. Defects in different DNA repair systems, such as nucleotide excision repair (NER), base excision repair (BER), and double-strand break repair (DSBR) can cause DNA damage [196–200]. For many years, DNA damage and defects in repair pathways have been the core of aging theories despite the adverse reports [167, 201]. Age-associated DNA lesions either could be due to extrinsic and intrinsic damaging agents directly or by defects of nuclear architecture known as laminopathies [202, 203]. Defects of the DNA repair process have been the leading cause of many progeroid syndromes [2, 204].

A broad range of nuclear genomic abnormalities ranging from point mutations to large chromosomal anomalies has been reported to be associated with aging [196, 205–208]. Several studies have shown the importance of artificial reinforcement or enhancement of nuclear DNA repair mechanisms delaying aging and onset of age-associated malignancies [209, 210]. Accumulation of DNA damage with age has not only been restricted to nuclear DNA but also found in mitochondrial DNA (mtDNA). The lack of histones and limited repair efficiency make mtDNA as a major target for age-associated somatic mutations due to the oxidative environment of mitochondria and replicative errors [211–214]. Mitochondrial dysfunction has been strongly correlated with the aging [215]. Many systemic diseases associated with aging have been reported to have a significant correlation with mutations of mtDNA [216]; however, evidence from genetic manipulation decreasing the load of mtDNA variations still does not explain their direct involvement in longevity. The role of mitochondrial biology regulators, such as polymerase ɣ and TFAM, has been very well studied and characterized for the longevity [217–219]. Although the oxidative stress-induced mtDNA damage and its association with age have been well documented, still the mouse models of mitochondrial aging (mutator mouse models) have shown the accumulation of somatic mutations instead of oxidative stress [217, 220–224]. The classical "free radical theory of aging" has also suggested the central role of mitochondrial ROS-induced DNA damage in the aging process. However, recent literature suggests that alteration of mitochondrial dynamics (fission and fusion), energetic balance (AMP to ATP ratio), mitochondrial metabolites (NAD^+), and mitochondrial calcium homeostasis could lead to mitochondrial dysfunction and establish the senescence phenotype [225–235]. Notably, mitochondrial dysfunction has also been linked to the pathogenesis of progeroid syndromes, and cells derived from patients have shown significantly reduced oxidative phosphorylation accompanied by reduced levels of oxidative phosphorylation proteins [2, 236–238]. Recently, mtDNA has also been identified to be epigenetically regulated by DNMT1 isoform 3-induced methylation [239], and it would be interesting to study the epigenetics of mitochondria with the aging and age-associated diseases.

The identification of mitochondrial and telomere interaction has helped in connecting the two dots of aging biology, mitochondria, and telomere. Telomeres are nucleoprotein complexes at the ends of chromosomes, which function to maintain

the integrity of chromosomes along with preventing the recognition as double-strand breaks by DNA damage repair machinery [240]. In the late 1960s, a Russian biologist Alexey Olovnikov proposed the telomere shortening as an explanation for Hayflick's limit of fibroblast cultures [241]. Later the absence and nearly absence of telomerase, an enzyme to maintain the telomere length, in differentiated cells were found to be the reason for Hayflick's limit [242]. Consequently, the decreased telomere length with age was also observed in in-vivo conditions as well [243–246]. Telomere length could be affected by a combination of various factors, such as age of donor, genetic and environmental factors, socioeconomic status, exercise, obesity, and smoking [247–254]. In proliferating tissues, telomere shortening may cause telomere dysfunction and activate the P53-mediated cell death, leading to organ atrophy [255–258]. Indeed, the first connecting link of mitochondria and telomere is telomerase (TERT) which under oxidative stress localizes to the mitochondrial matrix and regulates mitochondrial biology and sensitivity to oxidative stress [259, 260]. Chen et al. [261] have demonstrated that TIN2, another shelterin complex protein, localizes to mitochondria and increases the globular form of mitochondria [261]. Reduction of cellular levels of TIN2 by RNAi not only decreased the glycolysis and ROS production but also enhanced the oxidative phosphorylation and ATP production, suggesting a fundamental mechanism by which telomere proteins could regulate metabolism in aging and cancer [261]. Interestingly, fibroblasts from WS patients show increased telomere attrition leading to premature senescence, and this could be reversed by enforced hTERT expression [262]. Similarly, accelerated shortening of telomere in fibroblasts from AT patients could be rescued by the hTERT expression [33]. Moreover, mouse models of WS (*Wrn*-null) and AT (*Atm*-null) recapitulated degenerative pathologies associated with progeroid syndromes of humans when transferred to telomere-deficient mouse with shorter telomeres [263]. The observation could partially explain accelerated telomere attrition and genomic instability in WS and BS that both WRN and BLM directly interact with the TRF2 protein of shelterin complex and have potential to unwind the telomeric D-loops [263]. ATM kinase plays a central role in maintaining telomere length and is essential for the addition of new telomere repeats; thus diminished expression of ATM leads to telomere shortening and genomic instability in both mouse and humans [264].

11.4 Conclusion

Significant advancement in our understanding of the genetic and cellular abnormalities of the progeroid syndromes has mirrored many similarities to the "natural" aging process but also some differences. Molecular features like reduced replication potential increased oxidative stress, and telomere attrition is common in progeroid syndromes and a healthy aging process. Added to this are the proteins functionally involved in altering chromatin structure, interfering with the coordination of essential DNA replication factors causing replication stress and the creation of an imbalance in oxidative metabolism, being shared by both normal aging process and

different progeroid syndromes. However, due to the involvement of a single defective gene unlike multiple gene loci as in healthy aging, levels of senescence vary in these syndromes. The complex and variable nature of healthy aging limits the use of a single progeroid syndrome as a model to understand aging, yet they provide a reasonably suitable condition to study the particular facets of aging. One of the most exciting areas of current and future research remains that of developing and testing of the therapeutic interventions of premature as well as the normal aging process.

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Role of Stress and Hormones of the Hypothalamic-Pituitary-Adrenal (HPA) Axis in Aging

12

Ankush Gupta

Abstract

The hypothalamic-pituitary-adrenal (HPA) axis is one of the most important allostatic systems that allow a person to respond and adapt against diverse stresses by upregulating the glucocorticoids and adrenal androgens. However, its inhibition is equally important to prevent against deleterious effect of its overexposure to neuroendocrine and inflammatory stresses. This chapter aims to examine the effect of stress and aging on the dysregulation of the HPA axis. Chronic stress and aging are phenomenons that have complex interaction at the level of HPA axis; while chronic stress promotes aging, consequently aging leads to dysregulated stress management. The effects of the terminal regulators of the HPA axis like glucocorticosteroids (GCs; cortisol) and adrenal androgens (DHEA and its sulfate; DHEAS) are drastically opposite; while cortisol promotes neuronal cell death and degeneration, DHEAS plays protective role against neuronal impairment. With age there is a marked increase in the nocturnal as well as 24 h circulating cortisol secretion accompanied by clear flattening of the diurnal rhythm of cortisol secretion evident from both animal and human studies. Also, there is a clear dysregulation of the negative feedback inhibition of the GC secretion in chronic stress and aging. Besides, the androgenic steroids like DHEA/DHEAS secreted in response to ACTH also undergo marked depreciation in elderly subjects. Consequently, there is a marked increase in the cortisol/DHEAS molar ratio with physiological and pathological aging. Hence, the dysregulation in these two classes of steroids with aging and chronic stress and their manifestations are examined in detail in this chapter.

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12.1 Introduction

The intricate coordination between the nervous and the endocrine systems ("the neuroendocrine system") which comprise of the multiple glands located throughout the body is directly under the control of the central nervous system particularly through the hypothalamic-pituitary axis, and it controls the major processes of the human body. All the physiological or psychological stimuli are perceived by the central nervous system, and consequently the response is mediated through the neuroendocrine mechanisms. Hormones are the terminal effector molecules of the endocrine system, which not only control the optimal growth and development but are also responsible for healthy and normal functioning of the human body (homeostasis) as well as the gradual aging processes. Multiple glands located throughout the body, viz., the pituitary (brain), thyroid (front of the neck), parathyroid (neck), adrenal (on the kidneys), gonads (groin), pancreas (abdominal cavity), etc., produce a multitude of hormones that control various instructions and functions for the various organs in the body. The most important regulator of the entire endocrine system of the human body is the "master gland/regulator" called as the hypothalamic-pituitary axis [1, 2].

Aging is a gradual process of progressive decline in the cellular functions and overall mental and physical fitness of an organism that eventually leads to development of age-related cognitive decline, several diseases, and ultimately death of the subject [3]. Several diseases like diabetes [4–6], atherosclerosis [7], rheumatoid arthritis [8–11], osteoporosis [3], Alzheimer's disease [12], progeroid syndromes [13, 14], etc. are considered as hallmarks of the aging process. However, the process of aging itself has a high degree of variability, with some individuals exhibiting very little-to-no loss of function ("successful" aging), while others suffer significant loss of function ("unsuccessful" aging) [15].

Stress is any phenomenon by which a potentially harmful stimulus (real or perceived) leads to or threatens the physiological or psychological homeostasis or well-being of an organism. The physiological response to stress which is regulated by the brain occurs in two steps: first rapidly by the activation of numerous catecholamine-mediated (via epinephrine and norepinephrine) effects of the sympathetic adrenal medullary (SAM) pathway like increased blood pressure, heart rate, etc. and second delayed by triggering the hypothalamic-pituitary-adrenal (HPA) pathway by the hypothalamus-pituitary gland to release a surge of adrenocorticotrophic hormone (ACTH), which causes the adrenal cortex to release glucocorticoids (cortisol in humans and corticosterone in rodents as well as humans) and the androgenic hormones [3, 15]. While acute (short-lived) stress stimuli strengthen and prepare the immune and endocrine system positively in a "fight-or-flight" response, chronic

(long-term) exposure to these stimuli adversely affects and promotes the aging process of the body tissues probably by eliciting imbalances in reactive oxidation species (ROS) formation and DNA damage/repair processes and by inducing neuroinflammation [3, 16–22]. Thus, the real question is whether chronic stress response and, consequently, dysregulated HPA axis (glucocorticoid rhythmicity) result from the aging process of the brain regions that regulate the HPA axis or these are the processes that ultimately lead to the accelerated aging processes of the brain and other tissues. This chapter will focus on the role of chronic stress on the hormones of the HPA axis in the aging process.

12.2 The Hypothalamic-Pituitary (HP) Axis

The complex coordination between the nervous and the endocrine systems ("neuroendocrine system") in the maintenance of the homeostasis in the human body occurs through the hypothalamic-pituitary region located at the base of the brain. The pituitary gland (*hypophysis*) considered as the master gland is about 1 centimeter in diameter and 0.5–1 gram in weight, lies at the base of the brain, and is connected to the hypothalamus by the *pituitary* (or *hypophysial*) stalk [Fig. 12.1]. It has a bipartite structure; the anterior (*adenohypophysis*), which is glandular, and the posterior (*neurohypophysis*), which is fibrous, have different embryological origins. The anterior pituitary (glandular) has originated from *Rathke's pouch*, an embryonic invagination of the pharyngeal epithelium, and the posterior (fibrous) pituitary arises from the neural tissue outgrowth from the hypothalamus. Almost all the secretions arising from the pituitary gland are either under the control of nervous or endocrine signals originating from several discrete nuclei of the hypothalamus. The posterior pituitary secretes the hormones, viz.:

(a) *Antidiuretic hormone* (ADH also called *arginine vasopressin* (AVP); nine amino acids long) which stimulates the kidneys and promotes water retention.
(b) *Oxytocin* (nine amino acids long) which stimulates milk ejection from the breasts and uterine contraction during child birth.

The secretion of these hormones is under the nervous control of hypothalamus, mainly secreted by large neurons located in the *supraoptic* and *paraventricular nuclei* of the hypothalamus through the axoplasm of the nerve fibers that passes from the hypothalamus to the posterior pituitary gland through the *hypophysial* stalk.

In contrast to the posterior pituitary, the secretions of the anterior pituitary are under the hormonal control of the hypothalamus via; *hypothalamic-releasing* and *hypothalamic inhibitory hormones* (or *factors*) secreted within the discrete nervous nuclei of hypothalamus and transported to the anterior pituitary by the *hypothalamic-hypophysial portal vessels*. The major hormones from the hypothalamus and their corresponding pituitary hormones and their major functions are as follows:

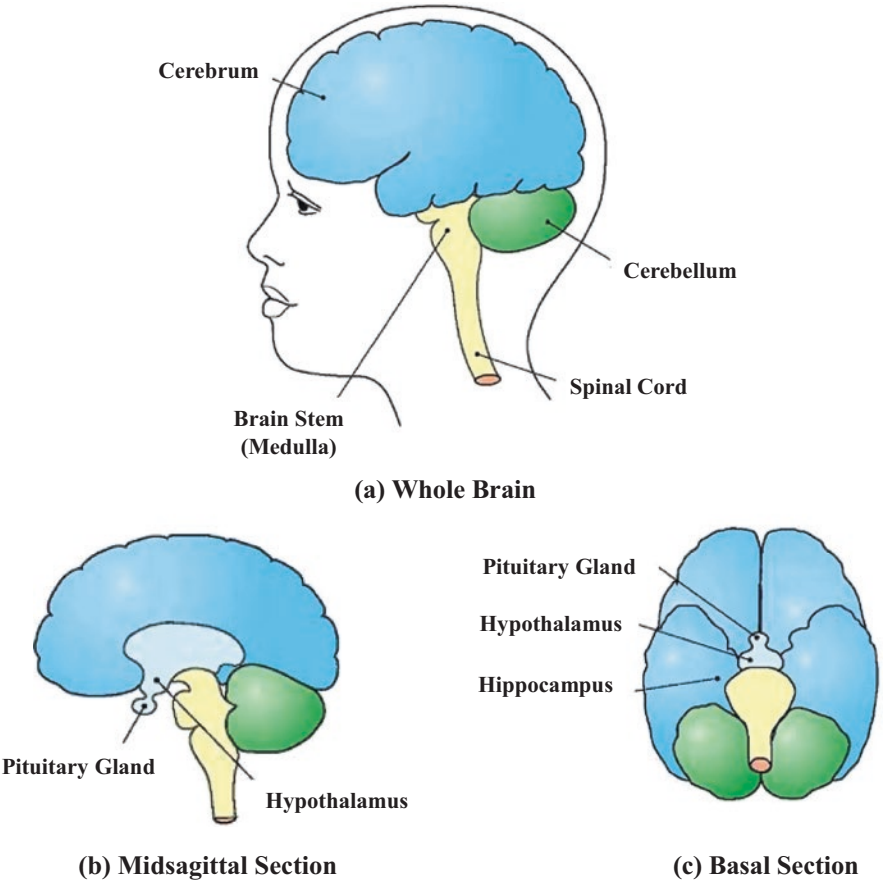

Fig. 12.1 Schematic representation of the different brain regions. (**a**) Whole brain. (**b**) Midsagittal section. (**c**) Basal section

(a) *Growth hormone-releasing hormone* (GHRH; polypeptide chain of 44 amino acids) causes the release of *growth hormone* (GH or somatotropin; polypeptide chain of 191 amino acids) that stimulates growth in the entire body by promoting protein synthesis, cell multiplication/ differentiation, and fat breakdown.
(b) *Growth hormone-inhibiting hormone* (GHIH; polypeptide chain of 14 amino acids), also called as *somatostatin*, inhibits release of the *growth hormone*.
(c) *Thyrotropin-releasing hormone* (TRH; 3 amino acid long peptide) causes release of *thyroid-stimulating hormone* (TSH; glycoprotein of 2 subunits, α (89 amino acids) and β (112 amino acids)) that causes the thyroid glands to release *thyroxine* which controls the basal metabolic rate (BMR) of almost all the chemical reactions occurring in the body.
(d) *Corticotropin-releasing hormone* (CRH; polypeptide chain of 41 amino acids) causes release of *adrenocorticotropic hormone* (ACTH; polypeptide chain of

39 amino acids) that causes the adrenal cortex to release glucocorticoids like *cortisol* (in humans) and *corticosterone* (in rodents and humans), key regulators of the stress response.

(e) *Gonadotropin-releasing hormone* (GnRH; polypeptide chain of 10 amino acids) causes release of the 2 gonadotropic hormones, *luteinizing hormone* (LH; glycoprotein of 2 subunits, α (89 amino acids) and β (115 amino acids)) which stimulates ovulation and corpus luteum formation in females and secretion of testosterone in males and *follicle-stimulating hormone* (FSH; glycoprotein of 2 subunits, α (89 amino acids) and β (112 amino acids)) which stimulates spermatogenesis in males and development of ovarian follicles in females.

(f) *Prolactin inhibitory hormone or factor* (PIH; dopamine which is a catecholamine) causes inhibition of *prolactin* (polypeptide chain of 198 amino acids) secretion which stimulates milk production.

(g) *Melanotropin-inhibiting hormone* (MIH) causes inhibition of *melanocyte-stimulating hormone* (MSH; 2 forms; 13 and 22 amino acids) secretion which causes skin color change in reptiles and amphibians but unknown function in mammals [1, 2].

One of the most important hallmarks of the hypothalamic-pituitary (HP) axis-mediated hormones is the negative feedback inhibitory regulation of their own secretion which helps to maintain the normal homeostasis of the body. Whenever the optimal secretion and function of the terminal hormones from the respective glands are accomplished, there occurs a negative feedback inhibition of the hormones from both hypothalamus and pituitary, respectively.

12.3 The Hypothalamic-Pituitary-Adrenal (HPA) Axis

The major neuroendocrine response to chronic stress adaptation is through the hypothalamic-pituitary-adrenal (HPA) axis. In response to physiological and psychological stresses and lower blood glucose concentrations, the parvocellular neurons of the paraventricular nuclei (PVN) of the hypothalamus are stimulated to synthesize the corticotropin-releasing hormone (CRH), which acts synergistically with arginine vasopressin (AVP), angiotensin II, and epinephrine on the anterior pituitary adrenocorticotropes to release the adrenocorticotropic hormone (ACTH) into the bloodstream [23].

Corticotropin-releasing hormone (CRH), a polypeptide chain of 41 amino acids, when released into the *hypophysial* portal blood further stimulates the type 1 CRH receptors in the anterior pituitary corticotrope cells to stimulate the transcription of *proopiomelanocortin* (POMC) gene [24, 25]. The *proopiomelanocortin* (POMC) is a preprohormone that gets cleaved into β-lipotropin and a pro-ACTH in the anterior pituitary which further gets cleaved into ACTH, joining peptide and amino-terminal peptide [Fig. 12.2]. ACTH can be further cleaved into *melanocyte-stimulating hormone* (MSH), and the β-lipotropin can be cleaved into γ-*lipotropin* and β-*endorphin* [26, 27]. Arginine vasopressin (AVP) potentiates

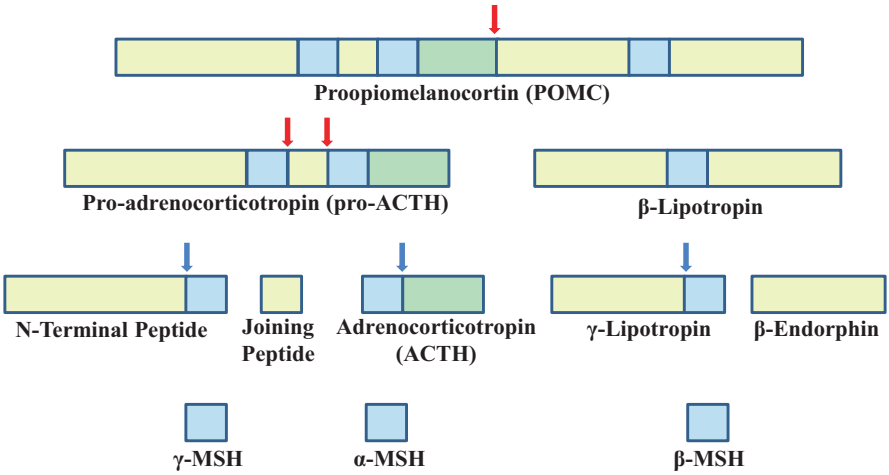

Fig. 12.2 Proopiomelanocortin (POMC): a preprohormone gets converted to β-Lipotropin and Pro-adrenocorticotropin (Pro-ACTH) which further gets cleaved into ACTH, joining peptide and amino-terminal peptide in the anterior pituitary. Cleavage of the joining peptide and the ACTH can produce γ-MSH and α-MSH, respectively while β-Lipotropin can be cleaved into γ-Lipotropin and β-Endorphin, respectively in the hypothalamus

CRH-mediated POMC gene transcription by acting on V1B receptor [28, 29]. Several pro-inflammatory cytokines like interleukin 1 (IL-1), IL-6, tumor necrosis factor (TNF)-α, and physical stresses can also induce POMC gene expression and hence ACTH secretion via CRH [30, 31].

The ACTH from the anterior pituitary stimulates the release of glucocorticoids (cortisol, a major glucocorticoid in humans, and corticosterone, a minor glucocorticoid in rodents and humans) [Fig. 12.3] [20] by activating G-protein-coupled, melanocortin-2 receptors (MC2R) on the adrenal cortical cells of the *zona fasciculata* (middle layer of adrenal cortex) [32] as well as the synthesis of all steroidogenic CYP enzymes (CYP11A1, CYP17A1, CYP21A2, CYP11B1) involved in glucocorticoid synthesis [33–35]. ACTH also stimulates the release of androgens like dehydroepiandrosterone (DHEA) and androstenedione from *zona reticularis* (innermost layer of adrenal cortex) of the adrenal cortex. Major mineralocorticoid like aldosterone and other minor ones are also secreted from the *zona glomerulosa* (outermost layer of adrenal cortex) that is regulated by the Renin-Angiotensin System (RAS) [12, 20] [Fig. 12.3].

12.4 Cortisol

Cortisol plays a major role in the body's response to stress by acting as a potent agonist of the glucocorticoid (type II) and the mineralocorticoid (type I) receptors located throughout the body prominently in the brain regions controlling the HPA

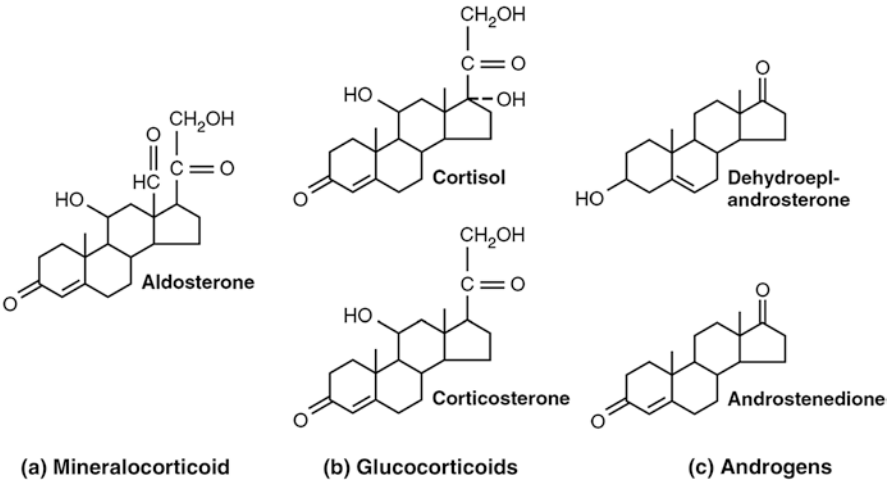

Fig. 12.3 Steroid hormones of the adrenal cortex; (**a**) mineralocorticoid (aldosterone); (**b**) glucocorticoids (stress hormones of the HPA axis; cortisol and corticosterone) and (**c**) adrenal androgens (dehydroepiandrosterone (DHEA) and androstenedione)

axis. The activation of these receptors mediates the tissue response of cortisol including the negative feedback of CRH and ACTH from hypothalamus and pituitary, respectively [36].

The average blood concentration of cortisol averages 12 μg/100 ml with an average secretory rate of 15 to 20 mg/day. However, the blood concentration of cortisol follows a diurnal pattern throughout the day, rising in the early morning (up to 20 μg/100 ml) and reaching its lowest level (up to 5 μg/100 ml) at about midnight or 3–5 h after the onset of sleep affected by the circadian rhythms [1, 12, 37]. The primary function of cortisol is to increase the blood sugar level through gluconeogenesis; to aid in the metabolism of fat, proteins, and carbohydrates; and to suppress the immune system [1, 38]. Cortisol stimulates liver and muscle glycogenolysis by activating glycogen phosphorylase mediated via epinephrine [39] and also delays glucose utilization by cells [1]. Elevated cortisol levels for longer duration lead to proteolysis, muscle wasting, and sometimes abnormal deposition of fat in the liver [40].

12.5 Dehydroepiandrosterone (DHEA) and Its Sulfate (DHEAS)

Dehydroepiandrosterone (DHEA also known as *androstenolone*), its sulfate (DHEAS), and *androstenedione* (C-19 steroids) are the major androgenic hormones that are secreted only in humans and few primates by the innermost layer of the adrenal cortex, the *zona reticularis*, in response to ACTH as well as *cortical androgen-stimulating hormone*, released from the pituitary. The average blood concentration of DHEA is 175 μg/100 ml with an average secretory rate of 20 mg/day

[1, 41–43]. The levels of these adrenal androgens are very low during the first year of life and are believed to be involved in the early development of male sex organs; however, their secretions begin to rise at the adrenarche, progressively till the third decade; after which it declines at 1–2% per year, remaining only 20–30% of their peak values till the seventh–eighth decade of life. During adrenarche, DHEA is known to impart androgenic characteristics like development of pubic and axillary hair growth, increased oiliness of hair and skin, mild acne, and adult-type body odor [44–46]. In spite of the interindividual variability, the DHEAS/DHEA ratio does not change with aging, and due to very low variations within subjects, they can be considered as highly specific individual markers [47–49]. DHEA and DHEAS are important adrenal precursor steroids that are converted to active androgens and/or estrogens in peripheral tissues. In males almost half of androgens are derived from the DHEA by several metabolizing enzymes, and in females almost 100% of active estrogens are derived from these steroids in the peripheral tissues and 75% near the menopause [12, 47, 50–52]. DHEA has very low affinity for the androgen receptors of testosterone and dehydrotestosterone (DHT) as well as estrogen receptor ERα; however, it has a high affinity for estrogen receptor ERβ [53–55]. DHEA acts as agonist of nuclear receptor like hepatic peroxisome proliferator-activated receptor alpha (PPARα) and modulate fatty acid metabolism as allosteric antagonists of GABAA receptor, thereby enhancing the neuronal and glial survival and improve learning/memory capacities [56, 57]. DHEAS is known to stimulate neuronal long-term potentiation and play a protective role against senescence [58].

12.6 The HPA Axis Dysregulation in Chronic Stress and Aging

Complex cross talk occurs between chronic stress and the aging phenomenon; while chronic physiological or psychological stresses accelerate aging, conversely aging causes stress dysregulation. Both chronic stress and aging have similar effects on the physiology of brain regions that regulate the HPA axis via neuroinflammation and altered neuronal metabolism. Numerous brain structures, like hippocampus, several discrete hypothalamic nuclei, prefrontal cortex, amygdala, and the bed nucleus of the *stria terminalis*, coordinate the physiological response to chronic stress [15]. Physiological stress stimuli, like severe pain; pleasant or unpleasant olfaction; altered concentrations of nutrients like glucose, electrolytes, and water; various hormones in the blood; and psychological stresses like anxiety, fear, or depression, ultimately excite or inhibit the various discrete hypothalamic nuclei which appear like the collecting center of information and thereby regulate the pituitary secretions that coordinate its response [1, 20]. Discrete areas of the brain are activated during stress, viz., amygdale during emotional response leading to the formation of emotional memories [59, 60]; hippocampus during learning processes, memory, and cognitive functions; and frontal cortex during planning and controlling actions [61]. Repeated stress mediates gradual decline in these command centers that further lead to hormonal dysregulation and ultimately appearance of the

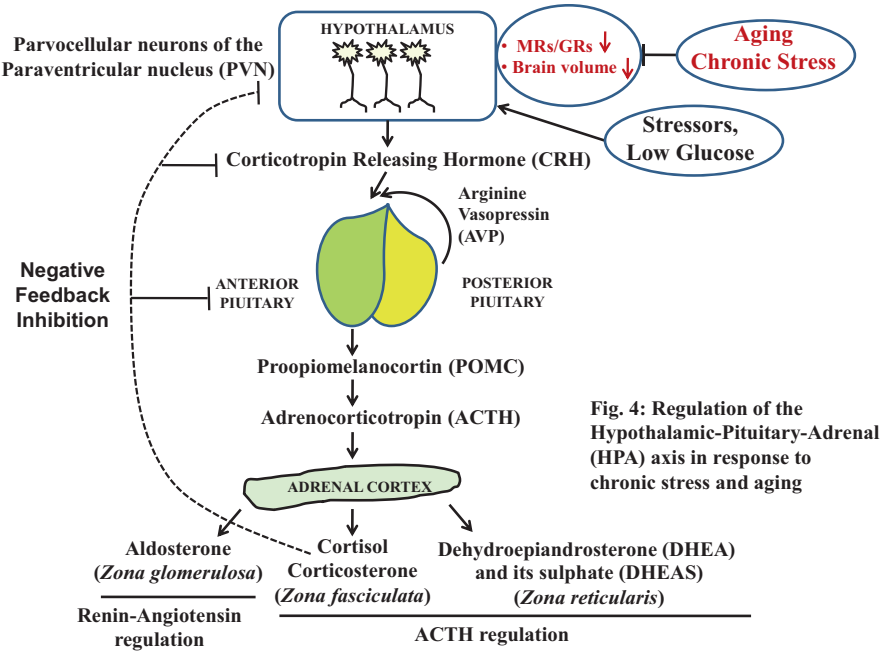

Fig. 4: Regulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis in response to chronic stress and aging

Fig. 12.4 Regulation of the hypothalamic-pituitary-adrenal (HPA) axis in response to chronic stress and aging. Physiological and psychological stresses ultimately stimulate the several discrete hypothalamic nuclei that regulate the pituitary secretions of POMC (and hence ACTH) which further induce the glucocorticoid (cortisol and corticosterone) and adrenal androgen secretions and thereby coordinate its response. After cessation of the stress stimuli, the glucocorticoids negatively regulate this response by feedback inhibition of the CRH and ACTH secretions from the hypothalamus and the pituitary, respectively. However, during aging or chronic stress, there is a clear dysregulation of the adrenocortical secretory patterns regulated by the ACTH, viz., marked increase in the nocturnal cortisol, upregulated 24 h cortisol secretion accompanied by clear flattening of the diurnal rhythm of cortisol secretion evident from both animal and human studies. Also, there is a clear dysregulation of the negative feedback inhibition of the GC secretion in chronic stress and aging due to substantial neuronal loss of the MRs and GRs and loss in the volumes of prefrontal cortex, hippocampus, and hypothalamus which are the key brain region controlling learning, memory, and cognitive function in the body. Besides, the androgenic hormones like DHEA/DHEAS secreted by the adrenal cortex in response to ACTH also undergoes marked depreciation in elderly subjects. Consequently, there is a marked increase in the cortisol/DHEAS molar ratio with physiological and pathological aging

symptoms of aging. Thus, aging begins with the senescence of the brain structures including the hypothalamic nuclei that behave like clock that regulates the rate at which the individual grows older. The effects of the two different kinds of steroids, glucocorticosteroids and androgens, are drastically opposite; while cortisol promotes neuronal death and degeneration, DHEAS plays protective role against neuronal impairment; hence the dysregulation in these two classes of steroids with aging and their manifestations are reviewed in this chapter [Fig. 12.4].

12.7 Cortisol Dysregulation

The hypothalamic-pituitary-adrenal (HPA) axis is one of the most important allostatic (adaptive) systems that respond and prepare an organism in the face of several stressful stimuli. Under normal physiological conditions, it is required for the mobilization of stored energy, maintenance of vasomotor tone, promoting behavioral adaptations, and regulating memory processes and is in turn regulated by a negative feedback inhibition to prevent further activation of the HPA axis as excessive or prolonged stimulation has many deleterious effects. The two major modulators of the HPA axis are the endogenous circadian rhythms and the acute and chronic stresses. Under a normal healthy person, the concentration of the terminal molecule of the HPA axis, i.e., cortisol, follows a diurnal pattern, rising in the early morning and reaching its lowest level at about midnight regulated by the circadian rhythms [1, 12, 37]. However, abnormally altered patterns of serum cortisol have been observed with psychological stresses like severe depression, anxiety, mood disorders, fear, etc. and physiological stressors such as hypoglycemia, illness, fever, any trauma, surgery, pain, sleep deprivation, physical exertion, infections, or temperature extremes [1, 62, 63]. The defect in the allostatic response of the HPA axis via feedback inhibition is also severely compromised during the aging process. Several studies have demonstrated that although a certain degree of circadian rhythmicity in the serum cortisol is maintained, there is an appreciable increase in the nocturnal serum cortisol levels with aging which is responsible for the significant flattening of the cortisol circadian profile in older subjects as compared with younger ones. Also, there is an overall increase in the 24 h serum cortisol levels leading to overexposure of the tissues to the deleterious effects of the heightened cortisol levels [47].

While stressors activate the HPA axis by their action on CRH and AVP leading to enhanced POMC (ACTH) and hence GC (cortisol and corticosterone) secretions, the GCs regulate their action through the glucocorticoid receptors (GRs) (type II) and mineralocorticoid receptors (MRs) (type I) located throughout the body and especially in brain regions that control their negative feedback inhibition like hippocampus, hypothalamus, etc. MRs are predominantly expressed in the limbic structures of the brain mainly hippocampus and some in hypothalamus [64, 65], whereas GR is more ubiquitously distributed in the brain mainly in parvocellular neurons of the hypothalamus [66–68]. Cortisol has a tenfold higher affinity for MRs than GRs; therefore binding of GCs to MRs plays an important role in negative feedback control and maintenance of basal HPA axis mainly during their lower night levels of the circadian rhythms [69–71], while binding of GCs to low affinity GRs plays an important role in feedback control of HPA axis during stress [72].

The response of GCs through MRs and GRs mainly occurs in two time domains: the "fast/membrane" and the "slow/genomic" effect. The "fast/membrane" mode of action occurs within minutes via non-genomic membrane-located low-affinity MRs and GRs leading to enhancement of glutamate in CA1 hippocampal neurons resulting in behavioral effects like aggressiveness in rats [73–77]. The "slow/genomic" mode of action occurs from minutes to hours even days via the intracellular high-affinity genomic receptors depending upon the duration of exposure to GCs [78–81].

12.7.1 Animal Studies

The feedback inhibition via GCs occurs by the inhibition of POMC gene transcription (hence, decreased ACTH secretion) in the anterior pituitary [82] and also by the inhibition of the CRH and AVP mRNA synthesis and secretion from the hypothalamus [83, 84] [Fig. 12.4]. There is reduction in the expression of GRs and MRs in the rat prefrontal cortex, hippocampus, and hypothalamus during aging and also the GC-mediated receptor translocation thereby resulting in reduced GC signaling and hence reduced/impaired negative feedback inhibition [85]. Global reduction in GR signaling in mice activates the HPA axis and leads to impaired cognition [86, 87], while forebrain-specific deletion of MRs leads to learning and memory impairments, whereas on the contrary, forebrain MR overexpression enhances memory and reduces anxiety [88–90]. Reduction of both type I and type II receptors in the hippocampus results in decreased inhibition of the PVN in the hypothalamus, resulting in attenuated feedback inhibition of the HPA axis in aged rodents [91–97]. Also, several studies have demonstrated that lesions in the hippocampus lead to enhanced GC production under acute stress condition due to aberrant feedback inhibition of the HPA axis [98–103].

Dexamethasone (DEX) a synthetic GC, 30 times more potent than cortisol, inhibits the HPA axis via negative feedback and decreases the level of endogenous cortisol. However, when it was injected in different brain regions of aged rats, it exhibited no suppressive response thereby indicating that negative feedback in aged rats is highly compromised as compared to younger ones [85, 104, 105].

12.7.2 Human Studies

The study of the HPA axis and its regulation in older humans is complicated because they generally suffer from multiple illnesses which might potentially alter the cytokine levels, viz., anxiety/depression, dementia, altered sleep patterns, obesity/weight loss, use of multiple medications, etc. However, in spite of the above variations, similar findings as compared to animal studies have emerged from several human studies which indicate an overall increase in the cortisol level and reduced resilience of the HPA axis with aging [47]. A significant nocturnal increase in the serum cortisol levels leading to clear flattening of the cortisol circadian profile [47, 106] and also an overall increase in the 24 h plasma-free and total cortisol levels in older subjects as compared with younger ones have been reported. This increase is correlated with increased GC production rate with no significant change in the cortisol-binding globulin (CBG) [107]. In aged individuals, the increased levels of early morning cortisol occurs earlier, thus exhibiting phase advancement [108, 109]. Also, a small and non-significant decrease in the plasma clearance rate (PCR) of cortisol occurs in aged humans [110, 111]. Salivary cortisol which is considered an important marker of free cortisol demonstrated an increased level at different times of the circadian profile with age indicating an increase in basal HPA activity with age [112].

A significant relationship between increasing cortisol over a period of 4 years and impairment of explicit memory and attention performances has been explained in a longitudinal study [113]. Along with impaired memory, 14% reduction in the hippocampal volume has also been reported in a study [114]. Administration of selective MR antagonist canreonate, although demonstrated an increased nocturnal HPA axis of the circadian rhythms in older subjects, was markedly less as compared to younger ones probably due to age-related MR impairments [115]. Alternatively, when MR agonists like fludrocortisone (FC) were used, significant inhibition of nocturnal cortisol levels of the circadian rhythms was demonstrated in younger subjects, while only minor reduction in HPA activity was seen in elderly subjects [116–118]. Thus, similar to animal studies, age-related impairment of the central MRs seems to be the causal factor leading to less responsive HPA axis.

Frailty is a pre-disability state where an older person, who is functioning normally when exposed to stresses, tends to have decreased ability to maintain their normal activities [119, 120]. As per Fried, frailty burden consisted of increased weight loss, exhaustion, decreased grip strength, decreased walking speed, and low physical activity [121]. In Women's Health and Aging Study, both evening and 24 h salivary cortisol were positively correlated with frailty burden [122]. Holanda et al. also reported smaller circadian variation and increased salivary cortisol in frail persons [123]. Several other studies have also reported a correlation between reductions in diurnal cortisol variability and increased frailty burden in older individuals marked by poor performance, viz., slow walking speed, standing balance test, handgrip performance, etc. [124–127].

12.8 DHEA/DHEAS Dysregulation

DHEA and DHEAS are one of the most abundant circulating steroids in humans, and in addition to their secretion from the *zona reticularis* of the adrenal cortex, they are also secreted by the gonads as well as the brain, therefore sometimes also referred to as "neurosteroids." Several studies have very clearly demonstrated an age-associated decline in the levels of circulating DHEA/DHEAS (the C-19 steroids) and especially in aged-demented persons, and since they are the metabolic precursors of the androgens and estrogens in males and females, respectively, hence a decline in their level has several deleterious effects associated with unsuccessful aging [47, 120, 128–130]. Older individuals exhibited overall decreased plasma and salivary DHEA levels, and with increasing age, the DHEA area under the curve was attenuated, and the decline became conspicuous [131, 132]. In fact, the decline in the DHEA levels has been morphologically correlated with the clear width reduction of the *zona reticularis* of the adrenal cortex with age [133]. In contrast to the cortisol secretion, which is normally maintained with age, there is significant impairment in DHEA/DHEAS secretion in response to exogenous ACTH, which indicates toward a probable unexplained defect in the cytochrome P450c17 desmolase activity in the *zona reticularis* of the adrenal cortex which converts cholesterol into pregnenolone, a rate-limiting step in DHEA (or any steroid) biosynthesis [134, 135]. Several studies have

demonstrated a small decline in the pregnenolone secretion with aging in both men and women and a marked decline in pregnenolone sulfate secretion after 60 years of age [49, 128, 130, 136, 137]. Additionally, the molar ratio of cortisol/DHEAS throughout the 24 h cycle was prominently higher in older individuals, especially if demented as compared to younger individuals. This ratio was further heightened at night time of the circadian rhythm in old-demented individuals indicating a deeper neurotoxic effect of the HPA axis dysregulation [47].

DHEA and DHEAS are prohormones with pleiotropic effects and are associated with the development and progression of degenerative disorders whose exact mechanism of action remains unclear [47]. However, recent evidences suggest that their mode of action involves several membrane-bound, cytosolic/nuclear hormone receptors and also receptors on the endoplasmic reticulum. In bovine aortic and primary human umbilical vein endothelial cells (HUVECs), through specific, unidentified G-protein-coupled receptors, DHEA activates the endothelial NO synthetase (eNOS) (eNOS/cGMP pathway) and increases the production of nitric oxide (NO), a key modulator of vascular function, by endothelial cells [138–141].

DHEA and its metabolites bind/activate a number of nuclear receptors like pregnane X receptor, androstanol receptor (constitutive), estrogen receptor-β, and peroxisome proliferator-activated receptors [142–145]. By activating the hepatic peroxisome proliferator-activated receptor alpha (PPAR alpha), DHEAS inhibits the activation of nuclear factor-κB and the secretion of interleukin-6 and interleukin-12, thereby exhibiting its anticarcinogenic and chemoprotective effects by modulating fatty acid metabolism and expression of peroxisomal enzymes [146–148]. DHEA is believed to be an agonist of sigma-1 receptor (sigma-1R) expressed on the endoplasmic reticulum of the heart, liver, kidney, and brain; and together with the Akt/eNOS signaling pathway, exerts vasculo-protective effects and improve cardiac functions [149–153].

DHEA exhibits potent antioxidant effects by inhibiting glucose-6-phosphate dehydrogenase and NADPH production which results in reduced oxygen free radical production via NADPH oxidase [154, 155] since chronic oxidative stress induces inflammation and plays critical role in the development of cancer, atherosclerosis, and Alzheimer's disease which are the hallmarks of the aging process [156–159].

Low levels of DHEA/DHEAS have been associated with host age-associated pathologies, viz., sexual dysfunction, mood defects, poor sense of well-being [160, 161], poor muscle strength [162, 163], poor mobility [164], frailty [165, 166], insulin resistance, obesity, and cardiovascular diseases [167, 168]. Better health and well-being are associated with higher levels of DHEAS [169], and rodent studies have also indicated improved immune functions and prevention from atherosclerosis, cancer, diabetes, and obesity [170]. However, current clinical modalities do not comply with evidence-based medicine, and further detailed clinical studies are required that can help us to better understand the clinical utility of DHEA in the management of age-related disorders.

Both human and experimental studies have indicated a considerable decrease in the prefrontal cortical as well as small decrease in the hippocampal and amygdalar volumes with aging accompanied by neuronal loss due to deleterious effects of

neurotransmitter imbalance, excitatory amino acids, adrenal steroids, etc. Also there is progressive enlargement of ventricular size indicating cerebral atrophy and volume loss. These reductions in the size of the different brain regions with aging are positively correlated with the nocturnal rise in serum cortisol as well as decline in the mean serum DHEAS concentration. Recent studies have also indicated that the prefrontal cortex is less able to constrain the HPA axis response to stress indicating a deterioration in the brain network that regulates the stress response [171–175].

12.9 Conclusion

The phenomenons of chronic physiological or psychological stress and aging have markedly similar effects on the regulation and control of the brain regions that control the HPA axis. While chronic stresses promote the aging process, the aging process itself complicates and dysregulates the stress response mediated by the HPA axis. During the aging process, there is clear distinction in the adrenocortical secretory patterns regulated by the ACTH, viz., marked increase in the nocturnal cortisol as well as upregulated 24 h cortisol secretion accompanied by clear flattening of the diurnal rhythm of cortisol secretion as evident from both animal and human studies. Also, there is a clear dysregulation of the negative feedback inhibition of the GC secretion in chronic stress and aging. Besides, the androgenic hormones like DHEA/DHEAS secreted by the adrenal cortex in response to ACTH also undergo marked depreciation in elderly subjects probably due to impaired cytochrome P450c17 desmolase activity and reduced pregnenolone secretion. Consequently, there is a marked increase in the cortisol/DHEAS molar ratio with physiological and pathological aging. It is well evident that both types of steroids, i.e., GCs and adrenal androgens, have markedly contrasting effects on the physiology of the brain; while GCs promote neuronal cell death and degeneration, adrenal androgens promote neuronal long-term potentiation and prevent functional impairment. During chronic stress or aging, the cumulative effect of the enhanced cortisol exposure promotes cell death and degeneration, and due to decreasing levels of the DHEAS, the protective and antiglucocorticoid effect is lost. This is evident from the substantial neuronal loss of the MRs and GRs and loss in the volumes of prefrontal cortex, hippocampus, and hypothalamus which are the key brain regions controlling learning, memory, and cognitive function in the body. Also, there is an age-related increase in the expression of 5-lipoxygenase (5-LOX) prominently in hippocampal and cerebellar neurons, stimulated by GCs and involved in the synthesis of inflammatory leukotrienes leading to neurodegeneration [176, 177].

The “glucocorticoid cascade hypothesis,” proposed by Sapolsky et al. in 1986 for aged mice, explains that exposure to GCs during stress temporarily downregulates GRs in hippocampus which is self-correcting initially; however chronic exposure leads to permanent loss of the GRs and impairment in the negative feedback inhibition. However, this hypothesis was not replicated in other animal and human studies [178]. Similarly, “glucocorticoid vulnerability hypothesis” states that high GCs during chronic stress leave an imprint on the hippocampus and prime the aging brain to

future metabolic stresses, but more studies are needed to prove this hypothesis [179]. In spite of all these hypotheses, the fact remains that chronic stress promotes or accelerates the aging mechanism prominently by dysregulation of the HPA axis leading to increased frailty and cognitive decline in aged individuals, and the aging process complicates the stress regulation by decreased resilience of the HPA axis. Hence, stress and aging which are mutually exclusive in the young age gradually become mutually complimentary with advancing age.

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Sex Steroids, Cognate Receptors, and Aging

13

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Abstract

Aging signals a gradual deregulation of physiological homeostasis. Steroid hormone actions are an important contributor to this deregulation because of their key involvements in the growth, metabolism, survival, and functional vigor of cells, tissues, and organs. Accumulated evidences show that aging is associated with reduced circulating levels of male and female sex hormones, i.e., androgens and estrogens, respectively, which in turn alter physiological milieu and lead to specific deficits in the organismal vitality. While a number of articles in the literature have provided a generalized description of age-related decline of physiological control mechanisms, in the current chapter, we have focused specifically on the role of sex hormones and sex steroid receptors in age-related bodily dysfunctions. Various segments of our article delved into the current understanding

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on influences of sex steroids and steroid receptors. Specifically, the roles of androgens, estrogens, and cognate sex steroid receptors in age-accompanied physiological and pathophysiological changes in gene expression and organ functions are discussed. In addition to citing our own studies, information from diverse fields of biology and medicine is taken into consideration in order to present a comprehensive view of sex steroid action with advancing age.

Keywords
Aging · Androgens · Estrogens · Steroid receptors

13.1 Introduction

Aging is a multidimensional process marked by progressive loss of cell and tissue functions, making organisms less fit for reproduction and survival [1]. A steady decline in bodily functions during aging is initially associated with a failure to establish homeostasis in response to stress. Thereafter, functional deficits set in even under rested, non-stressed conditions, thereby compromising workings of all vital organs at old age. Many countries in the twenty-first century will experience a large demographic shift toward the age 65+ population as a result of significantly increased longevity afforded by the progress in medical sciences and healthcare management. This shift in demography requires new measures that ensure less stressful aging with a goal to achieve reduced disease burden and improved cognitive and physical fitness along with active engagement in everyday life. Steroid hormones, which are important regulators of human metabolism, are likely to play a key role in these improvements [2]. The interplay between steroid hormones and aging is complex – the aging process impacting steroid hormone biosynthesis, while steroid hormones influencing aging at the molecular level. Elucidation of this interplay may uncover new therapeutics and novel approaches that afford prolonged health span in conjunction with extended life span.

13.2 Steroid Hormone Biosynthesis

Steroid hormones are lipid-soluble, low-molecular-weight compounds that are synthesized from cholesterol ([3], Fig. 13.1).

Steroid hormones are usually made by steroidogenic glands including the ovary, testis, and adrenals and by the placenta during pregnancy and then released into the bloodstream [3]. They are classified into three categories based on their physiological functions: the sex steroids, glucocorticoids, and mineralocorticoids. The sex steroids include androgens and estrogens. The Leydig cells of testis are the primary sites of synthesis of the principal male hormone, testosterone [5]. Three major sex steroids are also secreted by the ovaries, viz., the estrogens, androgens, and progestin [6]. The adrenal cortex produces three different classes of steroid hormones, namely,

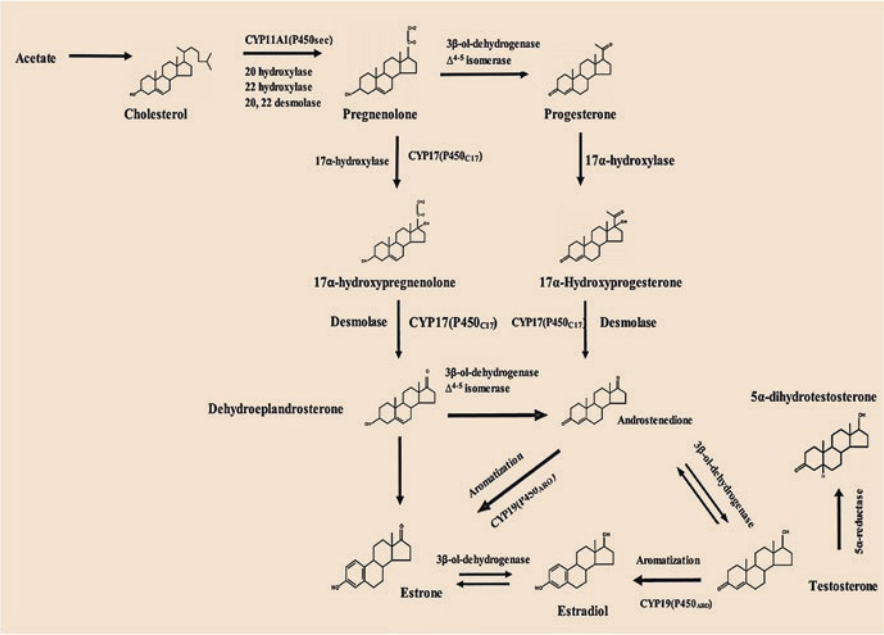

Fig. 13.1 Steroid hormone biosynthesis (gonadal cycle). A simplified pathway for steroid hormone biosynthesis is depicted. Key enzymes involved in this process are shown above the arrows indicating specific enzymatic reactions. (Modified from [3, 4])

glucocorticoids (cortisol and corticosterone), mineralocorticoids (aldosterone and deoxycorticosterone), and dehydroepiandrosterone (DHEA) and androstenedione, the precursors for the sex steroids androgens and estrogens. The zona glomerulosa zone produces the mineralocorticoids, whereas the zona fasciculata in the adrenal cortex produces the glucocorticoids. The zona reticularis of the adrenal cortex primarily produces the adrenal androgens [7]. The principal estrogen, 17β-estradiol, is secreted by the theca cells in ovary, which also secretes androgens such as androstenedione, DHEA, testosterone, and dihydrotestosterone. Androstenedione, the ovarian androgen, is synthesized by the theca cells and then transported to granulosa cells for estrogen synthesis. These cells also produce major progestins such as pregnenolone, progesterone, and 17-hydroxyprogesterone. Of these, pregnenolone is used as a precursor for the synthesis of all the steroid hormones (Fig. 13.1). The corpus luteum secretes progesterone and allows the fertilized ovum to be implanted for the maintenance of pregnancy, in the first 6–8 weeks of gestation. During pregnancy, placenta is the primary producer of progesterone [6].

Testosterone, a major androgen in circulation, is produced by Leydig cells of the testes [3]. The 5α-reduced testosterone, i.e., 5α-dihydrotestosterone (5α-DHT), is the active androgen in many androgen-targeted tissues including the prostate and liver. Androgens are also produced by ovaries in limited quantities. The androgens synthesized by the ovaries include DHEA, androstenedione,

Table 13.1 Major steroid hormones and their functions

Hormone(s)	Functions of steroid hormones
Progesterone (progestin)	Promotes the implantation of ovum and prepares mammary glands for lactation
Cortisol (glucocorticoid)	Controls metabolism; encourages gluconeogenesis; causes breakdown of fat and protein; produces anti-inflammatory actions; protects against stress; lowers immune responses; causes hypertensive actions; and maintains blood pressure
Aldosterone (mineralocorticoid)	Important for water and electrolyte balance. Increases reabsorption of sodium and maintains blood volume and blood pressure
DHEA and DHEA-S	Dehydroepiandrosterone (DHEA) sulfate affects many important physiological systems including aging, immunity, and development. Act as an important source of sex hormones, i.e., testosterone and estrogen in peripheral tissues
Testosterone (androgen)	Primary male sex hormone that promotes secondary male sex characteristics. It promotes sperm production and prevents bone resorption
Estradiol (estrogen)	Primary female sex hormone that regulates the female secondary sex characteristics. It controls estrous and menstrual cycle in females

Modified from [3]

testosterone, and dihydrotestosterone. Estrogen, the steroid hormone mostly associated with female-specific phenotype, is secreted primarily by the ovaries [8] and the placenta. It is also produced in lesser quantities by steroidogenic conversion, in the testes of men [8]. The amount of estrogen in women is approximately four times that of men [9]. Indeed, estrogens cause the development of female genital organs, endometrium growth, and inhibition of follicle-stimulating hormone secretion by the pituitary gland.

The hypothalamic gonadotropin-releasing hormone (GnRH) stimulates both synthesis and pulsatile release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the anterior pituitary [10]. FSH is required for sperm production; LH is needed for testosterone secretion. Major functions of steroid hormones are tabulated in Table 13.1.

13.3 Sex Steroid Receptors: Their Nuclear Localization

The sex hormones androgen and estrogen, being lipophilic, enter target cells by passive diffusion through the lipid bilayer membrane of cell. The full-length receptor to androgen, i.e., androgen receptor (AR), remains in the cytoplasmic compartment as an inactive protein in the absence of the hormone. The androgen-bound AR transitions to an active state, which enables the receptor to translocate to the nucleus and function as the mediator of androgen-responsive nuclear signaling [11, 12]. Using cells with the green fluorescent protein-labeled AR (GFP-AR), our study provided the initial evidence for the predominantly cytoplasmic residency of AR in the absence of androgen and its nuclear import to distinct foci when the cells were

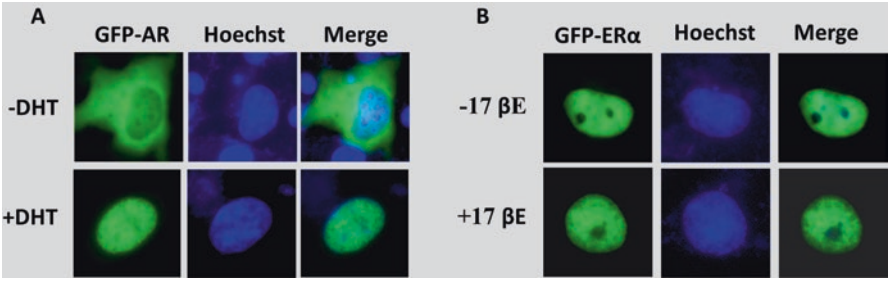

Fig. 13.2 Influence of sex steroids on receptor localization. (**a**) Androgen-dependent nuclear translocation and formation of nuclear foci of GFP-AR in transiently transfected cells. (**b**) 17β-estradiol (17βE)-dependent formation of nuclear foci of GFP-ERα. COS-1 cells, transfected with either 500ng of GFP-AR or GFP-ERα, were cultured in steroid-free medium for studying protein expression and localization. After 24 h of expression period, the cells were treated with DMSO:ethanol(1:1), or 10^{-8}M DHT or 10^{-8}M 17βE. After 2 h of DHT or 17βE treatment, cells were observed under a fluorescence microscope. Nuclear dye Hoechst was used to visualize the nuclei

exposed to the active male sex steroid 5α-DHT (Fig. 13.2; [13]). Conversion of AR from an inactive to active state and its role as a ligand-inducible transcription factor will be further elaborated in Sect. 13.4.

In contrast to AR, ERα is predominantly a nuclear protein in the absence of estrogen (17β-estradiol), and upon treatment of cells with estrogen, ERα localizes to distinct nuclear foci, which likely indicates hormone-dependent transition of the estrogen receptor to a transcriptionally competent active form (Fig. 13.2; [14]).

13.4 Androgen Receptor (AR), Androgen Action, and Aging

Diverse physiology, encompassing reproductive and nonreproductive processes, is regulated by the androgen receptor (AR), which is a ligand-inducible transcription factor and the initial conduit for transmitting androgen signaling to the transcriptional apparatus in the nucleus. Similar to other members of the nuclear receptor superfamily, AR has a modular primary structure, with each module representing a distinct functional unit [15–17]. In the absence of androgen (or other AR agonists/antagonists), the cytoplasmic AR remains sequestered as a multiprotein complex with protein partners that include molecular chaperones like HSP70, HSP90, HSP40, and co-chaperones like immunophilins (forskolin-binding proteins) and p23 [18, 19]. The ligand-bound AR, upon release from the multiprotein complex, undergoes a conformational rearrangement, which exposes its nuclear localization signal (NLS) for binding to importins, allowing nuclear translocation of the receptor (Fig. 13.2). Androgen-induced nuclear import of AR in living cells and its subsequent multiple rounds of nucleocytoplasmic shuttling was first reported in 2000 by us, using green fluorescent protein-labeled AR [13].

The transcriptionally active AR accumulates within microscopically visualized nuclear foci, which are thought to be the sites where AR interacts with target genes

via androgen-responsive DNA elements. These foci are also the convergence nodes for coregulators and other components of the transcriptional apparatus [20]. The pioneer transcription factor FOXA1, bound at AR-driven regulatory regions, facilitates AR binding to cognate elements. However, examples are also known where AR, rather than directly binding to an androgen-responsive element, is tethered to a second DNA-bound transcription factor. Coregulators, which associate with AR, relay signals to RNA polymerase II and the basal transcriptional machinery via a multiprotein mediator complex. The p160 coactivators (SRC-1/SRC-2/SRC-3), which physically associate with AR, generate a surface for assembling various classes of coregulators such as histone-modifying enzymes (e.g., acetyltransferases/deacetylases, methylases/demethylases, kinases/phosphatases, ubiquitinases/deubiquitinases), chromatin-remodeling proteins (such as SWI/SNF, INO80, ISWI, and CHD complexes, chromodomain proteins, bromodomain, and extra-terminal (BET) family proteins). Long noncoding RNAs (lncRNAs), such as enhancer RNAs (eRNAs) and other types of lncRNAs (e.g., HOTAIR), are essential components of a coregulator complex. These entities coordinate gene induction or repression by AR [21–23].

Androgen/AR signaling has also transcription-independent roles in cellular functions. Non-genomic AR signaling can initiate at cell surface via membrane androgen receptor (mAR) and/or in the cytoplasm. The response occurs rapidly (within seconds to minutes) in the presence of inhibitors of transcription and translation, and it regulates several pathways including MAPK/ERK, PKA, FAK, p38, p53, calcium, and zinc [24, 25]. The membrane-located AR (mAR) is distinct from the cytoplasmic/nuclear AR described above. Cross coupling of genomic and non-genomic androgen signaling may influence cellular proliferation, survival, and apoptosis [26]. In another example, AR associates with telomeres and plays a role in telomere stability independent of its transcription function [27]. Telomeres, which cap each end of a chromosome as repeats of a core nucleotide sequence, are essential for genomic stability. Without telomere repeats, each round of DNA replication would shorten the functional nucleotide sequences at chromosomal ends. Cells will eventually recognize shortened chromosomal ends as DNA damages and trigger chromosomal degradation.

13.4.1 AR Activity in the Liver and Its Age-Dependent Regulation

Normal liver functions and several liver diseases are influenced by AR activity [28]. For example, hepatic glucose and lipid metabolism are deregulated by liver-specific ablation of AR in mice [29]. AR regulates hepatic steroid, drug, and nutrient metabolism, as evident from the AR-mediated transcriptional regulation of certain cytochrome P450 phase I enzymes and the phase II sulfotransferase SULT2A1; additionally, the liver abundance of these enzymes shows gender differences [30–32]. AR confers a protective influence against high-fat diet-induced NAFLD (non-alcoholic fatty liver disease), since liver-specific loss of AR in a mouse model led to insulin insensitivity and type 2 diabetes [28]. Testicular feminized (Tfm) mice,

which lack functional AR, were resistant to liver cancer upon carcinogen exposure, which revealed a role for AR in liver cancer [33]. Notably, in a yin-yang relationship, AR promoted initiation of hepatocellular carcinoma (HCC) in mice but suppressed HCC metastasis [34]. Sorafenib, a multiple kinase inhibitor against HCC, was more effective in inhibiting HCC progression in the presence of functional AR in a preclinical HCC metastasis model [34]. Finally, in the context of normal aging, AR-ablated, not AR-intact, male livers developed microvesicle steatosis at advanced age in mice [29].

During physiological aging, male rodents show a gradual reduction in hepatic AR expression, and AR protein levels are undetectable beyond 20–24 months of age (equivalent to an ~80-year-old human) (Fig. 13.3, [35]). Loss of hepatic AR mRNAs parallels the loss of AR protein in old rats [35, 36]. Dietary calorie restriction, a proven means for extending invertebrate and vertebrate life spans and retarding age-related diseases, prevented AR loss and restored androgen sensitivity of the aging rat liver [36–38]. Age-associated steady increase in the liver expression of SULT2A1 sulfotransferase (a phase II sulfate-conjugating enzyme for bile acid) and DHEA (dehydroepiandrosterone) is a consequence of the relief of the *SULT2A1* gene from AR-mediated repression [30]. Consistent with this repression, calorie restriction prevented age-associated rise of SULT2A1 levels in the aging male rat liver (Fig. 13.4, [37]).

At the molecular level, positive and negative changes in specific transcription factor activities are linked to the loss of hepatic AR during aging. Roy, Chatterjee, and colleagues conducted detailed investigations of the underlying mechanisms for these changes [35, 39, 40]. The NF-κB transcription factor is a negative regulator of human and rat AR gene transcription [40, 41]. This negative regulation is consistent

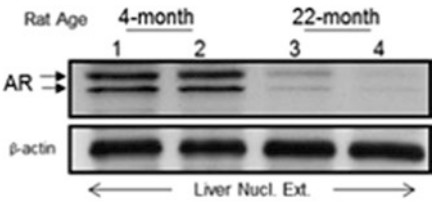

Fig. 13.3 Loss of AR in the livers of male rats during aging. Arrows in the Western blot indicate positions of AR and phosphorylated AR [35]

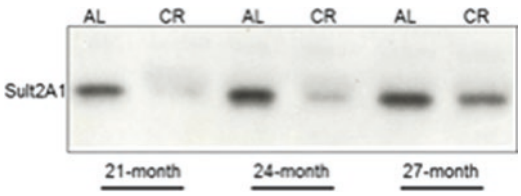

Fig. 13.4 Loss of SULT2A1 in livers of old male rats by calorie restriction (CR). Liver lysates from ad libitum (AL)-fed rats (21-, 24-, 27-month-old) had high SULT2A1 levels [37]

with the findings that the NF-κB activity in hepatic and extrahepatic tissues is elevated during aging in response to chronic inflammation and oxidative stress, which are hallmarks of physiological aging [40, 42]. In contrast, an age-dependent factor (ADF) gradually declines in activity during aging [39]. ADF can stimulate AR promoter activity by inducing a 20-base pair DNA element (ADF element) located in an upstream region of the rat AR promoter [35, 39]. The nuclear ADF activity was detected in hepatic and non-hepatic cells. AR gene repression is partly a consequence of the replacement of a PARP-1-associated, p/CAF-containing coactivator assembly at the ADF element by a corepressor complex, which associates with the p53 tumor suppressor protein. Beyond its classic role in DNA repair, PARP-1, i.e., poly (ADP-ribose) polymerase-1, can coactivate transcription factors [43], and p/CAF, a histone acetyltransferase, is a core component of coactivator complexes in many contexts. The corepressors mSin3A and Groucho/TLE-1 are part of the corepressor complex. They help stabilizing the corepressor complex due to association with p53. Age-associated switch from a coactivator to corepressor assembly at the ADF element is coordinated by the B-Myb and c-Myb transcription factors, which directly bind to the 20-base pair ADF DNA element. As core components of the ADF activity, B-Myb and c-Myb associate with PARP-1 (in the context of coactivator assembly), or p53 (in the context of corepressor assembly). The heterogeneous ribonucleoprotein K (hnRNPK), which associates with PARP-1, serves as a platform for the convergence of coregulators. The B-Myb level in the rat liver is markedly reduced during aging, and B-Myb is undetectable at the ADF-regulated chromatin region. In contrast, irrespective of age, c-Myb continues to occupy this region. Figure 13.5 depicts a model showing coregulator dynamics at the ADF-regulated AR promoter in young vs. old liver.

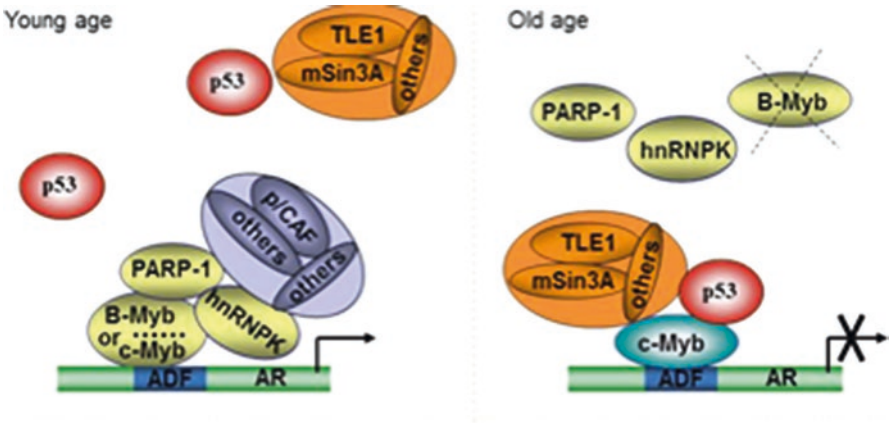

Fig. 13.5 Schema for AR gene regulation by ADF during aging. ADF-bound B-Myb/c-Myb and a coactivator complex, which contains PARP-1, hnRNPK, and p/CAF, mediate AR gene induction at young age. AR gene repression at old age is coordinated at the ADF site by c-Myb bound to a p53-associated corepressor complex, which contains Groucho/TLE1 and mSin3A corepressors. (Modified from [35])

Similar to aging, AR gene repression due to oxidative stress involves recruitment of p53, Groucho/TLE-1, and mSin3A to the ADF-regulated chromatin in exchange for the departure of PARP-1, hnRNPK, and p/CAF factors that occupy this region under normoxia. Unlike aging, however, the cellular abundance for B-Myb is not altered by oxidative stress, so that p53 is anchored to the regulated region by association with both B-Myb and c-Myb [35].

We speculate that AR gene suppression in old animals is triggered by the age-associated decline in PARP-1 activity. The intracellular level of NAD+ (oxidized nicotinamide-adenine dinucleotide), a cofactor for PARP-1, declines steadily during aging [44–46], which in turn reduces PARP-1 activity [46]. The reduced NAD+ level is detrimental to mitochondrial homeostasis and to the overall metabolic vitality of an organism. Restoration of NAD+ levels can reverse this decline and delay aging, leading to health span and life span extension [44, 46].

13.4.2 Androgen Dependence of Normal Prostate and Prostatic Diseases of Old Age

AR levels in the rat prostate were found to decline during aging [47]. Androgen action plays an obligatory role in the development and function of the prostate, which is an exocrine gland in adult males. Congenital defects for functional AR or 5-alpha-reductase in genetic XY males cause incomplete development or complete lack of this secondary reproductive organ. The alveolar-ductal structures of the prostate are embedded in a fibromuscular stroma, with the basement membrane providing the stroma-epithelia barrier. Androgen action in the stromal tissue is essential for prostate development [48]. AR-expressing luminal epithelial cells produce a multitude of proteins, including the prostate-specific antigen (PSA), which secrete into the ductal lumen. On the other hand, the basal epithelium contains AR-negative epithelial cells, neuroendocrine-type cells, as well as macrophages and lymphocytes. Embryonic development of prostate begins with the androgen-stimulated synthesis and secretion of various growth factors from AR-expressing stromal fibroblasts and fibromyoblasts. Glandular development subsequent to epithelial cell growth is in turn induced by the paracrine action of stroma-derived secreted factors. In adult prostate, direct androgen action on AR-positive luminal epithelial cells preserves glandular integrity. In castrated rodents, ~ 90% of luminal epithelial cells are lost due to apoptosis. Androgen replenishment restores prostate structure, when stem cell-like basal cells differentiate into AR-negative basal epithelial cells, which progress to mature, AR-positive luminal cells [49].

During aging, accumulated pathologic assaults from insults including chronic inflammation, oxidative stress, and genetic changes (oncogene activation, tumor suppressor inactivation) disrupt the homeostasis between proliferation and apoptosis and lead to prostate cell hyperstimulation that culminates in either prostate hypertrophy, which clinically manifests as lower urinary tract symptoms (LUTS) and benign prostate hyperplasia (BPH), or hyperplasia and dysplasia of prostate acini that progress to adenocarcinoma, viz., prostate cancer. Mechanisms that distinguish the path to noncancer prostate enlargement from malignant progression remain unknown.

The most prevalent prostate disease for aging men is BPH/LUTS, which usually begins from the fifth decade of life. BPH, manifesting as new glandular or stromal growth at the transition zone surrounding the upper portion of the prostatic urethra, causes disruptive urinary symptoms [50]. The disease is initially a quality of life problem; however, left untreated, the symptoms can grow into serious complications such as bladder and kidney damage, inability to urinate and urinary tract infections. Signs of BPH/LUTS begin in nearly half of all 50-year-old men, and by age 80, ~80% of all men have BPH. AR/androgen signaling is closely linked to BPH etiology. For example, eunuchs (castrated men) do not develop BPH, and 5-α reductase inhibitors (such as finasteride, dutasteride), which block testosterone conversion to the prostate-active potent androgen, viz., 5α-dihydrotestosterone (DHT), are effective in alleviating clinical symptoms of BPH in many (but not all) patients. Cellular senescence, which irreversibly blocks senescent cells at the G1/S cell cycle checkpoint, is thought to contribute to BPH etiology [51]. Normally, cellular senescence is a tumor suppression mechanism that prevents proliferation of the cells that are damaged by various insults such as oxidative stress arising from cell's normal metabolic activities, chromosomal instability due to shortened telomeres in replicating cells, and DNA damage from exposure to chemicals or ionizing radiation [52]. In a contrarian response, a senescence-associated secretory phenotype (SASP) of senescent cells, characterized by the secretion of pro-inflammatory cytokines, chemokines, growth factors, and certain proteases, generates a tissue microenvironment which promotes enhanced proliferation of nearby cells and recruitment of inflammatory cells, both leading to hyperplastic prostate growth. Prostate-originated epithelial cells proliferated faster in the presence of conditioned media from the prostatic fibroblasts of elderly donors (ages 63–81 years) compared to younger donors (ages 40–51 years) [53]. The molecular basis for BPH development is under active investigation.

Precancerous prostatic intraepithelial neoplasia (PIN), which over time can emerge as adenocarcinoma, is histologically distinct from BPH. The risk for prostate cancer increases with age. The median age of men at diagnosis of the cancer is >75 years. Prostate cancer in its early stage is androgen-dependent. Androgen deprivation therapy (ADT) involving medical or surgical castration is the standard of care for managing the disseminated disease when remission occurs due to cancer cell apoptosis. However, post-ADT relapse is almost a total certainty, and for about 70% cases, the re-emerged disease progresses to a therapy-resistant terminal stage within an average of 20 months [54]. Recurrent cancer, which arises in a castrated background, still depends on AR/androgen signaling for tumor growth. Sustained AR levels and elevated intratumoral de novo androgen biosynthesis in post-ADT patients have been extensively documented [55]. Second-generation AR antagonists (e.g., enzalutamide) and androgen biosynthesis blockers (e.g., abiraterone) are used to control post-ADT and post- (or pre-)chemotherapy tumor growth, although responses are non-durable (lasting 4–5 months on average) [56, 57]. An example of AR expression in nontumor and tumor tissue in a clinical specimen of primary prostate cancer is shown in Fig. 13.6.

In summary, using the prostate and liver as examples, this section highlights a role for androgen/AR signaling in the physiology and pathophysiology of reproductive and nonreproductive tissues and a role of aging in AR signaling.

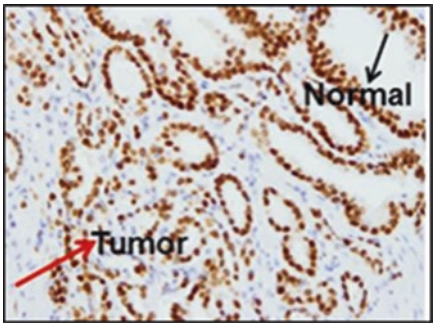

Fig. 13.6 AR-expressing prostate tumor and adjacent normal prostate acini of a primary human prostate cancer specimen. AR was detected in a paraffin section cut from a formalin-fixed prostatectomy sample by immunohistochemistry using a polyclonal rabbit antibody against human AR. Horseradish peroxidase conjugated anti-rabbit IgG and diaminobenzidine was used for staining. Magnification: 4× (B. Chatterjee, unpublished)

13.5 Androgen, Estrogen, and Aging

During aging, circulating androgen and estrogen levels decline in males and females, respectively (Fig. 13.7). Androgen deficiency in aging males (ADAM) is a common observation. Declining estrogen levels in females are apparent in the perimenopausal phase (by 36–45 years), and thereafter, due to cessation of ovarian function, estrogen levels fall precipitously in menopausal women (46–55 years of age) [4]. Physiological parameters such as muscle mass, bone strength, cardiovascular health and cognitive competency decline concurrently with reduced sex hormone levels [10]. Since women tend to live longer, they are more vulnerable to age-related health problems.

13.5.1 Testosterone in Aging Males

Androgens promote diverse male physiology including spermatogenesis, muscle mass, bone health, hair growth, nitrogen retention, and development of secondary sexual characteristics [58]. Testosterone levels, 95% of which are produced by testicular Leydig cells, gradually decline in aging males [59], whereas peripheral estrogen (as estrone) steadily rises [60]. The net effect is a reduced testosterone to estrogen ratio that leads to diminished musculature in men [61]. Studies with brown Norway rats, whose aging of the reproductive tract is similar to that of humans, showed that testosterone biosynthesis is lower in 23-week-old than 13-week-old rats. The age-related decline in testosterone production is thought to be due to accumulation of redox particles or other toxic materials that are byproducts of steroidogenesis [62, 63].

Reduction of testosterone in aging men has been documented in multiple ways: (i) taking samples directly from the spermatic vein in older men, (ii) meta-analysis of cross-sectional samples, and (iii) longitudinal investigations performed in healthy

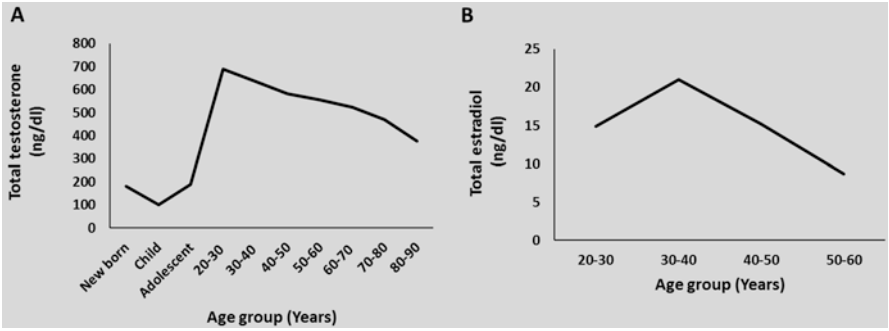

Fig. 13.7 Aging and sex hormone levels. With aging, total testosterone levels gradually start decreasing in males (**a**) after 30 years, and total estradiol levels begin decreasing in females (**b**) after 39 years. (Modified and compiled from [66, 67, 68])

groups or cohorts [64]. Plasma testosterone in males decreases significantly after the age of 50 years. Males in the 80- to 90-year age group have about 40 % less circulating testosterone compared to males under the 50-year age group. The mean plasma testosterone concentration for young males ranges from 5.0 to 8.5 ng/ml; for older males circulating testosterone falls in the range of 1.5–5.3 ng/ml [65]. It is estimated that total testosterone (free testosterone + steroid hormone-binding globulin (SHBG)-bound testosterone) levels decline approximately 1.6% per year, while free testosterone levels fall by 2%–3% per year [64].

A 15-year-long study conducted in New Mexico (USA) showed that total testosterone levels decline by ~110 ng/dL/decade in men older than 60 years of age [61]. A study in Massachusetts (USA) predicted a 0.8%–1.3% annual decrease in bioavailable testosterone concentrations; and a longitudinal study of aging in Baltimore (USA) predicted a yearly decline of 4.9 pmol testosterone/nmol SHBG (sex hormone-binding globulin) [69–71]. Medications, illness and comorbidity accentuate androgen deficiency [72]. The Baltimore study showed significant age-variant longitudinal effects of age in testosterone (T) and T/SHBG, after compensating for variables [61]. In humans, the urinary excretion of androgenic and non-androgenic neutral 17-ketosteroids is considered a fair measure of androgen production in the body. A gradual but definite decrease in the urinary steroid excretion in aging men was observed when the 24-h urinary androgen excretion was analyzed in 50 men in the age group of 30 and 80 years, using the capon's comb method [73]. Twenty percent of men aged over 60 years and 50% of men over 80 years had net testosterone below normal levels.

In women also, the urinary androgenic steroid excretion, which is about one-third of male excretion levels, declines with advancing years. This is likely due to reduced androgenic steroid production by the adrenal gland, which is thought to occur from age-associated reduction in testicular responses to gonadotrophin stimuli, along with hypothalamo-pituitary compensation for reduced testosterone levels [74].

13.5.2 Estrogen and Female Aging

The ovaries and placenta are the primary source for estrogens, which induce female features such as genital organ and breast development, endometrium growth, and inhibition of FSH secretion by the pituitary. Estrogen receptors (ERα, ERβ) mediate estrogen action in the nucleus. ERα is expressed significantly in the breast, ovary, uterus, prostate (stroma), bone, white adipose tissue, testes, muscle, liver, and neuronal tissue, whereas ERβ is expressed most substantially in the colon, bone marrow, salivary gland, testes, prostate, and vascular endothelium [75]. Estrogen is the primary mediator of normal sexual and reproductive behavior in women; additionally, it impacts many nonreproductive functions in men and women [76, 77]. Similar to non-genomic androgen action (discussed in Sect. 13.4), estrogens mediate rapid signaling by activating membrane estrogen receptor [78].

The female reproductive system offers the best example of aging in mammals. "Menopause" results due to a transition from complete ovarian function to ovarian non-function due to lack of estrogen synthesis in women close to 50 years of age. Besides its role in female reproductive tissues, estrogens influence functions of both sexes in diverse nonreproductive tissues such as the brain, adipose tissue, bones, skeletal muscle, vascular system, colon, and skin. Loss of estrogen action leads to increased incidence of osteoporosis, nervous system disorders, and cardiovascular diseases [79]. Within the first year of menopause, estrogen loss is approximately 80% in women [80]. This is accompanied by a speedy decline in muscle mass and strength [81, 82], caused by loss of estrogens [81]. Estrogens are also produced in men through aromatase-catalyzed conversion of testosterone to estradiol in the limbic system and brain [83]. Leydig cell development and function may also be estrogen-dependent [84].

How age-related changes in sex hormone levels affect a subset of age-related health issues is discussed below (Sect. 13.6).

13.6 Influence of Sex Steroids on Diseases Prevalent in Aging

13.6.1 Osteoporosis

Osteoporosis is the pathological loss in bone density and strength usually associated with aging. It leads to bone fractures, majorly in the hip region, vertebra, and forearm. Both estrogen and testosterone are required for normal growth and maintenance of bones. Lowering of sex hormone levels or impaired hormone function causes osteoporosis and minimal trauma fractures. The higher bone mass in men, as compared to women, implies that they are less prone to the development of osteoporosis [85]. The difference in age-related bone loss in men and women is depicted in Fig. 13.8.

Estrogen deficiency causes postmenopausal osteoporosis due to termination of ovarian function [88]. Bone cells are conspicuous estrogen targets since estrogen

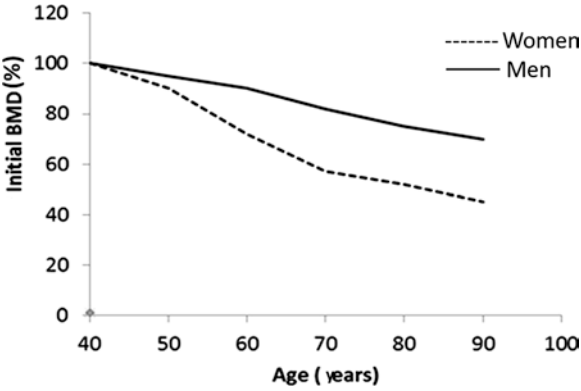

Fig. 13.8 Difference in age-related bone loss in men and women. Changes in bone mass 40 years onward in females (dotted line) and males (solid line). *BMD* = bone mineral density. (Adapted from [86] and [87])

administration prevented bone loss induced by oophorectomy in perimenopausal women [89, 90]. At menopause, women undergo an accelerated phase of bone loss, which becomes more pronounced during the subsequent decade when 20–30% of cancellous bone and 5–10% of cortical bone are lost [91]. This accelerated phase of bone loss in women continues into a phase of slow bone loss, which lasts indefinitely.

Bone loss in menopausal women can be prevented by estrogen therapy. In osteoblasts [92, 93] and osteoclasts [94], estrogen action takes place through high-affinity ERs to regulate bone turnover. During menopause, the control is lost and bone turnover increases. Also, estrogen deficiency makes the bones more sensitive toward parathyroid hormone (PTH) [95] causing further enhancement in resorption. Increase in urinary calcium excretion [96, 97] and decrease in intestinal calcium absorption [96, 98] prevent the outflow of calcium from the skeleton to the extracellular fluids. Although it is clear that estrogen deficiency leads to an acceleration of bone loss in early postmenopausal years in women, the role that the deficiency plays in causing secondary hyperparathyroidism and increase in bone turnover in late postmenopausal women is not clear. A comparative study by Mckane et al. [97], in three separate groups of women, pre-menopausal, untreated elderly, and treated elderly, showed no effects on serum PTH and bone resorption, due to aging after correcting for estrogen deficiency [97]. Also, it was demonstrated by Heshmati et al. [99] in postmenopausal women (with an average age of 69 years) that the reduction of the serum estrogen levels by an aromatase inhibitor, letrozole, to almost undetectable levels, blocks androgen to estrogen conversion in target tissues [97], resulting in a 15% increase in bone resorption markers [99]. This study proved that the minimal levels of sex steroids present in late postmenopausal women are significant in influencing bone turnover. In fact, estrogen affects bone turnover both directly by influencing bone cells and indirectly by affecting bone turnover, by its effects on calcium homeostasis. Age-related bone loss is caused by both increase in bone resorption and impairment in bone formation; however, at menopause, bone resorption exceeds bone formation [100, 101]. Estrogen deficiency during menopause increases both bone resorption and impaired bone formation. Decreased bone

formation during late post-menopause is usually attributed to age-related factors, such as growth factors [91, 102] or lower growth hormone and IGF-1 levels [103, 104]. However, estrogen itself might influence the production of IGF-I [105] and other hormones in osteoblastic cells in vitro [94].

A gradual and steady bone loss is also observed in aging men [91]. Bone mineral density in men depends on androgen levels [106, 107]. In young men, testosterone levels correlate with bone size [108], and hypogonadism or lowered testosterone levels are risk factors for hip fracture [109]. Testosterone treatment can improve bone density. Since, estrogen is produced by conversion of testosterone by aromatase, during androgen therapy for males, it is important to use an androgen which can be acted upon by aromatase for maximal beneficial effect on bones. Estrogen action is an additional determinant of bone density in males [110]. Men with deficiency of aromatase enzyme or mutant, inactivated estrogen receptors have below normal bone density, despite normal testosterone levels [111, 112].

13.6.2 Sex Steroids and Sarcopenia (Muscle Loss)

Aging is accompanied by a progressive decrease in muscle mass [113]. Decrease in the skeletal muscle is particularly significant as it is essential for locomotion. Sarcopenia, or loss of muscle mass, is different from muscle wasting and has its root in a Greek word meaning "loss of flesh" [114]. Age-related muscle loss can lead to disability, hospitalization, and death in older adults and creates a huge financial burden. Typically, in young adults, 50% of total bodyweight is lean muscle mass which decreases to approximately 25% of total bodyweight by the age of 75–80 years. The lowering of muscle mass in the lower limbs with aging is most important with regard to mobility status. The muscle cross-sectional area in quadriceps decreases up to 40% between 20 and 80 years [115]. The decrease in muscle mass is usually correlated with decrease in muscle function. However, studies demonstrate that decrease in muscle strength is more than what it should be on the basis of loss of muscle mass during aging [116], especially beyond 60–70 years [117]. This mismatch between muscle mass and strength results probably due to the deterioration of muscle quality. The reasons for deterioration of muscle quality are not known and may occur due to oxidative stress, mitochondrial dysfunction, a pro-inflammatory state, or metabolic problems. Also, non-muscle-related factors such as loss of motor neurons, changes in nerve-muscle communication, and hormonal changes may be other causes [118].

Androgen and Muscle Mass Androgen-induced changes on muscle mass have been documented [119]. For example, androgen deprivation therapy against prostate cancer induces a decrease in the skeletal muscle and lean tissue and an increase in body fat [120]. Randomized trials have demonstrated that higher than normal doses of testosterone in men lead to an enhancement of fat-free muscle mass, size, and strength [121]. Androgen treatment in older men with restricted mobility and low testosterone caused improvements in muscle strength and ability to climb stairs. Testosterone induces muscle protein synthesis probably by increasing the use of

cellular amino acids in skeletal muscles. Higher amounts of testosterone also increased expression of androgen receptor. Augmentation of the growth hormone (GH) axis may be beneficial as well, since dual therapy with GH and testosterone in older men enhanced lean body mass to a greater extent than the no-treatment control group or the group receiving either growth hormone or testosterone [122]. However, increased lean mass after treatment with testosterone and GH may not translate into improved muscle performance.

Loss of Muscle in Menopausal Women In women, muscle mass starts declining gradually after 30 years, and the decline is accelerated after 50 years of age, and after menopause, muscle mass is lost at 0.6% per year [123, 124]. In postmenopausal women, important factors that may contribute to the loss of muscle mass include physical inactivity, protein intake, and oxidative stress [125, 126]. Older individuals who had participated in resistance training exercises had higher levels of muscle mass when compared with their sedentary counterparts. Protein intake is important for maintaining muscle mass in postmenopausal women. In younger men and women, the recommended protein intake is 0.8 g/kilogram of body weight daily [127]; however, 1.2 g per kg body weight works better for older men and women. Interestingly, consuming essential amino acids containing proteins from animal sources leads to increase in muscle mass in elderly women [128]. This finding demonstrates the importance of protein intake in delaying sarcopenia. During menopause, oxidative stress increases [125] which in turn may provoke cell apoptosis [129]. The mechanism of apoptosis is not completely understood but may results from reduced energy production by mitochondria, provoking muscle fiber atrophy and muscle loss [80]. Another factor that plays an important role is vitamin D3 along with calcium [130]. Vitamin D3 production by the skin is affected by age, season, latitude, and skin pigmentation [131]. Vitamin D3 receptor is present on muscle cells, and association between vitamin D3 and muscle function has been demonstrated [132, 133]. Loss of muscle strength can be associated with estrogen loss that occurs at menopause [82, 134]. Estrogen can promote muscle buildup by stimulating IGF-1 receptors [135]. In addition, ERα and ERβ are present in muscle fibers, and their number is greater in children, men, and women as compared to postmenopausal women [136]. The transcriptional activity of estrogen receptors is triggered by both circulating estrogen and IGF-1 [137, 138]. Hence, estrogen receptor action may be mediated by both estrogen and IGF-I, and a drop in their levels at menopause can possibly affect muscle mass and strength.

13.6.3 Sex Steroids, Cardiovascular Diseases, and Aging

Cardiovascular disease and atherosclerosis are one of the major causes of morbidity and death worldwide, and there is a clear age dependence of the disease. Also, the disease is more common in males as compared to females [139], indicating a potential role of sex hormones in the occurrence of atherosclerosis.

Testosterone and Heart Disease Various epidemiological studies have found a correlation between coronary heart disease and low testosterone levels [140, 141]. Radiological studies suggest an inverse correlation between total and free testosterone and aortic atherosclerosis in men [142]. Epidemiological data showed association between low testosterone levels and atherogenic lipid parameters, such as lower HDL cholesterol and higher total as well as LDL cholesterol and triglyceride levels [143–145]. A negative correlation between testosterone and blood pressure also exists [146, 147]. Studies demonstrated reduced levels of testosterone in men with heart failure [148, 149], and administration of mechanical circulatory support in cardiac failure patients controls testosterone levels and other changes [150]. In summary, low testosterone levels are associated with cardiovascular problems, and testosterone treatment may alleviate some of the problems, depending on the patient.

Among women, cardiovascular disease (CVD) is rampant accounting for nearly 50% of female deaths [151]. Women develop CVD, around 10–15 years later in life as compared to men, and the risk increases after menopause [152]. This has led to much investigation regarding the role of estrogen as a potential cardioprotective agent. With increase in age, the difference in the number of cardiac events among pre-menopausal women versus age-matched postmenopausal women dramatically decreases. Also, there is no difference between the events occurring among postmenopausal and pre-menopausal women aged between 50 and 54 years [153]. After adjusting for age and smoking status, menopause does not seem to be associated with a higher risk for CVD [154]. Nevertheless, estrogen/progestin replacement to nearly 3000 postmenopausal women below 80 years of age, who were suffering from coronary artery disease, did not show significant differences in cardiovascular death, myocardial infarction, or any secondary outcome (including peripheral arterial disease, stroke, and congestive heart failure), between the treated and control groups after a follow-up of 4 years [155]. In fact, more women in the hormone-treated group suffered coronary events and thrombosis in the first year of treatment than the placebo-treated group. Therefore, to understand the role of estrogen in cardiac health, further studies are warranted.

13.6.4 Sex Hormones and Neuroprotection

Sex steroids play pivotal roles in the development and function of the central nervous system (CNS), and androgen, estrogen, and progestin are known to influence brain and CNS functions after binding to cognate receptors. The presence of sex steroid receptors outside of the CNS region and in the pituitary and hypothalamus indicates that sex hormones exert a wide range of influences on brain functions [156]. Sex steroids are also thought to play a role on the survival and programmed cell death of neurons. Neuronal diseases including dementia and Alzheimer's increase significantly with aging and causes disability among the elderly people worldwide [157, 158]. Gonadal and adrenal steroids regulate not only reproduction but also various neuronal and glial functions [159]. Studies predict that over 33% of

women and 20% of men aged 65 years and older may develop dementia [160]. Multiple factors such as genetic makeup and environment play a role in the development of these diseases [161]. Subtle changes occur with age in the nervous system, and treatment with pharmacological agents may reverse these effects. Steroid treatment may be useful in this regard since these hormones are neuroprotective and their levels decrease during aging [162]. Levels of different steroids and neurosteroids in the brain and nervous system have been measured by immunocytochemistry and GC-MS.

Estrogen and cognition Estrogen is thought to protect against the reduction of cognitive functions that occurs during aging. A low estrogen level negatively impacts learning and memory in postmenopausal females, and it increases susceptibility to neurodegenerative diseases. ERs are distributed in several parts in the hippocampus and in the brain frontal lobes that control verbal and working memory. Many neuronal functions are mediated by the gonadal steroid hormones [159]. Steroids called neurosteroids are synthesized in the nervous system, and sex steroids regulate their synthesis [163]. Differences in brain areas in females and males are largely dependent upon the action of sex steroids, and there is a difference in expression of steroid receptors in both sexes [164]. These differences in organization occur during development, and they remain permanent. Sex differences in brain development are well documented [165].

Within the CNS, specific receptors for estrogen are seen in different brain regions, and glial cells demonstrating the role of estrogen in controlling memory and cognitive functions in females [166]. The estrogen-induced genomic mechanisms have long-term effects on neurons including the synthesis and release of many neuropeptides and neurotransmitters [167]. For example, in the rat brain, there is differential expression of ERα and ERβ, providing evidence for distinct roles of each receptor subtype [168, 169]. In addition, rapid effects of steroid hormones in the brain via non-genomic mechanisms are observed [170] that are exerted through receptors present on the plasma membrane [171, 172]. Estrogen effects on the different nervous systems, such as, serotonergic, dopaminergic, cholinergic, and noradrenergic, may contribute to various aspects of brain function, including movement and cognitive function [173]. Estrogens also modulate brain opioid peptide mRNA levels and signal transduction [174, 175]. A biologically active opioid, called β-endorphin (β-EP), modulates behavioral, analgesic, temperature-related, and neuroendocrine properties. In older female rats, brain levels of β-EP are reduced with respect to fertile female rats [176]. The reduction in β-EP levels in plasma is proposed to play a role in the mechanism of hot flushes and sweating in postmenopausal women [177]. Lowered β-EP levels are also correlated with mood, behavior, and sleep disturbances that occur in older women [178]. Indeed, treatment with estrogens, as hormone replacement therapy, seems to alleviate some of these problems [179]. Oral estrogen replacement therapy, subsequent to menopause, increases circulating β-EP levels significantly [180].

The pineal gland secretes the hormone melatonin. A circadian rhythm is observed in melatonin synthesis and secretion, since production and release is low during the day time and high at the night time [181]. Melatonin is associated with antioxidant property [182]. The administration of melatonin to female mice undergoing senescence prevents the oxidative DNA damage caused by aging in the brain [183]. In humans, melatonin administration induces a tendency to sleep, reduces body temperature, and increases secretion of luteinizing hormone and prolactin [184, 185]. Melatonin reduces blood pressure in hypertensive individuals [186]. Aging influences production and biological responses of melatonin. Due to aging, melatonin levels and melatonin receptors are reduced in animals [177]. Studies in rat showed the presence of gonadal steroid receptors in the pineal gland, suggesting an influence of gonadal steroids on melatonin synthesis [187]. In female rats, estrogens regulate melatonin synthesis [188]; however, the effect of gonadal steroids on melatonin secretion in humans is still controversial.

13.6.5 Testosterone and Erectile Dysfunction

Aging males require adequate testosterone levels in order to maintain normal sexual behavior. Erectile dysfunction is a common problem in aging males. Over 70% of men, older than 70 years suffer from erectile dysfunction [189]. Erectile dysfunction can be treated by phosphodiesterase-5 (PDE-5) inhibitors in most but not all men [190]. Erectile dysfunction is prevalent in men with low testosterone levels [191]. In a study of healthy men, 60–75 years of age, spontaneous erections and libido are observed after testosterone treatment that produces normal testosterone levels [192]. For patients with low testosterone levels, who do not respond to treatment with PDE-5 inhibitors, testosterone treatment along with PDE-5 inhibitors improved erectile function [193, 194].

13.6.6 Estrogen and Skin Aging

Menopause in women results in a speedy deterioration of skin quality. Postmenopause, the skin becomes thinner. It has reduced collagen content and elasticity and increased dryness and wrinkling. Estrogen therapy increases skin hydration, elasticity, and skin thickness in aging women. Estrogen replacement can reduce skin wrinkle, improve collagen quality, and enhance vascularization. ERα and ERβ are expressed to similar extents in the skin of males and females. ERβ expression in the epidermis is significantly reduced in individuals over 70 years of age [195]. In postmenopausal women with estrogen deficiency, skin thickness reduces approximately 1.13% and collagen by 2% per year [196]. Type I and III skin collagen decrease by 30% in the first 5 years post-menopause [197, 198] paralleling the reduction in bone mass [196]. Skin thickness and collagen content in elderly females match with the duration of estrogen deficiency rather than age [196–198]. The collagen subtypes are also different in pre- and postmenopausal women [198]. In

response to estrogen therapy, the collagen content and composition improve. Skin wrinkling can be caused due to aging, but may also get enhanced due to hormones and environmental factors. Wrinkling occurs due to reduction in connective tissue and skin elasticity [199]. Topical estrogen administration thickens the elastic fibers and skin in postmenopausal women.

Another hallmark of aging is reduced wound healing. Estrogen might play a major role in skin physiology and wound healing. Studies indicated that estrogens influence wound healing by modifying the inflammatory responses, speeding up reepithelialization, and regulating proteolysis [200]. The healing of wounds in postmenopausal, estrogen-deficient women is slower as compared to young women, while in women taking estrogen replacement, it was comparable to women in younger age group [201].

13.7 Hormone Replacement Therapy (HRT) and Aging

Several studies showed that supplementation with male and female sex hormones such as testosterone, estrogen, progesterone, or growth and thyroid hormones (popularly referred as "fountain of youth") has the potential to improve the quality of life and prevent many age-related symptoms such as fatigue, depression, loss of libido, osteoporosis, and cardiovascular diseases. Recent studies showed various symptoms of menopause, and the risk of osteoporotic fractures decreased significantly by administration of HRT [202]. Though there are many studies supportive of hormone replacement therapy, the side effects in long-term use cannot be denied which may also result in the development of cancer [203, 204]. In consideration to the risk-benefit ratio, the most suitable method for the younger women is the lifestyle management in comparison to the HRT due to lack of proper strategy in use. HRT can be successfully used as a prevention strategy to decrease the severity of many age-related disorders like coronary heart disease, etc. [202].

13.8 Discussion and Conclusion

The primary sex hormones, androgens and estrogens, are vital for a number of physiological functions. Extensive evidences indicate that aging causes a general decline in both of these hormonal levels. Aging of the reproductive glands (ovaries or testes), which are source for the release of sex steroids, results in undesirable clinical manifestation. With aging both males and females suffer from the reduction of sex hormones, though the effects are much more evident in females with the onset of menopause. Age-related effects of hormonal changes on numerous biological processes are summarized in Fig. 13.9.

All steroid hormones are synthesized from cholesterol, the main source of which is LDL cholesterol [3]. The type of steroid hormone produced by a specific steroidogenic cell type depends on its response to tropic hormones and steroidogenic enzymes. Studies in aging rats have shown that the reason for lowering of steroid

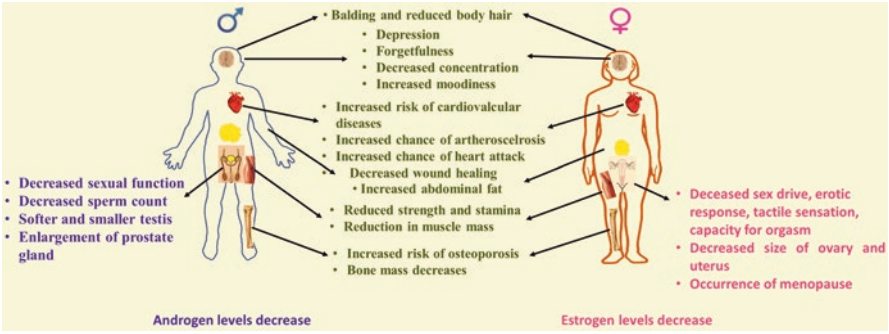

Fig. 13.9 Effect of aging on various biological processes and linkage with sex steroids and vice versa. Aging causes a decline in both male and female sex hormonal levels influencing the body and its various biological processes

hormonal levels is that the cholesterol is not transported in optimal amounts to adrenal and testes for the initiation of steroid biosynthesis, namely, cholesterol conversion to pregnenolone that occurs in the inner mitochondrial membrane. The reduction in levels of two important proteins StAR (steroidogenic acute regulatory protein) and PBR/TSPO (peripheral benzodiazepine receptor/translocator protein) which enable cholesterol transport to the inner mitochondrial membrane appear to be the cause for decline in cholesterol availability. Further studies have demonstrated that age-induced increase in production of ROS (reactive oxygen species) is responsible for the changes in expression of these proteins. "ROS" generation and consequent oxidative damage to macromolecules seem to be the primary determinants of longevity in mammals.

Biological macromolecules, including lipids, nucleic acids, and proteins, are susceptible to oxidative damage induced by free radicals [205]. As cellular membranes produce these free radicals, lipid peroxidation in membranes is the major cause of ROS damage during aging [206, 207]. This hypothesis is supported by the observation that lipid peroxidation is enhanced with age [208, 209] and leads to formation of lipofuscin, a fluorescent pigment that accumulates in tissues with age [208]. In steroidogenic cells, the risk of lipid peroxidation is especially high as they use molecular oxygen for steroid biosynthesis [210, 211] and other cellular functions [206, 207, 212, 213]. The cytochrome P450 enzymes required for steroid hormone synthesis use molecular oxygen to hydroxylate substrates and may lead to production of ROS such as hydroxyradicals, superoxide anions, and other oxygen free radicals [210, 211, 214, 215]. These oxyradicals cause oxidative changes leading to cell damage and death. Since lipid peroxidation in membranes could affect the structure, function, and composition of membranes, almost all the steps associated with cholesterol utilization and steroidogenesis which are entirely dependent on membrane integrity will be adversely affected [5]. As a result, steroidogenic cells are well equipped with various antioxidants. For example, in young rat adrenals, minimal lipid peroxidation and highest resistance to oxidative damage were observed [216]. However, aging causes many oxidative changes in adrenocortical

and testicular Leydig cells [217]. With age, the protective antioxidant system in cells weakens, causing excessive oxidative stress and decline of steroid production. It is not clear why the oxidative balance in steroidogenic cells cannot be maintained through expression of antioxidant enzymes. In many mammals, females live longer than males. For example, male Wistar rats have an average life span of 24 months, while females have average life span of 29 months; a similar gender difference exists in humans [218]. The difference seems to lie in the oxidative status of both species. Studies showed that levels of glutathione, a major cellular antioxidant, are approximately half in males compared to females. Levels of 8-oxo-deoxyguanosine (8-oxo-dG), an excellent indicator of oxidative damage, are fourfold higher in males than females [218].

Since the endocrine system is a major regulator of overall metabolism and growth, changes in steroid hormone levels are important contributors to overall aging. Little is known of the interrelations between oxidative stress, steroidogenesis, and aging at the molecular level. It is necessary to understand the molecular events that cause ROS generation and the mechanisms by which ROS reduce levels of proteins such as StAR and PBR/TSPO, which ultimately impact steroidogenesis. Precise roles of androgen and estrogen in aging, longevity, and quality of life need to be deciphered. Suitability of sex steroid therapy for reversing age-related accumulation of ROS needs to be explored. Therapy, however, should be directed specifically to target tissue(s) without interfering with vital physiological processes.

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Immunosenescence, Inflammaging, and Their Implications for Cancer and Anemia

14

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Abstract

Aging or senescence is a complex process that causes the progressive degeneration of physiological capacity, resulting in a greater probability of death. Senescence affects all cells and tissues of organism, including those of the immune system. The immune system of older people declines with age, and this phenomenon is known as immunosenescence. Immunosenescence results in greater susceptibility to pathology of various age-related disorders, a higher incidence of infections, neoplasia and autoimmune diseases, impaired response, susceptibility to chronic diseases, and weak response to vaccination. Aging affects cells of both innate and adaptive immunity. The cells involved in innate immunity show altered functions. Neutrophils, monocytes, or macrophages show reduced phagocytic ability and impaired superoxide production. Macrophages show reduced levels of MHC class II complexes. Dendritic cells show impaired migration and phagocytic capability and natural killer cells, a reduction in cytotoxicity. Mast cell number increases with age, and degranulation changes contribute to inflammatory responses in elderly. Aging characterized by a chronic, low-grade inflammation is termed as "inflammaging." Inflammaging is a highly significant risk factor for morbidity and mortality in the elderly people. This phase of inflammation is associated with many chronic human diseases, including allergy, arthritis, atherosclerosis, cancer, and autoimmune diseases. Aging can cause dysregulation of the adaptive immune system due to diminished number of naïve B and T cells but a reciprocal rise in memory B and T cells. This results in decrease in T- and B-cell diversity along with low antibody affinity and rise of autoreactive antibodies causing overall weakening of the immune system. An increase in inflammatory markers with age, along with decreased efficacy of

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immunological surveillance, a process where neoplastic cells are detected and destroyed, increases the risk of cancer with age. Anemia of inflammation (AI), also referred as anemia of chronic disease (ACD), is the most common cause of anemia in the elderly. Oxidative stress may cause erythrocyte damage and thus results into AI. The interplay of inflammation and oxidative stress is described in a number of age-related disorders, such as anemia, cancer, angiogenesis, and vascular diseases.

Keywords
Immune system · Immunosenescence · Inflammaging · Cancer · Anemia

14.1 Introduction

Aging is also known as senescence and leads to decreased metabolic activities resulting in progressive degeneration of physiological capacity and higher probability of mortality [1]. Aging is the prime cause of many types of diseases in humans. Aging shows profound direct or indirect effect which may have an impact on development and progression of chronic diseases including atherosclerosis, heart failure, type 2 diabetes, hypertension, osteoporosis, macular degradation, pulmonary insufficiency, renal failure, Alzheimer's, Parkinson's diseases, arthritis, cancer, anemia, and many more [2]. Death across the globe per day is approximately 150,000 among which 2/3 (100,000) deaths are due to age-related diseases. Aging leads to occurrence of various diseases, where aging and age-related disease relationship is complex and better understanding of how aging results in different diseases is needed. Senescence can be of two types functional and demographic. Functional senescence is the state involving decreased functional capabilities, commonly observed in organisms with time during the course of aging, and demographic senescence on the other hand refers to the increased chance of mortality in aged people, both observed due to effects on a variety of cells and tissues of organisms [3].

Age-associated changes in the immune system are known as immunosenescence. Immunosenescence causes profound effects on the immune cells and is therefore becoming an area of great interest among the scientific community as well as in health-care sectors. The human body frequently encounters a wide variety of pathogens, such as viruses, bacteria, or fungi. Different organs and cellular and soluble components of our body that comprise the immune system are able to potently fight against any infectious threat. Immunosenescence is associated with a higher incidence of infections, neoplasia and autoimmune diseases, impaired response, susceptibility to chronic diseases, and weak response to vaccination [4, 5]. Aging affects all cells of the immune system affecting both innate and adaptive immunity. Thus, immunosenescence is discussed below in detail for both branches of the immune system.

An insidious feature in most of the age-related diseases is chronic inflammation. Thus, aging characterized by a chronic, low-grade inflammation is termed as

"inflammaging" [6]. Inflammaging is a highly significant risk factor due to both morbidity and mortality in the elderly [7]. The transient immune response activated during acute inflammation helps host cells to repair and survive in the harmful microenvironment due to traumatic tissue injury or an invading pathogen. Aging leads to increase in chronic inflammation and inflammaging (low-level inflammation) with elevated level of inflammatory cytokines along with acute phase proteins in the serum. In older individuals, these altered inflammatory cytokines and proteins can support and develop autoimmune diseases [8]. Thus research has reported that aging exerts significant effect on all the immune cells with impact on number and function of different cell types leading to the deregulation of immune system with considerable complexity.

It is now well reported that a number of infections due to various pathogens such as bacteria, virus, or yeast lead to inflammation. A number of chronic diseases such as anemia [9], cancer [10], cardiovascular diseases [11], arthritis [12], neurodegenerative diseases [13], osteoporosis [14, 15], and type 2 diabetes [16, 17] can induce mild to severe inflammation and are also common outcomes in aging. Inflammatory process leads to reduction in cellular antioxidant capacity and induces oxidative stress [18]. Chronic inflammation is accompanied by oxidative stress conditions in most of the cases and is considered to be a major factor in lots of oxidative stress events. In the elderly the reasons for occurrence of nonspecific inflammation are not clear till now. Oxidative stress may be the leading cause of the chronic inflammation in the elderly. It is hypothesized in the free radical theory of aging that oxidative stress is a major detrimental factor leading to shorter life span, as it limits longevity. It is already reported in many studies that inflammation and oxidative stress are interconnected in several age-related disorders, such as anemia [19], cancer [18], angiogenesis, and vascular diseases [20, 21]. The effect of inflammation and oxidative stress in various age-related disorders is listed in Table 14.1, and its role in anemia and cancer has been discussed in detail below.

14.2 Aging and Innate Immunity

The innate immune system is characterized by the usage of receptors that recognize a broad array of pathogens. Upon recognition, an immune reaction is initiated without the need for prior sensitization. Therefore, innate immunity serves as a first line of defense against pathogen and invaders and contains various cell types and soluble factors. Cells of innate immune system are monocytes, mast cells, macrophages, dendritic cells (DCs), granulocytes, NK cells, etc., where as soluble factors are the acute phase proteins and the mannose-binding lectin (MBL). They are activated in a triggered-enzyme cascade and subsequently assist in opsonizing pathogens for phagocytosis, lyse them directly, or produce inflammatory peptides [22–24]. Secreted inflammatory cytokines and chemokines by macrophages and DCs allow cross talk between cells of the innate and adaptive immune system that is absolutely crucial for activation of the adaptive immune responses. The basal activity of the innate immune system seems to expand with age. Aging leads to increase in chronic

Table 14.1 Inflammaging- and immunosenescence-related diseases and their associated factors.

Diseases	Marker of diseases and factors involved	References
Anemia	Iron sequestration due to increase in IL-6, followed by increase in iron regulatory peptide, hepcidin, and the iron exporter, ferroportin	[9, 19, 105]
	Decreased erythropoiesis, increase in oxidative stress and free radicals	
Cancer	Activate transcription factors like NF-κB, AP-1, p53, HIF-1α, PPAR-γ, β-catenin/Wnt, and Nrf2	[10, 18, 20]
	Mutation and DNA damage due to increase in free radicals	
	Caspase-1 activation, which causes the maturation and secretion of pro-IL-1β and pro-IL-18	
Cardiovascular disease	Higher generation of free radicals	[11, 21]
	Activation of a variety of transcription factors including NF-κB, AP-1, and PPAR-γ	
Arthritis	Overexpression of cytokines, such as TNFα	[12]
	Imbalanced production of chronic inflammatory conditions	
	Deficiency of IL-10	
Neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, multiple sclerosis, etc.)	Extracellular β-amyloid (Aβ) plaque deposition	[13]
	Altered monocytic as well as T- and B-cell function	
	Increased numbers of endogenous Tregs Th1 lymphocytes and microglia	
	Formation of inflammatory demyelinated plaques	
Osteoporosis	Pro-osteoclastic cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, are elevated	[14, 15]
	Function of osteoprotegerin (OPG) regulator of osteoclastogenesis is altered	
	Heparin from mast cells plays important role in pathogenesis of age osteoporosis	
Type 2 diabetes	Nod-like receptor 3 (NLRP3) activation by ROS or inflammasomes leads to caspase-1 increase and decreased autophagy	[16, 17]
	Dysfunctional mitochondria, senescence-associated secretory phenotype (SASP) involving oxidative stress and endoplasmic reticulum (ER) stress	

inflammation and inflammaging (low-level inflammation) with elevated level of inflammatory cytokines such as IL-1β, IL-6, IL-8, and TNF-α along with acute phase proteins in the serum. In older individuals these altered inflammatory cytokines and proteins can support the development of autoimmune diseases [8]. Thus research has reported that aging exerts significant effect on all innate immune cells

with impact on number and function of different cell types leading to the deregulation of innate immune system with considerable complexity.

14.3 Age-Related Changes in the Innate Immune Cells

14.3.1 Natural Killer Cells in Aging

Natural killer (NK) cells are cytotoxic lymphocytes of the innate immune system, known for their vital role in host defense against various infections and diseases [25]. They are able to directly kill cells with release of perforin and granzymes in MHC-unrestricted recognition. NK cells maintain the balance of both activating signals by NK receptor-ligand active interaction and inhibitory signals by NK inhibitory receptor-MHC-I interaction. Research has shown that there is age-dependent increase in circulating NK cells in humans [26, 27]. In addition to that, phenotypical changes are reported in elderly from immature CD56 (high) NK cells to mature CD56 (low) NK cells. So, NK cytotoxicity is used as a biomarker of healthy aging [28, 29]. Very little is known about the expression and functional role of activating and inhibitory receptors of NK cells in the elderly. While the CD94-NKG2A signaling pathway remains unchanged, aging leads to increase in killer cell immunoglobulin-like receptor (KIR), decrease in CD94-NKG2A expression [30], decrease in ability of IFN-α and IFN-β to enhance cytotoxicity of NK, and a decrease in efficiency of IFN-γ production [31]. Even though aging decreases NK cell role in regulation and recognition against its target in infection and disease condition [32] by lowering NK cytotoxicity, healthy elderly can maintain NK cytotoxicity due to overall increase in circulating NK cells, constant $CD16^{-}$/FcγRIII-mediated NK activation, cytotoxic granule release, and TNF-α and perforin production [30, 33, 34]. High NK cytotoxicity is linked with healthy aging and longevity, while low NK cytotoxicity shows reverse function with rise of morbidity and mortality [35].

14.3.2 Monocytes in Aging

Monocytes are the cells of innate immunity found in peripheral blood and bearing high antimicrobial activity. They have potent capacity for phagocytosis and show high production of antimicrobial proteins. Based on distinct transcriptional and functional characteristics, three subsets of monocytes are known, which are classical monocytes ($CD14^{bright}CD16^{-}$), intermediate monocytes ($CD14^{bright}CD16^{+}$), and nonclassical monocytes ($CD14^{dim}CD16^{+}$) [36]. Intermediate and nonclassical monocytes perform an inflammatory role by producing pro-inflammatory cytokines upon activation and have characteristics of antigen-presenting cells. As monocytes reach peripheral tissues, they give rise to macrophages and dendritic cells. Chemokine receptors on monocyte cells determine their migration properties [37], and expression of CCR2, CCR5, and CX3CR1 classifies different monocytes [38]. Though overall changes in number of monocytes with age are debatable [39, 40],

$CD16^+$ monocytes show an increase, while classical monocytes show a decrease in number with age [41]. Overall elderly show a shift within monocyte subsets. Further, nonclassical monocytes show shorter telomeres and express β-galactosidase, a senescence-associated marker, in the elderly [42].
Defect in monocyte's function with age is confirmed by different studies evaluating impaired phagocytic potential of monocytes and macrophages [43], altered capability to process, and present antigens/peptides to T cells with decreased levels of major histocompatibility complex (MHC) class II molecules [44–46]. Furthermore, spontaneous production of pro-inflammatory cytokines such as IL-6 is increased in the older individuals [47], and expression and function of pattern recognition receptors (PRRs) is altered. Research on aging and its role on expression and function of toll-like receptors (TLRs) and cytokine responses have been extensively done. Monocyte subsets in the elderly are reported to show declined production of IL-6 and TNF-α upon TLR1/TLR2 stimulation, decreased IL-6 synthesis in response to a TLR7/8, and increased TLR5 expression and MAPK signaling leading to higher IL-8 production [48]. This leads to impaired phagocytic potential and function.

14.3.3 Dendritic Cells in Aging

Dendritic cells (DCs) are professional antigen-presenting cells that play a critical role in linking the innate and the adaptive immune system. As discussed earlier monocytes migrate to peripheral tissues to give rise to dendritic cells. They are the key players which can capture and process antigen and secrete a variety of cytokines. Efficiency of antigen presentation to T cell is not altered with aging, but due to the decrease of cell number as well as decrease in migration to lymph node due to impaired CCR7 marker (lymph node homing), there is a change in the phenotype and functionality of DCs. DCs show reduced IL-2 production, impaired capability to induce T-cell proliferation, and a decrease in expression of co-stimulatory molecules [49]. In the elderly, IL-10 that is known to inhibit DC maturation is found in elevated levels [49]. Phosphoinositide 3-kinase (PI3K), which has a critical role in both phagocytosis and migration in DCs [50, 51], shows decreased activation by impaired phosphorylation of AKT [52] in the elderly. All these changes affect DC function and antigen presentation by DCs to T cells.

14.3.4 Macrophages in Aging

Macrophages are one of the most important cells of innate immunity that are also known as pathogen sensors. These are involved in inflammatory response initiation, release of mediators, activation of a range of inflammatory cells, activation of adaptive immune response, elimination of pathogen, and repair of damaged tissue. During aging the production of prostaglandin E2 increases [53]. This increase leads to the suppression of expression of MHC-II molecules on macrophages and finally results in poor $CD4^+$ T-cell activation. Aging causes a decrease in number of

macrophage precursors in bone marrow [53]. Aging also causes a defect in production of superoxide anion and nitrous oxide, in TLR function with decreased expression of TLR1 and TLR9 on macrophages [54]. Further the level of macrophage-derived chemokines, MIP-1α, MIP-1β, MIP-2, and eotaxin, as well as phagocytic ability decreases in the cell [53] effecting both innate and adaptive immunity.

14.3.5 Neutrophils in Aging

Neutrophils are leukocytes that are most abundant in the peripheral blood compartment. They are the first innate immune cells to be recruited at the site of infection. Neutrophils are known for their role in host defense, are short-lived cells, and are active during inflammation and bacterial and fungal infections. Reports have shown that overall total circulating neutrophil numbers are not altered with age [55]. TLRs present on neutrophils play a vital role in the enhancement of phagocytosis, release of antimicrobial peptides, production of cytokines, and recruitment and activation of other immune cells at site of infection. Aging leads to defective function of neutrophils due to a decrease in signaling; alternation in membrane lipid raft structure, dynamics, and function [56]; and decreased expression and activation of GM-CSF receptor [56]. Aging does not affect chemotaxis and adhesion process [57] but leads to significant reduction in phagocytosis [58], expression of Fc receptor CD16 [59], and production of Fc receptor-mediated superoxide, thereby resulting in neutrophil dysfunction. Overall aging decreases neutrophil function which affects the first line of defense to infections and may lead to tissue damage.

14.3.6 Mast Cells in Aging

Mast cells are highly granulated tissue-dwelling cells, widely distributed throughout the body in connective tissue and adjacent to mucosal surface where they are frequently located in close proximity to blood vessels and periphery. Due to this strategic location, they are one of the first cells encountering environmental stimuli such as pathogenic microorganisms, allergens, and toxins. On their activation, they release a large number of pro-inflammatory and immune-regulatory mediators like lysosomal enzymes (β-hexosaminidase, cathepsin-D), vasoactive amines (histamine, serotonin), proteases (tryptase, chymase, carboxypeptidase-A), lipid mediators (leukotrienes, prostaglandins), chemokines, and cytokines. It is now well known that mast cell degranulation can regulate disease severity dramatically. Aging increases the levels of prostaglandin (PG) E2 which is known to contribute to inflammatory response in elderly. PGE2 does not trigger degranulation of dermal mast cells in young individuals but has ability to initiate mast cell degranulation, contributing to inflammatory responses in elderly. Mast cells in aging have attracted a huge attention recently, as there are many reports which support their connection with aging and state that mast cell numbers increase with age [60, 61]. The role of mast cells in skin aging is extremely important and has high significance as mast

cells are present in the skin and release compounds and active mediators from their granules. Degranulation of mast cells in papillary dermis may lead to extracellular matrix reconstruction (ECM), ECM remodeling, inflammation, and angiogenesis in the skin. All these factors are closely associated with the skin aging processes. Thus, the essential factors implicated in aging and tissue damage in the skin may possibly relate to mast cells. Skin aging process and the involvement of mast cells in accelerated skin aging are highly correlated. Overall proliferating cells, nuclear antigen-positive fibroblast-like cells show a decrease, while CD45-positive cells and mast cells gradually increase with progression of age in the skin [62]. Mast cell's mediators release has a prime role in activation of different innate and adaptive immune cells. Alteration in mast cell function with age therefore disturbs the overall function of other cells (Fig. 14.1).

14.4 Aging and Adaptive Immunity

Adaptive immunity is the second stage of defense against specific pathogens and disease conditions. It fully depends on diverse range of cells which rely upon somatic diversification to express different receptors. The development of these cells starts in the bone marrow. T lymphocyte and B lymphocytes are the major specialized cells of adaptive immune system [63]. Activation of T cells is achieved by recognition of peptides presented by MHC molecules on antigen-presenting cells (APCs), for example, DCs, by their T-cell receptor (TCR). Adaptive immunity has unique characteristics for tackling pathogens and infections in an antigen-derived process which finally leads to generation of antigen-specific memory cells. Memory cells are skilled to deal efficiently with pathogens during a second/subsequent attack. Aging can cause dysregulation of the adaptive immune system [64]. Aging leads to diminished number of naïve B and T cells, but a reciprocal rise in memory B and T cells, due to a rise in pathogenic and environmental threats, over a period of time. Due to this decrease in bone marrow and thymic productivity with growing age, levels of naïve B and T cells fall, whereas memory and effector T cells rise [65–67], resulting in decrease in TCR diversity. Identical to T cell, peripheral B-cell pool is also altered with aging. Aging replaces naïve B cells due to a large number of memory cells (antigen encountered) in the B-cell pool, which limit B-cell diversity [68]. Lastly negative selection declines along with function of B cells finally resulting in low antibody affinity and rise of autoreactive antibodies showing profound effects and overall weakening of the immune system (Fig. 14.2).

14.4.1 T Lymphocytes in Aging

T lymphocyte (T cells) is a type of lymphocyte that plays a central role in cell-mediated immunity. T cells can be distinguished from other lymphocytes, such as B cells and natural killer cells, by the presence of a T-cell receptor on the cell surface. Primary feature of T cells is their ability to discriminate between healthy

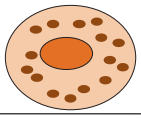
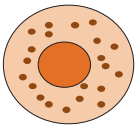
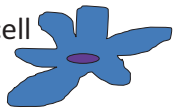
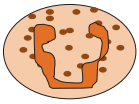
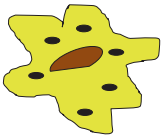
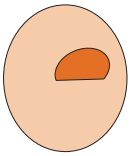
Cells	Changes due to Aging
Mast Cell 	Increase in prostaglandin E2 Increase in mast cell number Enhanced IL-6
NK Cell 	Decrease in CD94-NKG2A expression Increase in NK cell number Reduced IFNα, IFNβ,IFNγ, cytokines and chemokines production Reduced cytolytic ability, decreased CD1 expression
Dendritic cell 	Decrease in IL-2, PI3K activation Impaired CCR7 marker, Decrease in cell number Decrease in lymphocyte cytotoxicity, Reduced IFN production.
Neutrophil 	Reduced CD-16 receptor expression Decreased superoxide production Decrease in phagocytosis, chemotaxis, defect in apoptotic function
Macrophage 	Reduced MIP-1a, MIP-1b, MIP-2 Decreased MHC-II interaction Increased prostaglandin E2 Decrease in phagocytosis, cytokine production, defect in apoptosis activity
Monocyte 	Shift within monocyte subsets Impaired phagocytic potential Decrease in MHC-II level Increased IL-6 and IL-8 , altered PRR expression and function

Fig. 14.1 Age-related changes in cells involved in innate immunity. Aging results in differential receptor expression, altered cell number, defect in apoptosis and phagocytosis function, and defect in chemokine and cytokine production in various cells of innate immunity

and abnormal cells in the body. They respond initially through naïve T cells, followed by activated T cells in effectors function and finally by memory T-cell persistence. T cell contains different effector cells like $CD4^+$ helper, $CD8^+$ cytotoxic, and $CD4^+$ $FOXP3^+$ regulatory cells. T-cell development occurs in the thymus with help from stromal cells including the thymic epithelial cells. Aging has a major impact on the thymus over time where thymopoiesis declines and volume

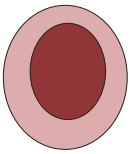
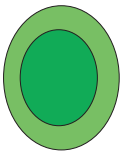
Cells	Changes due to Aging
B cell 	Reduced development, decreased mature B cells Absent of CD27 naïve B cells, decreased response to new antigens Reduced CD40, CD27 Isotype shift
T cell 	Decreased T-cell Diversity, Reduced naïve CD4+/CD8+ cells, Increase in memory and effector CD4+/CD8+ cells Decrease in IL-2, CD28, telomerase length Increased volume of thymus

Fig. 14.2 Age-related changes in cells involved in adaptive immunity. Aging results in altered cell number, cell diversity, development aberration, and receptor expression in cells of adaptive immunity

of thymus increases [69]. In general with age, thymic epithelial cells decrease and are not replaced due to impaired proliferation [70]. There is also a resistance to programmed cell death [71], restricted T-cell diversity [29], and decreased activity of regulatory T cells (Tregs) leading to increased inflammation and autoimmunity [48]. Aging causes a decrease in T-cell receptor sensitivity, defect in expression profile of CD28 co-stimulatory molecules and antigen-independent expansion of $CD8^+$ T cells [73]. An accumulation of immune risk $CD8^+CD28^-$T cells [74] leads to impaired immune function in the elderly. Even though naïve CD4 T cell is unaffected, naïve T cells show functional defects such as shorter telomerase, decreased IL-2 production, less diverse T-cell receptor repertoire, and defect in effector cell differentiation exiting the thymus [75]. Defect in CD154 (CD40L) link with loss of CD28 in $CD4^+$T cells results in decreased antibody production as $CD4^+$T cells normally enhance B-cell proliferation [71, 76].

14.4.2 B Lymphocytes in Aging

Aging affects the development of B cells with fewer naïve cell generation resulting in decreased mature B cells leaving the bone marrow and significant absence of CD27 in naïve B cells. Low levels of mutated and isotype-switched $CD27^-IgD^-$ cells are seen in healthy human adults, but in some autoimmune diseases and in the elderly, levels of these cells are increased. Even though the antibody levels are constant, the antibodies show low affinity due to shift in isotypes. With the help of T

cell, B cell undergoes switch of surface from IgM to IgG, IgE, and IgA [64, 77]. In case of memory B cells, their ability to undergo apoptosis decreases with aging which results in clonal expansion of determined B cells [78, 79]. Decrease in expression of co-stimulatory molecules such as CD40 or CD27 results in loss of B-cell action [80]. In elderly, $CD4^+$ T cells show decreased IL-2 production and CD40L expression. This affects B cell - T-cell interaction which ultimately results in decreased B cell expansion and altered antibody production. The molecular basis of these changes is not known. In aged mice somatic mutations in primary response to some haptens are known to decline. Impaired affinity maturation with a corresponding decrease in mutational frequency is also reported in older humans.

14.5 Inflammation: An Important Component of Innate Immunity

Inflammatory response is an important part of body's innate immunity. Inflammation is basically characterized by five cardinal signs: *rubor* (redness), *tumor* (swelling), *calor* (heat), *dolor* (pain), and *functio laesa* (loss of function) [81]. It can be classified as acute (immediate) and chronic (delayed) phase; acute inflammation is nonspecific, immediate, and a short-term response, whereas chronic phase is a long-term and specific response. Acute inflammation leads to increase in blood flow and vascular permeability which results in accumulation of fluid, leukocytes, and inflammatory mediators such as cytokines. The response during acute inflammation is usually beneficial as it involves wound healing at the site of tissue injury by infiltrating leukocytes. In contrast, chronic phase is a long-lasting active inflammatory response which results in tissue destruction and also attempts tissue repair. It is associated with several chronic human disease conditions including allergy, arthritis, atherosclerosis, cancer, and autoimmune diseases [82]. In totality, inflammation is a protective immune response which involves the development of evolutionary conserved innate as well as specific humoral and cell-mediated immune response against pathogens such as bacteria, viruses, and infective parasites, chemical irritants, and nondigestible particles. Innate immune response is a broad and nonspecific response that involves recognition of pathogen-associated molecular patterns (PAMPs), on invading pathogens, and damage-associated molecular patterns (DAMPs), the endogenous danger signals by germline-encoded pattern recognition receptors (PRRs) of immune cells.

The innate immune system activates inflammasomes in response to infections or stress-associated stimuli [83]. It further leads to caspase-1-mediated activation of inflammatory responses, which causes the maturation cleavage of leaderless proinflammatory cytokines, IL-1β and IL-18. A significant subset of NLR (nucleotide-binding domain, leucine-rich repeat-containing) proteins play a key role by activating inflammasomes [10]. Inflammasomes regulate initiation of an inflammatory form of cell death referred to as pyroptosis. Hence, inflammasomes act as key regulators of inflammation and play a crucial role during infectious and noninfectious inflammatory diseases [84].

14.6 Chronic Inflammation

Macrophages and lymphocytes act as primary cells during chronic inflammation. Macrophages play a crucial role in the clearance of effecter cells during inflammation, and the impairment of clearance leads to chronic inflammation. Apart from the basic allergic inflammation, mast cells are involved in different kinds of acute and chronic inflammatory processes by release of various chemical mediators such as cytokines and chemokines. Mast cells can also be activated by pathogenic antigens from bacteria and virus, growth factors, cytokines, and hormones to release distinct mediators differentially even without degranulation [85]. They have the ability to influence phagocytosis, chemokinesis, and also T and B cell by manipulating their pathways. Therefore, mast cells may act as central effectors or switchboards between the migrating and non-migrating cell types throughout the inflammatory process [86]. Tissue-resident macrophages and mast cells primarily recognize the infection initially and invoke production of a variety of inflammatory mediators such as chemokines, cytokines, vasoactive amines, eicosanoids, and products of proteolytic cascades. IL-6 acts as a major cytokine involved in acute phase of inflammation and persists throughout the transition from acute to chronic inflammation by changing the nature of leucocyte infiltrate (from polymorphonuclear neutrophils to monocyte/macrophages). In addition to this, IL-6 favors chronic inflammation by stimulating T- and B-cell responses [87]. The events leading to localized chronic inflammation, particularly in chronic infections and autoimmune diseases, are poorly understood, and little is known about the causes and mechanism of systemic chronic inflammation. Chronic inflammation is a common component in various age-related diseases including diabetes [12] and cardiovascular and autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus, colitis, gastritis, psoriasis and arthritis [88], allergic asthma, and atopic skin disease [89].

14.7 Inflammaging

Aging characterized by a chronic, low-grade inflammation is termed as "inflammaging" [6]. Inflammaging is a major risk factor due to both morbidity and mortality in the elderly people [7]. Acute inflammation plays a beneficial role as it facilitates the tissue repair and induces adaptation in host cells by invoking transient immune response to harmful microenvironment due to traumatic tissue injury or invading pathogens in the host. During aging, this response may be impaired and result in increased susceptibility to infection [90]. The probable reasons for this kind of resistance may perhaps be accumulation of the damaged macromolecules and cells (self-debris) during aging [91]; toxic products produced by the microbial constituents of the human body, such as oral or gut microbiota, which can leak into circulation and affect surrounding tissues [92]; age-related changes to the immune system [93]; and a variety of age-related diseases. The immune system of older people loses its efficiency with age, which results in greater susceptibility to pathology of various age-related disorders as a consequence of inflammation (Fig. 14.3).

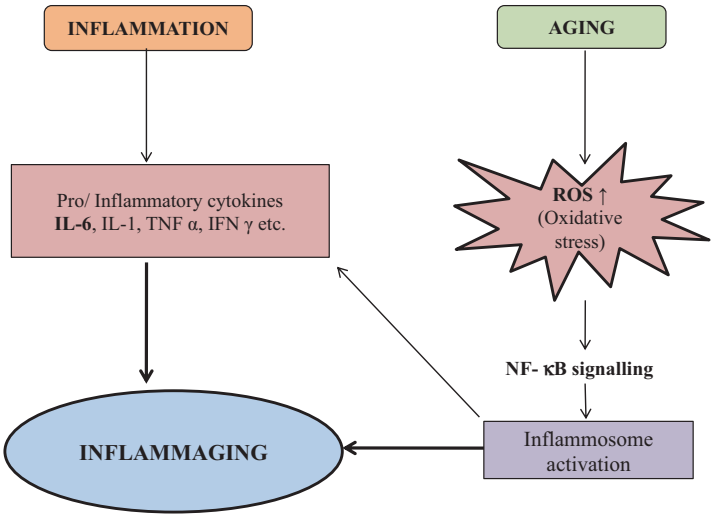


Fig. 14.3 Inflammaging. Schematic representation of critical interplay between cascade of downstream signaling pathways of inflammation and aging that leads to inflammaging

Despite numerous similarities, little is known about several inflammatory components and pathways that distinguish age-related pathologies from each other [94].

14.8 Mast Cells and Inflammaging

Mast cells are multifunctional, long-lived secretory cells that play a versatile role in hypersensitivity and innate and adaptive immune responses. Mast cells are traditionally known to be inflammatory as they recruit other cells and initiate inflammatory cascade by releasing various chemical mediators and cytokines. Now, a number of investigations have revealed the contribution of mast cells in either pro- or anti-inflammatory responses, depending on context. Pro-inflammatory role of mast cells in IgE-associated allergic disorders has already been extensively studied [95, 96], but there are reports which support the role of mast cells as anti-inflammatory and in host defense against parasites, bacteria, and perhaps even viruses [97]. Thus, it can be concluded that mast cells along with their wide range of mediators have the potential to be beneficial as well as dangerous consecutively or simultaneously as required [98].

It is now well known that mast cell degranulation can regulate disease severity dramatically. During inflammation, prostaglandin (PG) E2 is present in high levels. It has been reported that in young animals, PGE2 does not trigger degranulation of dermal mast cells while it acts as a potent mast cell stimulator in older mice. This shows that PGE2 has ability to initiate mast cell degranulation changes in the aging animal or contribute to inflammatory responses in elderly [99]. There are many reports which state that mast cell number increases with age [100, 101]. Studies in

elderly have indicated the importance of mast cells in the pathogenesis of osteoporosis [15], in accelerated skin aging [102], and stated that higher number of mast cells in older population makes them more susceptible for liver diseases than younger population [103]. The above observations may attract the attention of researchers to investigate the role of mast cells in aging in a more focused way, and further exploration of mast cell stabilizers may contribute to prevent or slow the aging process.

14.9 Oxidative Stress: Direct Correlation with Anemia and Inflammaging

Anemia is characterized by low numbers of circulatory erythrocytes in blood along with reduced hematocrit and hemoglobin levels. In recent years, anemia has been reported as the leading cause of disability, morbidity, and mortality in old age patients. Several chronic diseases in the elderly may cause inflammation. This inflammation may lead to anemia, called as anemia of inflammation (AI), and this condition of anemia is highly prevalent in the elderly [104]. Here, we will focus on anemia of chronic inflammation which is of great interest nowadays. Several pathogenic infections due to bacteria, virus, or yeast and inflammatory response during various chronic diseases like chronic kidney diseases, cardiovascular diseases, and cancer may lead to the onset of AI [105]. Many disease conditions associated with AI and aging are common. It is already reported that chronic inflammation leads to reduced cellular antioxidant capacity and results in oxidative stress condition [18]. A decrease in the expression of CD47 (eat me not signal) and CD147 has also been reported in aged erythrocytes under oxidative stress due to which they become susceptible for phagocytosis by macrophages [106]. The development of AI depends on the interaction of a number of factors. In spite of the association with a variety of life-threatening diseases, anemia of inflammatory response is in fact a protective and natural mechanism which is used by host to restrict the potentially harmful foreign antigen in the body by limiting the amount of available iron. Cells of reticuloendothelial system (RES) can also take up free iron; hence high level of iron retention by these cells under some circumstances reduces the iron availability for erythropoiesis [9]. Hepcidin an antimicrobial peptide (Hepc) plays an important role in the hypoferremia and is stimulated by major inflammatory cytokine IL-6 during AI. It is primarily produced by hepatocytes and binds to its receptor ferroportin (also called iron exporter) present at distal sites such as duodenal enterocytes and tissue macrophages. Increase in hepcidin levels leads to iron sequestration as it binds to ferroportin and hinders iron export during inflammation [105, 107]. It is now clear that chronic inflammation and oxidative stress are interconnected and can induce each other. In the elderly the occurrence of nonspecific inflammation is not clear till now, but the oxidative stress is considered to be the leading cause of the chronic inflammation and also acts as major contributor of AI. Reactive oxygen species (ROS) are continuously produced and cleared by the biological system to maintain homeostasis. The impairment of clearance mechanism results in high level of ROS which leads to the development of oxidative stress [108]. These highly reactive and unstable ROS ($O2^{-}$, OH^{-}, H_2O_2) trigger an unstable chain reaction and generate

more reactive OH^- in the presence of transitional forms of some metals, such as Fe^{3+}, which is normally harmless but potentially toxic. It is hypothesized in the free radical theory of aging that oxidative stress is a major detrimental factor leading to shorter life span, as it limits longevity. The continuous decline in cellular metabolism and loss of function during aging involves impairment of mitochondrial uptake or its degradation. This condition leads to increased ROS production and inflammasome's stimulation. The process of autophagy is also impaired during inflammaging which is regulated by several mechanisms. In normal conditions, autophagy is a housekeeping mechanism which is necessary for the removal of misfolded proteins and dysfunctional organelles and facilitates their degradation in lysosomal system and maintains the cellular homeostasis. Hence, the progressive decline in autophagy during aging disturbs cellular homeostasis [109]. The direct or indirect stimulation of NF-κB signaling via inflammasomes induces pro-inflammatory phenotype. Furthermore, autophagy can also be suppressed by inflammatory signaling. TNF-α, an inflammatory cytokine, can regulate the process of autophagy in NF-κB-dependent manner as it can induce as well as suppress this process. TNF-α activates mTOR (major autophagy inhibitor) during NF-κB signaling, and on the other hand, TNF-α can also stimulate the expression of Beclin 1 (enhancer of autophagy) in the absence of NF-κB signaling. Both the stimulatory and inhibitory responses are dependent on the TNF-α-induced ROS production [17]. Oxidative damage has the ability to accumulate over time; hence it is directly correlated with aging and leads to the accumulation of oxidatively damaged proteins, lipids, and nucleic acids. Oxidative stress may cause eryptosis; higher ROS accumulation leads to erythrocyte membrane/cellular damage along with high level of proinflammasomes and thus results into AI, which is the most frequent type of anemia in the elderly (Fig. 14.4). The interdependence of inflammation and oxidative stress is described and reported in many diseases linked to aging [18], such as anemia, cancer, angiogenesis, and vascular diseases [20, 21].

14.10 Critical Interplay of Mast Cells in Anemia

On the basis of the above discussion, it can now be said that anemia is a normal consequence of aging, but the cause of anemia is often unexplained in later age by nutrient deficiency, infection, autoimmune disease, inflammation, and many more [110]. The interdependence of inflammation and oxidative stress has already been discussed in the previous section in various aging-related diseases. Further, oxidative stress is also found to be directly linked to anemia in some reports [19, 111]. The interconnection of inflammation, anemia, and oxidative stress mediated by several immune cells is also reported in literature. Red blood cells or erythrocytes are highly susceptible to oxidative damage as they contain high oxygen and hemoglobin concentration and also lack the repair mechanism [112]. Erythrocytes are continuously exposed to adverse conditions in circulation which results in oxidative damage and their subsequent clearance during inflammation and aging leading to anemia.

Mast cells play a vital role during inflammation by releasing a broad spectrum of pro-inflammatory cytokines and chemokines upon cross-linking of their

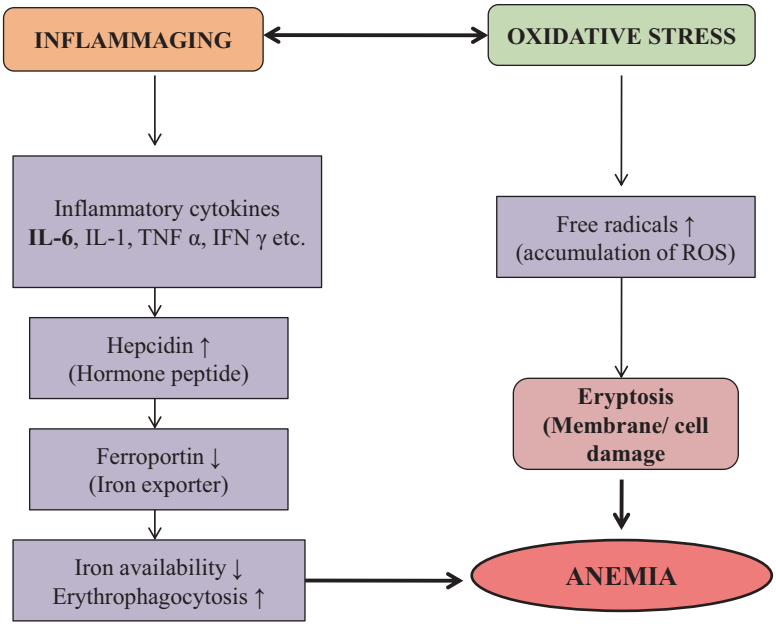


Fig. 14.4 Role of inflammaging in anemia induction. Schematic representation of cross talk between inflammaging and oxidative stress that is important for pathogenesis of anemia in the elderly

high-affinity IgE receptor, FcεRI [113]. In addition to their major participation during inflammatory process, mast cells can also play a crucial role in phagocytosis as nonprofessional phagocytes. Mast cells have been reported to phagocytize wide range of pathogens involving bacteria, yeast cells, and parasites and also particulate material like latex beads, gold, ferritin, aggregated IgE, and red blood cells [114–117]. A very early study revealed phagocytosis of red blood cells by rat mast cells from bone marrow and lymph nodes [116], and after some years, another report demonstrated evidence of erythrophagocytosis by neoplastic mast cells in cat tissues [115]. Now, a very recent report provided the evidences of scavenger activity of murine mast cells in vitro and in vivo. It has been demonstrated that mast cells show uptake of opsonized and oxidatively damaged erythrocytes but not normal erythrocytes and activated mast cells show significantly higher uptake of oxidatively damaged erythrocytes which mimics inflammatory conditions [118]. On the basis of these reports, it can be inferred that the multifunctional mast cell, in addition to their indirect influence on erythrocyte clearance during inflammation, may also contribute directly in erythrophagocytosis under special circumstances which can lead to severe or fatal anemia in elderly. Mast cell impact in this context has also been observed in some clinical studies. Direct correlation of mast cell with anemic condition was observed during mastocytosis (uncontrolled proliferation mast cells in one or more organs) in humans [119, 120]. Another study also stated that mast cell activation leads to increased release of neuropeptides which results into

neurogenic inflammation and hyperalgesia during sickle cell anemia; these observations led the authors to hypothesize the direct correlation of decreased mast cell activity with reduced pathophysiological consequences of sickle cell anemia [121], although direct clearance of erythrocytes by mast cells leading to pathology of anemia is still not clear under normal or during oxidative stress conditions and inflammation in either younger or older adults.

14.11 Aging and Cancer

Aging can be defined as the process by which organisms proceed through a physical deterioration of the body. Aging and cancer are entwined in an intricate and abstruse relationship. There are many reasons why cancer occurs more frequently in older persons [122]. Older persons have less resistance and longer exposure to carcinogens, decreased DNA repair, and defects in tumor-suppressor genes. Epidemiological data verify that both cancer and mortality increase gradually with age indicating aging as a major risk factor for cancer [123]. More than 75% of all newly diagnosed cases of cancer occur in persons above the age of 55. There is an increase in tumor development with age [124]. Cancer can be defined as a functional disease of aging, where the cells acquire the ability to hyperproliferate and migrate. The human body has the ability to renew somatic tissues for repair and restoration. But due to this, over time with age, oncogenic mutations may accumulate due to DNA replication increasing the cancer risk.

The increased risk of cancer with age could also be due to a decline in the immune and endocrine functions in the elderly. The inflammation shows an increase accompanied by a concomitant decrease in immune surveillance [125]. The increase in obesity with age may also contribute by increasing cytokines like IL-6 and may correlate with increased incidence of cancers including breast, prostate, and bowel [126–130]. Despite all these correlations and studies, the relation between age and cancer is still not very well understood. Recent studies have shown a decrease in cancer prevalence, metastatic rate, and cancer mortality but increase in incidental tumors in the oldest of old [131]. These findings further suggest that there may be further alterations in angiogenesis, capillary sclerosis, apoptosis, hormonal receptor expression, and immune responses in the very advance age [132, 133] that may lead to changes in tumor biology [125, 131, 134] (Fig. 14.5).

14.12 Future Insight for Inflammaging and Disease Management: A Concluding Remark

A number of human diseases are correlated with anemia as an outcome; hence it directly reflects the severity of the disease at a later age. In the modern times, it's a challenge for research scientists and laboratory-based physicians to understand the actual causes in order to treat anemia and its contributing factors in elderly. Above we have discussed iron-deficiency anemia (low hepcidin levels) in the context of

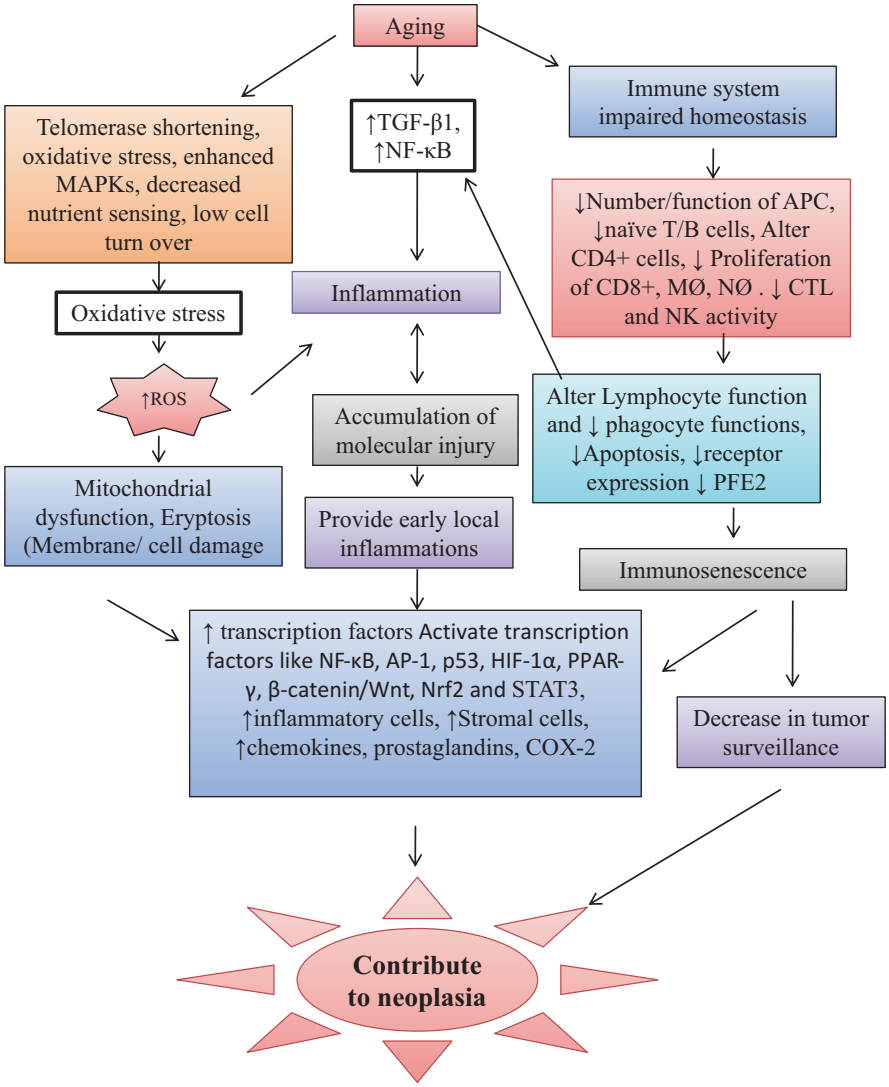


Fig. 14.5 Schematic representation showing interplay of aging, immunosenescence, inflammation, and cancer. Aging leads to impaired homeostasis, expression of different transcriptional factors, inflammation, and reduction in immune surveillance finally contributing to neoplasia

anemia of inflammation or chronic disease. An approach to prevent iron trapping using hepcidin-antagonizing agents directly or inhibiting IL-6 signaling pathway to lower hepcidin levels may be useful in preventing AI. An integrated nutritional/dietetic approach with nutraceuticals to manipulate oxidative stress and related inflammation might circumvent the onset of AI in the elderly population. Due to their huge spectrum of activities and ample potential, inflammaging has attracted

the focus of both basic and clinical researchers to explore its involvement in human pathology. On the basis of the above review, we hypothesize the direct or indirect involvement of inflammaging in the pathogenesis of anemia and cancer. It can be concluded that more focused research in the field of immunosenescence and inflammaging might be useful to prevent the development of severity of disease in elder population including anemia, and cancer, and lead toward an increased longevity, performance, and enhanced quality of life.

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Bone Marrow Stem Cells, Aging, and Age-Related Diseases

15

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Abstract

Bone marrow is a soft, gelatinous, and dynamic tissue present in the central cavity of long bones such as the femora and humeri. Bone marrow is a large reservoir of pluripotent stem cells such as hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and multipotent adult progenitor cells (MAPCs). Apart from stem cells, it also has bone marrow stromal cells comprising endothelial cells (ECs), osteoclasts, osteoblasts, fibroblasts, tissue macrophages, and adipocytes along with soluble components such as cytokines, chemokines, growth factors, and hormones. Bone marrow has a unique capability to proliferate and differentiate into unspecified lineage of all types of cells of the body and provide immunity to the body. Hence it serves as an organ of the immune system. However, the lifespan of tissues depends on the replacement of damaged cells and supply of new cells. With advancing age, bone marrow and stem cells are inefficient to maintain the homeostasis for the delivery of new cells, because of alterations in bone marrow and bone marrow stem cells, which lead to aging process. Aging is a universal process. All cells, tissues, organs, and organisms undergo changes with age. Age-related bone marrow alterations, which include deterioration of bone marrow cellularity, fat cell deposition, and contracted hematopoietic tissue are strictly associated with many age-related diseases such as cancer, altered B lymphopoiesis, osteoporosis, and age-related macular diseases. Various theories explain that the aging process is associated with the bone marrow. Theories such as stem cell theory of aging, gene expression theory, epigenetic mechanism, reactive oxygen species (ROS) theory, metabolic theory, and telomere theory of aging are helpful to understand the process of aging. However, the exact mechanism of aging is still unclear. The extreme consequences of aging are tissue failure, failure

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of regeneration processes, diseases, and lastly death. In recent years, advanced medical science such as bone marrow transplantation has increased health span and life-span and showed great potential towards the recovery from age-related diseases such as type-2 diabetes, osteoporosis, and Alzheimer's diseases. However, we have many questions such as the following: What is the principal rule of aging? Can aging be prevented? and How can we increase life-span? These questions are remaining to be answered.

Keywords
Bone marrow · Stem cells · Aging · Age-related diseases

15.1 Introduction

15.1.1 Bone Marrow

Bone marrow is a soft, gelatinous, and dynamic tissue, which serves as an organ of the immune system. Its amount fluctuates from person to person and within the same individual over time, i.e., hematopoietic marrow comprising higher cellularity and efficient hematopoiesis at childhood changes to yellow or fatty marrow holding more adipocytes and having less hematopoiesis in adulthood [1]. Therefore, bone marrow can be classified into the red bone marrow having active hematopoiesis and yellow bone marrow containing very less hematopoiesis activity [2]. It is confined in the medullary cavities of long bones such as the humerus and femur [3]. Bone cells such as osteoblasts are generated from stromal stem cells, while osteoclasts originated from HSC's monocyte and macrophage lineage. In addition, bone marrow cells also deliver some bone regulatory factors. Osteoblasts and osteoclasts provide secretary factors, sustain and maintain the microenvironment for particular functioning of the stem cells. Although bones and bone marrows are two different and separate tissues, they function as complementary systems and act as a single functional unit [4].

Bone marrow includes two components, parenchyma or hematopoietic component, which comprises HSCs and hematopoietic progenitor cells (HPCs) and stoma or vascular component, which receives non-hematopoietic progenitor cells such as MSCs and MAPCs [5]. Bone marrow is a large reservoir of pluripotent stem cells such as hematopoietic stem cells (HSCs) [6], mesenchymal stem cells (MSCs) [7, 8], and multipotent adult progenitor cells (MAPCs) [9]. Stem cells have unique ability to proliferate and differentiate into an unspecified lineage of cells of the body.

Bone marrow possesses cellular component and soluble component (Fig. 15.1). The cellular component encompasses stem cells such as HSCs, MSCs, MAPCs, and bone marrow stromal cells such as endothelial cells (ECs), osteoclasts, osteoblasts, fibroblasts, tissue macrophages, and adipocytes, whereas soluble component constitutes of cytokines, chemokines, growth factors, and hormones. Each cell type of bone marrow performs its particular function and maintains bone marrow structure and functions (Table 15.1).

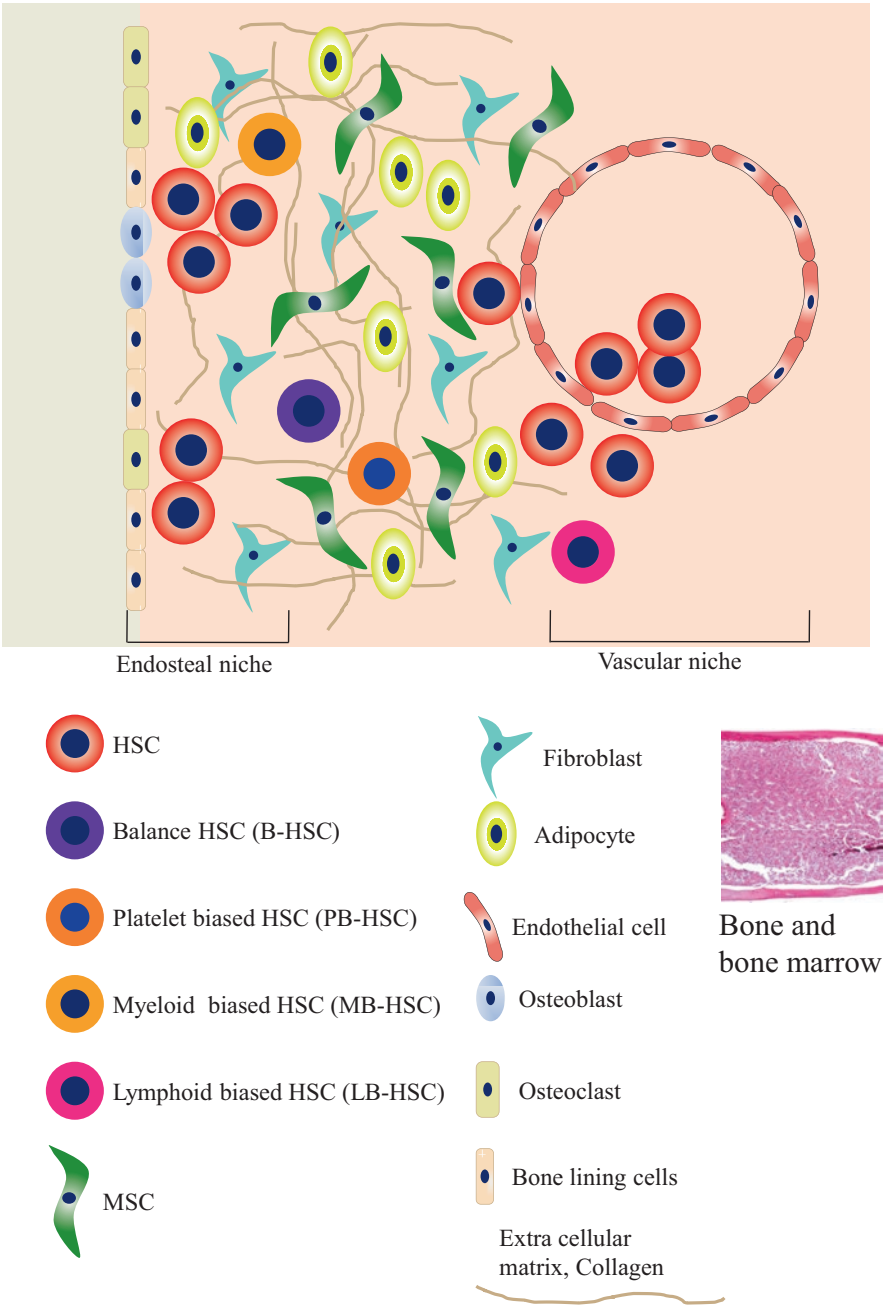

Fig. 15.1 Adult bone marrow structure and cellular organization
Bone marrow shows the structural organization of bone marrow cells and niches such as endosteal niche and vascular niche. Bone marrow comprises various kinds of cells including HSCs, MSCs, MAPCs, adipocytes, endothelial cells, osteoblasts, and osteoclasts. Each cell type performs its particular function

Table 15.1 Bone marrow cells and their functions

S. No.	Bone marrow cell	Properties and functions	References
1	HSCs	Self-renewal, differentiation, and production of all blood cells	[11]
2	MSCs	Differentiate into adipocytes, chondrocytes, and osteocytes, homing efficiency, release trophic factors, and immunomodulation capabilities	[8]
3	MAPCs	Differentiate into mesodermal (e.g., endothelial cells, adipocytes, chondrocytes, and osteocytes), endodermal (e.g., hepatocytes), and ectodermal (e.g., astrocytes and neurons)	[9, 24]
4	Endothelial cells	Line blood vessels, secrete insulin-like growth factor-binding protein, VEGF, and pleotrophin (PTN) and act as a regulator of stem cells' fate	[8]
5	Osteoblasts	Regulate activity and expansion of HSCs, secrete granulocyte colony-stimulating factor (G-CSF), angiopoietin, and osteopontin. The bone surface lining osteoblasts termed as SNO (spindle-shaped N-cadherin+ osteoblastic) cells	[8]
6	Adipocytes	A negative regulator of HSCs	[8, 27]
7	Osteoclasts	Produce PGE2 and involved in the regulation of erythropoiesis	[8]
8	Non-myelinating Schwann cells	Secrete TGF and regulate erythropoiesis	[8]
9	Sympathetic neurons	Involved in the regulation of erythropoiesis and release of CXCL12, act as a master regulator of hematopoiesis	[8]
10	BM macrophages	A major source of PGE2 and involved in the regulation of erythropoiesis	[8]
11	Megakaryocytes	Secrete RANKL, calcium-sensing receptors, NMDA-type glutamate receptors, TGF-β and TGF-β-receptors, and estrogen receptors, osteonectin and osteocalcin, and regulate bone remodeling	[4]
12	Pericytes	Regulate survival stabilization and maturation of other stromal cells and have contractile and phagocytic property	[8]
13	CAR cells	An essential component of the stem cell niche, maintain an undifferentiated state of HSCs, erythroid progenitor, and lymphoid progenitor and retain HSCs in bone marrow	[8]

15.1.2 Cellular Components of Bone Marrow

15.1.2.1 Bone Marrow Stem Cells and Function and Aging

15.1.2.1.1 Hematopoietic Stem Cells (HSCs) and Functions

HSCs are primary stem cells of bone marrow and capable of producing all hematopoietic lineages. HSCs exhibit certain surface markers such as murine HSCs that express Sca-1 and c-kit, while human HSCs showed CD133. HSCs exhibit a lack of lineage-specific markers.

HSCs exhibit expression of surface markers such as CD34, which is the first differentiation and most commonly used marker for isolation and enrichment of primitive human HSCs. CD133 is another surface marker for h-HSCs' selection. A more recent selection marker for h-HSCs is CDCP1 (CUB domain-containing protein). Selection markers of h-HSCs include c-Kit for hematopoietic growth factors that control cell proliferation and survival; VEGFR-1 (vascular endothelial growth factor receptor-1), involved in HSC cycling, and VEGFR-2, also known as KDR, maintain HSC viability. Further research is required to explore the exact surface markers' selection for isolation and characterization of h-HSCs. Similar to human, mouse HSCs (m-HSCs) express c-Kit, Sca-1, and Thy-1. Also, m-HSCs express CD34, which depends on activation status and developmental stage of the cells, and FGFR (fibroblast growth factor (FGF) receptor) marker is used for HSCs, which also show a low level of Sca-1 marker. Highly purified m-HSCs express CD201 or EPCR (endothelial protein C receptor) and endothelial cells also positive for CD201. CD201 marker is also expressed by h-HSCs. Also, m-HSCs' surface marker expression includes CD105, known as endoglin, which is involved in transforming growth factor (TGF) receptor (TGFR) signaling [10] (Table 15.2).

Bone marrow is the primary site of hematopoiesis, which is a continuously dynamic process of production and consumption of all terminally differentiated blood cells to operate various functions throughout a lifetime [11]. HSCs, having self-renewal capacity and reconstitution ability of hematopoiesis following transplantation, are differentiated into lineage-committed and multipotential progenitor cells. HSCs and progenitor cells are not randomly scattered; rather they are protected and resided in a highly organized bone marrow microenvironment or bone marrow niche [8]. In the steady state, hematopoietic progenitor cells (HPCs) manage daily hematopoiesis. HPCs are of two types – lymphoid progenitor cells (LPCs), which differentiate into lymphocytes and plasma cells and myeloid progenitor cells (MPCs), which differentiate into granulocytes, monocytes, erythrocytes, and platelets – while HSCs are mostly quiescent [11].

Recent studies have classified HSC subtypes such as platelet-biased HSCs (PB-HSCs), balanced HSCs (B-HSCs), myeloid-biased HSCs (MB-HSCs), and lymphoid-biased HSCs (LB-HSCs). PB-HSCs can make MB-HSCs and LB-HSCs, and MB-HSCs can generate B-HSCs and LB-HSCs. Biased HSCs hold different lineage differentiation potential. For instance, MB-HSCs ($CD150^{high}CD34^{-}$ LKS) contain greater self-renewal potential than B-HSCs and LB-HSCs which favor myelopoiesis over lymphopoiesis. As a result, MB-HSCs substitute all types of

Table 15.2 Cell surface markers and transcription factors of bone marrow-derived stem cells

Stem cells	Cell surface markers (A)	Expression of transcription factors (B)	Differentiation into cells (C)	References
HSCs	Positive – SCA-1, C-KIT, CD105, CD150, Thy-1, and CD34	Zfx, Bmi-1, Tel/Etv6, and FoxO	All immune cells	(A) [10, 50]
				(B) [51]
				(C) [8]
	Negative – Lin, FLT3, CD38, and CD48			
MSCs	Positive – CD29, CD44, CD73, CD90, CD105, and Sca-1	Oct-4, Rex-1, and Sox-2	Mesenchymal lineages such as chondrocytes, adipocytes, and osteocytes	(A) [11, 17, 19]
				(B) [52]
				(C) [8, 11]
	Negative – CD11b, CD14, CD34, CD45, and CD86 [19]			
MAPCs	Positive – SSEA-1, CD13	Oct-4, Rex-1	All three germ layers such as mesodermal (e.g., endothelial cells, adipocytes, chondrocytes, and osteocytes), endodermal (e.g., hepatocytes), and ectodermal (e.g., astrocytes and neurons)	(A) [24]
				(B) [24]
				(C) [9]
	FLK-1, SCA-1, and Thy-1			
	Negative – CD34, CD44, CD45, c-KIT, MHC-I, and MHC-II			

HSC population in the HSC hierarchy in the bone marrow during aging. LB-HSCs create more lymphoid lineage than myeloid lineage cells [11].

15.1.2.1.2 Hematopoietic Stem Cells (HSCs) and Aging

Hematopoiesis is a process in which hematopoietic stem cells and hematopoietic progenitor cells generate all mature cells that form the whole blood and immune system throughout the life-span [12]. Hematopoiesis is confined to proximal ends of long bones such as the femora and humeri. Hematopoietic cellularity of the marrow reduces with advancing age compared to young adults. However, peripheral blood count does not show alteration significantly with aging process [11, 13]. During aging, HSCs receive phenotypic and functional abnormalities including altered homing efficiency, mobilization properties, and repopulating ability. These changes in aging of HSC are due to aberrant chromatin modification, downregulated DNA repair mechanism, protein misfolding, and higher inflammatory and stress response. Moreover, an increased level of reactive oxygen species (ROS) inserts more DNA damage in aged HSCs of bone marrow. It was shown that aged HSCs dysregulate DNA methylation of differentiating genes controlling myeloid and lymphoid

balance, impaired histone modification, and disturbed cell polarity and activate mammalian target of rapamycin (mTOR) [11]. Also, HSCs exhibit abnormal differentiation and differential potential with advancing age. This abnormal differentiation of HSCs drives to the immunosenescence or "immunoaging" in which there is lack of function of lymphoid cells such as B cells, T cells, and NK cells. Previous studies have shown that B cells' generation and their diversity decrease significantly, while production of memory B cells and autoantibodies producing B cells, which lead to autoimmunity, expands more with advancing age. Additionally, T-cell production and their affinity toward antigen decrease with the aging process. Further, differentiated lymphocytes are inadequate to recognize the new pathogen. NK cells also exhibit decline of cytokine secretion and cytotoxicity with age. Erythropoiesis also decreases with age causing anemia. Furthermore, aged bone marrow microenvironment pushes young HSCs to produce more myeloid cells compared to the young microenvironment. Although myeloid cell number increases due to aged bone marrow microenvironment, and abnormal differentiation of HSCs, their functionality decreases causing inflammatory surrounding known as "inflammaging." Thus, both immunoaging and inflammaging motivate the deterioration of both adaptive and innate immune system, immunosenescence. The failure of the immune system (immunosenescence) with age induces high susceptibility to infection, disease including autoimmune disease, myelodisplastic syndrome (MDS), and cancer and also affects the entire body [11, 14]. Moreover, HSCs which generate blood cells such as B cells, T cells, monocytes, macrophages, and dendritic cells also have been revealed with impaired functional elevation with advancing age. Aged dendritic cells are unable to activate B cells and T cells and low expression of toll-like receptors on macrophages and monocytes, reduced secretion of cytokines and chemokines, and altered B-cell and T-cell compartments are also correlated with the aging process [15].

HSCs highly express TWIST which is a master transcriptional regulator that regulates HSCs' myeloid lineage development and HSC self-renewal. Furthermore, TWIST is also involved in the regulation of neural crest differentiation toward MSCs, cell lineage determination, induced expression of Stro1 (an MSC stemness marker), and development of MSC progenitors and plays a significant role in MSC differentiation, maintenance, and self-renewal [8].

15.1.2.1.3 Mesenchymal Stem Cells and Functions

A primary source of MSCs is bone marrow. Other important sources of MSCs are adipose tissue, umbilical cord, umbilical cord blood, amniotic fluid, dental pulp, periodontal ligament, skin, fetal tissues, and placenta. MSCs are significantly involved in tissue homeostasis and formation of bone marrow niche structure and organization. MSCs maintain immunomodulation properties and are capable of suppressing and regulating the immune system. MSCs are not immune cells, but they control both innate immunity and adaptive immunity. Therefore, to emphasize their role in modulating the immune response, MSCs are termed as "coordinators of the immune system." MSCs are a crucial component of stem cell niche. MSCs regulate differentiation, maintenance, and self-renewal of HSCs. MSCs deliver survival

signals, stemness, and proliferation of HSCs and their progenitor cells. MSCs also protect HSCs from the chemotherapeutic agent and cytotoxic effect [8, 16].

Mesenchymal Stem Cell Characterization

Mesenchymal and Tissue Stem Cell Committee (MTSCC) of the International Society of Cellular Therapy (ISCT) has been defining the minimal criteria for the characterization of MSCs, which include the following: plastic adherent property in culture; must be positive for MSC-positive markers such as CD29, CD44, CD73, CD90, CD105, and Sca-1 and negative for MSC-negative markers such as CD11b, CD34, CD45, CD14, and HLA-DR (human leukocyte antigen D related); and differentiation into mesenchymal lineage such as chondrocytes, adipocytes, and osteocytes. Additionally, fibroblast cells like spindle-shaped morphology [8, 11, 17–20]. MSCs are the heterogeneous mixture of a subpopulation of cells which may or may not fulfill the specified stem cell criteria. MSCs, those satisfy the criteria are termed as "mesenchymal SCs," and those do not, called "multipotent mesenchymal stromal cells" [8] (Table 15.2).

Origin of Mesenchymal Stem Cells

The origin of MSC is still a matter of conflict. Some researchers believe it is from mesodermal origin while others say neuroectodermal origin, even dual origin has also been proposed. MSCs can be differentiated into mesodermal lineages such as chondrocytes, osteocytes, and adipocytes in vitro, suggesting the MSC's mesodermal origin. Several reports proposed a neuroectodermal origin of MSC because of MSCs presence in all vascular organs, i.e., perivascular region. A previous report showed that endosteal localized MSCs expressed only CD271, whereas MSCs in the perivascular region showed CD146 + CD271+. Moreover, CD146 + MSC/pericytes were detected in hematopoietic microenvironment. Thus MSCs may be of mesodermal origin or neuroectodermal origin; it remains to be explore [8, 21].

Dual Effects of MSCs on Erythropoiesis

MSCs support HSCs and hematopoiesis, but they also inhibit the erythroid differentiation ability of HSCs by soluble factors. Therefore, MSCs have the dual role toward erythropoiesis. IL-6, interleukin secreted from stromal cells, inhibit erythroid development and favor expansion of myeloid progenitor cells in the peripheral blood. Further, previous research published that those MSCs initially with lower density or confluence of MSCs in culture discharge prostaglandin (PGE), which enhances erythropoiesis. As a result of growth, higher density or confluency of cells forms a monolayer of MSCs, which produce no PGE, resulting in inhibition of blast-forming unit-erythroid (BFU-E). Hence lower confluence of MSCs favors erythropoiesis, whereas higher confluency inhibits erythropoiesis [8].

Immunomodulation of MSCs

Immunomodulation property of MSCs includes immunosuppressive and systemic immunoregulatory properties. MSCs are not immune cells, but they play a critical role in innate and adaptive immune responses. Hence they are called "coordinator of

the immune system." The fundamental mechanism of MSCs' immunomodulatory effect in modulating immune response is remaining to be disclosed. However, murine experiments exposed that MSCs favor the generation of immunosuppressive immune cells' subset including B regulatory cells, NK regulatory cells (NKregs), CD4+ Tregs, and regulatory DCs (DCregs). Immunosuppressive regulatory cells make up a tolerogenic microenvironment, which actively suppresses the immune response. Apart from favoring production of immunosuppressive immune cells, MSCs simultaneously and forcefully overcome various pro-inflammatory immune cells [8].

The mechanism of an immunosuppressive capacity of MSCs is of two types, cell to cell contact-dependent and contact-independent mechanisms. In cell to cell contact-dependent mechanisms, MSCs control immunosuppressive or anti-inflammatory microenvironment by directly modulating immune cells, such as by inhibition of activated neutrophils; strong inhibition of NKC function and proliferation by cytotoxicity and cytokine production, such as prostaglandin (PGE2) and indoleamine 2,3-dioxygenase (IDO); inhibition of B-cell proliferation, activation and antibody generation, differentiation and function; inhibition of production of DCs from monocytes; by direct inhibition of DC proliferation, maturation, differentiation, antigen-presenting capacity and pro-inflammatory function; by strongly suppressing CD4+ T helper cells and cytotoxic CD8+ T-cells' proliferation and activation, and completely repressing Th1 and Th17 conversion. Whereas, independent mechanisms of MSCs performed by mainly soluble anti-inflammatory factors secreted by MSCs (specifically MSCs type-2 cells) such as chemokines, cytokines and hormones, which include IL 1 receptor antagonist (IL1RA), IL2, IL6, macrophage colony stimulating factor (M-CSF), hepatocyte growth factor (HGF), monocyte chemotactic protein 1(MCP1), and intracellular adhesion molecule 1, indoleamine, 2,3-dioxygenase, human leukocyte antigen (HLA), CCL2, PGE2, and TGF-β. Interestingly, human MSCs use IDO, whereas murine MSCs use nitric oxide (NO) to exert immunosuppressive response. IDO and NO act as a molecular switch, which suppresses recognition and activation of immune response by inhibition of activation of pro-inflammatory monocytes and macrophages and direct activation of regulatory cells such as Tregs. The anti-inflammatory environment is driving and converting MSCs to type-2 cells that secrete high level of chemokines, cytokines, and hormones [8, 22].

MSCs' immunosuppressive capability requires activation or "licensing" and is not intrinsic. Inflammatory microenvironment or inflammatory cytokines, such as TNF-α, IL1, IL17, and IFN, activate MSCs and increase the expression of anti-inflammatory molecules and adhesion molecules present on the cell surface of MSCs. Surface adhesion molecules promote close intercommunication between MSCs and immune cells and participate and increase the impact of anti-inflammatory signals. Induced MSCs release anti-inflammatory molecules such as IDO, an enzyme that metabolizes L-tryptophan and produces L-kynurenine. Kynurenine is an inhibitor of erythropoietin and toxic for NKCs and T-cells, whereas tryptophan shortage or starvation commands to cell cycle arrest in T cells. Hence MSCs potently suppress proliferation of lymphocytes. MSCs directly (cell to cell contact) transfer inhibitory signal to immune cells, with the help of surface molecule such as Fas

ligand and programmed death ligand 1 (PD-L1). Transferred inhibitory signal actively represses polarization of Th1 and TH17 [8].

Timing, kinetics of activation, ligand concentration, and type of activated TLR signaling are some significant factors that polarized MSCs toward either type-2 MSCs (anti-inflammatory MSCs) or type-1 MSCs (pro-inflammatory MSCs). Bone marrow MSCs showed higher expression of TLR-3 and TLR-4. TLR-4 signaling in MSCs is also required and supports proliferation and differentiation of HSCs. TLR-4 signaling polarizes MSCs toward type-1 MSCs (pro-inflammatory MSCs), whereas TLR-3 signaling polarizes MSCs toward type-2 MSCs (anti-inflammatory MSCs). In vitro study reveals that type-1 MSCs (pro-inflammatory MSCs) are capable of releasing pro-inflammatory cytokines, presenting antigen, and delivering chemokines for activation of inflammatory immune cells such as B cells and T cells. Type-2 MSCs (anti-inflammatory MSCs) discharge anti-inflammatory IL4, IDO, and PGE2 and inhibit lymphocyte proliferation and NKC function [8].

15.1.2.1.4 Mesenchymal Stem Cells and Aging

Similar to HSCs, MSCs also exhibit age-associated change with advancing age. Aged MSCs show declined proliferative capacity and clonogenic and differentiation potential. Moreover, MSCs have shown more differentiation toward adipocyte lineage, which leads to accumulation of adipocytes in the bone marrow with the aging process and turns red bone marrow (at birth) to yellow marrow (in elderly). The magnified deposition of adipocytes in aged bone marrow inhibits B-lymphogenesis and HSC function and positively regulates myelopoiesis. Age-dependent adipocyte differentiation (adipogenesis) of MSCs is not entirely understood. However, some speculation has been proclaimed including adipogenesis, which may dysregulate insulin/insulin growth factor 1(IGF1) receptor signaling (IIS) and decrease in bone formation and changes in the composition of extracellular matrix [11]. MSC dysfunction may cause a metabolic disorder such as accelerated aging-associated metabolic syndrome. Production of adipocytes consumed MSCs in this syndrome. Also, type-2 diabetes and prediabetes are more commonly observed in metabolic syndrome and may also lead to MSC dysfunction by generation of advanced glycan end-products. These glycan end-products are stored in the bone matrix and they induce ROS production, apoptosis and suppress proliferation of MSCs during aging. Similar metabolic alteration is detected in severe disease of accelerated aging such as Hutchinson-Gilford Progeria Syndrome (HGPS) [20]. One report showed that age-related switch promotes MSCs toward adipocyte differentiation, instead of osteoblasts' differentiation via NFATc (nuclear factor of activated T cell)/Maf and WNT signaling [15]. Previous literature published that aged MSCs are associated with higher level of ROS and nitric oxide (NO), impaired DNA methylation, dysregulation of histone acetylation, telomere shortening, and p53-mediated DNA damage [11].

15.1.2.1.5 Multipotent Adult Progenitor Cells (MAPCs), Functions, and Aging

Catherine Verfaillie's group, in 2002, isolated multipotent adult progenitor cells (MAPCs) from rat and mouse bone marrow. Bone marrow-derived MAPCs are adult stem cells and capable for inserting trophic effect and immunomodulatory

properties. Also, MAPCs are used for tissue regeneration, although new, originally isolated MAPCs exhibit robust differentiation toward neuro-dermal lineage and produce neuron-like cells [18]. However, MAPCs can differentiate into cells of all three germ layers such as mesodermal (e.g., endothelial cells, adipocytes, chondrocytes, and osteocytes), endodermal (e.g., hepatocytes), and ectodermal (e.g., astrocytes and neurons) [9]. MAPCs show robust endothelial expression as compared to MSCs. Human MAPCs exhibit higher expression of CD44, CD13, CD73, and CD90, while MAPCs did not express mature hematopoietic markers such as CD34, CD45, CD56, CD105, and CD271 [18]. Rodent multipotent adult progenitor cells (MAPCs) derived from the bone marrow muscle and brain show c-Kit+, CD9+, CD13+, and CD31+ and CD44-, MHC-I-, CD45-, and Thy1-surface marker profile [23]. Moreover, cultured mouse MAPCs (mMAPC) exhibit higher level of expression of stage-specific antigen (SSEA-1) and CD13; mMAPCs also express a low level of Sca-1, Thy-1, and Flk-1 and also exhibit no expression of CD34, CD44, CD45, c-kit, and major histocompatibility complex (MHC) classes I and II [24] (Table 15.2). mMAPCs are significantly smaller than the MSCs, and rodent MAPCs show resemblance with extraembryonic endoderm precursor cells and extraembryonic endoderm cells. MAPCs also show higher expression of pluripotency factors such as Oct4, Sox7, Sox17, and Rex-1 and endoderm-specific genes such as Gata4 and Gata6 [18, 24]. Previous studies showed that cells infused in irradiated animals (treated with a low dose of radiation), which showed a low damage of the blood-brain barrier than non-irradiated animals. An animal generates thymus lymphoma and spleen lymphoma, which is commonly seen in aging NOD/SCID mice. mMAPCs exhibit higher level of differentiation and engraftment in low-dose irradiated intestinal epithelium and hematopoietic system and impart their function to neoangiogenesis in host tissue [24].

15.1.2.1.6 Bone Marrow Stromal Cells: Functions and Aging

Apart from stem cells, endothelial cells (ECs) that line blood vessels in bone marrow also undergo aging process and lose their number and function with advancing age. ECs secrete insulin-like growth factor-binding protein, VEGF, and pleotrophin (PTN) and act as a regulator of stem cells' fate [8]. Previous studies have disclosed that aged ECs showed declined secretion of stem cell factor and ligand such as CXC motif ligand (CXCL) 12 that support bone marrow stem cells such as HSCs. Moreover, aged ECs show decrease in Notch signaling and NO production that are involved in vasodilation and mobilization of HSC. Thus, aged ECs might be linked with defective mobilization and maintenance of stem cells in the bone marrow with aging process [11]. Previous studies have shown conditional deletion of Atr gene of osteoblasts causing hair graying, osteoporosis, and alopecia (premature age-related phenotype). Moreover, Atr mutant mice show depletion of HSCs and progenitor cells and their regenerative potential. Hence, osteoblasts are also required in maintaining bone marrow niche with aging process [11].

MSCs are the primary source of osteoblast generation. Osteoblasts regulate HSCs' activity and expansion by secretion of granulocyte colony-stimulating factor (G-CSF), angiopoietin, and osteopontin. The bone surface lining osteoblasts is

termed as SNO (spindle-shaped N-cadherin+ osteoblastic) cells. Osteoblast interacts with HSCs via Notch signaling, Bmp-1/Bmp-1 receptor signaling, parathyroid hormone/parathyroid receptor signaling, and Wnt-catenin signaling and induces proliferation, migration, and quiescence of HSCs. Further, HSCs and other stem cells are also monitored by niche cells such as adipocytes, which are a negative regulator of HSCs. Osteoclasts produce PGE2, non-myelinating Schwann cells secrete TGF, sympathetic neurons release CXCL12, and BM macrophages are a major source of PGE2 and involved in regulation of erythropoiesis [8]. Megakaryocytes are required in the regulation of bone remodeling by secretion of RANKL, calcium-sensing receptors, NMDA-type glutamate receptors, TGF-β and TGF-β receptors, and estrogen receptors, osteonectin and osteocalcin [4].

Pericytes are stromal cells that communicate with other stromal cells through paracrine signaling or direct cell to cell contact. Pericytes regulate survival stabilization and maturation of other stromal cells such as ECs. Pericytes have contractile and phagocytic property. They are associated with a neurovascular unit as an essential component and blood-brain barrier. Differentiation capacity of pericytes makes them a "ubiquitous source of adult tissue stem cells." Nestin is a filamentous protein secreted by neuroepithelial neuronal precursor stem cells, which are involved in HSC maintenance. Nestin+ cells are mixed cell population including ECs, MSCs, endothelial precursor cells, and myofibroblasts. The endosteal niche includes Nestin+ MSCs as an essential cell component, which secretes CXCL12, a critical chemokine for HSC migration. Sympathetic nerves, surrounding arterioles, also contribute their role in cyclic release and migration of HSCs. Malfunction of nerve system leads to impaired hematopoiesis. Hence autonomic nervous system acts as a "master regulator of hematopoiesis." CXCL12+ abundant reticular (CAR) cells are identified as an essential component of the stem cell niche. Perivascular CAR cells have bi-lineage potential, i.e., they can differentiate into both adipocytes and osteoblasts. CAR cell-depleted (genetically engineered) mice showed a decline of HSCs. CAR cells are involved in the maintenance of an undifferentiated state of HSCs, erythroid progenitors, and lymphoid progenitors and retain HSCs in bone marrow [8].

15.1.2.1.7 Bone Marrow Niche and Functions

Bone marrow stem cells are not randomly located within the bone marrow. HSCs and hematopoietic progenitor cells (HPCs) are localized in extremely organized and specialized area such as the endosteum of the bone and around the blood vessels. Additionally, undifferentiated cells are localized in the endosteum area, while mature and differentiated cells reside toward bone marrow cavity [25]. Bone marrow niche is an extremely designed and supportive microenvironment within the bone marrow. Bone marrow microenvironment constitutes cellular component, which contains stromal cells and accessory cells, and a soluble component that is secreted by niche cells themselves. Bone marrow stromal cells include fibroblasts, bone marrow macrophage, endothelial cells (ECs), osteoblasts, osteoclasts, adipocytes, and MSCs. Accessory cells comprise myeloid-originated cells such as dendritic cells, circulating macrophage, and lymphoid-originated cells such as natural killer cells, B cells, and T-regulatory cells. Stem cell niche of bone marrow supports

growth and differentiation of stem cells, where they are confined. The niche also protects stem cells from damage and provides cytokines/stimulatory factors for accurate functioning. Almost all stromal cells originate from MSCs or HSCs or progenitor cells [8].

According to the current concept of bone marrow niche, there are several types of niche within the bone marrow including endosteal niche, vascular sinusoidal niches, perivascular arteriolar niche, and sinusoidal megakaryocytic niche. Endosteal niche, also known as osteoblastic niche or osteo-hematopoietic niche, includes HSCs and osteoblast cell population. The osteoblastic cell population is spindle-shaped, cadherin-positive, and located at the lining of the bone surface. Therefore, these cells are also termed as spindle-shaped N-cadherin+ osteoblastic (SNO) cells. Endosteal niche favors HSC quiescence. Moreover, early lymphoid progenitor cells of mice are predominantly found in an endosteal niche. Vascular niche or vascular sinusoidal niche or sinusoidal reticular niche includes the majority of HSCs and SMA + CD146 pericytes. Additionally, leptin-receptor+ and CAR (CXCL12-abundant reticular) cells are found predominantly in vascular niche and firmly associated with ECs and HSCs [8]. Thus, endosteal niche favors HSCs' maintenance and quiescence, and vascular niche provides a signal for differentiation of HSCs [25]. Hematopoietic stem and progenitor cells (HSPCs), nestin+ cells, leptin-receptor+ cells, pericytes, and CAR cells make up the perivascular niche or perivascular arteriolar niche or pericytic arteriolar niche. Mouse HSCs and early myeloid progenitor cells are predominantly located in perivascular niche. Sinusoidal megakaryocytic niche is composed of HPSCs and megakaryocytes that secrete CXCL4 and are involved in HSC quiescence and maintenance [8]. Translocation of megakaryocyte progenitors toward sinusoidal megakaryocytic niche induces platelet production and megakaryocyte maturation [25]. It remains to be disclosed how each niche communicates with others, how they work – whether they function as a single unit or work together or they are really separated niche – and how they respond toward disease or aging.

15.1.2.1.8 Bone Marrow and Aging

Aging is a universal process. All cells, tissues, organs, and organisms undergo changes with age. Age-related bone marrow alterations include deterioration of bone marrow cellularity, fat cell deposition, and decrease of hematopoietic tissue. Thus, age-related changes in bone marrow are reflected by a higher risk of myeloproliferative disorder, anemia, and declined immunity. With age, fat infiltration reduces space occupied by hematopoietic tissue from 90% (at birth) to 30% in old age (at age 70 years) in humans. Diminished volume of hematopoietic tissue in bone marrow produces small native lymphocytes which decline the adaptive immunity with age [26]. Accumulated bone marrow fat (BMF), which is different from subcutaneous or visceral tissues' fat, secretes adipokines such as leptin and adiponectin. Higher accumulation of BMF leads to osteoporosis and weak bone mass during aging [27] (Fig. 15.2).

Furthermore, HSCs also showed several age-related changes including skewed X-chromosome inactivation, telomere shortening, accumulation of mitochondrial

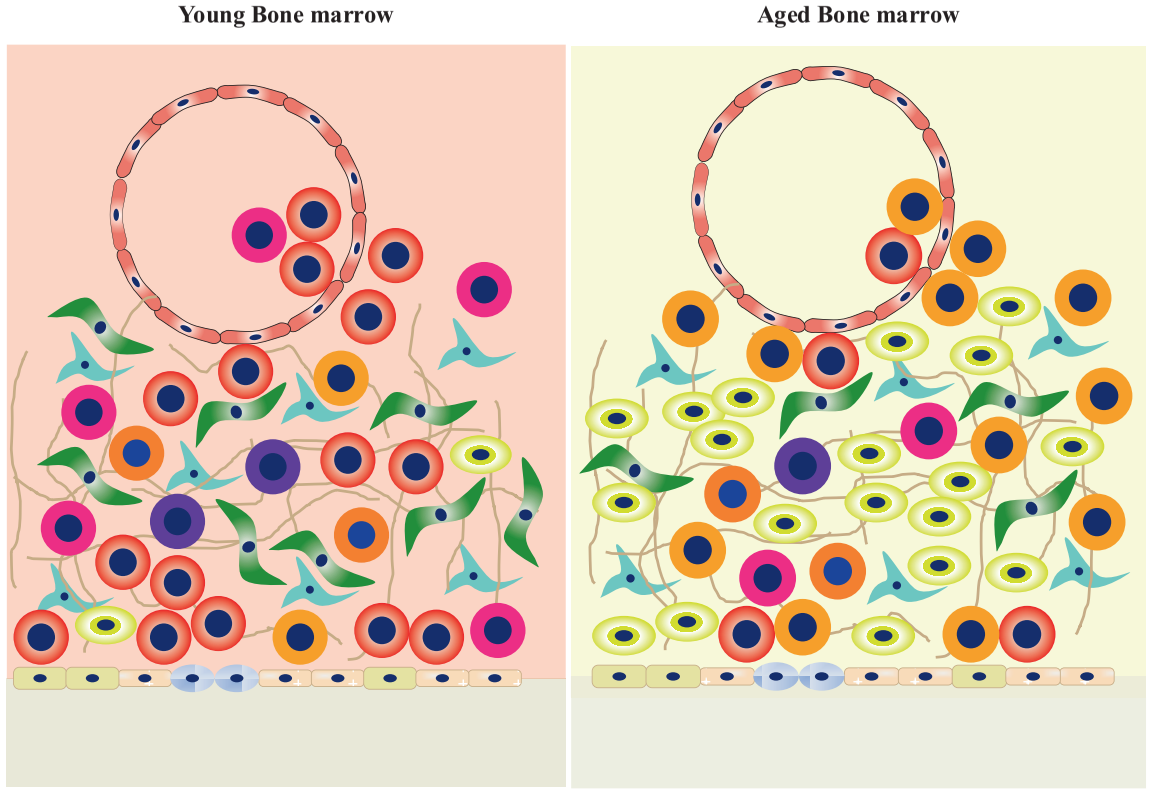

Fig. 15.2 Differences between young bone marrow and aged bone marrow over time
Young bone marrow, reddish in color due to high hematopoiesis activity, turns into yellow or fatty marrow, which has great adipocyte deposition and shows insignificant hematopoiesis activity, over time (age)

DNA mutations, and micronuclei formation. These age-related variations cause HSCs' dysfunction and inactive hematopoiesis. Impaired hematopoiesis declines production of red blood cells (RBCs) with advancing age, which causes anemia, a significant health problem in the elderly. However, the mechanism of age-related expansion of bone marrow fat and the reduction of hematopoietic tissue (declined cellularity) remain to be explored [26]. Moreover, previous reports disclosed that old rats treated with growth hormone showed decline of fat deposition within bone marrow and increase of hematopoietic tissue. Hence the decrease of growth hormone production with age, dysregulation of insulin growth factor signaling, and changes in the composition of the extracellular matrix may play a significant role in fat accumulation within the bone marrow [11, 26].

Almost all niche cells including accessory cells such as blood lineage and stem cells such as MSCs and HSCs undergo changes with age. However, the exact mechanism of this is still unclear. Published research explained that transplantation of young BMCs into old recipient mice showed reduction of B-cell generation, and transplantation of old BMCs into young recipient mice showed a decline of peripheral B lymphocytes. Further, decrease of homing efficiencies of engagements (BMCs' young to old mice, and BMCs' old to young mice) and generation potential toward myeloid lineage have been shown in both situations [14]. Previous studies indicate that aged HSCs showed higher CD150 expression, which favors the expansion of myeloid-biased HSCs, resulting in myeloid-biased HSCs' fraction to dominate the entire aged HSCs' pool in the bone marrow niche with the aging process. Age-associated myeloid bias could be due to cell-intrinsic modifications, which turn lymphoid-biased HSC clones into myeloid-biased HSC clones within the aged HSC pool in the bone marrow, i.e., clonal evolution, or clonal selection/expansion, in which myeloid-biased HSC clone expands more and dominates the entire aged HSCs' pool, i.e., clonal shift. Moreover, myeloid-biased HSCs showed higher self-renewal potential and long-term repopulating capacity than lymphoid-biased HSCs. Additionally, myeloid-biased HSC clone expansion reduces occupied space of lymphoid-biased HSC clone, resulting in a decreased number of lymphoid progenitor cells and lymphopoiesis. Thus, aging process favors expansion of homogeneous population (myeloid population) and reduces heterogeneity (lymphoid and myeloid cells population) of the bone marrow niche [11, 14]. The decrease in lymphopoiesis leads to several blood diseases such as anemia.

15.1.3 Soluble Components of Bone Marrow

Apart from the cellular component, a soluble component is necessary for proper functioning of bone marrow. The soluble component includes cytokines, growth factors, hormones, calcium, and chemokines. Stem cells and their progeny cells generate soluble factors. For instance, MSCs, ECs, osteoblasts, and CAR cells secrete stromal cell-derived factor-1 (SDF-1, also known as CXC12), which is a critical

chemokine participating in HSCs' maintenance and HSCs' homing within HSC niche [8]. Previous research showed that SDF-1−/− (gene knock out) mice's HSCs have colonization defect, and reinforced expression of SDF-1 in bone marrow vascular endothelial cells enhances colonization property of bone marrow by stem cells. Hence, bone marrow endothelial cells secreted SDF-1, which is essential for hematopoietic colonization of bone marrow. SDF-1 also induces expression of VCAM-1 on ECs and very late antigen (VLA)-4 on megakaryocyte [25]. Niche cells also release SCF and TGF-β, which are relevant to HSCs' regulation and maintenance. TGF-β secretion is associated with osteoblastic differentiation of MSPC [8].

15.2 Aging

15.2.1 Axis or Theory of Aging with Respect to Bone Marrow/ Stem Cells

People are trying to defeat aging since the millennium to achieve immortality and gave different theories or hypotheses for aging. A critical view of the bone marrow associated explanation for aging is given here (Fig. 15.3).

15.2.1.1 Stem Cell Theory of Aging

Millions of cells from the bone marrow, gut, and skin tissues are removed after completing their biological job or life-span. For example, after 100–120-days, erythrocytes are eliminated from blood tissue after finishing their life-span. The life-span of tissues depends on the replacement of these removed cells and supply of new cells. Tissue-specific stem cells sustain the supply and replacement of these cells throughout life. For instance, a pool of hematopoietic stem cells (HSCs) in the bone marrow of humans produce millions of new erythrocytes per second to maintain the homeostasis between lost erythrocytes and new erythrocytes for a lifetime. That is why the rate of the aging process of the tissue depends upon tissue-specific stem cells [28]. Moreover, these tissue-specific stem cells maintain the homeostasis in the tissue by differentiation of tissue-specific stem cells to produce the required cell types and producing more stem cells in the tissue to sustain and repair the tissues, protect tissues or prevent organ failure, and struggle with the aging process. However, tissue homeostasis is not constant because of the functional inability of tissue-specific stem cells during aging [14, 29], increasing number of progenitor cells, side population (SP) HSCs, and adipocytes instead of stem cells in old age [29] (Fig. 15.4).

15.2.1.2 Gene Expression Changes

During aging, genes that are regulated by age include genes, which are mostly involved in differentiation. For instance, lymphoid lineage-specific genes are deactivated, whereas myeloid lineage-specific genes, megakaryocyte-associated genes, and many proto-oncogenes are found to be more activated during aging. As a result, the risk of age-associated lineage-specific diseases increase such as myelodysplastic syndrome, myeloproliferative disorders, or leukemia, myeloid leukemia [11, 14,

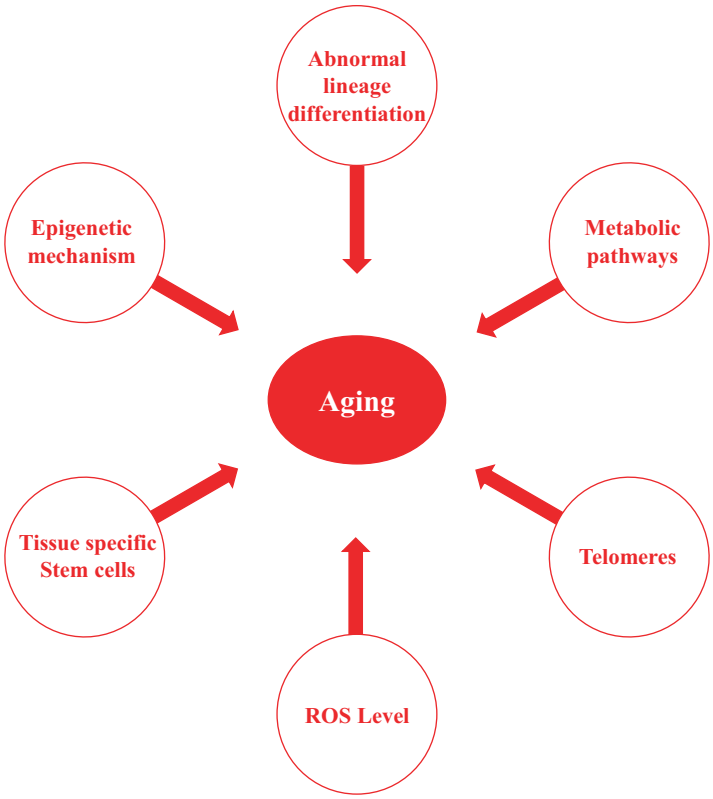

Fig. 15.3 Axis or theory of aging
An aging process, which includes various theory or axis such as stem cell theory, gene expression changes theory, ROS theory of aging, epigenetic mechanisms, metabolic pathways in aging, and role of telomeres in the aging process

30] (Fig. 15.5). Moreover, microarray study found that aging process activates inflammatory response genes and stress genes and deactivates some genes including DNA repairing genes, chromatin remodeling regulatory genes, and genomic integrity genes. Inflammatory response gene products lead to creating an inflammatory microenvironment within aging bone marrow and downregulate some genes such as P-selectin gene. P-selectin genes encode adhesion protein that showed crucial involvement in the mobilization and engraftment. Thus, mobilization and engraftment potential of stem cells reduce within aging bone marrow. Thus, epigenetic irregularities of genes can dysregulate the transcriptional activity across the genome and lead to abnormal cellular function. As a result, functional defects of stem cells increase, declining the regenerative capability of stem cells. These findings explore numerous and diverse changes at the molecular, cellular, tissue, and organism levels, and it also explains why we feel inflammation in our tissues or body during aging and why we face a high risk of diseases with advancing age [14, 29].

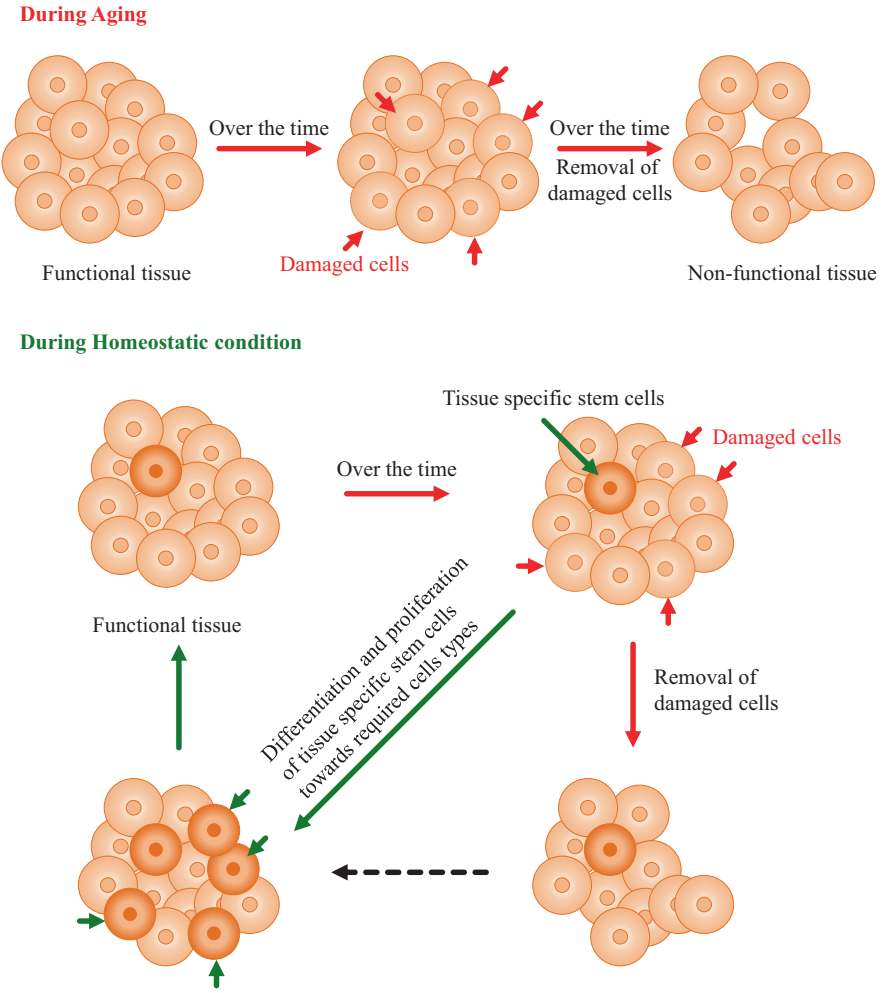

Fig. 15.4 Stem cell theory of aging
Tissue-specific stem cells maintain the homeostasis in the tissue by differentiation of tissue-specific stem cells to produce required cell types and by producing more stem cells and differentiated cells in the tissue to sustain and repair the tissue and protect tissue or prevent organ failure and struggle with the aging process

Upregulated inflammatory response genes, loss of DNA repairing genes, chromatin remodeling regulatory genes, and genomic integrity genes cause functional defects of stem cells, diminish the age-dependent stem cells, and reduce self-renewal ability and regenerative capability of stem cells. Hence gene expression changes explain the decline of bone marrow potential of aging bone marrow.

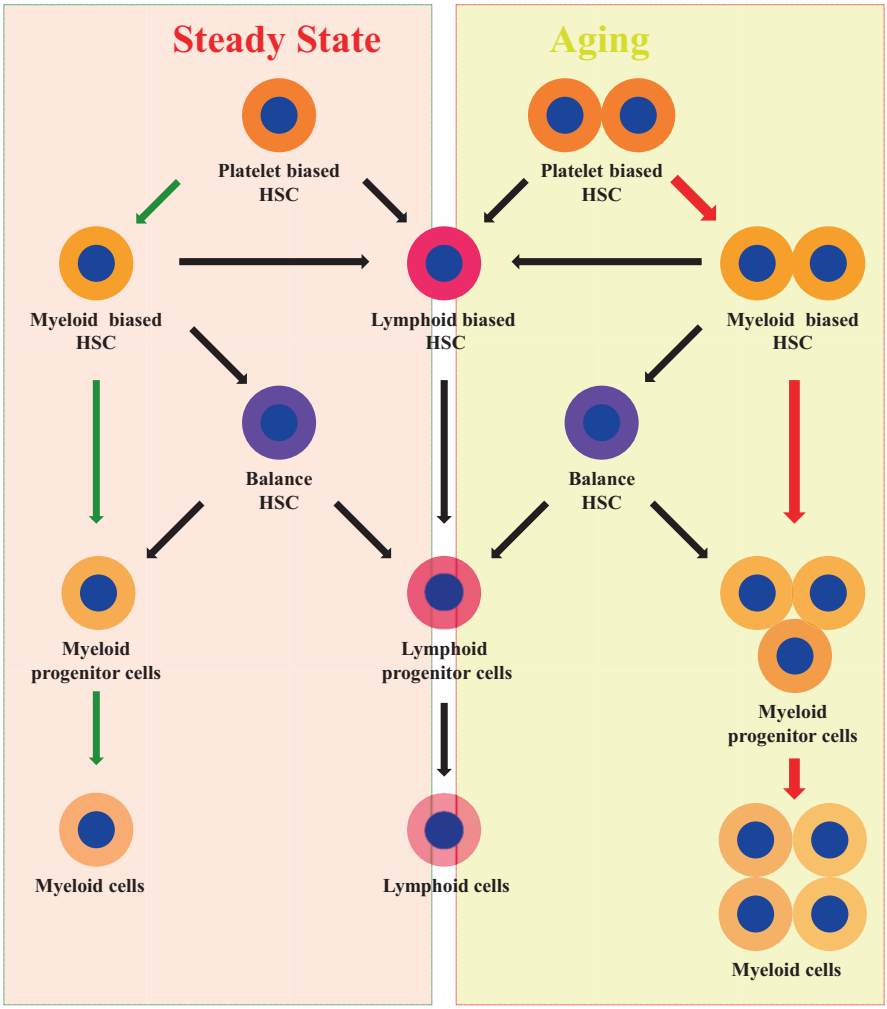

Fig. 15.5 Abnormal lineage differentiation (Image taken from doi: https://doi.org/10.3389/fimmu.2016.00502)
During aging, lymphoid lineage-specific genes were deactivated, whereas myeloid lineage-specific genes, megakaryocyte-associated genes, and many proto-oncogenes were found to be more activated with aging. As a result, myeloid cells increase in number as compared to lymphoid cells during the aging process. Dysregulation of lineage differentiation leads to abnormal cellular and bone marrow functions

15.2.1.3 Epigenetic Mechanisms

The previous report revealed that epigenetic mechanisms are linked with aging processes. Epigenetic modification such as DNA methylation is involved in many biological activities including gene imprinting, regulation of chromatin structure, and genomic instability. Global DNA methylation declines slowly and increases at

promoter regions during aging. Previous studies exposed that 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC), both epigenetic marks, coexist in the genome. 5mC is a stable DNA modification, but an epigenetic regulatory enzyme such as ten-eleven translocation (TET) family of protein enzymatically oxidizes 5mC to 5hmC, and then thymine-DNA glycosylase (Tdg) produces unmethylated cytosine [31]. Previous researchers have shown that catalytic activity of TET-2 is required for the maintenance of HSC progenitor development. In addition, mouse model study showed that somatic mutation in ten-eleven translocation-2 (TET-2) of HSCs generates atherosclerosis [32]. Hence, hydroxymethylation of 5mC is associated with silencing effect of 5mC and potential involvement in demethylation. Moreover, DNA methyltransferase-1 (DNMT1) has a lower affinity for 5hmC and is unable to maintain the methyl transfer to DNA during cell division [31] (Fig. 15.6).

Genome-wide research revealed that regulatory regions in embryonic stem cells and differentiated cells, gene bodies, and promoter showed high enrichment of 5-hydroxymethylcytosine (5hmC). During aging, depletion of 5mC and gaining of the 5hmC level exhibit an age-associated change in MSCs. Aged bone marrow-derived mesenchymal stem cells acquire global loss of DNA methylation. Thus, 5hmC-associated DNA demethylation has a crucial role in aging. Furthermore, 5hmCCpG sites, which occur in chromatin region marked by H3K4me1 are associated with poised enhancers in MSCs, whereas 5hmC is related to active enhancers marked by H3K4me1 (monomethylation of lysine 4 of histone H3) and H3K27ac (acetylation of lysine 27 of histone H3) in differentiated and embryonic stem cells. Interestingly, "stemness" of stem cells is associated with a change of 5hmC at H3K4me1, H3K27me3 (trimethylation of lysine 27 of histone H3), and H3k29me3 (trimethylation of lysine 29 of histone H3) regions of chromatin. Hence, low-density CpG region, intron, enhancer, cell adhesion, and morphogenesis-related genes showed the higher level of 5hmC, whereas embryonic stem cells and differentiation-related gene express a small degree of 5hmC. Moreover, proliferation- and development-associated related genes showed increased level of 5hmC in mouse brain, in combination with decreased level of 5mC during aging. Deregulation of 5hmC is involved in various diseases such as degenerative diseases and cancer [31].

15.2.1.4 ROS Affects HSC Aging

Aging of stem cells also depends on intracellular oxidative stress. This stress comes from reactive oxygen species (ROS) level within the cell, which causes DNA damage and decline of self-renewal capacity of stem cells. Impaired mitochondrial function and ATP generation in the cells elevate the level of intracellular ROS. Mitochondrial function, turnover, and biogenesis are associated with transcription factors such as FOXO transcription factors and Bmi1, which regulate the level of intracellular ROS [33]. The higher level of ROS activates MAP kinase (MAPK), which in turn activates Cdk (cyclin-dependent kinase) inhibitors, which block HSC division required for self-renewal and Atm expression involved in DNA damage-repair and genomic stability. On the basis of intracellular ROS level, HSCs can be divided into two subtypes. ROS^{high} HSCs lose self-renewal ability due to activation of Cdk inhibitors and differentiation capability because of damaged DNA, and ROS^{low} HSCs contain both higher self-renewal potential and repopulating

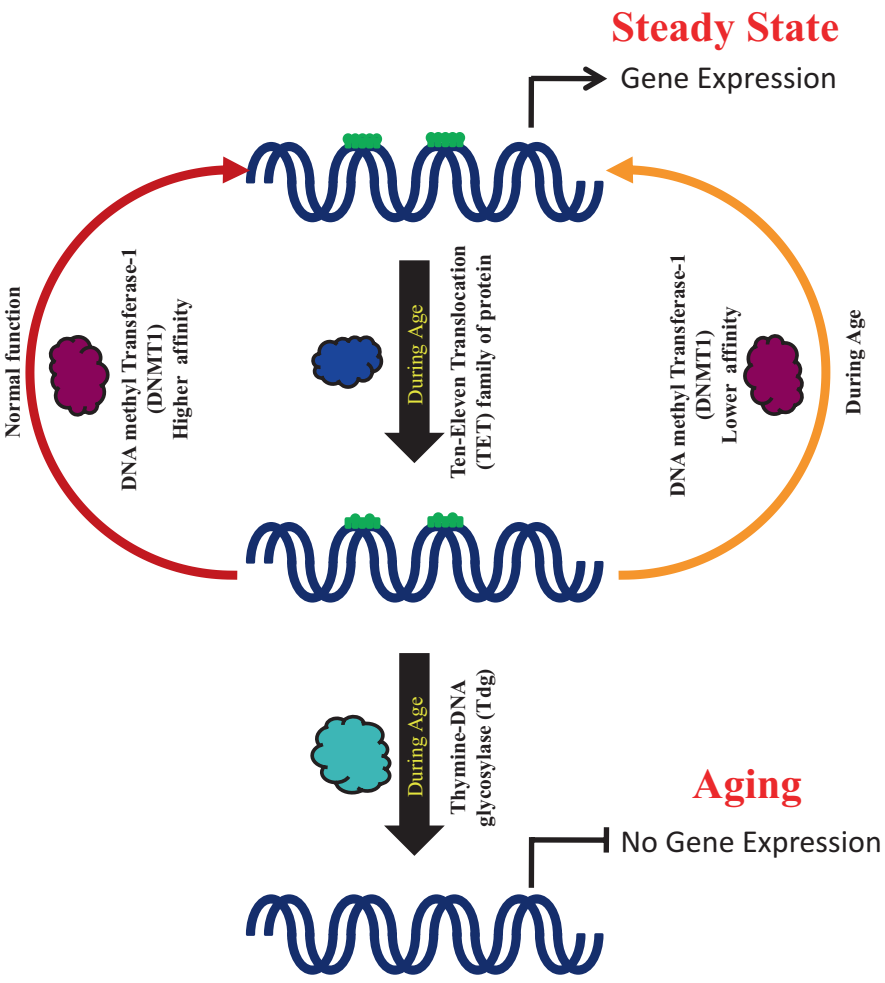

Fig. 15.6 Epigenetic mechanism of aging
Epigenetic modification such as DNA methylation has been implicated with aging. Global DNA methylation declines slowly and increases at promoter regions during aging. 5mC is a stable DNA modification, but an epigenetic regulatory enzyme such as ten-eleven translocation (TET) family of protein enzymatically oxidizes 5mC to 5hmC, and then thymine-DNA glycosylase (Tdg) produces unmethylated cytosine. Unmethylated cytosine is associated with inhibition of many genes and poised the enhancer in differentiated and embryonic stem cells [31]

ability. Hence, intracellular ROS levels regulate HSCs' activation, differentiation, proliferation, function, and homeostasis as well as aging of stem cells and hence raising the aging process [14] (Fig. 15.7).

15.2.1.5 Metabolic Pathways in Aging

The extreme consequences of aging are tissue failure, failure of regeneration processes, diseases, and lastly death. In recent years, advanced medical science, nutrition, and education have increased health span and life-span, but we still have many

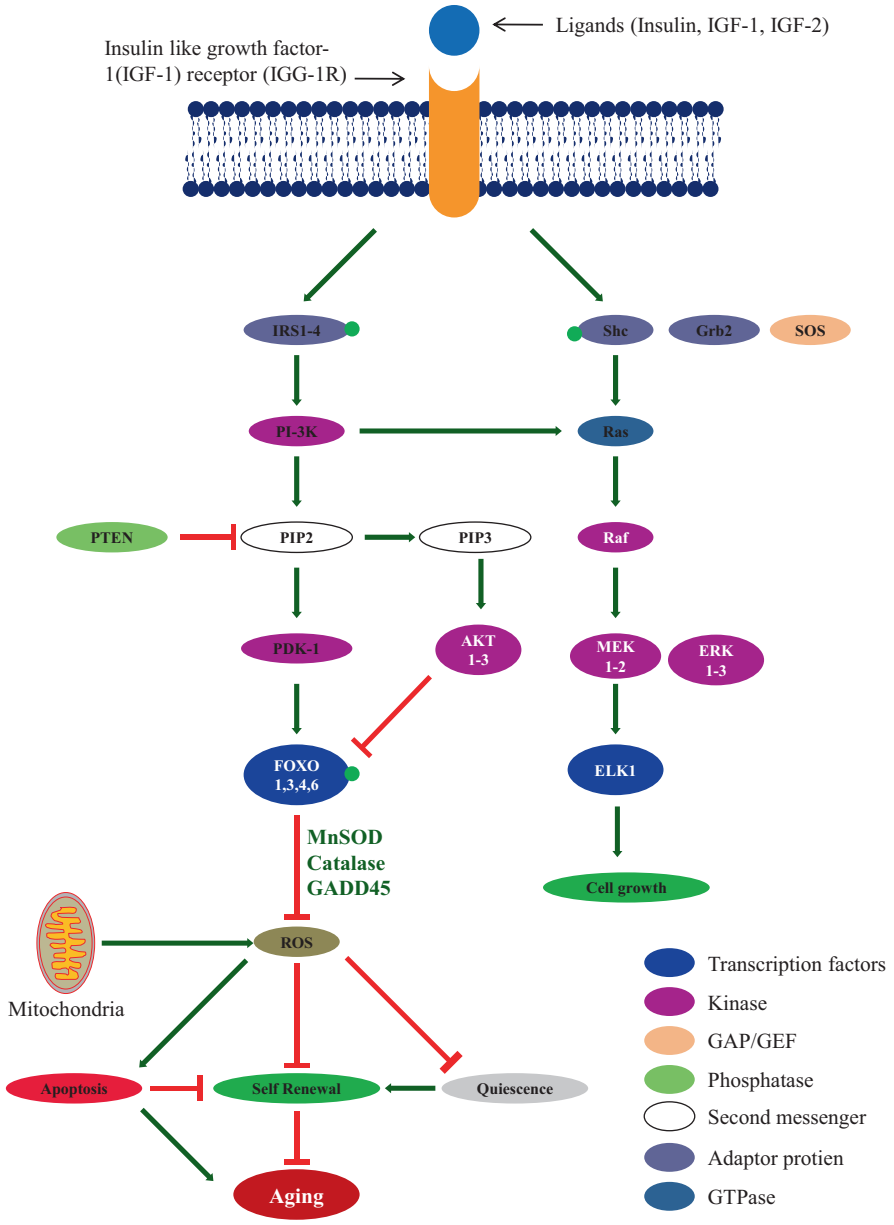

Fig. 15.7 Simplified description of metabolic mechanism of aging of stem cells in the bone marrow

Relationship between metabolic pathways and aging process. For example, Insulin and IGF1 signaling (IIS) activates FOXO transcription factors, which inhibit ROS production, the activity of p53, and enhance mitochondrial biogenesis and self-renewal. Hence metabolic pathway limits the aging process [35, 36]

questions such as what is the principal rule of aging, can aging be prevented, and how can we increase health span/life-span further?

The first breakthrough regarding this came in 1990, when the worm *C. elegans* having mutated daf-2 gene, which encodes insulin/insulin-like growth factor 1 (IGF1)-like receptor, showed a doubled life-span [33]. After that, various metabolic pathways are being searched, which delay aging process. For instance, Ames mice having a low level of IGF1 pathways showed more longevity. Decreased insulin and IGF1 signaling (IIS) activates FOXO transcription factors, which activate the catalase and antioxidant such as manganese superoxide dismutase (MnSOD). Moreover, FOXO transcription factors also improved mitochondrial biogenesis and function by activation of PPARγ co-activator 1α (PGC1α). FOXO transcription factors also inhibit the activity of p53, which is involved in other longevity pathways, such as mTOR and AMPK [33]. A previous study showed that FOXO-deficient mice had shown lymphoid development abnormalities, myeloid lineage expansion, and higher ROS level in HSCs compared to HSCs from bone marrow of normal mice. Moreover, FOXO-deficient bone marrow showed defective long-term repopulating activity; cell cycle arrest by regulation of downstream targets such as p27, p21, and cyclin protein of cell cycle; increased apoptosis; and depletion of HSCs. Hence, the association of evolutionarily conserved metabolic pathways (insulin/insulin-like growth factor 1 (IGF1)-like receptor→PI3K-AKT → FOXOs), stem cell depletion, and imbalanced HSCs' homeostasis in bone marrow indicates that it plays a significant function in the restriction of aging [34, 35]. Moreover, the complex metabolic system in mammals reduces the role of insulin/insulin-like growth factor 1 (IGF1) alone in aging. However, genetic and metabolic studies show that the conserved IIS pathway has a crucial role in human aging [36] (Fig. 15.7).

15.2.1.6 Telomeres and Aging

From the study of human fibroblasts emerged the role of telomeres linked to the aging process. Telomere, a repetitive TTAGGG sequence in humans, acts as a cap and protects chromosome ends from the damage [33]. A telomere is a highly conserved sequence from other organisms to humans [37]. Telomerase, an enzyme maintains elongation of telomeres and prevents senescence, a non-dividing state, in fibroblasts. However, the lack of an adequate level of telomerase results in the loss of or shorter telomeres with each round of DNA replication, which pushes the fibroblast cells to enter into a senescence state [33]. It is thought that the rate of telomere shortening is approximately 20bp per year, which is more gradual and has a continuous rate [13]. The loss of telomeres activates p53 via DNA damage pathway. p53, further, induces apoptosis, growth arrest, and senescence in stem cells. Thus the loss of telomeres acts as the molecular clock of stem cells during the aging process. Previous studies have shown that telomere shortening is strictly associated with age-related diseases. Moreover, a patient with dyskeratosis congenita having shorter telomeres, a premature aging syndrome, contains a mutation in TERC (the RNA component of telomerase) and TERT (the catalytic component of telomerase). Furthermore, functional loss of TERC and TERT is associated with several diseases such as bone marrow failure syndrome. Hence, telomeres' dysfunction declines

tissue's function, and organ failure particularly in the highly proliferative organ such as bone marrow, which promotes short life-span and aging. Thus, these studies speculate that telomere-based aging is primarily a bone marrow stem cell's defect caused by increased p53 activity, induction of growth arrest, senescence, and high level of apoptosis of stem and progenitor cells in the bone marrow [33].

Furthermore, inhibition of p53 by metabolic pathways, mild DNA damage, and lower level of p53 activation allow repair and maintenance of cellular functions resulting in the decline of aging process, whereas excessive DNA damage and p53 activation lead to p53-dependent cellular senescence and apoptosis, which accelerate aging. However, how players of aging such as mitochondria and p53 increase and decrease life-span remains to be clearly understood [33].

15.3 Aging- and Bone Marrow-Related Diseases

15.3.1 Cancer

HSCs show changes with age and produce either no cell population or uncontrolled cell population of a particular cell lineage (known as cell autonomous mechanism for functional decline) and decrease the fitness of stem cells and progenitor cells (termed as a non-cell-autonomous mechanism). The reduced fitness of stem cells and progenitor cells favor the oncogenic mutation, which encourages the initiation of cancer. For instance, fitness decline of aged B lymphopoiesis is connected with impaired receptor-associated kinase signaling. Moreover, impaired IL-7 signaling promotes selection of Bcr-Alb expression in aged B progenitors, which develop leukemias [38]. Altered bone marrow microenvironment, particularly stem cell niche including MSCs and their progeny leads to the uncontrolled growth of a particular population which produces several blood diseases. Most common malignant heterogeneous diseases of HSCs and progenitor cells are myelodysplastic syndromes (MDS), acute myeloid leukemia (AML), and acute lymphoblast leukemia (ALL) [8, 39, 40]. Previous literature showed that altered bone marrow niche, the particular stem cells' niche such as mesenchymal cells and their progeny, which have a strong immunomodulatory capacity, releasing trophic factors, and communicate with all other immune cells, involved in development and propagation of MDS [8]. Later on, MDS turns into AML [39]. AML is a most common cancer affecting older adults, and its incidence increases with advancing age. Moreover, rapid progress and more resistance to standard chemotherapy of AML in the elderly increase mortality rate in elder patients, if left untreated [13]. It is characterized by overproduction of abnormal white blood cells such as myeloblasts, monoblasts, and megakaryoblasts in the bone marrow and represses the generation of healthy blood cells. Unlike AML, overproduction and accumulation of lymphoblasts and immature cancerous white blood cells in the bone marrow drive ALL, most common in childhood. Although many age-dependent factors, including gene expression, epigenetic change, and cellular physiology of HSCs have been reported that lead to AML and ALL but the exact age-dependent molecular mechanisms of AML and ALL are still to be investigated [40].

15.3.2 Altered B Lymphopoiesis

Previous studies have shown that altered bone marrow microenvironment with advancing age inhibits B lymphopoiesis, the developmental process of B cells. Moreover, aged B cells are more pro-inflammatory in nature, which further reduces B-cell development. Thus, impaired B lymphopoiesis promotes decline in number, functions, and humoral immunity during aging process [41]. Microarray study reveals that antibody-secreting cells (ASCs) undergo alterations with advancing age such as a defect in energy production and higher ROS level has been identified [42].

15.3.3 Osteoporosis

BM-MSCs can differentiate into osteoblasts, chondrocytes, and adipocytes under the control mechanism/condition. Under the normal mechanism, RANKL (receptor activator of nuclear factor-kappa B ligand) binds to osteoclast's RANK (receptor activator factor kappa-B) receptor. Receptor-ligand binding activates osteoclastogenesis, resulting in the generation of osteoclasts. Aging process pushes bone marrow MSCs toward adipogenesis instead of osteogenesis. As a result, some osteoblasts and their activity decline, and a number of adipocytes increase. Moreover, osteoblasts and osteocytes mainly produce osteoprotegerin (OPG), a cytokine receptor, which mimics as RANK. RANKL-OPG binding inhibits the downstream signaling and blocks the osteoclastogenesis. Thus, the ratio of OPG/RANKL is an indicator of skeletal integrity and bone mass. Furthermore, OPG which is a member of the tumor necrosis factor receptor (TNFR) family interferes with activities of MSCs' differentiated osteoblasts and promotes MSCs' differentiation toward adipogenesis during the aging process. As a result, MSCs' differentiated adipocytes, instead of osteoblasts, accumulate in the bone marrow cavity, the primary characteristic of aged bone. Moreover, the increased number of adipocytes leads to osteoporosis in the bone with advancing age [43].

Moreover, previous researchers reported that cells expressing pre-adipocyte marker Pref-1 showed downregulation of osteoprotegerin, RANKL-positive, and exhibited a higher number of cells with advancing age. These cells generate osteoclasts from BM macrophage. Hence, cells at pre-adipocyte stage favor osteoclastogenesis and bone destruction with advancing age [44] (Fig. 15.8).

Previous *ex vivo* study showed that Interferon Regulatory Factor-1 (IRF-1) plays a crucial role in regulation and maturation of bone metabolism and is also involved in the activity of osteoblasts and osteoclasts. IRF-1 deficiency is closely associated with increased mineralization activity and also linked with decline proliferation of BM-derived osteoblasts. Irf-1−/− (gene knock out) mutant mice exhibit increased cellularity and cortical thickness, altered bone architecture, and bone morphology [45].

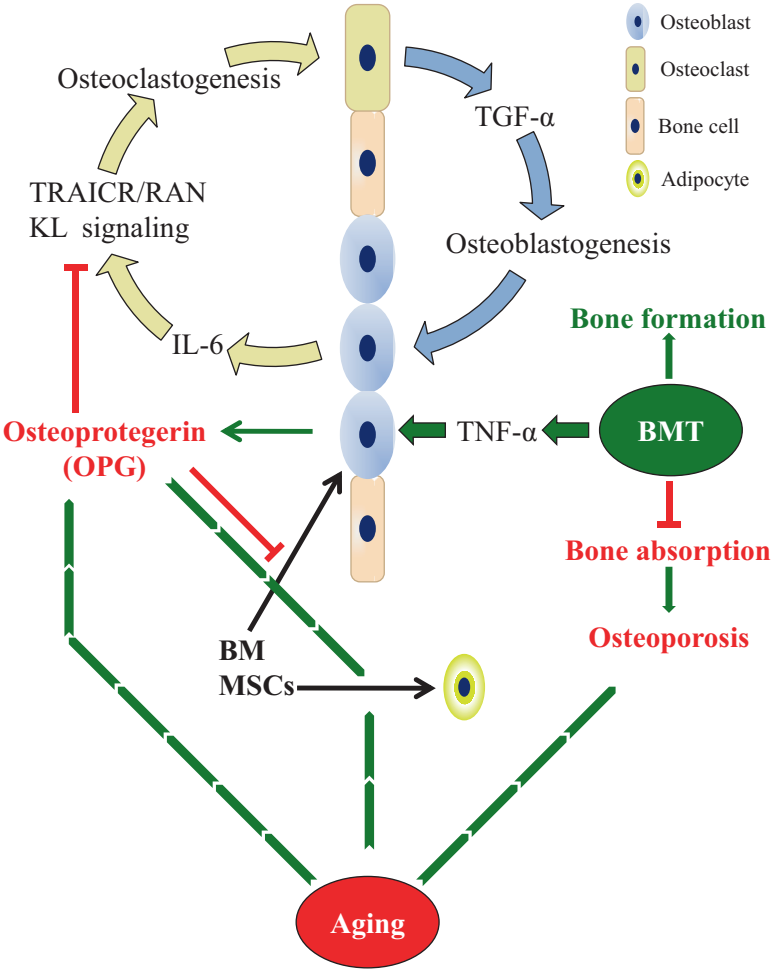

Fig. 15.8 Bone formation/osteoporosis/adipogenesis (https://doi.org/10.3389/fcell.2014.00016). MSCs can be differentiated into osteoblasts, chondrocytes, and adipocyte under the control mechanism/condition(s). Under the normal mechanism, osteoclasts secrete TGF-α, which in turn activates osteoblastogenesis, resulting in osteoblast generation. Osteoblasts release IL-6, which is more crucial for TRAICR/RANKL signaling in osteoclasts which are responsible for osteoclastogenesis. During the aging process, osteoblasts and osteocytes mainly produce osteoprotegerin (OPG), a cytokine receptor, which mimics RANK. RANKL-OPG binding inhibits the downstream signaling and blocks the osteoclastogenesis. Moreover, MSCs are differentiated into adipocytes, instead of osteoblasts, and accumulate in the bone marrow cavity, which leads to osteoporosis in the bone with advancing age. Bone marrow transplantation reduces bone absorption and inhibits osteoporosis [43]

15.3.4 Age-Related Macular Degeneration (AMD)

Vision loss disease such as age-related macular degeneration (AMD) is irreversible blindness and more common at the age of 60–70 that continuously rises. AMD is characterized by the deposition of drusen between the Bruch's membrane and basement membrane of the retinal pigment epithelium (RPE) [46]. AMD is associated with degeneration or death of cells such as choroidal endothelial cells (CECs), retinal pigment epithelial cells, and photoreceptor cells. Outer retina depends on the choriocapillaris for maintaining metabolic support and loss of endothelial cells of the choriocapillaris, which cause a severe problem. With advancing age, choroid and Bruch's membranes, which are an essential component of healthy vision, exhibit changes in the molecular composition and the structure of these tissues. Alterations in the tissues produce inflammatory environment and promote disease progression [47]. Tissue-specific stem cells may be unable to generate RPE-like cells during aging due to certain deficiencies, thus resulting in AMD. However, bone marrow-derived stem cells, embryonic stem cells (ESC), and tissue-specific stem cells may be able to produce RPE-like cells. Hence RPE transplantation could be a future technique for treatment of AMD [46].

15.4 Bone Marrow Therapy in Age-Related Diseases

15.4.1 Bone Marrow Transplantation

Accumulation of damage within the stem cells with age creates a deficiency of immune system, which leads to age-related diseases such as cancer, Alzheimer's disease (AD), Parkinson's disease, and osteoporosis. Previous research showed that bone marrow transplantation (BMT) is a choice for age-related diseases (Table 15.3).

15.4.1.1 Type-2 Diabetes Mellitus (T2DM)

Nutritional factors such as overnutrition, physical inactivity, genetic factors, and lifestyle cause obesity, which leads to age-related diseases such as T2DM. T2DM is a systemic, slowly progressing, chronic and life-threatening disease, if left untreated. T2DM incidences are increasing among aged people. The deterioration of insulin secretion by pancreatic β-cells and insulin-stimulated glucose uptake in highly active tissues such as adipose, muscle, and liver tissues is known as insulin resistance [48]. Stem cell transplantation therapy could be a useful approach for T2DM, which mainly results in decreased hyperglycemia, improved insulin sensitivity, and maintained normal blood glucose level in the body. Previous research showed transplantation of embryonic stem cell (ECS)-derived insulin-producing cells reduces hyperglycemia in streptozotocin-treated diabetic mice. Moreover, transplantation of human embryonic stem cell (ECS)-differentiated insulin-producing cells in the NOD/SCID diabetic mice decreases hyperglycemia. Since MSCs have immunomodulatory effect, homing properties, and can differentiate into insulin-producing cells, like the islets cells of the pancreas, it is a better choice for stem transplantation

Table 15.3 Bone marrow transplantation (BMT) therapy in age-related diseases, stem cell potentials and regenerative capabilities of the bone marrow-derived stem cells

Bone marrow transplantation (BMT) therapy			
S. no.	**Age-related bone marrow disease**	**Therapy**	**References**
1	Cancers	BMT	[8, 38–40]
2	Altered B lymphopoiesis	BMT	[41–45]
3	Osteoporosis	BMT	[15]
4	Age-related macular degeneration (AMD)	BMT	[49]
5	Type-2 diabetes mellitus (T2DM)	BMT	[15, 48]
6	Alzheimer's disease (AD)	BMT	[15]
Stem cell transplantation (SCT) therapy			
Stem cells	**Tissue regeneration models by cell therapy (A)**	**Stem cell potential, regenerative capabilities (B)**	**References**
HSCs	Ischemic myocardium	c-kit-1, Thy1.1^{lo}, Lin^{-}, Sca-1^{+} long-term reconstituting hematopoietic stem cells [53]	(A) [53]
	GFP-transgenic C57BL6/Ka-Thy-1.1 mice		(B) [53]
	C57BL/Ka-Thy-1.1 mice [53]		
MSCs	Myocardial infarction	Mouse MSCs derived	(A) [54]
	Immunocompetent Lewis rats	Angiogenesis and cardiomyocyte regeneration [54]	(B) [54]
	C57BL/6 mouse MSCs [54]		
MAPCs	Lymphoma in thymus and spleen	Play an important role in neoangiogenesis in mouse [24]	(A) [24]
	NOD/SCID mice		(B) [24]
	Mouse MAPCs [24]		

therapy for T2DM (Fig. 15.9). MSCs' transplantation favors PDX1 expression and protects islets' cells from pro-inflammatory effect and reduces hyperglycemia in diabetic mice. Thus stem cell transplantation decreased blood glucose level, increased insulin sensitivity, and restored islets β-cell function [15].

15.4.1.2 Osteoporosis

The most common age-related bone disease is osteoporosis, which is characterized by dysregulation of bone absorption and bone formation. The bone formation includes osteoblastogenesis process, which generates osteoblasts and osteoclastogenesis process, which produces osteoclasts. Both processes osteoblastogenesis and osteoclastogenesis are controlled by TNF-α, IL-6, and TGF-β. TNF-α provokes osteoblasts for producing IL-6, which in turn accelerate TRAICR/RANKL (TNF-related activation-induced cytokine receptor/receptor activator of nuclear factor-kappa B ligand) signaling and activate osteoclastogenesis resulting in the production of osteoclasts. Osteoclasts produce TGF-α, which regulates the osteoblastogenesis. SAMP6 (senescence-accelerated mouse prone 6) is a mouse model of age-related

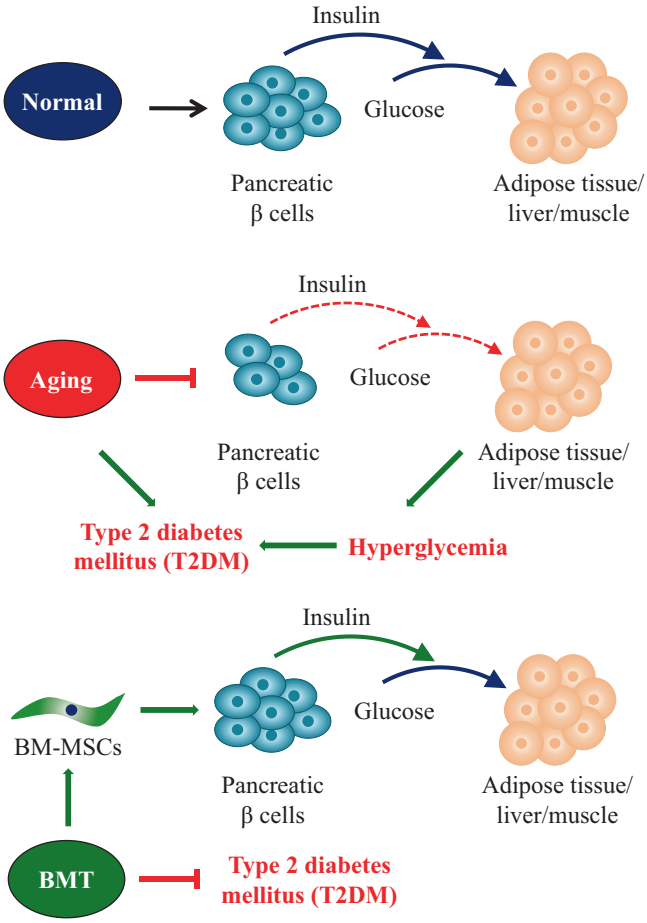

Fig. 15.9 Type-2 diabetes mellitus (T2DM) (https://doi.org/10.1038/nrd4275)
T2DM incidences are increasing among aged people. During normal condition, pancreatic β cells secrete sufficient amount of insulin into highly active tissues such as adipose tissue, muscle, and liver. The deterioration of insulin secretion by pancreatic β-cells increased with the aging process. As a result, hyperglycemia arises, which leads to T2DM. Stem cell transplantation therapy could be a useful approach for T2DM, which mainly involve decreased blood glucose level, increased insulin sensitivity, restored islets β-cell function, and normalized hyperglycemia or T2DM

osteoporosis disease. Previous researchers disclosed that bone marrow microenvironment of SAMP6, which showed dysregulation of TNF-α, IL-6, and TGF-β. Bone marrow transplantation increases the level of RANKL, IL-6, and IL-11, which control the imbalance of bone formation and bone absorption and prevent the osteoporosis in a mouse model [15] (Fig. 15.8).

15.4.1.3 Alzheimer's Disease (AD)

The most familiar age-related neurodegenerative diseases are Alzheimer's disease (AD) and Parkinson's disease (PD). AD is characterized by learning and memory loss, behavioral depression, brain atrophy, loss of neurons, neuronal synapses, neuronal dendrites, dendritic spines, and β-amyloid deposition in the brain. Previous research showed that SAMP8/SAMP10 (senescence-accelerated mouse prone 8/senescence-accelerated mouse prone 10) is a well-accepted mouse model for the study of AD, which reflects cognitive deficit similar to AD patient such as decreased catecholamine synthesis in the cerebral cortex, neuronal DNA damage, decreased hippocampal receptors, reduced neurotrophic factors, and elevated oxidative stress in the brain with the aging process. Bone marrow transplantation normalizes HO-1 (oxidative stress marker) level, IL-6, IL-1β, and iNOS, reduces β-amyloid deposition, and thereby prevents the progression of AD [15].

15.4.1.4 Bone Marrow Stem Cell Therapy for AMD

A preclinical study showed that bone marrow transplantation (BMT) could be used to treat vision loss or retinal dysfunction which is age-related degeneration. Stem cell transplantation approach is being discovered for regeneration of the retina, and the treatment can cure ischemic and degenerating retina. Stem cells are known for secreting paracrine trophic factors, which can reach multiple damaged cells and impart regenerative effect. Therefore, stem cells are directly transplanted into damaged tissue. Stem cell therapy has no limitations to AMD diseases. Stem cells such as mesenchymal stem cells are a more commonly administered cell type for treatment of diseases such as AMD, mainly because of their homing and trophic properties [49].

15.5 Conclusion

We have presented information about bone marrow, its structure, stem cells, their organization or distribution within the bone marrow, and how bone marrow, stem cells, and stromal cells change with aging. The different theories proposed to explain the aging process are discussed, but still, we do not know the exact process of aging. The tissue-specific stem cells that differentiate and proliferate into the required cell type could be one of the driving powers for ameliorating the aging process. Failure of epigenetic control might be considered for the aberrant gene expressions observed during the aging process. ROS level increases inflammatory microenvironment and contributes its role in the aging process. The lineage skewing phenotype is seen during generation of stem cells such as abnormal HSC differentiation with advancing age that seems to have a direct force on this process. Gene expression studies propose that sustaining genomic stability and DNA repair pathways could be one of the driving capabilities of the protection against the aging process. However, the exact mechanism of the aging process is still unexplained. Therefore, future studies will explain how bone marrow and stem cells change over time and how bone marrow transplantation and stem cell transplantation may control aging process and help preventing age-related diseases as well as provide better understanding of the aging process.

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Part II

Interventions for Healthy Aging

Infantile Radiation and Aging Stresses: Effects of Calorie and Dietary Restrictions

16

Yi Shang, Keiko Odera, Shizuko Kakinuma, Yoshiya Shimada, and Ryoya Takahashi

Abstract

Biological macromolecules such as proteins, lipids, and nucleic acids in all living things are always exposed to endogenous and exogenous stress. An accumulation of damaged macromolecules in cells can lead to a decrease in normal cellular function and eventual cell death and/or carcinogenesis.

Reactive oxygen species (ROS) are considered to be major endogenous stressors which cause damage to macromolecules such as DNA, proteins, and lipids during aging. By contrast, ionizing radiation is an exogenous stressor which causes damage indiscriminately and abruptly to macromolecules, especially DNA, and increases cancer risk.

Lifelong calorie and dietary restrictions (CR/DR) are well known to have preventive effects on the onset of many age-associated disorders, including spontaneous cancers, and extend mean and maximum life span in various animals. However, limited information is available on the effect of CR/DR on intensive damage during infancy and on cumulative damages during aging.

Recent studies suggest that the level of protein carbonyls is correlated with cell death and spontaneous mutation rate. Therefore, it is of great interest to determine whether CR/DR reduces the level of accumulated altered proteins with age and prevents carcinogenesis.

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Here, we describe our studies suggesting that CR initiated after infant radiation improves life span and reduces carcinogenesis and that short-term DR initiated even later in life attenuates the effects on accumulation of altered proteins, including carbonylated proteins.

Keywords
Childhood radiation exposure · Calorie restriction · Dietary restriction

16.1 Introduction

All living organisms are constantly exposed to a variety of endogenous and exogenous stressors during aging. Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen and are considered to be a major endogenous stressor [1, 2]. High levels of ROS result in damage to macromolecules such as DNA, proteins, and lipids; therefore, ROS have been implicated as a causative factor of aging and age-associated disorders including cancer [1, 2]. Indeed, oxidative modified molecules of DNA, proteins, and lipids have been found to be increased with age in a variety of laboratory animals as well as humans [1, 2]. Among them, protein modifications may be a root cause of the aging processes because repair and degradation of damaged molecules of DNA, lipids, and protein are accomplished by the action of a variety of enzymes and protein factors [3, 4]. This idea is supported by the fact that the level of oxidative carbonylation of proteins is correlated with cell death and spontaneous mutation rate [5, 6].

Altered macromolecules can be also directly or indirectly generated by exogenous stresses, such as ultraviolet irradiation and ionizing radiation [3, 4]. For example, ionizing radiation produces a wide spectrum of DNA lesions, such as nucleotide base damage and DNA single- and double-strand breaks, and promotes cell transformation and carcinogenesis through induction of gene mutation and chromosome aberration [7].

The dietary regimen most frequently applied to improve multiple parameters of health and to extend life span is daily calorie restriction (CR), which involves decreasing energy intake without lowering nutritional value [8]. Another dietary restriction regimen employed is dietary restriction (DR) which is a moderate reduction of food intake by reduction of daily food intake or by every-other-day (EOD) feeding [8]. Both lifelong CR and DR initiated early in life have been shown to extend mean and maximum life span and delay the onset of age-associated disorders in laboratory animals [8–13]. Lifelong CR/DR also protects against spontaneous cancers and carcinogen-induced cancers [14–22]. Thus, CR/DR is well established and the only nongenetic intervention that has consistently been found to delay the aging process of a variety of animals. However, available information is limited on the effect of CR/DR on intensive damage during infancy and on cumulative damage during aging.

In this chapter, we first describe our recent studies concerning the effects of CR on the life span and carcinogenesis after exposure to ionizing radiation during infancy in a mouse model. We next consider the implications of DR initiated during

old age on selected parameters known to change with age, focusing on our studies concerning the effects of short-term DR initiated during old age.

16.2 Effect of Adult-Onset CR on the Life Span and Lifetime Cancer Risk After Early-Life Exposure to Ionizing Radiation

It is well known that exposure to ionizing radiation increases cancer risk. Epidemiological studies on atom bombs and the Chernobyl nuclear power plant survival showed children are highly susceptible to radiocarcinogenesis [23, 24]. Recently, medical usage of radiation, for example, pediatric computed tomography (CT) examination or radiotherapy for childhood cancer, has increased [25, 26]. It is also reported that the use of CT in childhood diagnosis might increase the risk of leukemia or brain tumor [27]. Thus, limiting the cancer risk after radiation exposure is of great public concern. CR has been suggested as a potent cancer prevention strategy for almost 100 years. In 1935, McCay et al. reported that reducing calorie intake by 30–40% prolonged life span and decreased tumor development [28]. Later, the beneficial effects of calorie restriction were confirmed in *Saccharomyces cerevisiae*, *C. elegans*, mice, dogs, and nonhuman primates [14–20]. Using animal models, the cancer prevention effects of CR were detectible in spontaneous, chemical carcinogen as well as radiation-induced tumors.

However, CR is usually not appropriate for children because of its potent negative effects on growth, so that information on cancer prevention category for childhood exposure to radiation or other carcinogens is very limited. In this chapter, we summarize the CR/DR method and animal models using CR/DR-prevented radiation-induced tumors and discuss if adult-onset CR affects the lifetime tumor risk in mice after childhood ionizing radiation exposure.

16.2.1 Animal Models

Although exposure to ionizing radiation is well known as a cancer risk factor, studies on CR/DR-prevented radiation-induced cancer are very rare. In 1984, Gross and Dreyfuss reported that DR reduced X-ray-induced mammary tumor using 3–4-week-old Sprague-Dawley rats irradiated by whole-body X-ray, split into 4 weekly doses of 1.5 Gy for mammary tumor induction. Cutting ad libitum food intake by one-third significantly reduced tumor induction and prolonged life span of rats [29]. Another study by Gross et al. showed that fractional gamma-ray exposure (split into 4 weekly doses of 1.5 Gy) at the age of 3–4 weeks induced myeloid leukemia in C3H mice, with an incidence of up to 50%. Restriction of food intake to half ad libitum efficiently reduced leukemia to 4% [30]. However, DR in these studies consisted solely of cutting down food intake, and the influence of malnutrition on cancer development was unknown.

In the 1990s, Yoshida et al. reported the cancer prevention effects of CR on spontaneous and radiogenic cancers. This CR method restricted calorie intake by

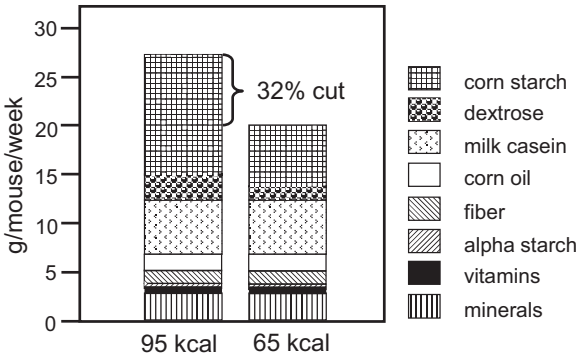

Fig. 16.1 Composition of diet. (Modified from Yoshida et al., Proc Natl AcadSci USA 1997 [20])

reducing the calorie proportion from carbohydrates, maintaining intake of other nutrients such as protein, fat, fibers, vitamins, and minerals (Fig. 16.1). Yoshida et al. reported that the incidence of spontaneous myeloid leukemia in C3H male mice was only 1%, and the incidence was up to 23.3% with exposure to 3 Gy of X-ray. However, leukemia incidence was significantly decreased by 30% CR. This was diminished to 7.9% when restriction was started before irradiation and 10.7% after irradiation, respectively. In addition, the first detection of myeloid leukemia in both CR groups was remarkably delayed compared with the standard diet group. CR also showed a significant life span extension in the groups that began CR after having been exposed to irradiation [31]. Using the same CR method, Yoshida et al. also showed its effects on suppressing spontaneous hepatoma development in C3H male mice [32].

16.2.2 Cancer Prevention Effects of Adult-Onset CR on Childhood Exposure to Radiation

The above reports were all carried out with adult animals. Recently, medical uses of radiation, such as pediatric CT examination and radiotherapy, were increased. There is an urgent task to focus on assessing risk and developing prevention strategies for radiation-induced cancer in children. CR has been known as a cancer prevention method for almost 100 years. However, CR for children is not appropriate because of the concern on malnutrition and disordered endocrine function. Since it is a long process of cancer development after radiation exposure, involving genomic instability and continuous inflammation, we proposed that adult-onset CR during the tumor promotion/progression process could be a functional strategy on suppressing cancer development after exposure to ionizing radiation at a young age and assessed the cancer prevention effects of adult-onset CR in a mouse model [33].

In this study, we chose male B6C3F1/Crlj mice (C57BL/6NCrlCrlj × C3H/HeNCrlCrlj). This strain is a standard strain for toxicological studies, and multiple tumors can be spontaneously developed or induced by radiation in this single strain

[34]. Mice were irradiated by 3.8 Gy of X-ray or sham-irradiated at the age of 1 week, weaned at 4 weeks, and fed with standard laboratory diet until 7 weeks old. Mice were then randomly divided into a 95 kcal group (95 kcal/mouse/week, standard calorie diet intake (SD)) or 65 kcal group (65 kcal/mouse/week, restricted calorie intake (CR)), using the same CR method as reported by Yoshida [31]. All mice were allowed to live throughout their entire life spans under experimental conditions, monitored for body weight change, autopsied at moribund or death, and examined pathologically for cancer type and grade. Life span analysis and cancer risk assessment were carried out. As described previously, all experiments were approved by the Institutional Animal Care and Use Committee of the National Institute of Radiological Sciences, Japan (Approval Number 07-1080), and all efforts were taken to minimize suffering [33].

Figure 16.2 shows the body weight change in function of age. The body weight differences between the SD and CR kcal diet in the sham-irradiated groups were observed from just 1 week after CR starting and throughout the whole life span of the mice. Growth inhibition of X-ray exposure was evident in the SD groups only. CR kept body weight at 30 g in both irradiated and sham-irradiated groups, more than 5 g lower than the standard diet, so that the growth inhibition of X-ray in the CR group was not detectable.

Figure 16.3 shows overall Kaplan-Meier survival curves of all four experimental groups. X-ray exposure induced life span shortening significantly, reducing overall life span by ~38%, irrespective of calorie intake (878.0–546.9 in 95 kcal groups, 1047.0–654.3 in 65 kcal groups). Meanwhile, life span extension effects of CR were up to 19.2% and 19.6% (878.0–1047.0 and 546.9–654.3) in sham-irradiated and irradiated groups, respectively. There was no difference detected on life span extension in sham-irradiated and irradiated groups, indicating that CR contributes to prolong life span through biological pathways that are independent of radiation responses.

Multiple types of tumor were spontaneous or induced by X-ray exposure in this B6C3F1 mouse model. Thymic lymphoma (TL) and early-occurring other

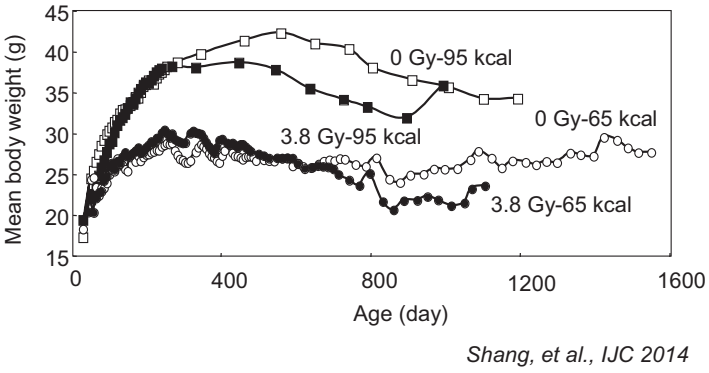

Fig. 16.2 Body weight change. Mean body weight vs. age for all experimental groups. Data represent the mean for 60–63 mice per treatment group. (Modified from Shang et al., Int J Cancer. 2014 [22])

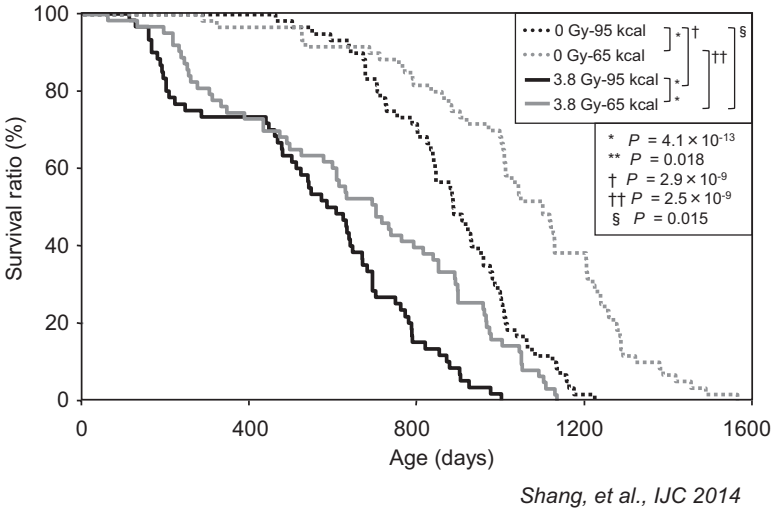

Fig. 16.3 Changes in overall survival ratio. Kaplan-Meier curves that represent overall survival ratios for all experimental groups. (Modified from Shang et al., Int J Cancer. 2014 [22])

lymphomas were the most common cause of death within 1 year of age, which were observed in the irradiated groups but not in sham-irradiated groups. An obvious cancer prevention effect of CR was not confirmed in these early-occurring types, although the mean life span of mice died by TL was slightly prolonged from 146.8 to 258.9 days ($P = 0.32$, student's t test). On the other hand, in cases of late-occurring lymphomas (tumor onset later than 430 days of age), hepatocellular carcinoma, and lung adenocarcinoma, CR significantly decreased tumor incidence and/or improved tumor-free survival in all four experimental groups (Table 16.1).

Next, using multivariate Cox proportional hazard analysis, we assessed the hazard ratio (HR) of cancer development (Table 16.2). Risks (HRs) of exposure to 3.8 Gy of X-ray were 4.22 for the SD group and 4.48 for the CR group, and radiation affected overall life span equally in both diet types. The liver was the most susceptible to radiocarcinogenesis (HR = 12.92 for the SD kcal group, HR = 20.89 for the CR group), and the risk of lung adenocarcinoma developing after radiation exposure was also high. Late-occurring other lymphoma was less associated with X-ray exposure (HR = 2.30 for the SD group, HR = 2.89 for the CR group). There were no TL and early-occurring other lymphoma observed in the sham-irradiated groups on either diet, and HRs could not be calculated for these groups. There is no doubt that TL and early-occurring other lymphoma were radiogenic.

CR extended whole life span in sham-irradiated mice (HR for life span shortening = 0.33) and to a much greater extension in tumor-free sham-irradiated mice (HR for life span shortening = 0.18) [33]. These findings suggested that the benefits of CR were much more remarkable for tumor suppression than for other death-causing disorders in this animal model. The tumors most suppressed by CR were late-occurring other lymphoma (HR = 0.10), lung adenocarcinoma (HR = 0.13), and

Table 16.1 Summary of tumor incidence (I), tumor onset (O), and mean life span (M) for major tumors

Type of tumor			0 Gy–95 kcal (N = 60)	0 Gy–65 kcal (N = 60)	3.8 Gy–95 kcal (N = 60)	3.8 Gy–65 kcal (N = 63)
Thymic lymphoma		I	0	0	20.0 ± 5.2 (12)[a]	14.3 ± 4.69 (9)[b]
		O			127	126
		M			146.8 ± 20.9	258.9 ± 41.5
Other lymphomas	Aarly occurring	I	0	0	8.3 ± 3.65 (5)	7.9 ± 3.4 (5)
		O			112	231
		M			189.4 ± 21.7	325.8 ± 37.7[c]
	Late occurring	I	48.3 ± 6.5 (29)	20.0 ± 5.2 (12)[a]	16.7 ± 4.8 (10)[a]	9.5 ± 3.7 (6)
		O	449	683	459	696
		M	901.3 ± 30.6	1072.1 ± 74.9[a]	703.0 ± 54.6[a]	874.2 ± 65.2
	Total	I	48.3 ± 6.5 (29)	20.0 ± 5.2 (12)[a]	25.0 ± 5.6 (15)[a]	17.5 ± 5.0 (11)
		O	449	683	112	231
		M	901.3 ± 30.6	1072.1 ± 74.9[a]	531.8 ± 74.2[a]	624.9 ± 84.5[a,b]
Liver	Hepatocellular carcinoma	I	13.3 ± 4.4 (8)	15.0 ± 4.6 (9)	46.7 ± 6.4 (28)[a]	31.7 ± 6.0 (20)[b]
		O	590	967	438	571
		M	829.0 ± 49.0	1191.1 ± 47.8[a]	690.6 ± 28.8[a]	861.9 ± 58.8[b,c]
Lung	Adenocarcinoma	I	20.0 ± 5.2 (12)	15.0 ± 4.6 (9)	25.0 ± 5.6 (15)	6.3 ± 3.2 (4)[c]
		O	459	994	476	518
		M	888.7 ± 54.0	1230.1 ± 66.3[a]	704.3 ± 35.3[a]	753.5 ± 142.0[b]

I incidence ± SE (%) (number of mice), *O* onset (days), *M* mean life span (days ± SE)
[a] $p < 0.05$, vs. 95 kcal–0 Gy
[b] $p < 0.05$, vs. 65 kcal–0 Gy
[c] $p < 0.05$, vs. 95 kcal–3.8 Gy

Table 16.2 Summary of Cox proportional hazard analysis for major tumors

			Radiation vs. 0 Gy		Calorie vs. 95 kcal		Radiation and calorie vs.
		Risk factor	95 kcal	65 kcal	0 Gy	3.8 Gy	0 Gy–95 kcal
Overall life span		HR	4.22	4.48	0.33	0.54	1.78
		95% CI	2.79, 6.38	2.90, 6.93	0.2, 0.49	0.37, 0.79	1.24, 2.57
		P value	8.39×10^{-12}	1.49×10^{-11}	1.40×10^{-7}	0.0015	0.002
Thymic lymphoma		HR	ND	ND	ND	0.65	ND
		95% CI				0.26, 1.66	
		P value				0.37	
Other lymphomas	Early occurring	HR	ND	ND	ND	0.88	ND
		95% CI				0.25, 3.02	
		P value				0.83	
	Late occurring	HR	2.3	2.89	0.1	0.18	0.37
		95% CI	1.06, 5.02	0.88, 9.43	0.04, 0.25	0.056, 0.55	0.15, 0.90
		P value	0.035	0.079	6.24×10^{-7}	0.0027	0.029
	Total	HR	2.88	4.29	0.1	0.35	0.65
		95% CI	1.42, 5.85	1.50, 12.29	0.04, 0.25	0.15, 0.79	0.32, 1.32
		P value	0.0034	0.0066	6.24×10^{-7}	0.012	0.24
Liver	Hepatocellular carcinoma	HR	12.92	20.89	0.23	0.25	3.86
		95% CI	5.68, 29.38	6.09, 71.63	0.063, 0.83	0.13, 0.48	1.70, 8.78
		P value	1.02×10^{-9}	1.34×10^{-6}	0.0251	3.04×10^{-5}	0.0013
Lung	Adenocarcinoma	HR	6.2	3.79	0.16	0.13	0.59
		95% CI	2.56, 15.05	0.66, 21.73	0.05, 0.47	0.039, 0.45	0.18, 1.94
		P value	5.50×10^{-5}	0.14	0.001	0.0013	0.39

HR hazard ratio, *CI* confidence interval, *ND* not detected

then hepatocellular carcinoma (HR = 0.23). CR also prolonged life span of irradiated mice (HR for life span shortening = 0.54); however, compared with the sham-irradiated groups, the effect was smaller, indicating that CR is less efficient for radiation exposure-associated disorders. This was mainly resulted from early-occurring radiogenic TL and other lymphoma, which were not influenced by CR much (HR = 0.65 for TL, HR = 0.88 for early-occurring other lymphoma).

Table 16.2 shows a comparison of sham-irradiated SD and irradiated CR groups to estimate whether CR could reduce risk of radiation or not. Our data showed that the increased risk of lung adenocarcinoma conferred by childhood radiation exposure was negated by CR. Moreover, the HR for late-occurring other lymphoma was 0.37, indicating that CR defeated the radiation exposure-associated risk of these tumors. The risk of hepatocellular carcinoma was also decreased by CR but obviated completely.

16.2.3 Mechanism of CR Effect on Cancer Prevention

Our study on these B6C3F1 male mice firstly demonstrates for the first time that adult-onset 30% CR can extend overall life span and reduce tumor risk after childhood ionizing radiation exposure in a tissue-dependent manner. Using these data, Tani et al. applied a multistage carcinogenesis mathematical model (Armitage-Doll model) to assess age-specific mortality. This mathematical model showed that CR decreased the age-specific mortality of solid tumors especially liver tumor across most of the life span, with mortality rate more associated with age due to again the number of "steps." Radiation exposure did not change the number of steps significantly, but increased the overall transition rate between the steps [35]. However, the mechanism of the cancer prevention effects of CR and the reason for tissue dependency are still not clear. Earlier reports demonstrated that the effects of CR on spontaneous and chemical carcinogen-induced tumors are not homogeneous. It is reported that liver tumors, skin tumors, and breast tumors show a greater response to CR than others [36, 37], while a small proportion of tumors such as histiocytic sarcoma and musculoskeletal tumor are less responsible to CR [34]. Our report also documented that CR effects differed between late-occurring solid tumors as well as late-occurring lymphoma and early-occurring radiogenic TL and other lymphoma. CR hardly effects on these radiogenic tumors. Though the mechanisms of CR are not fully understood yet, the tissue dependence of the tumor prevention results is thought to be considered with distinct tumor initiation and promotion conditions and/or events in various tissues. For example, CR remarkably increases corticosterone levels, and, importantly, adrenalectomy eliminates the suppressive effect of CR on skin and lung tumors [38, 39], but this is not seen in breast cancers [40]. The prevention effects of CR on breast cancers are more associated with downregulation of insulin-like growth factor (IGF)-1 than circulating corticosterone [40]. IGF-1 is known as a growth factor produced in the liver, and the signal from IGF-1 activates the downstream phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway, so that it regulates energy metabolism and cell growth

and controls body size and longevity [41]. IGF-1 upregulates the cell growth rate and suppresses apoptosis in various cancer cells [42]. Epidemiological reports have also shown that upregulated serum concentrations of IGF-1 are correlated with increased risk of colon, prostate, breast, and lung tumors [43]. Thus, long-term CR reduces serum IGF-1 levels in rodents and plays a crucial role in suppressing carcinogenesis [44], and tumors with PI3K activation, such as *PTEN*-null tumors, are resistant to CR [45]. Conversely, CR-mediated suppressive effects against leukemogenesis in rats are abolished by IGF-1 infusion [46]. Other mechanisms such as Sirt 1 upregulation and inflammation suppression are also thought to be important in CR effects. As mentioned above, since radiation carcinogenesis mechanism differs among tissue types, it is not surprising that the power of CR on cancer prevention differs. Further investigation into the underlying mechanisms of CR effects on different tissues is needed to better understand the tissue dependence as well as to develop possible CR mimetics. Targeting a major pathway of CR, such as IGF-1 and/or the Akt/mTOR pathway, may be more essential for cancer prevention in humans, especially in children than CR.

16.3 Effect of CR/DR Initiated During Old Age on Life Span and Selected Aging Parameters

Although the effect of lifelong DR on the protein turnover (synthesis and degradation) has been investigated [8, 9, 47, 48], the efficacy of DR when introduced during old age has not been well studied [9].

We summarize the effects of CR/DR initiated at old age on longevity and some age-related changes, focusing on our studies concerning the effects of short-term CR/DR on altered protein accumulation and protein metabolism.

16.3.1 Effect of CR/DR Initiated from Old Age on Life Span of Animals

In 1975, Stuchlikova et al. found that 30–40% CR significantly increased both the mean and maximum life spans of three types of rodent (mice, rats, and golden hamsters) when CR was started at 1 year old [10]. Weindruch and Walford reported that approximately 45% CR beginning at about 1 year old increased the mean and maximum life span of two strains of mice (long-lived C57BL/6J and extremely long-lived B10C3F1) [11]. The efficacy of CR/DR on life span extension has also been observed in rats by EOD feeding when the dietary regimen was introduced at middle age [12]. However, the effect of CR/DR in middle-aged or older animals has not been always constant. For example, it has been reported that EOD feeding could not extend life span in mice when treatment was initiated at older age (>10 months). Furthermore, the results of rat studies on Long Evans [49] and F3BNF1 [50] rats showed that there is no significant effect of DR beyond late middle age (>18 months) on longevity.

For more than 20 years, the National Institute on Aging at the NIH has investigated the effect of CR in nonhuman primate (*Macaca mulatta*). They reported that CR initiated from old age (16–23 years) did not show a significant effect on longevity [51].

Thus, the magnitude of the life span extension by CR/DR considerably varied in different experiments. Such variation might be caused by differences in regimen, start age of CR/DR, and genotype (sex, strain, and species). Further carefully designed CR/DR studies on aged experimental animals are necessary in practical application for humans.

16.3.2 Effect of CR/DR Initiated from Old Age on Parameters Known to Change with Age

As mentioned above, lifelong CR/DR is well known to improve health and delays the onset of age-related disorders in most animals [14–22]. However, such a lifelong diet is not realistic for human application. One of the greatest concerns is how CR/DR introduced to aged animals influences the age-related changes that are already underway. The following states the implications of DR initiated from old age on some parameters known to change during aging, focusing on our studies regarding the effects of DR for a certain period of time in later life.

Accumulation of altered proteins in various tissues of aged animals might be due to the decrease in protein turnover [3]. We investigated the effect of short-term DR during old age on altered protein accumulation and protein degradation.

We found that the proportion of heat-labile aminoacyl tRNA synthetases in the tissues of mice significantly increased with an increasing mortality rate [52]. Six weeks of DR initiated from old age resulted in a dramatic reduction of the level of heat-labile enzymes in the liver and brain [53]. While the mechanism of the decrease of altered proteins remains unclear, the decrease might be a result of an increase in protein degradation of altered proteins in old animals. In fact, we have found that degradation rates of introduced proteins and radiolabeled endogenous proteins in primary cultured hepatocytes of old mice were increased by short-term DR from old age [54, 55]. These results suggest that an increase in protein degradation is considered to be one of the causes of the reduction of altered proteins by DR in tissues of old animals.

The ubiquitin (Ub)-proteasome proteolytic pathway is responsible for selective degradation of damaged proteins in cells [56, 57]. Therefore, it is of interest to examine if the proteasome activities are activated by DR. We found that the activities of peptidylglutamyl-peptide hydrolyzing (PGPH) activity in proteasomes were significantly decreased in the liver of old rats [58]. Interestingly, the PGPH activity that declined remarkably with age was restored significantly by weekday EOD feeding initiated from 26.5 months of age. A similar effect was observed in the skeletal muscle and tendon of DR of old rats [59]. Thus, the reduction of altered proteins by DR from old animals appears to be partly due to the activation of proteasomes.

Reactive oxygen species appear to be involved in the generation of oxidative modified altered proteins in cells and tissues of animals. We found that the content of protein carbonyls increased in mitochondria isolated from the liver of old rats and that the level of mitochondrial protein carbonyls in old rats was reduced to the level in young one by weekday EOD feeding [60].

Gene expression profiling analysis revealed that even short-term CR of old mice is sufficient to reproduce the major effects of long-term CR from young rats [61]. It is conceivable that DR initiated even in later life can improve health span. Interestingly, a monkey study by the NIA demonstrated that CR from old age improved several measures of health, although CR old monkeys did not live longer than controls [51]. Thus, later life CR/DR seems to be a realistic and beneficial manner for promoting quality of life [62].

Finally, alteration of proteins, especially proteins and enzymes involved in repair and turnover of macromolecules in cells, could lead to a decrease in normal cellular functions and eventual cell death and/or carcinogenesis. Recent studies have revealed that the accumulation of oxidative proteins in cells decreases the replication fidelity and repair rate of DNA, resulting in increased mutation and cell death [5, 6]. Our studies suggest that CR initiated after infant radiation improves life span and reduces carcinogenesis and short-term DR initiated even from later in life attenuates the effects on accumulation of conformationally and chemically modified proteins (Fig. 16.4). Although the exact mechanisms of the beneficial effects of CR/DR conducted in our study are not yet known, these effects may be partly explained by the reduction of altered proteins generated directly or indirectly by infantile radiation and lifelong aging stresses. However, further, more detailed studies of the mechanisms underlying the improvement of stress-induced damages by CR/DR are necessary in view of human application.

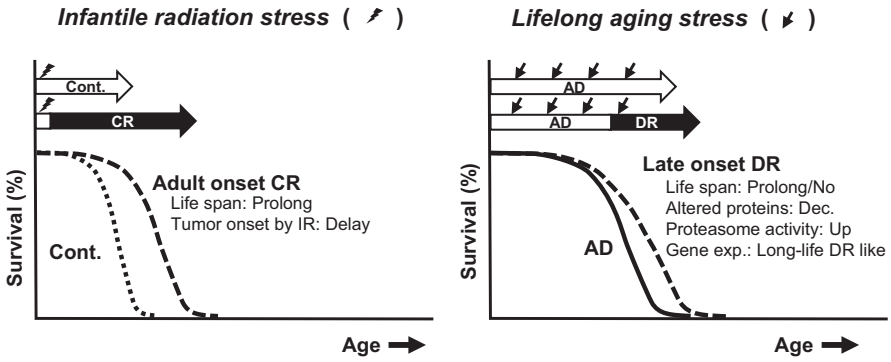

Fig. 16.4 Effects of calorie and dietary restrictions on infantile radiation and lifelong aging stresses. Infantile radiation (IR) exposure resulted in significant life span shortening. However, CR delays the tumor onset by infantile radiation and extends overall life span (left). Extension of life expectancy progressively decreased in older animals when a similar level of restriction was imposed on them; however, DR initiated even relatively late in life can restore an animal's youthful condition in terms of some molecular parameters known to change with age (right)

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Mechanisms and Late-Life Intervention of Aging

17

Sataro Goto

Abstract

The author's general thoughts on aging are described in the first sessions referring to definition and hallmarks of aging, animal models of human physiological aging, physiological vs. pathological models of aging, and private and public mechanisms of aging. In the second sessions, the interventions of aging are discussed focusing on dietary or caloric restriction (DR/CR) and regular exercise based mainly on the findings of the author and his collaborators. In the first sessions, selected matters of debate in the biogerontological research such as the selection of model animals are discussed. For the intervention, the results on the effects of late-onset regimens on the mechanisms of aging are presented for the DR/CR and regular exercise. It was found that the DR/CR initiated in old rodents at the ages equivalent to 50–60 years of age in human reduced the altered enzymes and increased the turnover rate of cellular proteins of old animals. Also, the nuclear and mitochondrial DNA oxidation as well as the protein oxidation was reduced in middle-aged or old animals after the regular exercise regimens. We have suggested a hormesis-like mechanism due to mild oxidative stress for these potentially beneficial effects that can reduce biological aging as well as postpone age-associated diseases.

This article is based on the oration for Prof. Kanungo Lifetime Achievement Award in the Indian Aging Congress, 18th Biennial Conference of Association of Gerontology (India) held in November 9–11, 2016 in New Delhi, India. The contribution is dedicated to my mentor Prof. Emeritus University of Tokyo, Dr. Den'ichi Mizuno, who had guided me to aging science in graduate school and passed away in October 1, 2017, at the age of 97.

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17.1 Introduction

At around 60 years of age, we begin to recognize progressive decline of our bodily functions and signs of aging not evident in the young and middle ages. The changes are gradually manifested as an increased frailty and risk of diseases that are collectively called geriatric syndrome, resulting in higher probability of death. Geriatricians often state that aging provides risk factor(s) for many diseases such as cardiovascular disorder, heart attack, stroke, dementia, atherosclerosis, sarcopenia, osteoporosis, and so on [1, 2]. This implies that aging may not be a disease by itself but biological aging underlies the etiology and development of these diseases despite some researchers and clinicians may claim differently. It is, therefore, important to understand the mechanisms of biological aging in order to slow down the process and retard onset of age-related diseases.

Figure 17.1 illustrates how much our life span would be prolonged if major age-associated diseases could be eliminated or biological aging could be retarded [3]. Additional years by the elimination of major diseases that kill old people such as cancer, cardiovascular diseases, or stroke would only be 1 or 2 years each, while the slowdown of biological aging could potentially increase many more years as estimated from the statistics on the Japanese centenarians. This suggests that reducing the rate of biological aging would be more beneficial than to eliminate individual age-related diseases for prolongation of life span and health span, although combating these diseases is obviously important as well to ameliorate immediate burdens of elderly people.

We discuss here molecular mechanisms and intervention of aging based on our findings in studies of mainly aging animal models in an intention to achieve longer healthspan.

17.1.1 Definition and Hallmarks of Aging

Biological aging could be most simply defined as the progressive decrease in homeostasis with advancing age. Homeostasis is often viewed as physiologically stable state that should essentially be unchanged for life, but it is actually changing with age, i.e., the homeostatic states of childhood, adolescent, and old age are different. Walter Cannon, the advocate of the idea of homeostasis, states that homeostasis is not a fixed stable state, but when it is challenged by internal or external stresses, the maintenance of homeostasis does not necessarily mean to restore the state before it was affected but rather establish a different stable state in new settings [4]. We should, therefore, realize that rejuvenation is not always what we should seek for to cope with age-associated changes to intervene aging processes.

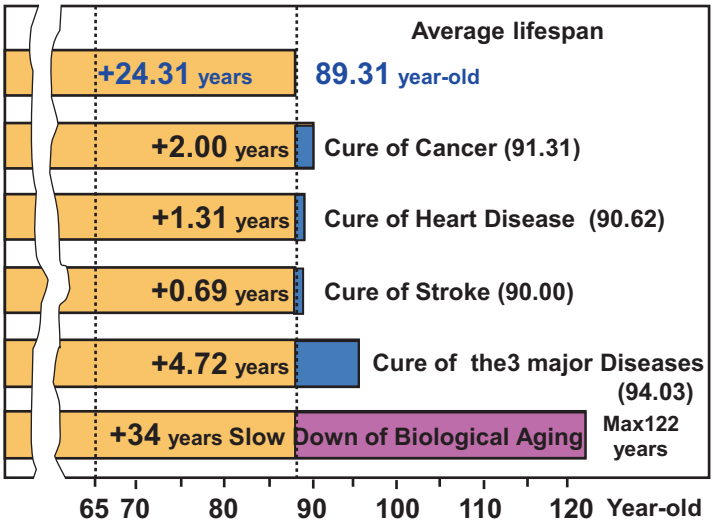

Fig. 17.1 Expected additional years of life in 65-year-old female, if the major geriatric diseases would be cured or the biological aging could be slowed down
The figure is drawn based on the Lifespan data of the Ministry of Health, Labour and Welfare (2015, Japan), and the report on the Tokyo Centenarian Studies (Hirose N, Gondo Y et al. 2001) for the maximum potential additional years of life estimated from the survival data of Japanese centenarians. (The original version of this figure is found in Goto S (2006) Jap J Geriatr 43:288 (in Japanese))

Biological aging is manifested as an increased probability of death of an individual with time. Each species has genetically fixed average and maximum life span. The rate of aging also appears to be specific to each species or subspecies, although it is modifiable to some extent as in the cases of intervention by dietary/caloric restriction (DR/CR) or otherwise as we shall discuss later in detail. Thus, the longevity and decline of bodily functions with age can be influenced by predictable or unpredictable environmental factors and chance.

To evaluate the progress and rate of aging in model animals and human, a number of parameters have been suggested to be candidates of hallmarks of aging. Suggested hallmarks have constituted the basis of hypotheses of biological mechanisms of aging such as the mutation theory of aging which proposes accumulation of DNA damage during aging could drive aging or the free radical theory of aging which claims that the increase in molecular damage due to oxidative stress is an important player leading to aging [5]. The other hallmarks of aging include genomic instability, protein alteration, biomembrane change, impairment of signal transduction, decline of nervous systems, decreased immune function, vascular changes, and so on. Geriatric physicians point out that we are as old as our blood vessels. Vascular change may be an important marker of both physiological and pathological aging but not readily become apparent in common animals as worms and insects since they do not have vascular systems.

The age-associated change of the skeletal muscle is another good hallmark of aging. The age-related shrinkage of the muscle (sarcopenia) and the muscle weakness occur with age in many species of multicellular model organisms including nematode [6, 7], insects [8], fish [9], and rodents [10]. It is a common major cause of age-related physiological decline in these animals and human [11]. It has been well-established that hand grip strength, a marker of sarcopenia, is a good predictor of all-cause mortality [12] and related to decline of cognitive function in elderly people [13]. Sarcopenia is attenuated by dietary restriction that retards aging in rats [14] and rhesus monkeys [15]. Thus, sarcopenia is a common hallmark of aging in humans as well as model animals.

17.1.2 Animal Models of Human Physiological Aging

Various animal models of aging have been used to understand human aging, including unicellular organisms such as yeast and paramecium, invertebrates such as nematode and fruit fly, and vertebrates such as fish and rodents. These organisms are widely used as models because of their usefulness due to their short life span, easy handling, and possible genetic manipulation, among others. We should be aware that the definition of aging and life span determination can be very much diverse in these models. For example, aging of single-celled budding yeast is measured by the ability of producing daughter cells (counted by the number of budding scars in a mother cell) or the numbers of viable days in post-replicative state, while aging of multicellular models such as nematode, fruit fly, rodent, and human is measured by how long the organisms can live. It is, therefore, important to realize that what we call biological aging may not be the same despite apparent similarity and possible common mechanisms.

It is often argued that mechanisms of aging should be conserved in model animals and human that can be separated by hundreds of millions of years in evolutionary history such as between yeast and nematode (about a billion of years) or between insect and mouse (several hundred millions of years). If true, a question is how fundamental mechanisms of aging would have been conserved for several hundred millions or a billion of years. The genes for aging phenotypes that appear late in life should have little chance to be inherited from one generation to the next. The idea of genes for the antagonistic pleiotropic phenotypes in aging that are beneficial when the animals are young enough to give rise to progenitors but the same genes can be detrimental in old ages may overcome this difficulty [16]. The question of how aging phenotypes have been inherited from ancient unicellular organisms up to the current variety of animal species and human, if they would have been conserved, is one of the most interesting yet unanswered questions in the biology of aging.

I would like to cite an important argument that age-related pathologies in model animals such as nematodes or fruit flies should be explored to see if one can regard them as good models for human aging or not in understanding causes that limit their longevity [17].

17.1.3 Physiological vs. Pathological Models of Aging

It has long been debated whether aging is a disease or not [18, 19]. In fact, it is not easy to distinguish between phenotypes of biological aging and age-associated diseases because biological aging underlies most, if not all, age-related pathologies. Nevertheless, the age-related decline of physiological functions cannot be avoided even in healthy individuals with no apparent disorders [20]. This suggests that the biological aging proceeds without apparent pathologies.

A variety of naturally occurring or genetically modified models with accelerated or premature aging phenotypes partly resembling normal aging have been reported, e.g., the sub-strains of the senescence accelerated mouse (SAM) models with a variety of defined phenotypes [21, 22] and the Klotho mutant mouse [23, 24]. These models are, however, more like models of age-associated diseases because only a part of physiological aging phenotypes usually seen in normal aged animals is manifested and may be regarded as disease models rather than those of physiological aging. It should, therefore, be cautious not to mix them with normal aging.

In human aging, a group of diseases called progeria syndromes exhibits premature or accelerated aging phenotypes that are partially manifested in unaffected old people. These include Hutchinson-Gilford progeria syndrome, Werner syndrome, the disorders defective in DNA repair, etc. George Martin has proposed to name these diseases segmental progeroid syndromes because the diseases exhibit a part but not all of age-associated phenotypes earlier in life usually seen in healthy old people [25]. It should, however, be noted that mechanisms underlying the apparently similar symptoms may be different from normal aging as in the cases of animal models of premature or accelerated aging mentioned above. For example, the expression of the RecQ helicase gene of which loss of function mutation is responsible for Werner syndrome is not reported to decline with normal aging except perhaps the reduced replicative capacity with age seen in normal human aging [26]. Also in Hutchinson-Gilford progeria syndrome, the expression of the dominant negative mutant form of lamin A/C gene from which a truncated translation product of the transcript with a specific deletion causes the disease is reported to be over 160-fold lower in late-passage fibroblasts of unaffected individuals than the counterparts from the patients, suggesting that the mutant form would not increase with normal aging in such a level that might cause aging due to the defect [27]. Thus, both animal and human progeroid models might not represent premature or accelerated forms of physiological aging for the purpose of understanding the cellular mechanisms operating during normal aging.

17.1.4 Private and Public Mechanisms of Aging

Mechanisms of aging can be classified in two categories as those that may be true only in limited species of animals or in specific cells/tissues/organs and those that can be generalized for any animals and cells. The two categories may be named as private and public mechanisms, respectively [28, 29]. Private mechanisms may

include the reduced acquired immune mechanisms with age and the decline of endocrine mechanisms with age that can cause impairment of bodily functions, for example. The mechanisms are important age-related changes in certain animals but cannot be true in animals such as worms and insects that lack or have only primitive forms of the systems.

Public mechanisms include the free radical theory or the proteostasis theory of aging that could be true for any animal models or human and cells in them. Naturally, public mechanisms are not mutually exclusive but can include each other as a part of the theory. Private mechanisms of aging are possibly caused by a combination of public mechanisms common to any animals and cells.

17.2 Intervention in Aging

Retardation of aging is highly demanded in aging societies that are suffering from medical as well as social and economic burdens due to ever increasing population of frail or diseased elderly people. Many studies have attempted to reduce the physiological decline with age, extend life span, and health span as well as retard onset of age-associated diseases. We discuss here selected topics of interventions of aging that include administration of chemical substances, dietary/caloric restriction (DR/CR), and practice of regular exercise, primarily in animal studies.

When it comes to the mechanisms of aging and the interventions of aging, age-related changes and effects of age on metabolic processes in energy metabolism often become major concerns [30, 31]. In this review, I would rather argue that processes such as changes of proteins and the turnover of proteins that can influence any cellular function(s) might be basically more important. Furthermore, our interest in the interventions of aging has focused on the brain, liver, and skeletal muscle tissues, which mostly contain the nondividing cells and are likely to be the major contributors to the organismal aging, focusing on the later stages of life.

17.2.1 A Note on Pharmacological Intervention by Defined Chemicals

A variety of natural and synthetic chemical compounds as well as plant extracts have been tested for life span extension of experimental animals or promotion of health of elderly people. Antioxidants have been most extensively studied in the past several decades since the free radical theory of aging by Denham Harman was proposed in 1956. He himself tested several chemicals for life span-prolonging effect in mice to reduce potential deleterious effects of oxygen-derived radicals (reactive oxygen species (ROS)) as he thought them being most harmful radicals in our body [32, 33]. Since then many studies were conducted to extend the life span of experimental animals or to ameliorate age-related diseases in humans; both were supposed to be due to ROS. The antioxidants, e.g., vitamin C and E, or natural products, such as polyphenols and carotenes, were used. However, the results were

rather disappointing in the human clinical trials, which attempted to reduce the risks of age-related diseases. The antioxidant supplements were reported to be effective in the experimental animals [34]. In human studies, it has been reported in a systematic review and meta-analysis of randomized trials with a total of more than 23 thousand participants that antioxidant supplements (β-carotene, vitamins A and E) might even significantly increased rather than decreased all-cause mortality [35]. In animal studies, the popular "antiaging" polyphenol resveratrol, which is not necessarily supposed to act as an antioxidant, has failed to extend life span of mouse strains with genetically heterogeneous background in multiple laboratories, when used as models of actual mixed human population [36]. Thus, it has not been successful to retard aging by antioxidants. It should, however, be mentioned that hormetic effects of ROS may promote health [37, 38]. Plant polyphenols such as catechin are suggested to promote health not directory as antioxidants but rather indirectly via signal transduction pathways [39] or possibly generating ROS in cells for hormetic effects [40]. Other chemical compounds that have been studied for the intervention in aging include rapamycin [41], metformin [42], and resveratrol [43], among others, but this review may not include them to be discussed in details in terms of effects of these chemicals on aging. The readers are advised to refer to comprehensive articles on the topics cited above and many others. It should, however, be stressed that any single chemical may not extend health span as a whole since elderly people often have multiple health problems not necessarily with common or related etiologies.

17.2.2 Intervention by Dietary or Caloric Restriction

Lifelong DR/CR is a well-known and perhaps the most reproducible non-genetic means to prolong life span and reduce the occurrence of diseases associated with aging including cancer and renal disease in experimental animals [44, 45]. The life span of DR/CR rodents are 30–50% longer compared to control animals provided with food ad libitum, if calories are restricted for life by 30–40% initiated at early stages of life [46]. Findings that many age-related biochemical and physiological changes are retarded by the regimen suggest that it may influence fundamental mechanisms of aging. To cite a few examples, which include papers reporting the reduced induction of heat-shock proteins [47], the decrease in the number of dopamine receptors [48], the retardation of onset of renal disorders [49], etc with age. Interestingly, it can ameliorate pathologies associated with genetic defects in experimental animals as well that may be regarded as not being influenced by lifestyles such as DR/CR. Spontaneous hypertensive rats (SHR) with the average life span of 18 months can live nearly as long as wild-type animals (30 months) by the CR, reducing the incidence of cardiac and renal lesions [50]. In short-lived SAM mice with genetic defects, the immunological decline with age was delayed by the CR [51]. More recently, the DR initiated early in life reportedly extended the life span of a mouse model of Cockayne progeroid syndrome (life span 4–6 months) with nucleotide excision DNA-repair enzyme deficiency by 200% [52]. Thus, the DR/

CR can prolong life span of animals even with genetic defects, ameliorating disorders responsible for the short life span.

In many studies including those cited above, DR/CR was started early in life, i.e., immediately after weaning or at initial stages of growth. On the other hand, the life span of mice dietary restricted from 1 year of age was extended by 10–20% with reduced incidence of cancer although the percentage extension was not as big (ca.35%) as in the lifelong CR animals [53]. The mice subjected to the CR during 14–25 months of age showed improved cognitive functions [54]. The other investigation, however, found that rats subjected to the CR starting at 18 or 26 months of age up to the end of life showed no significant change in the mean life span, cancer, and other pathologies compared to ad libitum-fed control animals [55]. It has been reported that CR for 8 weeks, started at 19 months of age, reduced incidence of tumors, resulting in a significant extension of both average and maximum life span in the mice [56]. These reports have suggested in general that the DR/CR initiated during later part of life is also able to extend the life span, thus retarding the onset of age-related diseases and bodily impairments.

We have been interested in the adult-onset DR/CR rather than lifelong regimens in mice and rats because if findings in animal studies were to be extrapolated to people, early onset studies might not give useful information as the restriction of food from childhood could cause growth retardation and other deleterious consequences, and therefore would not be recommended even if the regimens might result in life prolongation and beneficial effects in old ages.

The average life span of people, males and females included, in developed countries is currently around 80 years of age. Frequently used laboratory rats and mice have an average life span of about 30 months in well-controlled SPF conditions that are likely equivalent to daily life environments in developed countries. The comparison of relative rate of aging of model animals and human in physiological decline such as cognitive functions [57, 58] and onset of age-associated diseases like cancer [59] and sarcopenia [6, 8] as well as molecular markers such as protein oxidation [60] and mitochondrial DNA mutation [61] showed that the rate of decline is inversely correlated with the average life in both human and experimental animals. Based on the relative rate of age-related changes of the parameters cited above, it is suggested that laboratory mice and rats age approximately 30 times faster than human [5], Fig. 17.2). The middle-aged people (50–60 years of age) who might practice the DR/CR may, therefore, be equivalent to the rodents of 20–24 months of age. We have used mice and rats aged around these ages for our studies of intervention in aging. For the DR studies, the middle-aged animals were housed individually and subjected to either stepwise feeding of restricted food down to the level of 60% of the ad libitum consumption ([62, 63], see Fig. 17.3b) or every-other-day feeding in the case of rat experiments which resulted in a similar percentage reduction of body weight as in the stepwise dairy restriction of mouse studies [64].

We have studied the effects of the DR on the alteration of proteins and the protein turnover based on our previous findings in ad libitum-fed animals ([65, 66], Fig. 17.3a). The alteration of proteins was evaluated by the heat-lability of enzymes and oxidative modifications. Such proteins likely not only have reduced biological

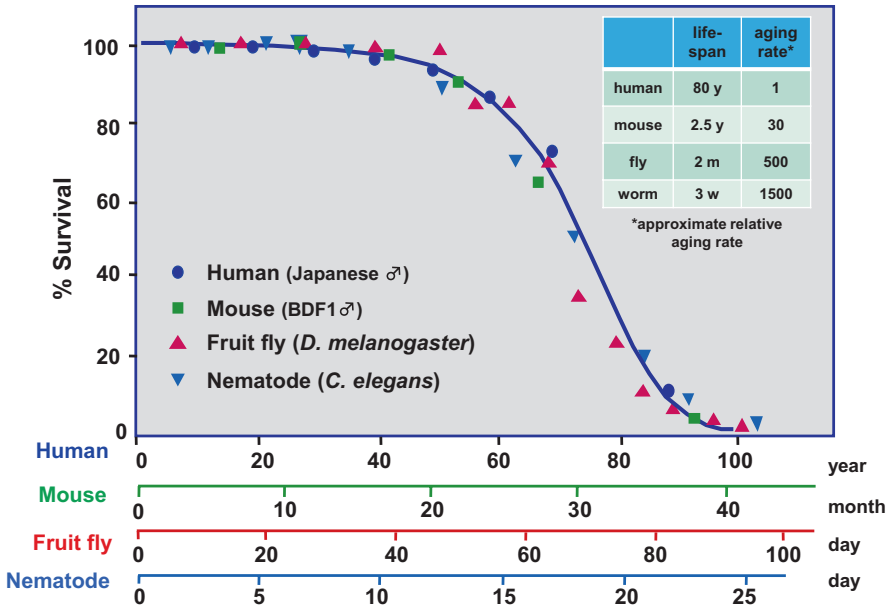

Fig. 17.2 Survival curve and the rate of aging of human and model animals. (Reproduced from Ref. [5])

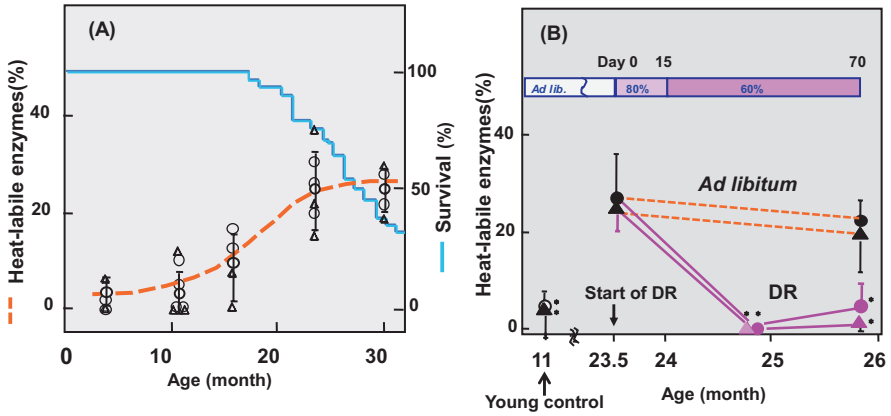

Fig. 17.3 Increase in heat-labile altered enzymes in the liver with age and the effect of late life DR in mouse

(**a**) Leucyl- and tyrosyl-tRNA synthetases of the liver of mice (BDF1) were studied. Mean lifespan: 28 months; Left ordinate: percentage heat-labile leucyl (triangles) and tyrosyl (circles) tRNA synthetases. Average (gothic circles) for the two enzymes. Right ordinate: percentage survival. Abscissa: age in month. (Adapted from Ref. [65]). (**b**) Effect of the late life DR for 70 days on the heat-labile enzymes starting at 23.5 months of age compared with the level of young animals (11-month-old). Broken lines: Ad libitum fed animals; Solid lines: DR animals. The data for young animals (11-month-old) are shown as control. (Adapted from Ref. [60])

activities but also may gain harmful functions, thus potentially contributing to the decline of physiological functions with age. The percentage of heat-labile aminoacyl tRNA synthetases in the brain and liver increased with age in mice from 5 to 15% at 4–6 months of age to 30–40% at 25 to 30 months [67]. Interestingly, it decreased to the levels observed in young adults within 2 months of the DR starting at the age of 23.5 months, suggesting that the altered enzymes were replaced by new molecules by either metabolic or cellular turnover ([62], Fig. 17.3b). It is more likely that the increase protein turnover is responsible for the replacement rather than the cell turnover that is very infrequent in adult rodent livers [68].

The effect of the DR on the protein turnover was studied in culture of nondividing liver parenchymal cells isolated from old mice subjected to the regimen [63]. The half-life of exogenous proteins introduced into or pulse-labeled in the cells was reduced by 40–50% to the levels of young animals after the 2 months of DR (Fig. 17.4). This was also true for oxidatively modified proteins. Thus, the DR appears to promote the degradation of altered forms of proteins in old animals. In addition to the increase in protein turnover, the decrease of the altered enzymes by the DR is also likely to be due to reduction of oxidative stress because the heat-labile enzymes were generated by exposing the enzyme fractions to a ROS-producing system [69]. In accordance with the reduced oxidative stress by the DR in old age, it has been shown that after 3.5 months of the DR, the mitochondrial protein carbonylation in the livers of old rats (30-month-old) was reduced to the levels observed in the ad libitum-fed young animals (10-month-old) [64]. It is,

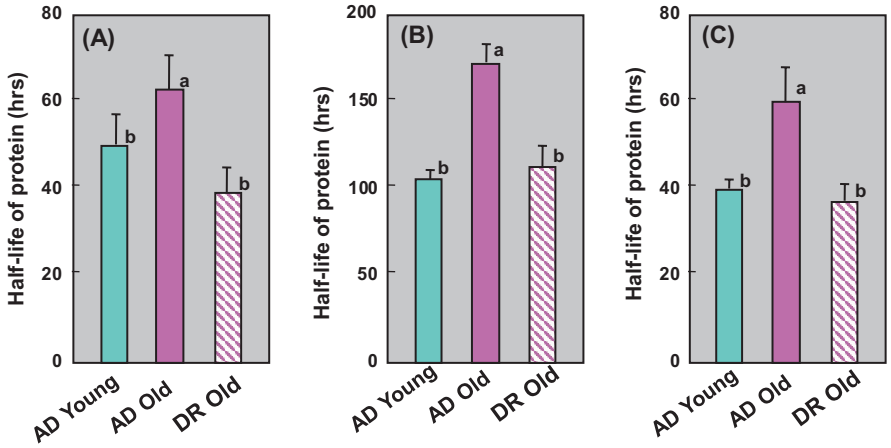

Fig. 17.4 Effect of dietary restriction on the half-life of proteins introduced into or pulse-labeled in hepatocytes in primary culture from old mice. (**a**) Horseradish peroxidase (**b**) ovalbumin, and (**c**) pulse-labeled endogenous proteins

Animals: SPF male mice (BDF1), mean lifespan 28 months. *AD* ad libitum fed, *DR* dietary restricted. Young: 3–6-month-old; Old: 23-month-old; old DR: the restriction was initiated at 23 months of age and continued for 2 months. Ordinate: Half-life of proteins (hours) (**a**) significantly different from young animals, (**b**) significantly different from old animals, Av ± SD (n = 3–4). (Adapted from Ref. [61])

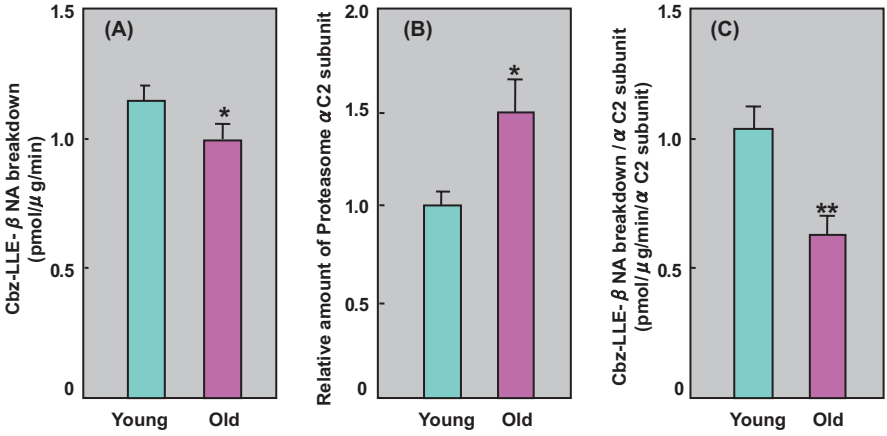

Fig. 17.5 Age-related changes of rat liver proteasome activity
(**a**) Enzyme activity; (**b**) relative amount of subunit (αC2); (**c**) specific enzyme activity: enzyme activity/relative amount of subunit. Young rats: 13-month-old; old rats: 31-month-old Av ± SD ($n = 4$) $*p < 0.05$, $**p < 0.01$ (Takahashi R, Hayashi T, Takano A, Goto S, unpublished)

therefore, likely that the reduction of altered proteins in old animals by the DR is caused at least partly by the increased protein turnover and reduced oxidative stress.

The degradation of soluble proteins in the cell is supposed to be catalyzed mainly by lysosomal cathepsins and/or proteasomes. The lysosomal inhibitors, such as ammonium chloride, did not inhibit the degradation of proteins introduced into the hepatocytes, but it was significantly reduced by a proteasome inhibitor Z-Leu-Leu-NVaH. It is therefore likely that the proteasome is mainly responsible for the degradation of introduced or pulse-labeled proteins in the cells. In other experiments using aging rat livers, we have shown that both 26S and 20S proteasome activities decline with age [70]. The age-related decline is, therefore, likely to be responsible for the reduced proteasome activity. It is interesting to note that the amount of the proteasome increased significantly with age as determined by the antibody against a proteasome subunit despite the decline in the activity. The specific enzyme activity per unit amount of the subunit decreased (Fig. 17.5). This suggested that there is a qualitative rather than a quantitative change in the enzyme during aging. Thus, altered proteasomes are possibly involved in the formation of a vicious cycle of the accumulation of altered proteins with advancing age. The finding that the late-life DR upregulated the proteasome activity is consistent with the reduced accumulation of the altered proteins in animals subjected to the regimen [66]. Other studies have also demonstrated that proteasome activity declines with age [71], supporting the decreased proteasome activity being involved in the accumulation of altered proteins in aged tissues. Interestingly, the 26S proteasome can reversibly be dissociated into the 20S catalytic form and 19S regulator by oxidative stress [72]. As the 20S proteasome has been shown to degrade oxidatively modified proteins selectively [73], the dissociation of the enzyme may be due to adaptation to the stress.

In this context, it is interesting to note that the longevity of the naked mole-rat, an extremely long lived rodent model, appears to be due to higher resistance to oxidative stress and more efficient protein turnover with high proteasome activity compared to short-lived laboratory mice [74].

In addition to the degradation of altered soluble proteins by proteasomes, the autophagic process has been reported to be involved in proteostasis particularly in the aging brain in which the accumulation of insoluble aggregated proteins or peptides can cause Alzheimer's and Parkinson's diseases due to the accumulation of β-amyloid and α-synuclein, respectively [75]. The DR/CR induces autophagic processes in model animals, thus contributing to the reduction of abnormal aggregated proteins and damaged organelles [76].

Interestingly, long-term DR/CR likely promotes removal of damaged proteins by upregulating the quality control mechanisms including the increase in heat-shock proteins and components of autophagic machineries in human skeletal muscle [77]. Thus, effects of the DR/CR on the proteostasis appear to be common in both model animals and human.

The impaired metabolism of triglyceride and lipoprotein can cause atherosclerosis and other age-associated vascular disorders [78]. The storage of triglycerides as an energy source in adipose tissues and their mobilization for utilization in other tissues are dependent on the plasma lipoproteins. The effects of the late onset of DR on the plasma lipoproteins and their mRNAs were studied in the liver of fasting mice. We have reported that the apolipoprotein A-IV (apo A-IV) in the plasma and its mRNA in the liver showed remarkable increase following 2–3 days of fasting in the young (6 months) but very little in the old (25 months) mice [79]. The apo C-II mRNA increased similarly but the apo C-III mRNA decreased significantly after fasting. The apo A-IV is present in the chylomicron and high-density lipoprotein (HDL). It activates lipoprotein lipase (LPL) in the presence of apo C-II, which promotes mobilization of fatty acid [80]. LPL is inhibited by apo C-III. Since expression of these proteins is modulated in response to fasting, this can enhance fatty acid mobilization to meet the energy demand when the food supply is limited in the young mice. In the old animals, however, apo A-IV was induced to a little extent, and apo C-III mRNA did not decrease significantly due to fasting. This suggests about impairment of fatty acid mobilization. When the 22-month-old mice were subjected to the DR for 3 months, it showed higher induction of the plasma apo A-IV as well as its mRNA in the liver of the DR-fasted old animals compared to the 25-month-old animal-fed ad libitum (Table 17.1, [81]). The apo C-III mRNA was reduced due to fasting in the old DR-animals. These results suggest that the triglyceride metabolism can be shifted by DR for higher use of fatty acids in response to fasting. This can happen by upregulating the LPL activity, and possibly this may also happen in non-fasting conditions. It is, therefore, conceivable that the late-life-DR may help reduce the risk of vascular disease such as atherosclerosis by enhancing lipid metabolism.

Histones make the chromatin structure to be compact due to the abundant basic amino acids, Lys, and Arg residues. These residues are posttranslationally modified by acetylation, methylation, and other modifications that can change transcription,

Table 17.1 Effects of age, dietary restriction, and fasting on the amount of hepatic apo A-IV and apo C-III mRNAs per unit amount of total RNA in young, old, and old DR mice relative to the unfasted levels *F*(0) of the ad libitum fed young animals

	Apo A-IV mRNA		Apo C-III mRNA	
	F(0)	*F*(3)	*F*(0)	*F*(2)
Young	1.00	52.3 ± 11.3	1.00	0.75 ± 0.05
Old	1.62 ± 0.75	1.95 ± 0.61[a]	1.57 ± 0.19[c]	1.43 ± 0.22[e]
Old-DR	0.97 ± 0.16	9.64 ± 1.73[b]	1.01 ± 0.22[d]	0.89 ± 0.08[d]

Young: 6-month-old; old: 25-month-old; old-DR 25-month-old (after 3 months of DR). The ad libitum fed or DR animals were fasted for 2 or 3 days before sacrifice. *F*(0): fasting day 0, *F*(2): after 2 days fasting, *F*(3): after 3 days of fasting

[a]young vs. old, $p < 0.001$

[b]young vs. old-DR, $p < 0.01$

[c]young vs. old, $p < 0.05$

[d]old vs. old-DR, $p < 0.005$

[e]young vs. old, $p < 0.01$ (mean ± SE, $n = 6$). Adapted from Ref. [79]

DNA replication, and DNA repair. The modifications of histones has attracted considerable interest as these epigenetic changes occur during embryonic development, differentiation of cells, and aging of cells and organisms as well as in many disease conditions. Protein carbonyl, a marker of oxidative stress, is reported to increase with age in a variety of animals including human [60], likely causing functional decline of cells with age. We found that rat liver histones except H4 are carbonylated in vivo to variable extents, H1 and H2A/2B being more carbonylated than H3. It seems histones H3 and H4 present in the inner core of the nucleosomes may be more resistant to the modifications. Interestingly, histones in the young (5 month) rat liver were significantly more carbonylated compared to their old (30 month) counterparts. This is contrary to the general tendency of the increased modification with age [82]. Since carbonylation of proteins mostly occur in the basic amino acid residues, the modifications of Lys and Arg residues of histones, which mask the positive charge, can reduce their interaction with the DNA, thereby influencing recruitment of the other proteins such as RNA polymerase, transcription factors, and repair enzymes by reducing their interaction with the DNA. It is, therefore, possible that the age-related decrease in the histone carbonylation might lead to reduced chromatin functions. This unexpected but possibly reasonable finding prompted us to study the effect of DR/CR on histone carbonylation. After 2 months of DR in 28-month-old rats, the histone carbonylation was increased to the level close to that of the young animals [82]. If we assume that histone carbonylation is due to oxidative stress and carbonylation of cellular proteins in general is attenuated in the DR-animals, this finding is paradoxical [83]. One possible explanation could be that DR may relax the chromatin structure and thereby contribute to activation of chromatin functions such as transcription and DNA repair in the aged tissue [84, 85]. These findings may point to a new physiological role of protein carbonylation not necessarily causing oxidative stress [86].

The oxidative damage to the nuclear and mitochondrial DNA is generally increased with age [87, 88]. The DNA base modifications in the nuclei can induce

cancer and possibly change gene expression. The oxidative damage to the mitochondrial DNA may impair energy metabolism, forming a vicious cycle of functional decline of the organelles and thereby possibly enhancing the rate of aging. The effect of DR was studied on the extent of 8-oxo deoxyguanosine (8-oxodG) in the nuclear DNA of the liver of old rat livers. DR started at 28 months of age significantly reduced the amount of 8-oxodG after 2 months (Nakamoto H & Goto S, our unpublished results). The repair enzyme OGG1 (8-oxo guanine DNA glycosylase/AP lyase) for the oxidative DNA damage was upregulated in the old (30 months) animal-fed ad libitum compared to the young (5 months) controls. The DR lowered the activity. These findings may be interpreted to suggest that the activity of the repair enzyme was increased in the old animals to reduce the damage, but the higher activity was no longer required after 2 months of DR when the damage was decreased.

Thus, the late-onset DR/CR can have beneficial outcomes in a variety of cellular functions, ameliorating potentially harmful age-related changes in rodents [89]. It should, however, be mentioned that effects of the DR/CR on lifespan can be variable among different strains of rodent models from increase to no change and even decrease [90]. Therefore, we have to be cautious of the effects particularly in terms of the lifespan since longevity is often affected by susceptibility to particular diseases that can be different in different strains.

When it comes to apply the regimens to human, we must be aware of the potential harmful effects of the DR/CR particularly in elderly people because they tend to eat less than when they are younger and therefore may lack in nutrients such as proteins and essential micronutrients. Also, we human would mostly not eat as free as laboratory animals but control by ourselves so that they may already be restricted to some extent in daily meals. In the DR/CR study of nonhuman primates, it was conducted by the National Institute on Aging, USA, that slightly food-restricted animals were used as controls [91]. The 30% DR/CR monkeys did not live significantly longer than the control animals, suggesting a possibility that the severe restriction of food as in rodent studies may not be needed to have beneficial effects in primates including human, although the recent report on the DR/CR studies in nonhuman primates concluded that the regimen was beneficial to prolong life span and health span [92]. Furthermore, the studies for 1 year of the DR/CR in nonobese people have shown harmful outcomes in that the bone mineral density [93] and muscle mass are reduced [94]. Thus, the DR/CR does not likely prolong health span at least in nonobese people despite potential benefits of the regimens that may be extrapolated from animal studies [95, 96].

17.2.3 Regular Exercise

Habitual exercise is well-known to have health benefits in reducing risks of age-related diseases and retarding progressive decline of physiological functions with age that affect quality of life in middle-aged [97] and older people [98]. In a seminal report of a large-scale prospective study of the relationship between physical fitness and mortality in cardiovascular diseases and cancer, it has been shown that the

negative correlation was more pronounced in elderly people aged above 60-year-old than in younger people (20 to 59-year-old) [99], pointing that the physical fitness is more effective to maintain health in elderly people. When talking about the effects of exercise on health, people tend to associate it with muscular systems, but we are more interested in systemic effects of the regimens.

While regular exercise is reported to be beneficial for health, it is often argued that too much exercise can be harmful due to possible excessive oxygen uptake and massive generation of ROS. In fact, extensive physical activities cause the oxidative damage of nucleic acids, proteins, and membrane lipids in multiple tissues of unprepared sedentary animals [100, 101]. The apparent contradictory outcomes due to exercise suggest that ROS can have dual (harmful and beneficial) roles depending on the extent, duration, and timing of oxidative stress on cells and tissues. It has been hypothesized that moderate regular exercise can be beneficial by upregulating the protective enzymes against oxidative stress [102, 103].

We studied the effects of regular exercise on oxidation of proteins in the brain and cognitive function of rats [104]. Young (4 weeks) and middle-aged (14 months) animals were subjected to regular swimming exercise, 5 days per week, for 9 weeks. Exercised animals showed improved cognitive functions in the two age groups. These changes were associated with reduction of protein carbonyls in the brain. The proteasome activity in the brain was upregulated, suggesting that the enzyme is responsible for the reduction of the oxidatively modified proteins. A mild oxidative stress due to the exercise regimen might have increased the enzyme activity as an adaptive response. It is reported that ROS stimulates neural cell proliferation both in primary culture and in vivo that was abolished by the treatment with α-lipoic acid, a potent antioxidant [105]. These findings might be relevant to exercise-induced beneficial effects to the brain in which changes of the redox state might be involved. In this context, it is interesting to note that physical activity increases neurogenesis in the brain [106, 107]. Furthermore, it has been reported that the thickening of the brain cortex without significant changes in the neuronal cell counts, a parameter reflecting better neural plasticity, was observed in the rats as old as 30 months of age kept in an enriched environment for a few months. This allowed the animals to stay active [108]. In epidemiological studies, physically active people who practice habitual walking have reduced risk of dementia [109]. We have suggested that modest increase of ROS would be a mechanism for the beneficial consequences by regular exercise [110].

Whether regular exercise has beneficial influence in other tissues in aged animals is an interesting question. We studied the effects of regular treadmill exercise (4 times a week, for 8 weeks) on the oxidative status in the liver of middle-aged (18 months) and old (28 months) male rats. In both age groups, the maximal oxygen uptake was increased by about 40% [111]. The ROS level in the liver extracts was significantly elevated in the old sedentary animals than in their middle-aged counterparts. The regular exercise attenuated the age-related increase in the oxidative stress marker [112]. The redox status evaluated by glutathione level showed more than twofold increase of the reduced form (GSH) in the exercised groups. Thus, it appears that the cellular milieu is changed to more preventive state against oxidative stress, suggesting a beneficial effect of exercise. The binding activity of NF-κB, the transcription factor for

the expression of genes of inflammatory proteins, such as inducible nitric oxide synthase (iNOS), interleukin-6 (IL-6), cyclooxygenase-2 (Cox-2), and tumor necrosis factor α (TNF-α), in the nuclear extracts with a deoxyoligonucleotide probe from the response element increased with age as was expected due to the increased oxidative stress. The exercise reduced the binding of NF-kB to the probe with concomitant increase in the amount of I-κB, the inhibitory protein of NF-kB. Thus, the regular exercise ameliorated or reversed changes with age that would exacerbate inflammatory processes. Glucocorticoids (GC) have anti-inflammatory activities and are used to suppress inflammation in chronic diseases such as asthma and rheumatoid arthritis. GC inhibits gene expression of pro-inflammatory cytokines including interleukins and TNF-α as well as enzymes or receptors responsible for inflammatory processes such as iNOS and Cox-2. The GC receptor (GR) is a transcription factor that regulates directly or indirectly on the gene expression of the inflammation-related proteins. We showed that binding activity of GR to the responsive DNA-element is significantly decreased in the liver of aged animals (28–33 months), but 8 weeks of the regular exercise reversed the change (Abe R & Goto S, unpublished results; [113]). No significant difference in the amount of GR protein was detected between young adult and old animals, suggesting that the quality rather than the quantity of GR is altered with age. The level of GC in the serum was increased in the exercised old animals compared to the sedentary controls. In view of the anti-inflammatory activities of GC, these observations support the view that regular exercise may have an ability to reduce inflammation through the GR pathway in old rats. Interestingly, GR can directly interact with NF-κB, modulating the activity of transcription of genes associated with inflammation [114, 115]. It is likely, therefore, that transcription factors like GR and NF-κB can synergistically ameliorate the expression of genes related to inflammation by exercise in old rats.

Regular exercise is reported to reduce the risk of a variety of age-related diseases such as cancer, cardiovascular disorders, type 2 diabetes, dementias, etc. We studied the effect of regular exercise on the oxidative damage to the nuclear and mitochondrial DNA and its repair in aged rats, which can correlate with these diseases [116]. Rats of 21 months of age were subjected to regular treadmill running for 8 weeks. The amount of 8-oxodG in the nuclear and mitochondrial DNA of the liver in sedentary old animals was 2- and 1.5-fold higher in the two organelles, respectively, compared to their younger counterparts (11-month-old) (Fig. 17.6). The mitochondrial DNA showed tenfold higher content of the oxidative lesion than the nuclear DNA as expected for higher oxidative stress in mitochondria. The 8-oxodG content was reduced to the levels of the young animal in both nuclear and mitochondrial DNA after the exercise. The activity of the repair enzyme OGG1 was downregulated in the nucleus but not in the mitochondria with age. It was upregulated significantly by the exercise in the nucleus but downregulated in the mitochondria. Thus, the repair activity was differentially regulated in the two organelles by exercise. The upregulation of OGG1 activity in the nucleus suggests that the reduced oxidative damage to the nuclear DNA is at least partly due to the increase in the repair activity. The reduced oxidative damage to DNA could be one of the mechanisms of anticancer and antiaging effects of physically active lifestyle [99, 117]. The reason for the

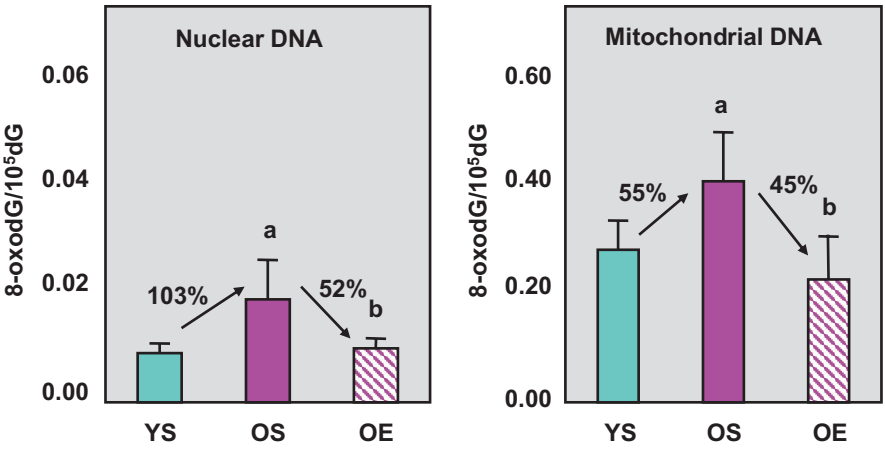

Fig. 17.6 Effect of regular exercise on the oxidative modification of DNA (8-oxo dG) in rat liver nuclei and mitochondria

Animals: F344 male rats. Young sedentary (YS): 11-month-old; old sedentary (OS): 23-month-old; old exercise (OE): 23-month-old after 2 months of regular exercise. Means ± SD ($n = 5$~8 for nuclei), means ± SD ($n = 4 \sim 5$ for mitochondria). $^{a}p < 0.01$(YS vs. OS), $^{b}p < 0.01$(OS vs. OE). The percentage change is shown for old rats vs. young animals and for exercised old rats vs. sedentary old animals. (Adapted from Ref. [114]).

downregulation of mitochondrial OGG1 activity despite the reduction of DNA oxidation is not clear. In accordance with our finding that the activity of mitochondrial OGG1 is downregulated by exercise, the mitochondrial antioxidant enzyme GSH peroxidase is reported to be downregulated in chronically trained rats [118], suggesting that it is also due to reduced oxidative stress by training.

Thus, while too much physical activity is obviously harmful due to massive generation of ROS and other detrimental influences, if the body is not conditioned to minimize the damage, modest and regular exercise can induce cellular defense against stronger oxidative stress, promoting health and reducing risk of diseases as a form of hormesis [110, 119]. Importantly, such adaptation can occur even at old ages apparently systemically at least in model animals [113, 120]. It has remained to be demonstrated whether it is also true in older people.

Apart from beneficial effects of exercise-induced mild oxidative stress, it is interesting to note that alcohol consumption, if modest, promotes health despite it causes oxidative stress. It has been reported that the prior intake of alcohol can attenuate ischemic/reperfusion oxidative damage in the brain DNA of model animals due to ROS generated in NADPH-oxidase-catalyzed reaction [121]. Adaptation to oxidative stress might be induced by intake of chemicals such as polyphenols that can be prooxidants rather than antioxidants as has been usually regarded [122, 123].

The adaptive response to increased resistance to oxidative stress by aerobic exercise has been suggested also in humans. It has been shown that antioxidant vitamins C and E ameliorate the beneficial effects of training in humans, suggesting that ROS generated by the exercise had caused the upregulation of health-promoting

parameters such as plasma adiponectin level and glucose infusion rate [124]. We found that the increased oxidative stress in middle-aged citizens with an active lifestyle compared with sedentary people, as evaluated by urinary 8-oxodG, was accompanied by the increase in the antibody (IgM) against 4-hydroxy-2-nonenal, an oxidative stress marker of membrane phospholipids, the level of which is apparently positively correlated with the protection against atherosclerotic disorders [125]. Other researchers have also reported that the middle-aged adults who practice habitual aerobic exercise showed higher resistance to oxidative stress [126].

To add good news to the beneficial effects of regular exercise, the short life span of mice-harboring mutator gene for mitochondrial genome [127] is extended by regular exercise, suggesting that genetic defects may be ameliorated by exercise although mechanism of this is not clear [128].

Thus, it is likely that habitual exercise promotes health via increased generation of ROS, upregulating protection mechanisms against oxidative stress in both animal models and humans, possibly contributing to the beneficial effects of exercise. Undoubtedly, the mechanism(s) of systemic beneficial outcomes of regular exercise are much more complex and poorly understood, waiting for the development and integration of research in different disciplines [129].

17.3 Concluding Notes and Perspectives

Aging is a very complex biological process of which mechanisms have not been fully solved despite decades of extensive research. Many theories have been proposed [5], suggesting conserved mechanisms that are claimed to be true for very short-lived single-celled organisms such as yeast and complex multicellular animals such as rodents and humans [30]. In my personal view, any pathway that appears to drive aging should constitute a part of the whole mechanisms of the maintenance of life. It would not, therefore, be possible to ascribe single molecules or pathways to explain biological aging. We might be able to explain a part but not the whole of aging by focusing on specific mechanisms. Therefore, to think of the intervention in aging, we have focused on means that can affect a variety of processes in a body, i.e., the DR/CR and regular exercise. Both means, however, would not necessarily be easy to practice for people who naturally wish easy life, regardless of their potential motivation. The DR/CR mimetics [130] and exercise mimetics [131] might be alternatives, but it would be hard, if not impossible, to find one that can substitute the whole benefits of the DR/CR and/or regular physical activity regimens to enjoy longer healthspan [132].

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Healthy Ageing and Cancer in Humans

18

Sen Pathak

"It is one of the worst aspects of our present developmental stage in medicine, that the historical knowledge of things diminishes with each generation of students."
Rudolf Virchow (1821–1902)

Abstract

Birth and death are the only two facts of life. A person who is born has to die because all of us have an expiration date. Ageing and cancer development are related because cancer is a disease of old age. A newly born baby may have cells in her/his body (biological ageing) equivalent to the cells of a 70-year-old human (cellular ageing). Such cells acquire genetic instability due to many factors including the attrition of their telomeres, a very specialized and repeated complex region of DNA and protein, present at the termini of eutherian linear chromosomes. Telomeres are highly repeated (TTAGGG)n sequences which are conserved throughout the vertebrate genomes, including human. Cancer originates in the organ-/tissue-specific stem cells. The origin of cancer stem cells may be from the original tissue-specific normal stem cells or from the differentiated induced pluripotent stem (iPS) cells. Aneuploidy is one of the characteristic features of most human cancers, especially the solid malignancies. Our recent research shows that both primary and metastatic cancer cells in solid tumors originate from the aneuploid giant cells through the process of budding. Cancer cells activate some meiosis-associated genes to become long-lived (immortal?) and acquire stem cell-like features; thereupon, they undergo a special type of cell

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division called meiomitosis. It is, therefore, very important to protect the guardians, telomeres, of individual chromosomes which can be done by following a Sattvic lifestyle including vegetarian diets, Pranayama and Yoga, and a stress-free spiritual life.

Keywords
Ageing · Cancer · Telomeres · Meiomitosis · Somatic cell nuclear transfer (SCNT) · Induced pluripotent stem (iPS) cell · Somatic cell reprogramming (SCR) · Sattvic lifestyle · Pranayama and Yoga

18.1 Introduction

We are all familiar with the phrase "Health is Wealth," but how many of us really do something about it? The life of an organism including that of humans is bounded by birth and death, which are connected by the process of cellular or organismal ageing. Every human being has an expiration date. Human life, like any other life, takes its origin from a single cell after fertilization. Normal somatic cells in the human body, with the exception of germ cells found in the testes in males and in the ovary in females, contain 46 hereditary strings called chromosomes, 23 of which are contributed from each parent during the fertilization of a mature ovum with a sperm cell. Since the father produces two types of sperms, X-bearing and Y-bearing (heterogametic), and the mother, only one type of X-bearing ovum (homogametic), it is the father who determines the gender of the baby. In the female genome, the Y-chromosome is replaced by an X-chromosome, and the autosomes are the same with 46, XX constitution, whereas in the male, it is 46, XY. In rare cases a mosaic or chimeric individual may have more than one chromosome constitution and may exhibit cell selection in vivo; its positive role in developmental anomalies and possible link with cancer pathogenesis is not fully appreciated [11]. Although the somatic cells in each organ have the same number and morphology of chromosomes, there is division of labor because each organ performs differently in a human body. It means the genes that are specifically active in the lung may not be active in the liver and vice versa. However, the housekeeping genes may be the same in all human organs.

The life of an organism including human originates from the embryonic stem (ES) cells. Recent advances in cell biology and embryology have shown that every organ in the human or murine body has its own reserve supply of stem cells. Each ES cell is totipotent and capable of giving rise to progeny of its own kind and can also differentiate into any of the specialized cells in the adult tissues. Following organogenesis, each adult organ maintains a small pool of its own stem cells which are organ-specific with their own cell surface markers. That cancer originates in the organ- or tissue-specific stem cells will be discussed later in this chapter [34, 38].

Ageing and cancer development are related because cancer is a disease of old age [4, 10, 30, 50]. If cancer is a disease of old age, then why do newly born babies

contract cancer? Here, I am talking about cellular ageing and not the age of the whole human body (phenotypic). A newly born baby, who is diagnosed with cancer, may have cells equivalent in age to the cells of a 60–70-year-old human. We age because of the attrition of a specialized substructure present at both ends of linear chromosomes called the telomere [29]. Telomeres are highly complex DNA and protein structures, repeated many times with the sequence (TTAGGG)n [27, 28]. Although children with Down syndrome (DS) have an extra copy of chromosome 21 (trisomy), they contain much less telomeric DNA compared to an age- and sex-matched normal child. Most human syndromes are known to have reduced amounts of telomeric DNA, a characteristic that induces genetic instability and predisposes such individuals to various diseases, including cancer [35, 36].

The purpose of writing this book chapter is to discuss the cellular relationship between ageing, somatic cell transformation, cell selection in vivo, predisposition, and the development of cancer. In addition, evidence-based human family pedigree-based data will be presented to show how an ailment-free and healthy body can be maintained following certain precautions in our daily routine so as to enjoy a cancer-free happy human life.

18.2 What Is the Purpose of Human Life?

Let me start this section with a story. I was traveling to India in October 2003, with a group of scientists including the then president, John Mendelsohn, of MD Anderson Cancer Center (MDACC) at Houston. While we were still in the air, I asked John, "What is the purpose of life?" Of course, he was very surprised to face such a rare question and asked me to reply. My reply was, "To find out one's uniqueness and share it with others." Now the answer to this question may vary from person to person. However, we all want happiness in life which cannot be purchased from a shop, because such a shop does not exist anywhere in the world. A person who has realized his/her uniqueness will never get depressed. A time comes in life when you derive extreme and everlasting happiness by giving away more than receiving. Our desires are the root cause of miseries in life. It is, therefore, very important to have control over your desires. Vedic Rishis and Sages of ancient India (Bharat Varsh) have shown humanity how to be contented in life. It is becoming evident that a happy person may have very few health problems, can cope easily in adverse situations, and heals faster than an unhappy one. Human nature is such that if desires are not fulfilled in time, one gets depressed. Recent discoveries have shown that depression is the most potent risk factor for cellular as well as organismal ageing. It may have its effect even on a single cell level and on a single chromosome by inducing attrition in the telomere.

18.3 Environmental Exposure and Human Diseases Including Cancer

Cancer is the ultimate result of an interaction between the genetic makeup and the living environment of a person. Here, I have used environment and lifestyle as synonyms to present my viewpoints. The genetic makeup or genotype of a person is a loaded gun; the lifestyle pulls the trigger. Our lifestyle, which includes the quality and eating habits of food and drink, the quality and the amount of air to breathe, tobacco smoking habits, place of living and the quality and duration of daily sleep, sedentary or active life, and many others, is responsible for our healthy and disease-free body. A healthy person is one who is spiritually, mentally, and socially happy and not merely disease- or ailment-free. According to Vedic literatures, the five elements that constitute the human body – earth (Chhiti), water (Jaal), sky (Gagan), wind (Sameera), and fire (Pawak) – are polluted to a great extent, except the fire [39]. In the name of modernization, humans have played a major role in polluting our environment. In olden days, people used to take more "live food," but today, most of us survive on "dead food." The meaning of live food is freshly cooked food and raw vegetables and fruits, while dead food includes processed, canned, and frozen food items which are mostly devoid of enzymes. Today, we have forgotten the famous phrase proclaimed by Hippocrates centuries ago – "Let food be thy medicine and medicine be thy food" [1]. We live today, in over-toxic and undernourished environments, causing our immune system to overwork and making us susceptible to various microbial infections.

The majority of our generation is suffering with chronic inflammation, which is responsible for different types of diseases including heart attack, joint and muscular pain, early onset of ageing, risk of depression, mental disorders, and cancer. In olden days, people used to walk long distances, but today our lives have become more sedentary because of TV programs, videos, air-conditioned housing, riding in cars, and flying in planes, which has negatively affected human health and longevity. We have multiplied our never-fulfilling desires responsible for making us depressed. Humans need more and more and are never satisfied with what they already possess in life; thus, they are never contented. Today, we have forgotten the real human instinct which is "Live and let live." Most of us practice the animal instinct – "Survival of the fittest" – in our society [39]. Some of the current lifestyle factors which lead to malignancies are intake of a high-fat diet and junk food, increased consumption of sugar and salt, chewing and smoking of tobacco products, excessive consumption of alcoholic beverages, exposure to industrial and automobile pollutions, late marriage and late pregnancy, use of birth control pills, lack of breastfeeding, and many more. Some lifestyle interventions could prevent 60–70% of cancers in our populations. For example, obesity seems to increase the risk of breast and prostate cancer, smoking causes lung cancer, red meat consumption increases the risk of colon cancer, and sun bathing increases the risk of skin cancer including melanoma.

As mentioned earlier, overweight and excessive body fat is a risk factor for breast and prostate cancer. Healthy lifestyle may prevent the development of cancer in

family members, even with constitutional genetic mutations. Later, I will briefly describe such a family story where, with almost identical mutations, one member has not yet developed cancer, whereas others have been diagnosed with multiple neoplasia.

18.4 Genomic Alterations and Neoplastic Transformation

Cancer cells are known to accumulate genetic arrangements which are of two types: (1) numerical chromosome alterations (NCA) and (2) structural chromosome anomalies (SCA). Most cancers exhibit both these anomalies during their lifetimes. Genetic instability, one of the salient characteristics of cancer cells, is caused by NCA and SCA [19, 20]. Now the question is whether NCA is followed by SCA or vice versa. In a recent report, Janssen et al. [22] demonstrated that, at least in in vitro experiments, NCAs appear first, and then the SCAs follow. However, this may not be the universal phenomenon for all types of cancer. In a series of publications, our group has reported the loss of telomeric DNA first, causing telomere associations (TAs) and the production of aneuploidy [40, 42, 43, 46]. During subsequent cell divisions and with the formation of dicentric chromosomes, cells follow the breakage-fusion-bridge cycles [26, 45], undergoing senescence and cell death or with the activation/upregulation of telomerase to stabilize the telomeres and activation of tumor suppressor/proto-oncogene(s) acquire neoplastic transformation and invasive phenotypes [8, 23]. In the most recent publication, we have published in vitro data, using confocal microscopy and time-lapsed recording, of single cells of Hey, SKOV3, and OVCAR433 human ovarian cancers treated with paclitaxel (PTX) to show the formation of polyploid giant cells that undergo cell budding and give rise to highly malignant and resistant cancer stem cells [31].

Cancer initiation and development of metastasis are considered stepwise acquisitions of genetic changes that convert primary cancer cells into highly invasive ones [16, 52]. However, most recent observations indicate that massive genetic rearrangements can be acquired in a single catastrophic event, converting normal stem cells into cancer stem cells with highly invasive phenotypes [32, 33]. Using next-generation sequencing (NGS), Stephens and associates [51] demonstrated such a phenomenon of massive gene mutations and chromosomal alterations and termed it chromothripsis (chromo for chromosome; thripsis means shattering into pieces). Telomere attrition in one chromosome of a stem cell or genome-wide reduction in somatic cells induced by extrinsic or intrinsic factors may generate double-strand DNA breaks and naked loci for recurring genomic alterations [2, 49]. This may bring about massive remodeling in the developing cancer clones in very short periods. Chromosome deletions, duplication, gene amplification, and oncogenic fusions or bringing master genes juxtaposed to “slave genes” may result in massive mutations, causing the development and metastasis of cancer.

18.5 Cancer Is Not a New Disease

Cancer is as old as the origin of mankind. While writing the history of cancer, a pathologist from the Harvard Medical School, Boston, Massachusetts, USA, Professor George Th. Diamandopoulos [12] stated that "*The Ramayana*, a manuscript from ancient India (about 2000 BC) is the first recorded instance in which mention is made of tumors and their treatment, either by cutting them out or by applying to them ointments containing arsenic." Arsenic is a chemotherapy medicine still prescribed in some countries for cancer treatment. The original four Vedas – Rigveda, Yajurveda, Samveda, and Atharvaveda, composed approximately 6000 years ago – contain references to human ailments, including cancer and its treatment. The other Veda, called fifth Ayurveda (Science of Medicine), has provided much useful information about the treatment of human diseases, including cancer. Two famous Vedic Rishis, Charaka and Sushruta, in their Samhitas (700 BC) have discussed the equivalent of cancer as granthi (benign neoplasm) and arbuda (malignant neoplasm). These entities could be inflammatory or noninflammatory based on the involvement of three doshas – Vatta, Pitta, and Kapha. According to Ayurveda, a person is healthy who has balanced coordination of these three doshas. Ayurveda is called a holistic treatment because it deals with the body, mind, senses, and soul. Last year, a Memorandum of Understanding (MoU) was signed between the US National Cancer Institute (NCI) and the AYUSH (Ayurveda, Yoga, Unani, Siddha, and Homeopathy) Ministry of India, New Delhi, to promote research for the treatment of cancer, not only by allopathy but many other pathies, including homeopathy, naturopathy, divine pathy, Yoga and Pranayam. Some efforts are already being made in this direction to bring and develop an integrated medicine program between the Indian Traditional and Allopathic Professionals [48].

18.6 Lifestyle and Cancer

The incidence of cancer is soaring every year, particularly in developing countries such as China and India. Although the overall incidence of cancer in the USA is extremely high, where one out of three individuals will be diagnosed with cancer, the current rate is flat and extremely low compared to the above mentioned developing countries. According to the US National Cancer Institute (NCI), India will become number one in the incidence of cancer within the next 15–20 years. The question is, why? Some physician scientists think it is because of better diagnosis. I think it is not the diagnosis of cancer but its actual incidence is increasing. At the same time, the incidence of birth defects is also increasing in India by leaps and bounds. It is well-known that the incidence of both cancer and birth defects is caused by chromosomal alterations, which may be alarmingly very high in Indian population (Pathak, under preparation). My short reply to this question is: "Indians are becoming Westernized and Americans are becoming Indianized." There has been a huge change in Indian lifestyle [37]. In early days in India, life was slow, people were more active, and their diets were very healthy and balanced. Today,

most Indians have cars and motor bikes which have greatly reduced their physical activity; junk foods, excessive alcohol consumption, and tobacco products (Pan masala and Pan parag, Gutka, Bidi, chewing tobacco, cigarettes, and many more) have become a upper-class status symbol and modern fashion in the country. The unregulated burning of coal, use of poor quality gasoline in automobiles, and industrialization have polluted the air of the country. In addition, excessive use of pesticides and chemicals as fertilizers in agriculture products has resulted in the province of Punjab running a "cancer train" for patients.

Cancer cells need glucose and fatty products to survive. With the increased consumption of sugar and junk, fast and more salty foods that cause obesity, we are providing fuel to cancer cells. To decrease the risk of all kinds of cancer, we must control glucose intakes in our daily diets, exercise regularly, maintain an average body weight, and lead cheerful happy lives. A well-balanced diet consisting of colored vegetables and fruits containing antioxidants, vitamins, minerals, and enzymes which will energize our immune system to fight against cancer cells and foreign invaders must be used in our daily habit. In rural India where there is no check available for the presence of excessive chlorine, arsenic, and several other carcinogens in drinking water, such monitoring must be enforced by both state and central governments.

In recent years, the consumption of tobacco and its products and of alcoholic beverages has increased steadily. Late marriage, late pregnancy, no breastfeeding, and use of birth control pills have become the norm in native Indian females, and because of it, they are experiencing a very alarming rate of breast cancer. In males, the incidence of prostate cancer is also on the rise, mainly because of unhealthy diets including processed meat, frozen food products, and obesity. Vedic Rishis have already laid down three principles of diet (Ahaar): Hit Bhukh, Mit Bhukh, and Rit Bhukh, meaning "consume (eat) whatever is beneficial to your body; consume in moderation (lesser quantity) and eat whatever (fruits and vegetables) is available in that particular season." By doing so, a human being can drastically reduce the occurrence of acute and chronic diseases and may lead a healthy and cheerful life. Stress and depression are the two important factors in pathogenesis in a person. A person who is stressed invites diseases because of a weak immune system. In the battlefield of Kuru-kshetra, Lord Krishna told Arjun to perform his duty without hoping for the results. An action without a desire is stress-free. Stress comes only when one desires positive results and does not get it. We must do our duty and leave the results in His hands. This is true in the case of cancer treatment also. We should also keep in mind that the outcome of any and every treatment is predetermined. Out of approximately 22,000 employees working and *Making Cancer History* at The University of Texas MD Anderson Cancer Center at Houston, TX, USA, there are a substantial number of cancer survivors. The lifesaving mantra is: Be Happy, Do not Worry.

18.7 Modern (Allopathy) and Ancient (Ayurveda, Yoga, and Pranayama) Modalities in Cancer Management

Today, allopathy has become the "King/Queen" of treatment of various human diseases, including cancer. The gold standard for cancer treatment in allopathy is surgery, chemotherapy, and radiotherapy. In addition, other modalities, such as immunotherapy and cell therapy (stem cell transplant), are also offered in some specialized cancer centers. For cell therapy, stem cells are collected from the bone marrow or from the umbilical cord and placenta, which has become a routine procedure in the USA, and grafted in the recipient patient, kept in isolation for some time. In a country like India, where the rate of childbirth is so high, stem cells can be collected from the umbilical cord and placenta and frozen down for future transplants.

Pranayama and Yoga are gifts to humanity from ancient India. Pranayama teaches us the correct methods of breathing, which is required for a healthy life. The meaning of Yoga is union: union with the inner self, union with God, and union with other creatures of the world. Pranayama and Yoga have become integral parts of complementary and alternative medicine (CAM). The use of CAM, which includes ancient Indian Pranayama, Yoga, Ayurveda and Chinese herbal, acupuncture, and natural medicines, is still in its infancy. The University of Texas MD Anderson Cancer Center (MDACC) at Houston has, however, taken a lead in this respect. Under the Global Academic Program (GAP) of MDACC, an agreement has been signed with SVYASA (Swami Vivekananda Yoga Anusandhan Samsthan) in Bengaluru, India. Trained Yoga teachers from SVYASA have been hired by the MDACC and every cancer patient, when taking chemo- or radiotherapy, is being taught Yoga and Pranayama. The preliminary results on breast cancer patients at MDACC have brought a substantial amount of research grants from the National Cancer Institute to conduct further research, including lung cancer patients. Participating cancer patients have better quality of life compared to nonparticipating, and they can tolerate side effects much better and are able to sleep properly during therapy.

18.8 Evidence-Based Cancer Prevention

The popular phrase "Cancer Prevention is Better Than Cancer Cure" is very well-known to all of us. Unfortunately, very little attention is directed toward the prevention of this disease in many developing countries, including India. As stated earlier, somatic cell mutations, induced or spontaneous, generate genomic instability and aneuploidy which may result in serious medical conditions, including cancer [25]. Identification of tumor-associated genetic alternations, therefore, is important for cancer prevention, early cancer diagnosis, and successful treatment.

I describe below only two examples of many such cases, classified as cancer family syndromes, where a change in lifestyles, including vegetarian dietary intakes and early genetic diagnosis, has prevented the occurrence of cancers in some members of the families:

Family A In this Asian family of four brothers and one sister, different histopathologic types of cancer have occurred. Of four brothers, one died at the age of 72 years with pancreatic cancer; a second brother who is 74 years old was diagnosed with breast cancer at age 64 and prostate cancer at 70 years; a third brother who is 68 years old has been diagnosed with pancreatic cancer and is undergoing treatment in the USA; and, finally, a fourth brother who is 72 years old is very healthy with no symptoms of any disease, including cancer. Their sister died at the age of 55 years due to stomach cancer. Because of the family history, gene sequencing was performed on the total DNA collected from the peripheral blood samples of the third and fourth brothers. The third brother, who is diagnosed with pancreatic cancer, is heterozygous for BRCA2 c.3362C G, BRCA2 c.2892A T, MSH6 c.-16 C A, and POLD1c.- 46A G. The fourth brother, who is healthy and asymptomatic currently, is heterozygous for BRCA2 c.3362C G, BRCA2 c.2892A T, MSH6 c.-16 C A, and PTEN c.-965G A. All those brothers who have been diagnosed with cancers are non-vegetarians and smokers; only the fourth brother, who is very healthy and still asymptomatic, is strictly vegetarian, a nonsmoker, does not drink, and leads a spiritual lifestyle. Approximately 10 years before his gene sequencing data became available, we had studied the chromosome constitution in his mitogen-stimulated peripheral blood lymphocytes (PBL). In a large majority of his PBL metaphases, the chromosome constitution was 46, XY. However, 2.0–3.0% of his metaphases showed aneuploidy with both numerical and structural chromosome abnormalities as shown in Fig. 18.1. The structural abnormalities included chromosome/chromatid breaks and dicentric formations. Our group has earlier reported many such cases

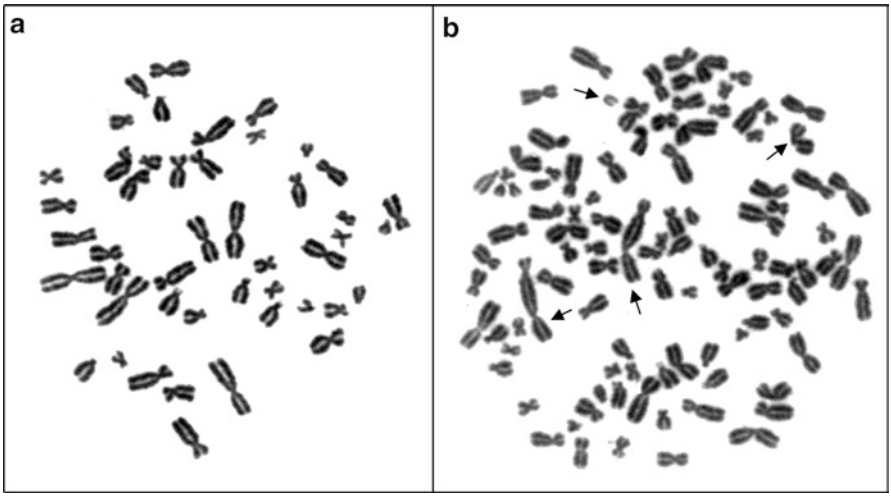

Fig. 18.1 Giemsa-stained metaphases from the peripheral blood lymphocytes of an asymptomatic member from a cancer family syndrome. A large majority of his metaphases show normal 46, XY chromosome constitution (**a**); however, 2–3% of his metaphases show abnormal chromosome numbers (polyploidy) and structural abnormalities including dicentrics and breaks, as marked by arrows (**b**). Because of leading a Sattvic lifestyle, he is still free of cancer at the age of 72

where specific chromosome abnormalities were observed in the PBL samples of not only untreated cancer patients but in some of their asymptomatic family members who, later on, developed a specific neoplasm [32, 36, 38, 46]. The genetic constitution of man is a loaded gun, and the environment (external as well as internal), which affects the lifestyle, pulls the trigger.

The lesson learnt from the study of family A is as follows: Although the third and fourth brothers inherited identical gene mutations in BRCA2 and MSH6 genes, only the third brother developed pancreatic cancer, and the fourth is still asymptomatic. Could it be because of their lifestyles? This author strongly believes "Yes." The genetic constitution of both brothers is a loaded gun; the lifestyle pulled the trigger in the third but not in the fourth brother. The fourth brother is currently undergoing regular evaluation and physical examination for his health. He does regular Pranayama and Yoga under the supervision of a trained Yoga teacher and takes balanced vegetarian diets and leads a very spiritually oriented life.

Family B One morning in the early 1990s, I had given a talk on the use of PBL karyotyping to identify cancer-predisposed individuals, a project very close to my heart. That afternoon, a colleague of mine, who was a Professor in Cancer Biology, came to my office and told me that he did not want to know if he would be predisposed for a particular type of cancer and, therefore, would not provide his blood sample for such study. It was his wish, and I respected that. Two years later, his son, a high school student, was diagnosed with lymphoma. My friend then came to my office and requested to be examined for his chromosome constitution and provided the PBL sample. When his Giemsa-stained cytogenetic preparations were analyzed, we found that 4.0% of his stimulated PBL metaphases showed chromosome/chromatid breaks involving chromosome 5, where the APC gene implicated in colon cancer is mapped [3, 6, 21, 32, 41]. I advised my colleague to go for a colonoscopy, which he did. The attending physician found nine adenomatous polyps, which were growing in his colon wall. These polyps were surgically removed, and the patient was advised to stop eating red meat and take more fibrous vegetables and fruits. In other words, a change in his lifestyle was recommended. Following the change in his food habit and lifestyle, he is even today free from colon cancer and leading a healthy and happy life.

These examples strongly suggest that leading a healthy lifestyle may prevent the occurrence of diseases, including cancer, despite being predisposed for such an ailment [34, 35].

18.9 Frequently Asked Biological Questions About Cancer

Are all cancer cells aneuploid? Does cancer originate in a strictly diploid cell? Are metastatic cells present in the primary tumor? How do cancer cells acquire resistance to chemo-/radiotherapy? Some of these questions are being reconsidered in

the light of newer findings in cancer cell biology. Most hematologic malignancies find their origin in a diploid somatic cell. However, this may not be true for solid tumors, where chromosome numbers may vary from almost haploid to more than 100. Most human epithelial malignancies in general are aneuploid [45]. There is compelling evidence to support the notion that tetraploid cells can give rise to aneuploidy: (1) some solid tumors have near tetraploid cell populations; (2) an increase of tetraploid cells is noted in the initial stage of tumor development in animal models; and (3) most cancer cell divisions exhibit multipolar mitosis. Recent observations using flow cytometry, light and focal microscopy, and fluorescent-labeled single cell time lapse videography have shown that solid tumors initiate and develop metastases in polyploid/aneuploid giant cells [31, 53]. As stated earlier, organ-specific stem cells or even iPS cells when exposed to intrinsic or extrinsic insults undergo attrition in their telomeres, the organelles that protect every chromosome in the nucleus [7, 8, 44]. Loss of telomere repeats, recognized as double-strand DNA breaks, brings about the arrest of such cells in the G2 stage of the cell cycle. Because of mitotic failure and the duplication of chromosomal DNA, the nucleus acquires more than the diploid chromosome number, thus giving rise to polyploid giant cells. The significance of polyploidy in normal development and in the causation of cancer has been described earlier [7]. Most of these polyploid giant cells undergo senescence and die, a phenomenon thought to be irreversible by some, but some cells survive. In a recent paper, Leikam and associates [23] showed that prolonged expression of the oncogene N-RAS in pigmented melanocytes dedifferentiated such cells and triggered the emergence of cancer stem cells with fast-growing metastatic tumors. Similar reports using different systems were published by others as well [15, 17, 24]. Recent reports show that tumor aneuploidy may correlate with markers of immune evasion and with reduced response to immunotherapy [9, 55]. We recently published observations on the emergence of smaller cells through the process of "budding" from the polyploid giant cancer cells (PGCCs) in three ovarian cancer cell lines [31]. Here we concluded that the giant cell cycle may represent a fundamental cellular pathway which involves the initiation of genomic reprogramming to generate new tumor-initiating cells in response to chemotherapy-induced stress and also may contribute to cancer relapse. Such reprogrammed daughter cells are the precursors of metastatic and resistant tumor cells. Cancer cells also follow multistep evolutionary adaptive mechanisms to invade and survive [16, 54]. A number of publications from the laboratory of the present author [33, 46, 47] and others [13, 14] have shown that cancer cells follow evolutionary pathways, as do different species in nature during speciation.

Now the burning question is: Why is germ cell-like differentiation important in tumor development? It is a must for self-renewal. Only germ cell systems are better equipped to produce cells with infinite lifespan. Cancer cells are known to survive much longer compared to normal somatic cells. Germ cells have the highest level of telomerase activity in adult testes. Mature human sperms have the longest telomeric DNA. Tumor cells differentiate along the germ cell pathways to become immortal due to telomerase activation/upregulation, at the cost of meiosis protein expression and genomic instabilities.

18.10 More Females in Animal and Cell Line Are Needed in Biomedical Research

Progress in biomedical research is solely dependent on animals, mostly small rodents – mice, rats, and sometimes hamsters for in vivo experimentation – and human and animal cell lines for in vitro experiments. Recently, it has come to our attention that experimental animals and human- or animal-derived cell lines of either male or female origin do not give similar results. Results obtained on animals and cell lines of male origin may not be reproduced on the animals or cell lines of female origin. The US National Institute of Health (NIH) in 2014 announced that it will require disclosure of not only the gender of animals but also a balance of both sexes in future grant applications. This is also true for the cell lines used in cultures, because such data provide guided therapies to be used for humans. Male (XY)- and female (XX)-derived cell lines could be quite different from each other in their biological characteristics. Until recently, most researchers have preferred using male and avoiding female animals because they are considered highly variable because of their estrous cycles. For others, it does not matter, whether cells are derived from a male or female. A large body of papers published in high impact journals do not even report the gender of animals used in their experiments; therefore, reproducibility of their results by another group becomes difficult. It is well-known that females (human/animal) respond differently to stress and survive longer as compared to males. As a matter of fact, the cell biology of female (XX)- and male (XY)-derived cells has not been studied thoroughly. New results are showing considerable variations between the two sexes. According to Margaret McCarthy, a neuroendocrinologist, almost 80% of genes in the human liver are expressed differently in male and female cells, as noted by Couzin-Frankel [5]. Today, more cancer cell lines derived from female patients are available as compared to male patients. HeLa was the first human cancer cell line derived from a 31-year-old black mother, Henrietta Lacks, of five children ever established in cell culture [18].

According to Vedic Sages, mother is our first guru. We are what our mothers ate and drank when we were in their uteri. Even after birth, a child learns more from his/her mother than it does from the father. A healthy and properly nourished pregnant mother delivers a healthy child. Underweight babies are born to a mother who is not healthy and properly nourished. Although such babies look normal phenotypically, they contain less than the minimal number of stem cells in their heart, kidney, lung, pancreas, and other organs, which may affect their adult body with different diseases [39]. This could be true for most Indians, including the author of this chapter, who was born as an underweight child and suffered cardiovascular disease. Others may suffer with diabetes as well.

18.11 Cancer and Ageing-Related Unanswered Questions

There are many questions in the field of human ageing and cancer that are waiting to be answered. Some of these questions are: (1) Can weakness of mind, not having a positive attitude, and lack of control over emotions increase the susceptibility to develop cancer? (2) Can our thoughts control the biology of our cells? (3) Can the genes in our cells be modified by our thoughts? (4) Do individuals with a positive attitude cope better with cancer and have increased survival? (5) Can mind management reduce the aggressiveness of the disease or cure it? (6) Why females survive longer than males do? (7) Why do we age anyway? (8) What is the purpose of human life? These are not only my questions but are asked by many others as well.

According to some, cancer is due to the imbalance of three gunas of the human mind: **Sattva, Rajas,** and **Tamas**. Today, we are talking about personalized cancer therapy which is based on genome sequencing. A single genetic variation rarely determines the personality. My recommendation is: Questionnaires should be developed to determine the personality of a cancer patient based on his/her gunas, which, then, should be considered for her/his customized cancer treatment.

18.12 Conclusions

Cancer is a disease of aged cells which have acquired/induced aneuploidy. Telomere attrition is one of the many factors that induces genetic reprogramming in the genome. Cancer originates in the organ-/tissue-specific stem cells. Genetic instability due to the loss of telomere repeats, transforms cells, and induces a massive mitotic catastrophe in the genome. Both primary and metastatic cancer cells in solid tumors originate from polyploid giant cells through the process of "budding." Cancer cells activate some meiosis-associated genes (Spo11 linked with depolyploidization, NANOG and Sox2) to become immortal and acquire stem cell-like features, therefore, undergoing a special type of cell division called meiomitosis. It is, therefore, very important to protect the guardians, telomeres, of individual chromosomes, which can be done by following a Sattvic lifestyle including vegetarian diets, Pranayama and Yoga, and stress-free life.

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Nutrition for Elderly

19

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Abstract

Ageing is a biological process that cannot be avoided. Being a little careful may prevent or slow down many ailments which affect our health as we grow old. Many chronic diseases like hypertension, diabetes, obesity, osteoporosis, cardiovascular disease (CVD) and cancer are known to be influenced by diet and hence can be kept in check and even prevented by regulating our diet. Good nutrition plays its most significant role during youth and middle age in the prevention of diseases which otherwise ultimately manifest as serious ailments among the aged. The fact that micronutrients play an important role in maintaining health and prevent non-communicable diseases has got noticed along with the diet/disease debate. In the elderly, micronutrient deficiencies are often common due to their decreased food intake and a lack of variety in their diet. A few studies indicate that dietary intervention may delay or prevent age-related health disorders and cognitive decline. A good way to sustain optimal level of health is to eat a balanced diet which caters to the specific nutritive need of the elderly. This article is a compilation of important information from literature and is not a specialized research document.

Keywords

Ageing · Nutrition · Elderly · Diet · Micronutrients · Antioxidants

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19.1 Introduction

Ageing is a universal experience for humans with diversity in meaning and interpretation. Imahori (1992) defined ageing as the "regression of physiological function accompanied by the advancement of age". It refers to a person's interactions and performance in a sequence of socially prescribed roles, accumulation of experience and changes in the physiological systems as well as in perceptual, cognitive, emotional and other psychological processes. It is a normative process and not a fixed dimension of the life cycle. As people grow older, changes are witnessed in the physical, cognitive and social realms. Ageing is generally recognized as a biological process controlled by multiple factors and among which the basic feature is programmed senescence of billions of cells in various tissues and organs which are responsible for physiological systems and which finally results in functional decline [1]. Increased life expectancy has made it necessary for the development of strategies to maintain health and well-being in later years. Active ageing denotes a satisfactory quality of life for an individual throughout the lifespan [2]. Basically, ideal nutrition for an elderly person in good health is not very different from that of younger individuals assuming in both cases that caloric intake is proportional to energy expenditure.

Nutrition has proved to be a major modifiable decisive factor of chronic disease and age-related decline. In addition to influencing present health, dietary adjustments also determine whether or not an individual will develop diseases like cancer, cardiovascular disease (CVD) and diabetes much later in life.

The term "population ageing" indicates the growing share of older persons in the population. It is set to become one of the most remarkable social transformations of the twenty-first century with consequences for nearly all sectors of the society including labour and financial markets, the need for goods and services such as transportation, housing and social protection as well as family structures. As the world population experiences a demographic transition from 12.3 % in 2015 to 16.5 % in 2030, the proportion of the elderly over the age of 60 years is expected to reach 1.4 billion by 2030 and nearly 2.1 billion by 2050. In 2015 just five countries taken together, Russia, China, Japan, India and the United States accounted for half of the world's population aged 60 years or over. To ensure progress in development towards reaching the goals outlined in the 2030 Agenda for Sustainable Development, it is essential to prepare for the economic and social shifts related to an ageing population. For the goals on removing poverty, protecting life and well-being at all ages and promoting gender quality and productive employment, population ageing is particularly relevant. During the second World Assembly on Aging, the 2002 Madrid International Plan of Action on Ageing (MIPAA) was adopted, and it focused on the need to consider elder people in development planning, highlighting that older persons should be able to take part in and benefit from the fruits of development to secure their health and well-being and that societies should provide enabling environment for them to do so [3].

19.2 WHO Guidelines

The proportion and number of older persons defined as 60 and over are increasing in all countries. WHO has developed a policy framework [4] in order to achieve the final goal of healthy and active ageing, with respect to the following aspects:

- Checking and reducing the burden of disabilities, chronic disease and premature mortality
- Decreasing the risk factors linked with non-communicable diseases and functional decline as individual age while increasing factors that protect health
- Educating and providing training to formal and informal caregivers
- Ensuring the safety, protection and dignity of ageing individuals
- As people age, enabling them to maintain their contribution to economic development, to activity in the formal and informal sectors and to their communities and families

19.3 Elderly Women Health

In all countries, women make up the majority of the older population mainly because globally women live longer than men. The number of older women is expected to be 373 million in Asia and 46 million in Africa by the year 2025. This involves its own special nutritional needs and patterns of malnutrition, for example, the incidence of osteoporosis in older women. A major cause of illness, disability, medical expense and death is caused by osteoporosis and related fractures. Women suffer 80% of hip fractures. By 2050, the annual number of hip fractures may touch 6.3 million. Their lifetime risk for osteoporotic fractures is at least 30% and in contrast the risk is only 13% for men. Women are at greater risk because their bone loss accelerates after menopause. Prevention is possible with hormone therapy at menopause. Lifestyle factors especially diet, physical activity and smoking are also linked with osteoporosis which opens the way for primary prevention. By increasing the bone mass at maturity, by preventing subsequent bone loss or by restoring bone mineral, many fractures can be prevented. During adolescence and young adulthood, it is important to include adequate intake of calcium and physical activity.

19.4 Common Ailments in the Elderly

In the elderly, resistance to disease declines. The incidence of some ailments in the elderly are degenerative in nature such as diabetes, cataract, arthritis (joint diseases), osteoporosis, osteomalacia, cardiovascular (stroke, heart diseases) problems, neurological (Parkinson's, Alzheimer's) and psychiatric (dementia, depression, delirium) disorders and cancer. Besides these, the prevalence of respiratory, gastrointestinal tract (GIT) and urinary tract infections is common among the elderly.

An ageing individual goes through a gradual decline in almost all body functions mainly in the cardiac, respiratory and renal functions, muscle strength, sensory faculties, nerve impulse conduction, agility, endurance and ability to maintain coordinated muscular effort, all of which may be due to structural and functional changes. As a result of external environmental stress, the process of ageing also results in reduced ability to maintain an internal environment. The thermoregulatory efficiency is also impaired to a certain extent since the ageing process affects the various factors regulating heat loss and metabolic heat production of the body. As age advances it is also accompanied by deterioration of most psycho-physiological functions and the capacity to do both physical and mental work. As a result the prevalence of cardiovascular, pulmonary, musculoskeletal and mental disorders among the elderly is more. Notable decrease in aerobic power, impaired thermoregulation, slower reactions and decreased acuity of the special senses are also commonly observed. [5]

19.4.1 Food-Related Factors and Ageing

The interaction between nutrition and ageing can be seen in two ways:

- With the biological ageing and associated physiological and structural changes in the body, the food preferences, food and nutrition intake and at the same time the nutritional requirements undergo considerable change. These are the impact of the ageing process, their food intake and their consequences.
- Another approach is to explore whether specific foods and their nutrients can either slow down the ageing process or accelerate it.

For example, most of the polyphenol compounds which are consumed with food are greatly metabolized in the gastrointestinal tract. For example, phloroglucinol is a polyphenolic phytoconstituent catalysed by the gut microbiota to acetate, butyrate and CO_2 and is also known to produce antibacterial activities. Thus it can alter the gut microbiota ecology which is known to be an integral part of human physiology. The gut microbiota ecology and the gut brain axis play major roles in changing central sensitivity of almost all inflammatory diseases. Recently, probiotics are also reported to reverse the deteriorated brain functions and cognitive performance in diabetic rats. Dietary manipulation is an emerging strategy for the management of type 2 diabetes [6].

Some herbs and spices are rich in antioxidants and are commonly used as flavouring agents in Indian food. Most hot and pungent active principles of spices are both antioxidant and mildly antimicrobial. Spices have excellent beneficial effect in many health-related problems like gastrointestinal curative and discouraging the formation of mutagens resulting in carcinogenesis. Recent studies in Japan have shown that daily oral intake of capsaicin which is present in chillies in the form of lozenges results in significant improvement in upper respiratory reflexes in older people. This can prevent aspiration and pneumonia. Ginger and its extracts are used

as a carminative stimulant in the gastrointestinal tract. Ginger can help cancer patients to avoid nausea induced by chemotherapy. Ginger and pepper extracts enhance the bioavailability of drugs with which they are administered. Curcumin present in turmeric has cancer-preventing properties due to its anti-inflammatory and antimicrobial action. It has been reported that consumption of turmeric helps in combating Alzheimer's disease. Fenugreek seeds are useful in lowering blood glucose levels and controlling diabetes. Extracts of garlic and onion inhibit platelet aggregation and help in lowering cholesterol levels. Spices like celery, dill, fennel, cumin, parsley and coriander contain bioactive compounds like phthalides which have anticancer properties. Lemon grass, asafoetida, basil leaves and poppy seeds have anticancer properties. [7]

The physiological and structural changes during ageing process which affect nutrient intake are as follows:

- Reduced metabolism in ageing including reduced basal metabolic rate, lack of physical activity and lack of appetite mostly due to lack of interest in food lead to significantly reduced food intake causing energy deficiency and various types of vitamin and mineral deficiencies.
- Loss of teeth and difficulty in mastication lead to altered food intake with preference for liquid and soft mashed diet. This leads to considerable alteration in nutritional intake causing deficiencies of vitamins, minerals and fibre. Avoidance of vegetables and fruits is a common feature of diets of elderly individuals. Fruit juice is preferred over fruits.
- Taste buds atrophy leads to alteration of threshold of certain tastes leading to altered food preferences, e.g. more sweet and salty foods. Elderly individuals, it must be remembered, are the common victims of hypertension and diabetes, the two conditions in which sugar and salt are usually harmful.
- Reduction in gastric volume associated with hypochlorhydria leads to altered meal pattern and gastric dysfunction. Delayed emptying of stomach and altered gastro-oesophageal reflex result in various gastric morbidities leading to altered food intake and avoidance of many foods.
- Atrophy of gastrointestinal tract musculature leads to reduced intestinal motility resulting in various types of gastrointestinal disorders and restriction of food intake and nutritional deficiencies. Constipation and flatulence are the major complaints due to reduced intestinal motility and which drastically alter dietary pattern and intake.

The production of a number of potentially health-promoting metabolites including short-chain fatty acids, conjugated linoleic acid and bacteriocins has been associated with bifidobacteria. They constitute a major portion of the microbiota throughout a healthy adult life, playing an important role in gut homeostasis and health. These are one of the most abundant genera present in the healthy infant gut. Ageing is a natural process which entails changes in the GI tract, immunosenescence and in some cases malnutrition. Also, changes in lifestyle, diet and medication have an unavoidable effect on the elderly microbiota and function. During later

years and with several diseases, the levels of *Bifidobacterium* and its species diversity decrease. These bacteria may provide a very important biomarker for certain diseases in future [8].

As mentioned earlier, physiological ageing corresponds with the biological process that occurs during ageing. Many physiopathological changes occur during this long biological process, spanning several decades, which gradually convert the physiological into pathological ageing. The commonly cited example is the hardening of the walls of blood vessels, a contributory factor for the onset of hypertension and cardiovascular disorders. This undesirable transition is accelerated if there is dietary excess of those nutrients which are recognized now to be responsible for diet-related degenerative clinical disorders like saturated fats, salt, sugar and cholesterol. On the other extreme, the most prevalent elderly diet consisting of liquid and soft mashed foods might be devoid of vegetables, fruits and fibre and is thus unable to supply vitamins, minerals and flavonoids which provide protective action in the ageing individuals. A vegetable-based diet with generous amounts of vegetables and fruits cooked without excessive use of spices and condiments and avoidance of frying will be a suitable diet for the elderly. It is in this context that food-based dietary guidelines for the elderly individuals will be of great benefit as a component of information dissemination strategy.

Both old age quality of life and longevity are generally impacted by nutritional and dietary interventions. Alterations in protein intake and limiting the dietary intake of certain essential amino acids are commonly observed dietary restrictions which are reported to increase lifespan. Studies indicate that rodent diets containing either lower methionine content or tryptophan appear to elicit beneficial effects. Maybe limiting these vital molecules may delay the onset of age-associated diseases and improve quality of life at older ages [9].

19.5 Dietary Guidelines

Usually elderly persons are less active and basal metabolism is lower so they require less energy [10]. Therefore instead of two big meals like lunch and dinner, they can be given 3–4 small meals by incorporating snacks in between meals. The daily intake of oil should be 15–20 gms. Protein-rich foods like pulses, milk, paneer (cheese) eggs, etc. can be included. Since the elderly are prone to nutritional deficiencies, they need foods rich in calcium, micronutrients and fibre. Apart from cereals and pulses, they need daily 200–300ml milk and 400 gm vegetables and fruits to provide fibre, micronutrients and antioxidants. Inclusion of these items in the diet improves the quality of the diet and bowel function. The foods should be cooked well, be soft and have less salt and spices. Less quantities of food should be consumed at regular intervals, and enough water should be consumed to avoid dehydration, hyponatraemia (low sodium) and constipation. [11]

19.5.1 Meal Planning

A typical meal plan for an elderly person may be as follows:

Morning 7.00 a.m. Water (1 glass), tea/coffee (1 cup), with 2–3 biscuits.
Breakfast 8.30 a.m. Oat dalia (porridge) or wheat dalia with milk and some dry fruit (raisins, cashew nuts) cooked well. Flavours like cardamom or saffron can be added to dalia – 1 bowl and 1 banana/apple/papaya (any one fruit) and a glass of milk. Sometimes suji (semolina) upma, poha (rice flakes), mung dal cheela (crepes) and dosa can also be served with sauce or chutney.
Mid-morning 11.00 a.m. Bael (wood apple) sherbet/lemon water/tender coconut water/lassi. Sometimes an orange or some grapes, custard apple, ripe guava, watermelon, muskmelon and pomegranate can be given.
Lunch 12.30–1.00p.m. Rice/khichri (1 cup), arhar/masoor/moong dhuli dal (1 cup), vegetables (1 cup) (any one or two) like bottle gourd, bitter gourd, tori (ridge gourd), parwal (pointed gourd), lady finger, sweet potato, tomato, potato, brinjal, beans, onion, ginger, garlic, spinach, fenugreek leaves, carrots, cauliflower, cabbage, fresh peas and fresh curd (1 cup). Sometimes dosa, idli, sambhar or chutney can be given. Cucumber, onion, tomato and carrot either diced or grated and salt and 1 tsp of oil can be added and eaten as salad.
Tea time 4.00 p.m. Tea maybe green tea/black tea/glass of milk with some savoury snacks or biscuits.
Evening 6.00 p.m. Puffed rice or roasted chiwra (rice flakes)/khandvi/dhokla.
Dinner 8.00 p.m. Chapati (two) with dal and some vegetable/paneer.

Desserts like fruit custard, rice kheer, fruit salad, etc. may be given once in a while after lunch. Besides rice and wheat, other cereals like ragi, bajra, sorghum and maize can also be included in the diet. While planning meals, regional food preferences and personal food choices should be considered to make the meals more acceptable. For example, if someone is from North India, they will prefer chapati made from atta (wheat flour), while people from the south will prefer rice-based dishes like dosa, appam, idiyappam, puttu, etc.

In planning meals for the elderly, one has to keep in mind that foods should be well cooked so that they can be chewed easily. Whole pulses like rajma, chana, whole mung dal, urad dal and masoor dal can be soaked and cooked well so that they can be digested easily. The temperature of foods served should not be too hot or too cold. A mixture of fennel seeds (saunf) and ajwain (Carom seeds) can be roasted lightly and kept in a small bottle. About ¼ tsp can be taken after meals. This will help in digestion and prevent flatulence. Triphala is a mixture of harida (Indian gallnut – hareetaki), baheda (belliric myrobalan – vibheetaki) and amla (Indian gooseberry) fruit which is dried and made into a powder. This is useful for preventing constipation, a common complaint among the elderly. About ¼ tsp in a cup of water can be taken after meals once in a day.

Plates used to serve can have a rim so that it is easy for the person to pick up food. Cups and glasses may be filled only 3/4 with liquids it may spill because of tremor in hands. It will be easier to lift the cup and drink. They can be lightweight and handles should be easy to grip.

The body needs a healthy diet in order to function well at all stages of life. The basic components of any diet should include a combination of protein from meat, egg, fish and pulses: five portions of fruits and vegetables per day and carbohydrates from cereals and root vegetables. It is also important that we try to include other specific micronutrients in our diet which help the body to remain healthy as we age. Older persons are particularly vulnerable to malnutrition. Since both lean body mass and basal metabolic rate (BMR) decline with age, their energy requirement per kg of body weight is also reduced. Basal metabolic rate is the number of calories required to keep your body functioning at rest, also known as metabolism. BMR is relative to body mass, age, weight and height. It is also affected by gender; therefore men need more calories than women. The process of ageing also affects other nutrient needs. The requirement for some nutrients may be reduced, while for other essential nutrients it may rise in later life. In elderly people, micronutrient deficiencies are often common due to a number of factors such as reduced food intake and a lack of variety in the foods they eat. Deficiencies of folic acid, zinc, pyridoxine, vitamin A, vitamin C and vitamin E result in impaired cell-mediated immunity and reduced antibody responses. Foods rich in micronutrients may be expensive which further lowers their consumption. Another factor which contributes to this group's increasing morbidity and mortality is that older people often show a progressive loss of immune function. Other age-related changes include the loss of cognitive function and deteriorating vision all of which hinder good health and dietary habits in old age.

Changes in diet seem to affect risk factor levels throughout life and may have even greater impact on older people. Moderate reductions in saturated fat and salt intake which would reduce blood pressure and cholesterol levels could help in reducing the burden of cardiovascular disease.

The intake of some nutrients [12], which become particularly important as we grow older, includes the following:

Calcium – It is required for the upkeep of healthy bones, but as we grow older, it starts to be reabsorbed back into the body from the bones. This condition is called osteoporosis and eventually leads to weakening of the bone tissue which leaves bones brittle and fragile. Good sources of calcium include milk and dairy foods such as yoghurt and cheese, green leafy vegetables and calcium-fortified cereals.

Fat – Older people who are within a healthy weight range should reduce saturated fat intake to improve heart health.

Fibre – As we age there are bowel problems resulting in constipation. Fibre-rich foods like wholegrain cereal, fruit, vegetables, pulses and nuts should be included in the diet, and intake of enough fluids should be encouraged to help the gut to function properly.

Fluid – The body's ability to conserve water gradually decreases as we grow older, and the perception of thirst becomes less sensitive. However dehydration can result in tiredness, dizziness, constipation, drowsiness and confusion among other side effects, so it is important to keep hydrated throughout the day even if we do not feel thirsty. Fluid intake includes water, tea, coffee, fruit juice or soups.

Iron – Good sources of iron include green leafy vegetables, meat and dry fruits. Iron is used to make haemoglobin which helps to store and carry oxygen in the red blood cells from the lungs to the rest of the body. Iron deficiency leads to anaemia when the organs and tissues receive less oxygen than they usually would be leading to tiredness and lethargy.

Vitamin C – Vitamin C is needed in the formation of collagen, which is essential to heal wounds and repair teeth and bones. It is also needed to make ligaments, skin, blood vessels and tendons. It is high in antioxidants which help to prevent heart disease and cancer. Citrus fruits like orange, lemon and vegetables are good sources of vitamin C.

Vitamin D – Vitamin D aids the body to absorb calcium thus slowing the rate of calcium loss from bones. Exposure to sunlight helps in formation of vitamin D from ergosterol present in our body. Still it is important to supplement our diet with foods which are rich in the nutrient such as oily fish, eggs and fortified cereals. As we grow older, we may need a vitamin D supplement to our diet as the body may be unable to process enough from the sunlight and your diet alone.

Zinc – Zinc is essential for maintaining a healthy immune system and is most commonly found in meat, shellfish, whole meal bread and pulses. Other nutrients contained in the mixture, vitamins C, E and B_6, beta-carotene, selenium and zinc are needed for normal immune response. A combination of antioxidants and B vitamins has been shown to improve immune response in the elderly.

B vitamins – Vitamins B_1 and B_{12} are needed for optimum brain function. Reports indicate that serum levels of B_{12} decline with age. Many cases of low serum B_{12} are associated with malabsorption due to gastric atrophy.

The elderly have frequent changes in sodium and potassium levels; therefore these levels should be monitored and maintained as per proper medical advice.

Vitamin E supplementation indicated lower incidence of infectious disease in older subjects and is also shown to reduce lung viral titres following influenza infection. Mental symptoms such as emotional irritability, cognitive impairment or other neurological signs are well known in case of vitamin deficiencies. To optimize brain function in older persons, the intake of a wide variety of foods and possibly supplements is necessary in maintaining a close interplay of nutrition, mood and behaviour. Antioxidants are of particular interest since brain ageing is associated with oxidative stress. A high intake of dietary Vitamin E may protect against the occurrence of Parkinson's disease. As ageing continues, carotene and carotenoids, ascorbic acid and alpha tocopherol influence brain function [13].

In older persons nutrients can affect cognitive function, mood and behaviour in several ways. Stroke and related disorders contribute significantly to disability in old age. The intake of protective nutrients like antioxidants can lower the risk of

stroke and related disorders by keeping the vascular system functioning adequately. It is likely that bioactive non-nutrients like phytochemicals also play an underestimated role in supporting vascular function. Foods with high antioxidant properties can also protect against the damage caused by free radicals which is a significant cause of brain ageing and loss of cognitive function. However older persons are often unable to maintain a varied enough diet to obtain necessary micronutrients in appropriate amounts. Supplementation or food fortification may thus be required for at risk populations.

Dietary antioxidants like vitamin C (ascorbic acid), vitamin E (tocopherols and tocotrienols) and carotenoids (β-carotene, α-carotene, lycopene, lutein, zeaxanthin and β-cryptoxanthin) may be of major importance in disease prevention. Fruits and vegetables contain many antioxidants that are useful to us. The role of antioxidants in slowing the progression of certain neurological disorders has also been suggested as oxidative stress may be a causative factor in several disorders of the nervous system which is common in old age. Phytochemicals with antioxidant properties are mostly phenolic acids and flavonoids. These compounds are found in garlic, onion, ginger, oats, soybean, tea; oilseeds like mustard, canola, sesame and rapeseed; herbs likes oregano; and spices like black pepper, turmeric and cloves. Among the components of the human diet, polyphenols from berries are essential micronutrients that have been particularly found to improve cognitive function. The major polyphenolic classes found in berries – flavonols, anthocyanins and stilbenes – focusing on resveratrol have been found to have beneficial effects on health and age-related cognitive decline as associated neurobiological processes in humans [14]. Resveratrol is a polyphenolic compound found in grapes which is known for its anti-ageing and anti-tumourigenic properties. It is known to provide neuroprotection against diet – induced neuro –inflammation and cerebral vascular dysfunction [15] Tables 19.1 and 19.2.

Table 19.1 Non-nutrient antioxidants in food

Soybean	Isoflavones, phenolic acids
Green tea/black tea	Polyphenols, catechins
Coffee	Phenolic esters
Red wine	Phenolic acid, resveratrol
Citrus and other fruits	Bioflavonoids, chalcones
Onion	Quercetin, kaempferol
Olives	Polyphenols
Turmeric	curcumin
Oats	Dihydrocaffeic acid, phospholipids

Source: Diet and Ageing – Exploring some facets, Ed. Kalyan Bagchi and Seema Puri (1999) Society for Gerontological Research, New Delhi

Table 19.2 Food sources of antioxidant vitamins

Vitamin E	
Vegetable oils	Peanut, palm, soya, corn, safflower, sunflower, cottonseed
Vegetables	Mustard leaves, turnip greens, pumpkin
Other sources include chicken, liver, salmon, shrimp, wheat germ, almonds, peanuts	
Vitamin C	
Fruits like orange, lemon, sweet lime, guava, amla, strawberries, melon, pomegranate, papaya	
Vegetables	Tomatoes, leafy vegetables, chillies, cabbage
Sprouted pulses like whole moong and chana	
Vitamin A (β carotene)	
Yellow/orange vegetables like carrot, pumpkin, sweet potato	
Yellow/orange fruits like apricot, oranges, papaya, cantaloupe melon	
Green leafy vegetables like spinach, mustard leaves, radish leaves, fenugreek leaves, broccoli	

19.6 Body Weight and Metabolism

Body weight and shape are a balance of energy intake (dietary calorific content) against output, i.e. calorific burn from exercise and activity. The body needs calories for functioning of internal organs. Our body produces calories by metabolism from the food we eat and burns it too. If energy intake is less than expenditure, weight is lost. When the calories consumed are in excess of expenditure, the body saves them in the form of fat. Thus weight is gained. Physical activity helps the body to maintain healthy level of insulin and blood sugar. Various parameters are used to assess health and fitness. The most widely used parameter is the body mass index or BMI. At all ages we need to maintain our BMI at 23 to be in good health [16].

The body mass index is a measure of the body weight relative to height that applies to both adult men and women. The BMI is calculated by measuring the weight in kilogram and dividing it by height in meters squared.

Example: Weight = 70 kg, Height = 170 cm (1.70 m)
Calculation: BMI = 70/(1.70 × 1.70) = 24.22

Lifestyle diseases are on the rise with increase in number of overweight and obese adults. We must try to achieve ideal body weight and maintain it by eating a balanced diet and exercising regularly. Regular exercise has enormous physical and emotional benefits too. Besides reducing the risk for high blood pressure, high blood cholesterol, diabetes, obesity and osteoporosis, regular exercise can be helpful in reducing the risk of depression.

Immune responses or the lack thereof have been implicated as potentially causative factors in ageing. Immune responses decline with ageing, while autoimmunity, i.e. the production of antibodies by the host against antigens produced by the same host, increases. Manipulation of two elements of the environment, body temperature and nutrition, has been shown to influence the relation of immune responses to age [17].

19.7 Can Nutrition Modulate the Ageing Process?

Studies have reported the role of various nutrients which delay the process of ageing in one way or the other. For example, calorie restriction is an important measure for healthy ageing. Epidemiological studies in human beings have also indicated that obesity is definitely a life hazard for elderly individuals and is associated with earlier mortality. It may induce other diet-related non-communicable disorders like diabetes, coronary artery disease and even malignancies. There are references of foods like yoghurt, honey and many other foods or drinks promoting good health, and certain nutrients have specific roles in the physiological process which modulate ageing. The following is a brief outline of several nutrients and their role and benefits in the ageing process (Table 19.3).

The damage done by free radicals in the ageing body can be overcome by antioxidants present in vitamins and minerals in food or by non-nutrient antioxidants present in many foods like onion, turmeric, olive, tea and soybean. Green leafy vegetables, fruits and spices are excellent sources of antioxidants by acting as scavengers of free radicals and by strengthening and regenerating the enzyme systems in the body which are protective and promote human health. During the biological process of ageing, there is also a generalized reduction of various enzyme systems in the body along with the atrophy of various tissues.

Anxiety and depression are commonly found in the elderly. Certain plants and their extracts have been found to be useful in helping to deal with these conditions. Ashwagandha (*Withania somnifera*) root powder and Kapikacchu (*Mucuna prurita*) dried fruit powder [18].

Recommended Dietary Allowances (RDA) serve as guidelines for the amount of each nutrient to be taken in a balanced diet. The table below gives RDA for adult men and women. While the basics remain the same, some nutrients which are required in higher amounts for adults above 60 years of age doing sedentary work are also given (Table 19.4).

Table 19.3 Nutrients and their roles

Nutrient	Physiological role	Specific role in ageing process
Iron	Immunocompetence	Preventing vulnerability to infectious disease
Iron/ folate	Prevention of anaemia	Maintaining appropriate level of activity
Calcium	Bone calcification	Preventing osteoporosis and fractures through falls
Selenium	Antioxidant	Preventing free-radical damage in the biological process of ageing
Vitamin A	Antioxidant	Preventing free-radical damage in old age, atherosclerosis, cataract, prevention of malignancies and free-radical damage on other tissues and enzyme system
Beta carotene	Antioxidant	
Ascorbic acid	Antioxidant	
Vitamin E	Antioxidant	
Lycopene	Antioxidant	

Table 19.4 Recommended dietary allowances for Indians [19]

Nutrient	Man	Woman	For adults above 60 years by WHO [13]
Body weight (kg)	60	55	
Energy (kcal/day)	2320	1900	1.4–1.8 multiples of BMR
Protein (gm/day)	60	55	0.9–1.1gm/kg per day
Visible fat (gm/day)	25	20	30%
Calcium (mg/day)	600	600	800–1200 mg/day in presence of adequate vitamin D
Iron (mg/day)	17	21	10 mg/day
Vitamin A (μg/day) retinol	600	600	600–700 μg/day
β-carotene	4800	4800	
Thiamine (mg/day)	1.2	1.0	
Riboflavin (mg/day)	1.4	1.1	
Niacin (mg/day)	16	12	
Pyridoxine (mg/day)	2.0	2.0	
Ascorbic acid (mg/day)	40	40	60–100 mg/day
Dietary folate (μg/day)	200	200	400 μg/day
Vitamin B_{12} (μg/day)	1.0	1.0	2.5 μg/day
Magnesium (mg/day)	340	310	225–280 mg/day
Zinc (mg/day)	12	10	
Vitamin D (μg/day)	10	10	10–15 μg/day
Vitamin E (mg/day)	8–10	8–10	100–400 IU/day or 12 mg/day
Vitamin K (μg/day)	55	55	60–90 μg/day
Phosphorus(mg/day)	700	700	600–800 mg/day
Copper (mg/day)	1.3	1.3	1.3–1.5 mg/day

A variety of microbial communities and their genes (the microbiome) exist throughout the human body with basic roles in health and disease. To study these microbial communities and their interaction with their human hosts, the Human Microbiome Project Consortium has got together many scientific experts. This resource may help in the development of novel prophylactic strategies such as the application of prebiotics and probiotics to improve human health.

This reference can be used in studies with a main focus on disease for comparative purposes including detecting shifts in microbial taxonomic and functional profiles or identification of new species not present in healthy cohorts that appear under disease conditions. The catalogue described in this study may be the most comprehensive reference set of human microbiome data associated with healthy adult individuals. The data can be analysed to identify new organisms, gene functions and

metabolic and regulatory networks as well as correlations between microbial community structure in health and disease [20].

One of the main causes of cellular and organism ageing is the build-up of DNA damage which is an unavoidable side effect. When DNA repair is compromised, it leads to constant DNA damage causing age-related disorders and reducing lifespan. Studies have shown that initial loss in weight related to planned dietary restriction actually enhances physiology. Adaptation in metabolic regulation along with changes in the function of energy producing organelles called mitochondria may also shift cellular metabolism towards roles that protect the genome from damage [21].

19.8 Dietary Restriction

A dependable environmental manipulation known to increase active and healthy lifespan in many species is dietary restriction (DR). Reduction of particular or total nutrient intake without causing malnutrition is termed dietary restriction (DR). This includes caloric restriction (CR) in which total caloric intake is reduced, as well as dietary interventions involving the restriction of specific dietary components like carbohydrates, protein and lipids [22].

Most et al. (2017) have reviewed that dietary restriction has beneficial effects during ageing through cellular nutrient sensing mechanisms that promote proteostasis, genome stability, stress resistance and stem cell function. Diet quality modifications along with caloric restriction markedly decrease the incidence of CVD, cancer and diabetes and attenuate age-related neurodegeneration, sarcopenia and auditory loss [23]. Short-term dietary restriction has been found to help in treatment of chronic cancer treatment more efficiently and preventing the occurrence of secondary malignancies as well as protecting from the acute stress of surgery [24].

During intermittent fasting (IF), a person can go from 16 to 48 hours with little or no energy intake and intervening periods of normal food intake, on a recurring basis, while periodic fasting (PF) is IF with fasting from 2 to as many as 21 days.

Emerging findings reveal that cellular and molecular mechanisms by which intermittent fasting (IF) increases the resistance of cells, tissues and organs to stress and common diseases linked to ageing and sedentary lifestyle. Studies show that intermittent fasting may protect against metabolic syndrome and associated disorders like diabetes and cardiovascular disease. Fasting in humans has proved efficient subjects for weight loss and enhancement in multiple health indicators including insulin resistance and reduction of risk factors for CVD. The molecular and cellular mechanisms by which IF enhances health and prevents disease processes involve activation of adaptive cellular stress response signaling pathways that enhance mitochondrial health, DNA repair and autophagy. Periodic fasting also promotes stem cell-based regeneration as well as long-lasting metabolic effects [25].

With ageing the incidence of CVD increases due to decline in arterial function manifested as arterial stiffness or endothelial dysfunction. It also contributes to chronic systemic disorders of ageing, e.g. cognitive and motor impairments. This is because appropriate blood flow/pressure and nutrient supply are essential for active

muscles and organs. Thus arterial dysfunction leads to tissue-level stress that may precipitate neurodegenerative diseases, kidney disease and likely many other age-related conditions. Therefore there is major interest in specific healthy lifestyle interventions which include dietary intervention strategies for preventing or reversing age-related arterial stiffening and endothelial dysfunction. Fruits, vegetables, whole grains, nuts, legumes, seeds, low-fat dairy products, moderate amount of lean meat and fish, limited consumption of refined/sugary foods, cocoa, olive oil and less salt help in maintaining a steady level of potassium, calcium and magnesium which are important electrolytes to maintain arterial health. Energy restriction without malnutrition is associated with beneficial effects on many physiological parameters with ageing. Calorie restriction slows down age-related arterial dysfunction by stimulating major cellular signaling networks that reduce oxidative stress/inflammation and increase nitric oxide availability. These energy-sensitive networks which tend to become impaired with ageing may be novel targets for alternative nutritional therapies, such as nutraceuticals [26].

These are many aspects to be taken into consideration when we consider nutrition for the elderly. The epidemiological and social aspects, the effect of physical activity, evaluation of the nutritional status of older persons, nutritional guidelines for healthy ageing and prevention of chronic non-communicable diseases and guidelines for promoting physical activities among older persons need to be considered. This will help them towards healthy ageing. New clinical studies of healthy ageing are required and quantitative biomarkers are an essential component, especially tools which can measure enhanced physiological integrity at every stage of life supposed to be a major catalyst to a long and fruitful life [27].

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Care of Older Persons in India: Scope of Policy and Technology Tools

20

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Abstract

India has not remained untouched by global population ageing. In the next three decades, every fifth Indian will be aged 60 years or more. The response of the State to face the challenges of such a momentous change in the society has been rather tepid in the previous century. Several affirmative actions of the State have made life easy for older Indians in the last decade. Matching the pace of population ageing, advent of newer technology in various aspects of old age care has been impressive. This article summarizes various aspects of old age care in India.

Keywords

Policy · Program · Legislation · Long term care

20.1 Introduction

With more than a hundred million people above the 60 years, India has started facing the challenges of population ageing. Older persons, particularly the very old, belong to the fastest growing segment in the population of India. While the Indian population would rise by 60% between 2000 and 2050, the number of older people would rise to 323 million in 2050 accounting for more than 20% of the population [1]. These proportions and numbers have important implication for every institutions of the society, apart from the financial and pension services. This altering landscape of the demography means that it is necessary to adopt newer and more novel approaches in order to find optimum solutions. This ranges from formulation of newer policies and programs for older persons to implementing newer technologies to improve the

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well-being of the older individuals. It was in the 1980s that the First World Assembly of United Nations on Ageing was held in order to develop newer strategies. Developed countries experiencing rapid population ageing reacted early to the phenomenon of population ageing, while the developing countries, including India, who were still battling with issues related to high population growth rates, infant mortality and maternal mortality, were slower in realizing it.

20.2 State Interventions

The Indian Government of India could appreciate the challenges of a large ageing population only in the 1990s. The first legislation, The National Policy on Older Persons, was adopted in 1999. This was followed by the enactment of the Maintenance and Welfare of Parents and Senior Citizens Act in 2007 which defined the responsibilities of the family and the State in care of the senior citizens. While policies and the law provided the framework, the most visible intervention in old age care was the launching of the National Program for Health Care of the Elderly (NPHCE) in 2011. Social welfare is a State subject in India, and the central government depends on the State governments for implementation. As a result, uniform implementation of these welfare measures for senior citizens is seldom achieved.

At a more local level, geriatric care in India largely consisted of conventional hospital-based healthcare with most of the social and economic care largely taken care by the joint family culture so prevalent in India. This approach appears inadequate, and greater will, both political and scientific; needs to be seen. Unsurprisingly, senior citizens experience the best quality of life in the familiar surroundings of their own home provided that they retain their independence and are not dependent on their families. Hence it makes sense promulgating approaches to enable them to be independent and improve the quality of life in their homes rather than focusing on setting up newer nursing homes where there are issues like privacy, inadequacy of care and limited time with caregivers. Financially, home healthcare is less expensive than live-in facilities for similar one-to-one service levels. The impact of this technology can be at both the global (national) level and the local level of the older individual.

20.3 Policies and Programs on Older Persons in India

Several policies and programs are in place in India for the welfare of older persons. Some of these policies are specific to old age issues while others generic in nature addressing the whole population. For example, National Policy on Older Persons is specific for old age, while National Disability Policy addresses old age issues also. Similarly there are specific and generic programs particularly in health sector directed at older population. While National Program for Health Care of the Elderly is an overarching program, National Blindness Control Program addresses a common disability, the issue of cataract in old age.

20.3.1 National Policy on Older Persons

The first policy document for senior citizens in India was the National Policy on Older Person (NPOP) [2], which was adopted in 1999. It was in keeping with the national constitutional provisions (Article 41) as well as the UN resolution to observe 1999 as International Year of Older Persons. Based on lessons learnt after implementation, certain modifications were made, and a newer policy was announced in 2011. Some salient provisions common in both these policies are the following:

- Promote and sustain dignity in old age by providing income security, building homecare services, and increasing access to old age pension and healthcare insurance.
- Acknowledged the importance of healthy ageing at home and emphasized the importance of preventive healthcare and early diagnosis.
- Building an accommodative age friendly society as envisaged in the Madrid Plan of Action of which India was a signatory.
- Recognizes the need for affirmative action in favor of elderly. Special attention will be necessary to older females so that they do not become victims of triple neglect and discrimination on account of gender, widowhood, and age.
- Considers 60+ years as a phase when the individual should have the choices and the opportunities to lead an active, productive, and satisfying life.
- The national policy identified the challenges in old age care and placed great emphasis on role of primary healthcare and the need to strengthen it and make it geriatric inclusive by reducing the rural-urban divide of resources and greater public sector outlays.

Like most policies in social sector, implementation of NPOP was not satisfactory though several changes were noticeable on the ground.

20.3.2 National Program of Healthcare for the Elderly

The Ministry of Health and Family Welfare launched National Program of Health Care for the Elderly (NPHCE) during the financial year 2010–2011 as a part of the 11th Five Year Plan initiative [3]. Vision of NPHCE was:

1. To provide accessible, affordable, and high-quality long-term, comprehensive, and dedicated care services to an ageing population
2. To create a new "architecture" for ageing;
3. To build a framework to create an enabling environment for "a society for all ages"
4. To promote the concept of active and healthy ageing in the health system of India

The specific objectives of NPHCE were to provide an easy access to health services through community-based primary healthcare (PHC) approach; to identify health problems and manage them; to provide referral services to district hospital and regional geriatric centers; to build capacity of the medical and paramedical professionals as well as the caretakers within the family; and to achieve convergence with National Health Mission, AYUSH, and Ministry of Social Justice and Empowerment for coordination of services.

While the NPHCE addresses most of the health problems in an institutional healthcare system, it completely neglects the home-based care of an older person in the family and has failed to incentivize elderly care. The NPHCE is a good and new initiative to take care of a fast-ageing population but requires more attention in the implementation and coordination so that the program becomes a reality [7]. In 2014, the Government of India included additional features to NPHCE for the very old (75+) with a new name.

20.4 Social Welfare System Functioning in Supporting Older Persons

The social welfare of the elderly is looked after by the Ministry of Social Justice and Empowerment, Government of India, through legislative measures like the Maintenance and Welfare of Parents and Senior Citizens Act 2007 [4] and the Integrated Program for Older Persons [5]. The policies and programmers mentioned above were effective in many sectors. Most visible impact was in the areas of financial security. The New Pension Scheme (NPS) was rolled out in 2004 with universal eligibility. Pension schemes from non-banking financial institutions and mutual funds in private sector are now emerging as popular financial security among urban Indians. Since adoption of the National Policy on Older Persons, several tax reliefs have been provided to senior citizens. The main legislative measures are summarized below in Table 20.1.

Table 20.1 Affirmative measures for older people

Affirmative measure	Description
National Population Policy (1999)	The first attempt by the government to ensure financial security, healthcare, shelter, and other needs of older persons in order to improve the quality of life
National Program of Health-Care for the Elderly	Aim of the NPHCE program is to provide separate, specialized, and comprehensive healthcare to the senior citizens at various levels of healthcare delivery system
Maintenance and Welfare of Parents and Senior Citizens Act, 2007	Attempts at providing social security by making it obligatory to take care of parents/senior citizens by children/relatives. Other measures include establishing old age homes and penal provision for abandonment of senior citizens
Integrated Programme for Older Persons (IPOP)	Encourages productive and active ageing through support for capacity building in institutions of civil society and maintaining old age, mobile care units, and day care center.

20.5 Assessing the Problem: National Sample Surveys

The National Sample Survey (NSS) is one of the oldest continuing household sample surveys in the developing world. There have been 74 rounds of survey till now with the first one being held in 1950. The Sixtieth Round of National Sample Survey conducted in 2004 focused on morbidity, healthcare, and the condition of the aged [6]. The survey was carried out between January and June 2004 covering 73,868 households from all over India with the exception of some interior areas. Some of the important observations of the report are summarized below:

- Old age dependency increased steadily in the previous two decades reflecting the population ageing.
- The sex ratio (number of females per thousand males) was also steadily rising in favor of older women.
- Five percent of older persons lived alone, though nearly 90% lived in family with spouse and children. Sixty-five percent of older persons were economically dependent on spouse or children.
- Thirty percent of older persons (age 60 years or more) reported an ailment during the last 15-day period. 6.4% of older persons were hospitalized at least once in the previous 365 days compared to 2.5% for all age groups.
- Eight percent of older Indians were confined to home or bed at the time of this survey. The proportion increased to 27% after the age of 80 years, and women were more likely to be bedbound than men.
- The perception of health, which is a determinant of health-seeking behavior, was an important observation of this study. Excellent to fair perception of health was reported by 83% of older persons without illness and 56–66% of those with an illness. This possibly indicates acceptance of illness as a part of ageing process.

A comparative analysis across the National Sample Survey Organization (NSSO) 42nd, 52nd, and 60th rounds of select health parameters was carried out by the Ministry of Health and Family Welfare, Government of India, in collaboration with WHO Country Office for India in 2007. It concluded that the problems of the aged may increase owing to the increase in the proportion of nuclear families, especially in the urban areas. With the advancement of age, the proportion of elderly either confined to home or to bed increases as can be seen in the chart above. Care of the elderly can be a challenge for any society, where social security is not universal [7]. There is very little data on health issues of 80+ people in hospital as well as community setting. This is possible because there were not many octogenarians and nonagenarians in Indian society in the preceding decades. In the abovementioned ICMR Task Force project [8], less than 10% of subjects were above 80 years of age, with a mean age 84.37 (±5.69) years.

20.6 Home Care and Long-Term Care

Older Indians prefer to live and age at the place where they have spent most of their lives. It has been a common observation in clinical practice that older people prefer to die in their homes rather than in a hospital or hospice. A recently concluded ICMR study at All India Institute of Medical Sciences revealed that 70% of patients who died after attending the emergency department died in their home indicating the importance of developing home care services for sick older persons [9].

The care of the older person is usually taken up by the family. This is a challenge at multiple levels, especially if he/she is bedbound. Three issues are of great importance in long-term care: (1) assistance in activities of daily living, especially personal hygiene; (2) treatment of chronic diseases and disabilities; and (3) acute unanticipated health problems. In recent years, care giving has turned into a commercial venture. Despite attempts to financially support institutions for training of caregivers by the government, they have largely failed to take off and continue to be poorly sustained and regulated. There have been innovations by private/NGO sector although they are few and sketchy. Some of the initiatives include programs managed by the Nightingale Medical Trust in Bengaluru, [10] Apollo Group of Hospitals [11]. Alzheimer's and Related Disorders Society of India (ARDSI) has four institutions for dementia patients in Kerala [12]. Max Group of Hospitals in Delhi run a home healthcare program in Delhi [13]. One Sama Nursing Home in Delhi also has a long- term care program for patients requiring long-term medical and nursing interventions [14]. Most of these services with the exception of ARDSI are heavily medical intervention oriented and in reality mean prolonged post-acute care rather than long-term care.

20.7 Application of Technology in Home Care/Long-Term Care

Technology has permeated every facet of our life and is today an undeniable part of everyday life. There has been a remarkable growth of technology in the field of healthcare over the last two decades. A MEDLINE search for papers on the use of technology in home healthcare published in 2003 found 556 papers; a decade later, the number was 1390 [15]. Along with the growth of the technology, there has also been an increase in the evidence base supporting it. While caregivers play the most significant role in keeping the elderly at home, home care technology now provides essential tools to permit this caregiving at a cost-effective scale. Many technologies have been developed to improve patient outcomes and lower the overall cost of care delivery while the patients remain at home [16]. Technology adopted for use can be active or passive. Someone must operate an active technology while passive technologies are, for example, cameras, sensors, or other devices embedded in the residential infrastructure that allow an individual to be monitored without requiring that individual or another person to operate them [15]. The main impact areas of technology in furthering home care are the following:

1. Remote health monitoring: Remote monitoring or tele-health is an all-inclusive term that describes the use of high-tech technologies to support long-distance care for patients. The monitoring can occur continuously in real-time or periodically [16]. Such systems can record and monitor a wide range of items such as an electrocardiogram (ECG), pulse oximetry, vital signs, weight, and blood glucose. They can also be used to monitor patient-related activities like falls, mobility, and security. Sensors can be used, either on the older person or through wearables like bands, to monitor locomotor parameters apart from vital parameters. They alert caregivers if the older person doesn't get out of bed or falls. These are becoming popular by the day, and these technologies accounted for $35.2 billion in sales worldwide in 2014, according to Kalorama Information with a good deal of that demand coming from home treatment and health facilities [17].
2. Real-time patient monitoring: GPS-tracking technologies allow family members and caregivers to locate older individuals when they are outside the confines of their homes.
3. Mobile phone technology (mHealth): mHealth most commonly refers to the usage of mobile communication devices, such as mobile phones, tablet computers, and PDAs, for health services and information. Mobile technology could assist in providing a variety of services, such as health education, communication, consultation, monitoring, diagnostics, and training to maintain autonomy and increase the quality of life of users [18]. It is expected to become an integral part of India's healthcare system [19]. In January 2016, four mHealth initiatives were launched that were aimed at educating pregnant mothers and Accredited Social Health Activist (ASHA) workers, helping tobacco users quit tobacco, and helping users with information on TB [20]. Broadly speaking, the role of mHealth in older care is summarized in Fig. 20.1 and Table 20.2.

The Ministry of Science and Technology, Government of India, has been funding innovative interventions for elderly and the differently able persons through a scheme "Technology Initiative for Disabled and Elderly (TIDE)" for the last one decade or so. Under the scheme information on all issues related to health and long-term care is provided through a website www.oldagesolutions.org.

20.8 Future of Health and Long-Term Care of Older Persons in India

Implementation has been slow due to multiple factors, lack of resources being the most important among them. With increasing participation of private sector in healthcare since adoption of neoliberal economic policies in the 1990s, it appears that most of the health and long-term care in foreseeable future will be provided by private sector. The role of public sector institutions will possibly be limited to human resource development and establishment of evidence base through rigorous research.

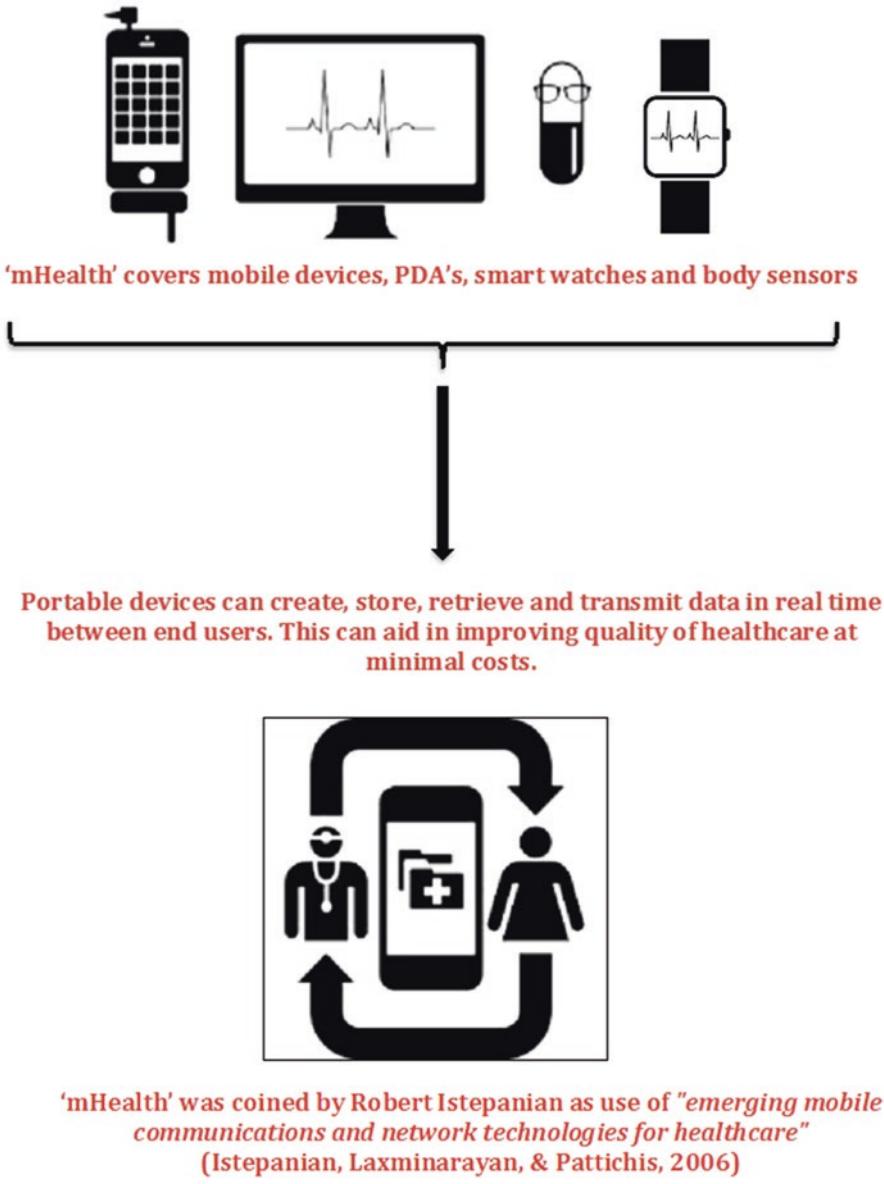

Fig. 20.1 mHealth definition and capabilities

Table 20.2 Range of services to be provided by mobile technologies

1. Safety and security monitoring, e.g., gas sensors, fire detectors
2. Remote health monitoring: Vital health information (blood pressure, pulse rate, temperature, etc.) as well as monitoring activity (number of steps, gait stride, and length). The data acquired can be stored and used to identify appropriate response that can be taken for falls risk and other geriatric syndromes [21]
3. Health information and services, e.g., tele-consultations, short message service (SMS) reminders for appointments and prescribed medicines, as well as educational text messages. Videos can be shared and disseminated, without a word limitation, as in SMS. They are easy to use with good penetration at every level of society
4. Health maintenance: Maintaining and enforcing prescriptions as well as follow-up of health variables at every visit like weight gain/loss, smoking, or alcohol cessation
5. Patient monitoring: Patients can be followed up with regularly after discharge. Certain scores can be used to predict increased risk of readmission
6. Development of risk prediction models for the young elderly, in which various risk prediction scores and models can be used in the form of applications for determining the likelihood of a person developing certain conditions later on in life.

There has been a shift in State policies in welfare sector, since the current National Democratic Alliance Government has taken over in 2014. There is a perceptible decline in social sector allocation. There is a policy shift toward encouragement of public private partnership and regulation of public and private services. In old age care, this will reflect in development of long-term care institutions in private sector with regulation of standards and quality control.

Opening of insurance sector to foreign equity may help development of health insurance and long-term care insurance in the same pattern as New Pension Scheme on the early 2000s. It may be anticipated that sooner than expected, more effective health insurance systems would evolve to reduce the pressure of out of pocket speeding by families for healthcare. This may help families to take care of older family members without the financial stress.

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