Pramod C. Rath *Editor*

Models, Molecules and Mechanisms in Biogerontology

Physiological Abnormalities, Diseases and Interventions

Springer

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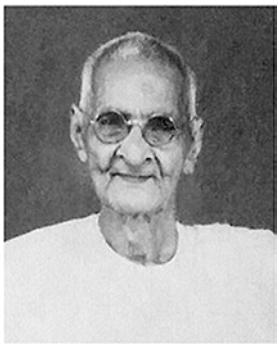

Purna Chandra Rath
(08 October 1889–01 May 1982)

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%).

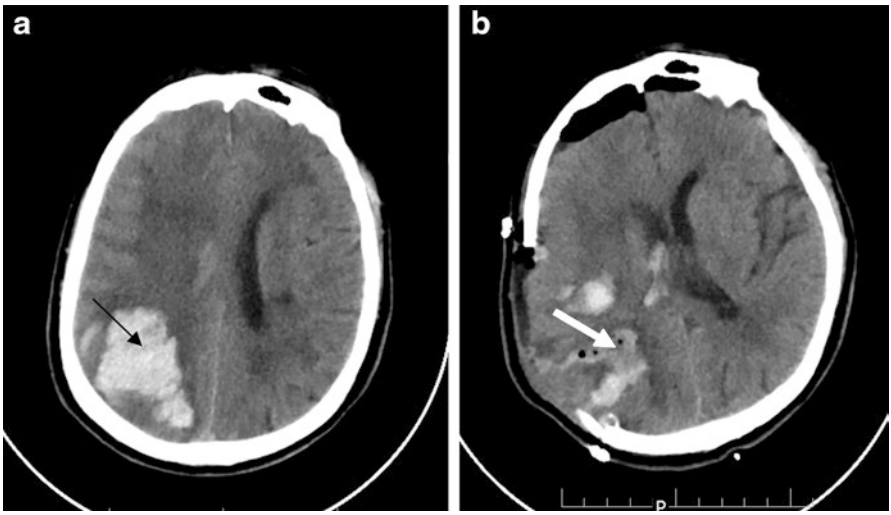

Fig. 1.1 (**a**) CT scan of the brain showing a blood clot (arrow) in the right side of the brain. (**b**) CT scan of the brain of the same patient after a few days of decompressive surgery, showing the absence of bone flap and less amount of clot remaining (white arrow)

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

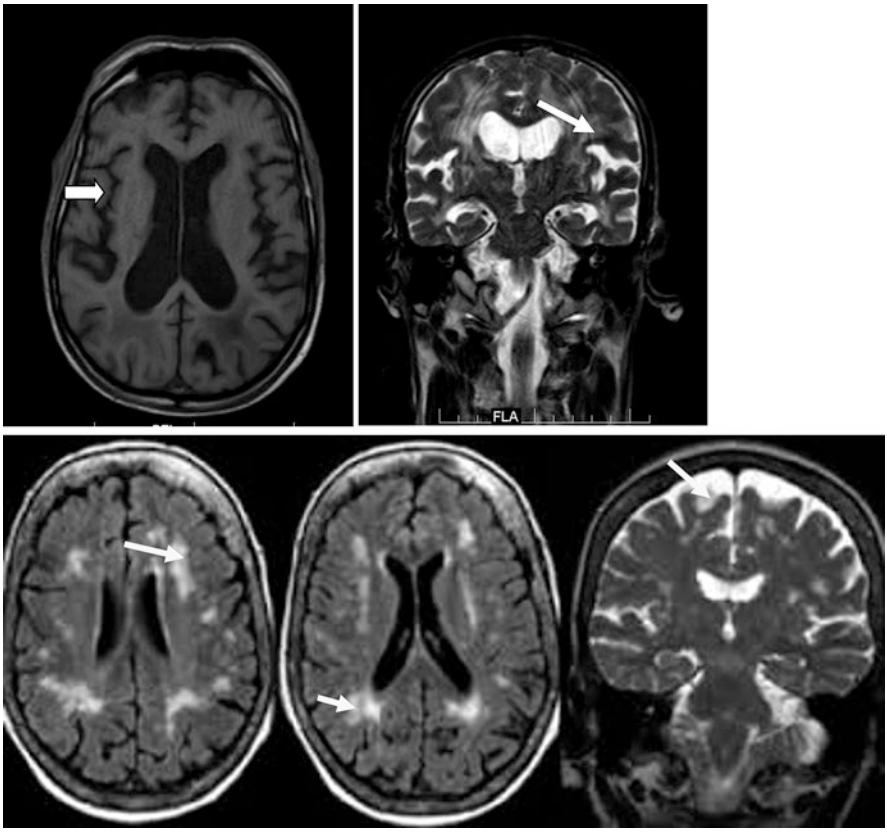

Fig. 1.2 MRI of the brain of patients with dementia showing bilateral ischaemic spots (white arrow) and shrinkage of the brain

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

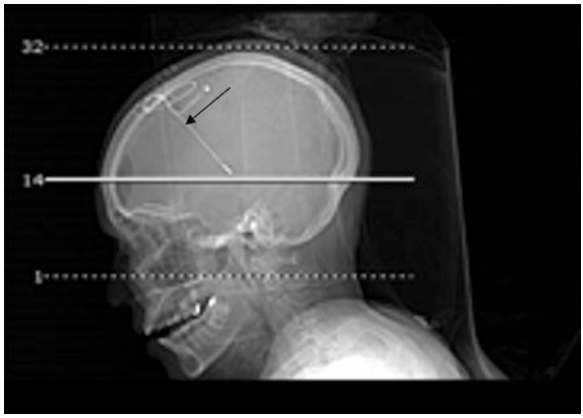

Fig. 1.3 X-ray of the skull (lateral view) showing the microelectrode (arrow)

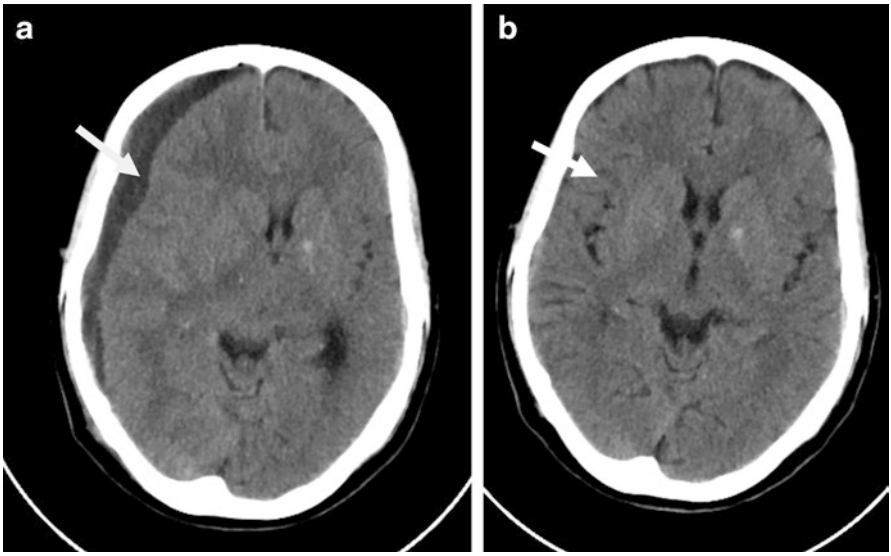

Fig. 1.4 (**a**) CT scan showing CSDH (white arrow) causing mass effect, pressure on the right side of the brain and brain shift. (**b**) CT scan after surgery showing good result with no collection of blood (white arrow)

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.

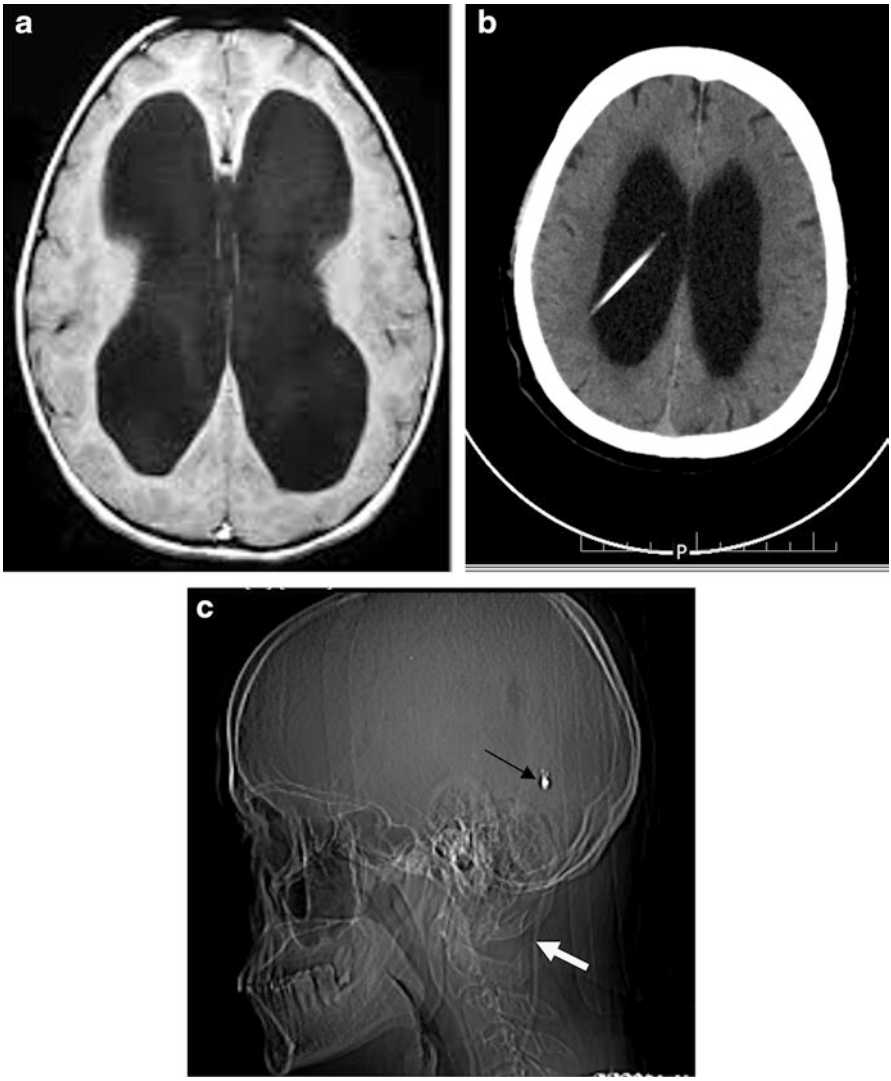

Fig. 1.5 (**a**) MRI brain showing hydrocephalus (left). (**b**) CT scan showing shunt in situ. (**c**) Postoperative X-ray of the skull showing the programmable valve (black arrow) and the connecting tube of the VP shunt (white arrow)

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

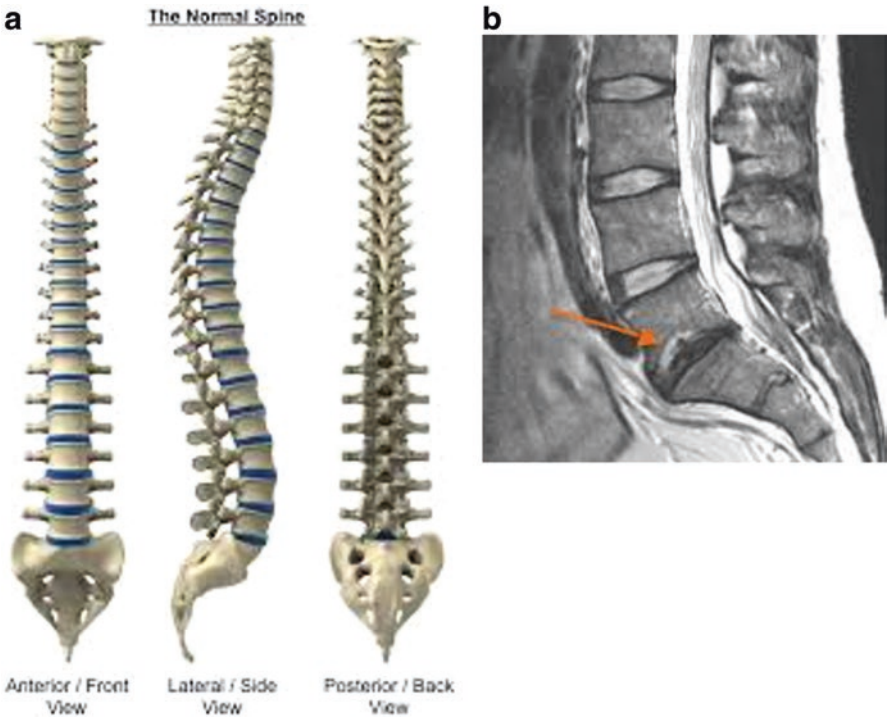

Fig. 1.6 (**a**) Appearance of normal spine (reference from the Internet). (**b**) MRI of lumbar spine with degenerative changes (arrow)

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,

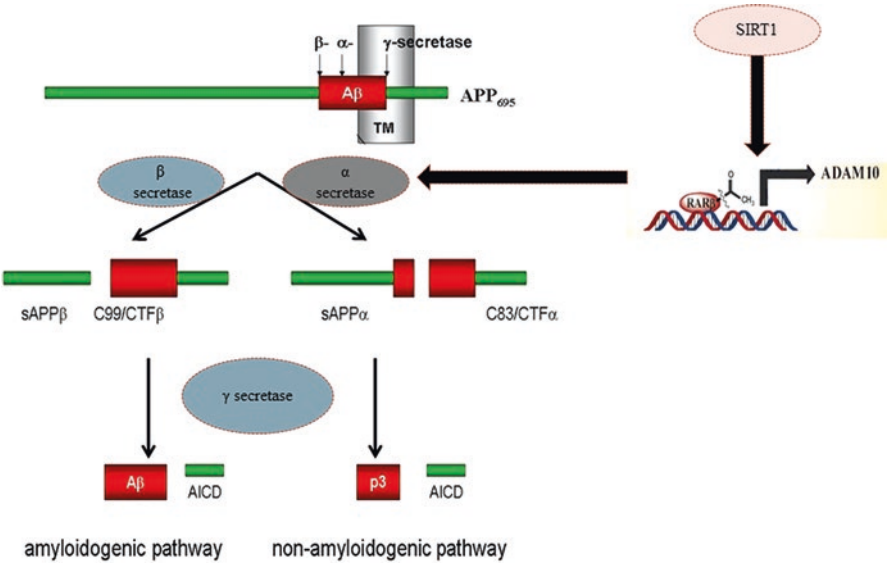

Fig. 2.1 Non-amloidogenic and amyloidogenic pathway of amyloid precursor protein

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).

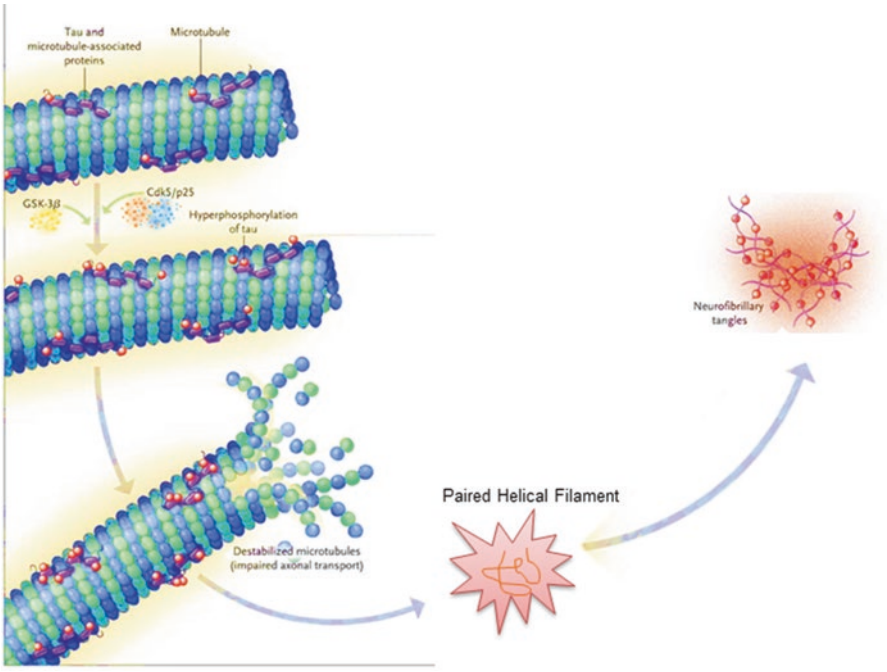

Fig. 2.2 Mechanism of neurofibrillary tangle formation by tau hyperphosphorylation

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