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BRAIN SENESCENCE-OMICS

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Abstract: Brain senescence is as obvious as death. It is associated with structural as well as functional compromises of the brain. Decline in cognitive function can also be associated with aging brain. Both genomics and proteomics study of the aging brain should help in deciphering the process of senescence. Results from this kind of "omics" studies, mainly proteomics, reveal that there is always a correlation of brain senescence with neurodegenerative diseases like Alzheimer's disease (AD), though successful aging without neurodegeneration is possible. Changes at the proteome level can reflect normal aging to mild cognitive impairment to a neurodegenerative disease like AD. Some additional factors are also identified which can enhance or reduce the senescence process. This article is a brief account of those and a mandate towards what could be done to understand the mechanism of brain aging and its correlation with neurodegeneration.

Keywords: Brain senescence; aging; genomics; proteomics; neurodegenerative diseases.

Introduction

Aging is one of the most complex biological processes, which, by definition, is intrinsically related to its phenotype. Human aging is associated with a wide range of physiological changes that not only make one more susceptible to death, but also limit one's normal functions. Apart from the diseases it causes, normal aging also has great biological consequence. Most of the age related phenotypes are irreversible resulting in loss of viability and increase in vulnerability of many cells, tissues or organs and may influence, directly or indirectly, the functions delivered by them. In fact, one hallmark of aging in humans and many other species is an age-related increase in mortality rates. Since, the number of pathological events increase with age, chance of death due to disease upon aging is higher. However, on the contrary, age related changes do not have pathological consequences. Normal aging is characterized by changes in appearance, such as a gradual reduction in height and weight loss due to loss of muscle and bone mass, a lower metabolic rate, higher reaction times, decline in certain memory functions, decline in sexual activity and many others. The word "aging", therefore, refers to the biological process of growing older in a deleterious sense, collectively known as "senescence".

Senescence in the brain is associated with cognitive decline, the major factor for most of the neurodegenerative disorders like Alzheimer's disease (AD) (Keller 2006). In a successful aging also, where there is no or minimum cognitive impairment, several changes in different regions of the brain take place. Some researchers argue that the only part of mammalian body in which senescence takes place is the brain and all the phenotypes we observe during aging is mainly the effects of the physiological alterations of that organ (Mattson *et al.* 2002). Among the mammals, there is a positive correlation between brain/body size ratio and lifespan, suggesting a pivotal role for the brain in determining lifespan. Since almost every task, if not all, is controlled by different parts of the brain, understanding the mechanism of normal aging of the brain is of immense importance. Though the first thing that comes to mind when talking about aging brain is AD, these

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two processes are distinct. The synaptic circuits of the brain in normal aging are compromised functionally whereas in case of AD, neuronal death takes place to compromise cognitive functions (Hof and Morrison 2004). Several studies have been done to determine differential genomic and proteomic expression level for aging brain with respect to that of young as well as in different regions of the mammalian brain. In this review emphasis is on the changes that take place during normal brain senescence and the contribution of 'omics' based research.

Alterations in the Aging Brain

During aging, the most vulnerable areas of the brain are the hippocampal and neocortical circuits (Hof and Morrison 2004). These circuits are affected due to synaptic alteration and this compromise in the hippocampus and neocortex may lead to age associated cognitive impairments. These symptoms, to some extent, are similar to the symptoms associated with neurodegenerative diseases but they are different morphologically. In fact, neurofibrillary tangles (NFT) and senile plaques (SP), which are the characteristics of AD (Mudher and Lovestone 2002), are absent in successful senescence. No such morphological marker for normal aging is characterized yet. The neurons providing the projection from entorhinal cortex to the dentate gyrus and pyramidal cells within CA1 appear to be the most vulnerable cell classes with aging (Morrison and Hof 2003). The pyramidal cells providing corticocortical links between the association regions of temporal, prefrontal and parietal areas are also highly vulnerable. These are the major cellular circuits that are exaggerated during normal aging and structural damage to these parts with senescence leads to different age associated impairments.

Another important factor controlling the normal functionality of the brain is the spine number and spine density. There are evidences in human that both the spine number and spine density are decreased upon aging. A 46% decrease in spine number and spine density has been reported in humans older than 50 compared with younger individuals (Hof and Morrison 2004). Electron microscopy study in monkeys noticed 50% loss in spines on the apical dendritic tufts of pyramidal cells in prefrontal cortex of old animals (27–32 years old) compared with young ones (6–9 years old) (Nimchinsky *et al.* 2002).

Thus the same circuits, vulnerable to degeneration in normal aging, are likely to lead to age associated memory decline. There are evidences in non-human primates that synapses are structurally intact during normal aging (Hof and Morrison 2004). But the functional decline of the synapses does occur during aging (Sato *et al.* 2005). Comparison of the hippocampal synaptosome proteins in young-adult (9-week-old) and aged (30-month-old) rats identified 19 proteins, which are associated to the cytoskeleton, neurotransmission, signal transduction and energy supply (Sato *et al.* 2005). Change in expression level of these proteins may deteriorate the synaptic activity in the hippocampus, affecting age-associated declines of brain functions including learning and memory.

Alteration of brain structure and functionality during aging - relevance to memory

Aging of the brain is associated with gradual decline in cognitive ability, including memory performance, cognitive speed and flexibility (Is *et al.* 2008). The human brain is uniquely powerful in its cognitive abilities, yet the hippocampus and neocortical circuits, which mediate these complex functions, are highly vulnerable during aging (Hof and Morrison 2004). Changes in brain morphology include a decline in total brain volume, cortical thinning and gyral atrophy (Raz *et al.* 2004, Uylings and de Brabander 2002). Several neuroimaging studies have confirmed that there are age related changes in morphological characteristics of the brain (Good *et al.* 2001, Hof and Morrison 2004, Jernigan *et al.* 2001, Sowell *et al.* 2003). White-matter degradation, in the form of hyperintensities, reduced white-matter integrity and volume loss, is also commonly observed (DeCarli *et al.* 2005, Head D. *et al.* 2004). Functional Magnetic Resonance Imaging (fMRI) and Diffusion tensor Imaging (DTI) study on two groups of older adults, that differ in their memory performance on tests of episodic memory, demonstrated neuroanatomical and functional differences

associated with longitudinal decline in episodic memory performance (Persson *et al.* 2006). Older adults with declining memory performance showed differences in DTI measures of anterior white matter as well as reduced hippocampus volume compared to older adults with preserved memory performance. Under certain conditions, some frontal regions are relatively more active in older than younger adults (Logan *et al.* 2002, Reuter-Lorenz *et al.* 2000, Rosen *et al.* 2002). This kind of over activation may be due to the compensatory action to get a better memory performance (Reuter-Lorenz and Lustig 2005). Presence of additional frontal activation may be a marker for cognitive decline (Persson *et al.* 2006).

Commonly studied animal models of human brain aging include rats, transgenic mice, and nonhuman primates (Head E. *et al.* 2005). Neuronal loss and degeneration in the hippocampal area have been considered signs of aging in earlier studies of rats. It was initially thought that the decline in learning with age in rats was due to cell and synapse loss in the hippocampus, but functional, rather than structural changes may also be involved. Neuronal dystrophy, neuron scattering and granulovacuolar degeneration (GVD) can be used for investigation of age-related neuropathological changes indicating neuronal degeneration (Is *et al.* 2008). Within intracellular and extracellular matrices various biochemical mechanisms play roles in the occurrence of neuronal dystrophy (Charalampopoulos *et al.* 2006). These age-related biochemical mechanisms are associated with neuronal degeneration, resulting in neuronal loss (Carroll 2002).The aging spine and synapse may be the key to age-related memory decline (Morrison and Hof 2003). The neuronal circuits are vulnerable to sublethal age-related shifts in morphology, neurochemical phenotype and synaptic alterations and these might impair normal brain function as well. Aging has been shown to alter the expression and distribution of *N*-methyl-D-aspartate (NMDA) receptors in many different brain regions, including the hippocampus (Clayton *et al.* 2002). This biochemical alteration of the synapse, i.e. shifts in NMDA receptors, may also contribute to memory impairment. Many studies examining cellular substrates of the age-related cognitive decline have focused on the hippocampal formation because its structural integrity is crucial for normal learning and memory as they are known to be vulnerable in the process of aging (Geinisman *et al.* 2004). It has been thought for many years that age-associated learning and memory impairment is associated with neuronal death of the hippocampal region of the brain. However, recent studies using modern stereological methods for counting nerve cells have convincingly demonstrated that the number of pyramidal cells in hippocampal fields CA1, CA2 and CA3, as well as granule cells in the hippocampal dentate gyrus is preserved in aged rats, mice, monkeys and humans (Geinisman *et al.* 2004). Quantitative analyses of immunohistochemical labeling for the presynaptic vesicle marker synaptophysin suggest, however, that the number of CA1 stratum radiatum synapses is preserved in aged rats with impaired spatial learning (Smith *et al.* 2000).

The process of successful memory formation likely requires coordinated patterns of neural activity among a distributed network of brain regions (Miller *et al.* 2008). During successful periods of encoding, healthy young individuals show a reciprocal relationship of medial temporal lobe (MTL) activation and parietal deactivation. Normally, the individual memory performance is decreased with aging. But there are evidences of cognitively normal older individuals who can display a wide range of performance on memory tasks, compared with young adults. With the help of fMRI data, it is demonstrated that young and old adults show a similar magnitude and extent of hippocampal activation during successful associative encoding. Successful encoding requires the coordination of neural activity in hippocampal, prefrontal, and parietal regions, and that age-related memory impairment may be primarily related to a loss of deactivation in medial parietal regions (Fox *et al.* 2005). Both young and old adults engage the hippocampus during successful encoding, but old adults, particularly those on the lower end of memory performance within the continuum of normal aging, fail to deactivate medial parietal regions (Miller *et al.* 2008).

Changes in brain morphology and brain volume upon aging

Several fundamental indicators of normal brain development, namely, volumes of intracranial space, whole brain, gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) are very much useful to understand age related decline in brain functions. Both postmortem and in vivo imaging studies have demonstrated that, with advancing age, brain weight or volume, GM, and GM-WM ratios decrease and CSF volume increases (Courchesne *et al.* 2000). The study of the brain volume with the help of longitudinal MRI in older adults suggests the shrinkage of the whole brain upon aging (Resnick *et al.* 2003). There are experimental evidences that both the ventricular volume and the intracranial CSF volume increase during normal brain development and aging (Courchesne *et al.* 2000, Resnick *et al.* 2003). Both cross-sectional and longitudinal MRI observation indicates that the orbital and inferior frontal cortex and the mesial temporal cortex, although to a lesser extent, are also vulnerable to longitudinal tissue loss (Resnick *et al.* 2003, Resnick *et al.* 2000). There are evidences that suggest cross-sectional declines in global grey but not white matter volume during adulthood (Courchesne *et al.* 2000, Good *et al.* 2001).

Changes in the hippocampus and cortical regions

The hippocampus is one of the most frequently affected regions of the brain in normal aging and pathological memory disorders like Alzheimer's disease (Is *et al.* 2008). Neuronal loss and degeneration in the hippocampal area have been considered signs of aging in earlier studies of rats. It was initially thought that the decline in learning with age in rats was due to cell and synapse loss in the hippocampus, but functional, rather than structural changes may also be involved. Functional connectivity of the hippocampus is defined as the correlation between activity in the hippocampus and activity in the rest of the brain, as measured by neuroimaging (Grady *et al.* 2003). There has been considerable interest over the past few years in assessing age-related changes in hippocampal structure and function in humans. A number of studies on structural changes in the hippocampus with age have been performed, but the results have been variable. Although correlations between hippocampal volume and memory performance have not always been found (Petersen *et al.* 2000, Tisserand *et al.* 2000, Ylikoski *et al.* 2000), there are evidences of fMRI and neuroimaging studies which suggest a strong relation of hippocampal volume, plasticity, and functionality with aging (Grady *et al.* 2003, Is *et al.* 2008, Persson *et al.* 2006) and since the memory and cognition is dependent on the hippocampal formation, the age related memory decline is also obvious due to loss of hippocampal integrity. Therefore the integrity of the hippocampus structure is very crucial for successful learning and memory. In the absence of a loss of synapses, spatial learning impairments that occur in a subpopulation of aged rats may be due to other synaptic alterations in the CA1 stratum radiatum (Geinisman *et al.* 2004). In fact, the number of morphologically definable synapses may remain constant, while functional synaptic contacts may be actually decreased in numbers. This possibility is suggested by the discovery of postsynaptically silent synapses. The presence of functional NMDA receptors (NMDARs) and AMPA receptors (AMPARs) also play crucial role in learning and memory (Geinisman *et al.* 2004). If enhancement of synaptic transmission is necessary for successful learning (Martin *et al.* 2000), a diminished expression of AMPARs in both perforated and nonperforated synapses may have a deleterious effect on the cognitive function even when synapse number is constant. Deterioration of synaptic plasticity in the hippocampus is also evident from experiment with cultured hippocampal neurons of senescence-accelerated mouse prone 8 (SAMP8) (Lopez-Ramos *et al.* 2011).

Changes in cortical thickness are important to the study of aging because they provide a local measure of alterations in gray-matter morphology that can be made continuously across the cortical surface (Salat *et al.* 2004). Consistent with many volumetric studies, marked thinning was noted in prefrontal cortex. Prefrontal cortex has received much attention in the field of cognitive aging as it has been noted that older adults can perform poorly on tasks that require executive functions presumed to rely on prefrontal cortex, among other structures (Greenwood 2000). Thus, it is

possible that early age-related alterations in this region could contribute to age-related declines in executive processing tasks such as working memory tasks (Salat *et al.* 2002).

Lesions in the hippocampus (HC), the entorhinal cortex (EC), and the prefrontal cortex (PFC) are associated with impairment of episodic memory - reduced HC volume is linked to memory declines in dementia and decline in EC volume predicts progression from mild cognitive impairment to dementia (Rodrigue and Raz 2004). Poor memory performance with aging is associated with greater annual rate of shrinkage of the EC than HC or PFC. Volumetric shrinkage has been linked to neuronal loss both in HC and EC (Bobinski *et al.* 2000, Kordower *et al.* 2001).

"Omics": its Importance and Relevance to Brain Senescence

Modern research demands high throughput data that should be effective in predicting biological pathways, mechanisms of a particular phenomenon, or reasons behind some common diseases. To develop a theory in biology, a huge volume of data is required, which are statistically significant in a short time period. The "omics" research fulfils this demand.

Among all the "omics" techniques, particularly proteomics has great contribution in the study of brain senescence. Over the past few years, many proteomic studies have described variation of expression of certain proteins in adult rodent and human brains upon aging (Carrette *et al.* 2006, Seefeldt *et al.* 2006, Wang *et al.* 2007). The proteome describes the ensemble of proteins expressed by a cell, tissue, or the whole organism at a specific point of time. Proteomics tries not only to identify proteins, but also to determine protein localization, post-translational modifications, interactions and protein function. Moreover, the analysis of a proteome represents an important supplementation to the genome analysis. This proteomic study can be very useful in determining biomarkers for normal aging as well as neurodegenerative diseases. Biomarkers are biochemical features that can be used to measure a normal biological process as well as the progress of disease or the effects of treatment (Schulenborg *et al.* 2006). A biomarker should provide sensitivity and specificity to diagnose a disease or the particular biological process in very early stages. The proteomics approach provides this quality of developing a biomarker. In normal senescence of the brain also, proteomics can play a very crucial role. The differential expression of the specific proteins upon aging can be treated as potential biomarkers and correlating the proteins' functional implication with age can help us to understand the aging process better and also the age associated impairment in memory or cognition.

Keeping in mind the advantages of proteomics technology, in 2001, the German Federal Ministry of Education and Research (BMBF) initiated the National Genome Research Network (NGFN; www.ngfn.de) as a nation-wide multidisciplinary networking platform aiming at the analysis of common human diseases and aging. Within the NGFN the Human Brain Proteome Project (HBPP; www.smp-proteomics.de) focuses on the analysis of the human brain in health and disease (Hamacher *et al.* 2008). Under the Human proteome Organization (HUPO), the HBPP has two funding period. In the first funding period, HBPP1, running from 2001 to 2004, necessary technologies were established and optimized. In HBPP2, which started 2004 and will end in May 2008, the developed technologies are used for large-scale experiments, offering new links for disease related research and therapies. The Brain Pilot Project (BPP) initiative was launched in 2003 with the ambitious goal to characterize human and mice brain proteomes (Hamacher *et al.* 2004).

The brain is the most complex organ in mammals, both in the variability of the cell types and the number of circulating macromolecules. Therefore, characterization of the brain proteome, defined as the complete set of proteins expressed in the brain at one point of time, is an enormous and challenging task. Establishing a brain proteome database may accelerate pre-and clinical development of more specific diagnostic and prognostic disease markers and new, more selective therapeutic interventions. In particular, both in normal aging and in neurological disorders, multiple underlying pathogenic mechanisms may require a large repertoire of

molecular targets and biomarkers rather than individual proteins to better define a complex disease. Thus studying differential brain proteome will be of huge help in understanding the changes take place in case of brain senescence.

Along with the proteomics studies, several studies of the gene expression level in the brain aging have contributed during the recent years using DNA microarray (Galvin and Ginsberg 2005, Prolla 2002). But these data of genomics are not confirmatory to conclude about which genes are responsible for the aging process or age related memory impairments. Study of the parallel changes in gene expression in aged human and mouse cortex (the extent of similarity between age-related alterations of levels of specific mRNAs in either species has been compared) has concluded that in both these species, the degree of expression of a relatively limited number of cortical genes, around 300, is significantly altered during senescence (Sharman *et al.* 2005). There is also a linear correlation in terms of gene expression level in both the species. This is very significant finding in terms of the future research regarding brain senescence because this will allow the experimentalists, be it genomics experiment or proteomics, the use of mouse strains to study general age-related genetic events associated with human aging process. Thus, the two "omics" platform can complement themselves very well.

Changes in the proteomic levels due to brain senescence: some examples

It is obvious that when the senescence of the brain is taking place due to normal aging, the protein levels expressed and functioning in the brain tissues will be changing continuously with time. The HUPO brain proteome project (HUPO-BPP) was developed to establish standards and guidelines for proteomic studies of the brain and to develop a reliable database of brain proteome (Hamacher *et al.* 2008). Several laboratories are involved in the study of brain proteome. The same brain samples from mouse of different developmental stages were distributed to different laboratories for the analysis of the brain proteome and these laboratories have analyzed the samples using two-dimensional gel electrophoresis (2D-GE) followed by mass spectrometry (MS) of the protein spots from the 2D gel and identification of proteins (Carrette *et al.* 2006, Seefeldt *et al.* 2006, Wang *et al.* 2007). There are also other groups, who carried out proteomic comparison of brain tissue samples from postmortem young and old brains (Chen *et al.* 2003), comparison of hippocampal synaptosomal proteins in young adult and old rats (Sato *et al.* 2005), and analysis of the mouse cerebellum proteome (Beranova-Giorgianni *et al.* 2002). Several proteins from different brain parts were found to be differentially expressed. Some of them will be discussed here in this review.

Changes in the expression of proteins related to neurogenesis and synaptosome

Proteomic analysis of the brain from neonatal (7 days) and adult (8 weeks) C57BL/6 mice revealed differential expression (higher expression in the neonatal mice that adult) of some of the proteins involved in the process of neurogenesis between the two groups of mice (Carrette *et al.* 2006). Two of them- the Dihydropyrimidinase-related protein (DRP) and the brain Fatty Acid Binding Protein (FABP) are involved in the neuronal migration during central nervous system development. Brain FABP is also required for the establishment of the radial glial fiber system in developing brain, a system that is necessary for the migration of immature neurons to form cortical layers. Another protein which is expressed higher, not only in the neonatal brain than the adult mice brain but also down-regulated in the old postmortem human brain than the young brain (Chen *et al.* 2003), is the Stathmin which is another developmentally regulated neuronal protein which binds tubulin and regulate microtubule catastrophe (Liu *et al.* 2005). In addition to its involvement in cell proliferation and differentiation, stathmin may also be related to the regulation of differentiated cell functions according to its expression profile in human and rat brains (Carrette *et al.* 2006). The decrease of these proteins is in agreement with the age-dependent decline of neurogenesis.

γ-enolase, also called neuron-specific enolase (NSE), has a protective role for neurons and is used as a marker of brain damage (Anand and Stead 2005) and it is over-expressed with aging in mice (Carrette *et al.* 2006). Since apoptosis is a

critical cellular event during several stages of neuronal development and especially in aging, it is not surprising that annexin V, which is an apoptotic marker, is up-regulated in adult mice. *In vitro* studies of primary cultured neurons have shown that annexin V is essential for the survival and neurite outgrowth of developing cortical neurons. This protein is also implicated in the survival of glial cells, and protects neurons and glial cells against peroxide and hypoxia injuries occurring upon aging (Han *et al.* 2004).

Expression of the synapse-related proteins, synaptosomal-associated protein, 25 kDa (SNAP-25), was found to be increased and α-synuclein (α-SYN), was found to be decreased during aging (Sato *et al.* 2005). SNAP-25 is located on the presynaptic membrane and is required for neurotransmitter release. Thus, the amount of neurotransmitter release might change during aging. α-SYN is expressed abundantly in presynaptic terminals and is associated with synaptic vesicles in cultured hippocampal neurons. Down-regulation of α-SYN in knockout mice and in primary neurons suggested that α-SYN regulates synaptic neurotransmission. Gene mutations of α-SYN have been associated with autosomal-dominant hereditary Parkinson's disease (PD) patients. The slight decrease in the amount of α-SYN in aged rats raises the possibility that it is relevant to these deteriorations of brain functions in aged people.

Changes in the proteins related to basic metabolism

Isocitrate dehydrogenase is an enzyme involved in catalytic process of the citric acid cycle. It catalyzes the third step of the cycle, producing α-ketoglutarate and CO_2. Proteomic study of the whole brains from mice of three developmental stages [embryonic day 16 (E16), postnatal day 7 (P7), and 8 weeks (W8)] has revealed a five-fold decrease of this enzyme from stage E16 to W8 (Frohlich *et al.* 2006). A higher expression of isocitrate dehydrogenase in neonates and lower developmental stages is consistent with the higher metabolism of ketone observed in the brain during neonatal and initial developmental period (Morris 2005). Several other enzymes involved in the intermediate metabolism of carbohydrates, such as creatine kinase B, glutamine synthase, and triosephosphate isomerase were expressed at higher level in adult brain (Carrette *et al.* 2006). During development of mammal brains, there is a switchover in the respiratory fuels. Young developing brains utilize both ketone bodies and glucose, whereas adult brain metabolism is dependent on glucose only. Therefore, one can expect a decrease of the expression of many glycolytic enzymes in the neonatal brains compared to adults. In a proteomic study, glutamine synthetase was also found to be up regulated in aging mice, and was associated with an increase of the protein oxidation (Poon *et al.* 2006). The level of α-enolase is decreased during aging in the hippocampal synaptosome (Sato *et al.* 2005). α-enolase participates in the glycolytic pathway and creatine kinase catalyzes the phosphate group transfer from creatine phosphate to ADP and produces ATP. Thus, energy metabolism in the synapses might decrease in aging. Further, α-enolase is present on the surface of rat neuronal cells, where it serves as a plasminogen receptor. Thus, the decrease of α-enolase in aged rats reduces its receptor-like functional role in the aging process.

Changes in the cytoskeletal or structural proteins

The dihydropyrimidase-like 2 (gene name: Dpysl2), the dihydropyrimidinase- related protein 3 (gene name: Dpysl3), and dihydropyrimidinase-related protein 5 (gene name: Dpysl5) showed significant stage-dependent abundances in the study of the brain proteome with the E16, P7, and W8 developmental stages (Frohlich *et al.* 2006). The intensities of three Dpysl2 protein spots increased in state E16 to W8 brain samples. A similar result has been observed in a comparison between neonatal and adult rat brains (Carrette *et al.* 2006). Besides other functions, the Dpysl2 protein promotes microtubule formation and serves as a mediator of Semaphorin3A (Sema3A, formally collapsin) signaling, being associated with growth cone collapse (Frohlich *et al.* 2006). Dpysl3 protein is known to be strongly expressed in early embryonic post-mitotic neuronal cells and evidence exists for its involvement in neuronal differentiation, axonal outgrowth, and neuronal regeneration. The Dpysl5 protein is known to be highly expressed in fetal and neonatal brains and

may play an important role in neuronal differentiation and synapse formation. In accordance with these findings, two Dpysl5 protein spots showed significantly decreased abundance in W8 as compared to the E16 and P7 stages (Frohlich *et al.* 2006).

In another study done recently, three proteins, namely, pyridoxal phosphate phosphatise (PLPP), collapsin response mediator protein 2 (CRMP-2) and a-internexin, all of which are known to be involved in brain cytoskeleton formation and associated with acute and chronic neurodegenerative conditions, were found to be increased in the aging brain of SAMP10 mouse – a model of age related cerebral degeneration (Furukawa *et al.* 2010).

Two other structural proteins showing age related differential expression are T-complex 1 and β-actin. The amount of β-actin was increased during aging and that of T-complex 1 was decreased in the hippocampal synaptosome (Sato *et al.* 2005). T-complex 1 is essential to folding of actin and is included in the chaperoninTriC/CCT. The expression of β-actin mRNA in rat hippocampus was reported to decrease in the aging process, which suggested that the synthesis of β-actin decreases in hippocampal synaptosomes during aging. It was reported that synaptic actin turns over rapidly in cultured hippocampal neurons (in less than 1 min). Thus, the increase in β-actin may thus be due to a decrease in the rate of degradation rather than an increase in expression. Another possible explanation is that translational efficiency of β -actin may be changed in aged brain.

Other actin-related proteins that changed during aging were coronin 1A (whose amount decreased) and septin 2 (whose amount increased). Septins are guanosine triphosphatases that associated with actin via anillin. Septins are responsible for actin bundling and are required for cytokinesis and exocytosis. Coronin 1A is related to phagocytosis and cytokinesis. Thus, these changes may reflect changes in vesicular transport at synapse during aging (Sato *et al.* 2005).

β-actin is a major cytoskeletal protein composed of actin filaments, which are abundant in synaptic areas, such as pre-synaptic nerve endings and post-synaptic dendrites. In these areas β-actin and its related proteins form a submembranous cytoskeleton and are involved in neurite growth, cell adhesion, synapse formation, and exocytosis of neurotransmitters. Age-dependent changes in actin and actin-associated molecules may be related to the neuronal dysfunction associated with aging.

Changes in expression of oxidative burden and proteasome related proteins

The over-expression of specific enzymes such as UCH-L1 and glutathione-S-transferase (GST) in the C57BL/6J mouse pituitaries (Marzban *et al.* 2002) seems to be the manifestation of an increased response to general age-derived cellular alterations such as increasing oxidative burden, accumulation of damaged proteins, and elevated protein synthesis. The expression of GST was increased in old pituitaries. Furthermore, ubiquitin carboxyl-terminal hydrolase (UCH-L1) was significantly up regulated in senescent C57BL/6J mouse pituitaries. Since GST is involved in antioxidative defense and UCH-L1 is part of the ubiquitin/proteasome system, which is responsible for the removal of damaged proteins, these results suggest increased oxidative burden and an increased activity of the ubiquitin system.

The enzyme superoxide dismutase [Mn], a protein related to oxidative burden, is also over-expressed in the older mouse (W8) compared to the E16 mouse (Frohlich *et al.* 2006). This higher expression of Mn-SOD may be to account for higher oxidative damage upon aging. Another antioxidant enzyme in human, peroxiredoxin 2 appear to be involved in the redox-regulation of cellular signaling and differentiation and it is down-regulated in old brains compared to young brains (Chen *et al.* 2003). This gives us the idea that oxidants may play an important role in brain aging.

The heat shock protein 60 kDa (HSP60) and protein disulfide isomerase- two folding-related proteins were found to be decreased during aging (Sato *et al.* 2005). Decrease in these proteins might lead to an increase in misfolded proteins during aging. This kind of protein misfolding is thought to be linked to the pathogenesis of many age-

associated neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) etc.

Other proteins

There are some other functionally important proteins, which show age-related differential expressions in mouse, humans, and monkeys. Among them, the proteins which show over-expression in the W8 stage of mouse compared to the E16 stage are- cytochrome c oxidase (Vb subunit), creatine kinase chain B, electron transfer flavoprotein alpha-subunit, glycerol-3-phosphate dehydrogenase 2, cytoplasmic malate dehydrogenase, mitochondrial ATP synthase alpha chain, mitochondrial aconitase 2, carbonic anhydrase 2, septin 5 etc. and those which are expressed lower in the older stage are- epidermal fatty acid binding protein, nucleoside diphosphate kinase B, 58 kDa glucose regulated protein, proteasome subunit alpha type 1 etc. (Frohlich *et al.* 2006).

Another protein apolipoprotein-A1 is found to be down regulated in human old brains. The age-dependent decline in abundance of this protein may contribute to brain aging and underlie the higher risk for developing diseases in older individuals (Chen *et al.* 2003). The β-secretase enzyme activity is also increased with aging in human, monkey, and mouse brain (Fukumoto *et al.* 2004). This protein is responsible for the generation of amyloid beta (Aβ) from the amyloid precursor protein (APP) and this Aβ deposition in the brain is the major cause of AD.

It is believed that all the proteins that show age-dependent variation in their expression level have some impact on aging, age-related memory decline or loss of other functionality. These alterations in the proteomics level due to brain senescence also can make platform for the different kinds of brain disorders and neurodegenerative diseases to occur.

Proteomic alteration in brain senescence: is it related to neurodegenerative diseases?

Most neurodegenerative disorders are region-specific. For instance, in the most common geriatric dementia, Alzheimer disease (AD), neuronal loss is predominantly found in the cerebral cortex and hippocampus (Heese and Akatsu 2006), whereas in the most common disabling movement disorder, Parkinson disease (PD), neurodegeneration largely centers on the brainstem at least during the early phase of the disease (Braak *et al.* 2003). Thus, identification of proteins unique to each brain region, those associated with neurodegenerative mechanisms in particular, could yield opportunities to overcome major obstacles in the development of new protective and restorative therapies for prominent neurodegenerative diseases.

The proteomic identifications of the cortical proteins and CSF proteins have revealed that many of the cortical proteins are involved in catalytic activities and protein binding. In addition, a significant number of proteins have transport, signal transduction, and cytoskeletal functions. The finding is strikingly similar to the results from a recent proteomics study on the proteins from human temporal lobe. A great portion of the CSF proteins are known to be specifically involved in or associated with specific central nervous system functions. Some of these proteins are implicated in the mechanisms of a few neurodegenerative diseases. A few examples of those are: neurofilament triplet H protein, cystatin C precursor, protein NDRG2, Apolipoprotein E precursor, cathepsin D precursor, proteolipid protein1 etc. (Pan *et al.* 2007). A very recent study of differential expression of proteins in SAMP8 mouse brain compared to that of the SAMR1 mouse have obtained many age specific proteins to be up/down –regulated which were previously reported to be involved in AD (Zhu *et al.* 2011).

Glucose metabolism is the main source of energy for brain under normal conditions, and it also plays an important role in maintaining normal synaptic function. The necessity for glucose in brain function had been considered solely due to ATP production. Any alteration in the protein levels that regulate the glucose metabolism may lead to cellular dysfunction such as impaired ion-motive ATPase to maintain potential gradients, operate pumps, and maintain membrane lipids asymmetry, etc. dysfunction of which are physiological hallmarks of AD (Sultana *et al.* 2007). A feature of many neurodegenerative

diseases is the accumulation of abnormal proteins in hallmark pathologic inclusions; in AD these are predominantly amyloid-β (Aβ) in senile plaques (SPs) and tau in neuro-fibrillary tangles (NFTs) (Hardy and Selkoe 2002). A dominant model of several common and uncommon neurodegenerative diseases is that these are diseases of protein misfolding (Montine *et al.* 2006) and thus the changes in the expression level of those proteins related to protein folding with the neurodegenerative disorders can be correlated.

Where do genomics stand?

A very recent study with microarray analysis of aging brain astrocyte transcriptome has revealed altered regulation of genes associated with the actin cytoskeleton, proliferation, apoptosis, and ubiquitin-mediated proteolysis and altered regulation of intracellular signalling pathway and MAPK pathway in individuals with APOε4 allele indicating a role of astrocyte dysfunction in the pathogenesis of neurodegeneration in the aging brain (Simpson *et al.* 2011). Another study, comparing the young with the normal aging and AD-like aging has revealed different genes belonging to metabolic pathway, particularly protein synthesis, which showed opposite regulation pattern in the healthy old with respect to AD-like animals (Abdel Rassoul *et al.* 2010). They also suggested for the existence of compensatory mechanisms during pathological brain aging that disappear in AD-like aging. Thus use of the techniques of genomics in combination with proteomics can result in more relevant hypotheses regarding normal brain senescence and neurodegeneration.

Some positives factors

Mitochondria are both a major source of oxidants and a target for their damaging effects, and, therefore, mitochondrial oxidative stress appears to be a cause, rather than a consequence, of cell aging (Sastre *et al.* 2003). Mitochondrial function and morphology are impaired upon aging, as judged by a decline in membrane potential as well as by an increase in peroxide production and size of the organelles. In view of the age-related decreases in mitochondrial protein synthesis, mitochondrial transcripts, and expression of genes involved in mitochondrial turnover, the rate of this turnover might determine its susceptibility of mitochondria to oxidative damage and mutation, thus controlling the rate of cell aging. The data available for nematodes and flies provide a compelling case for the view that the accumulation of oxidative damage to specific mitochondrial proteins leads to the progressive dysfunction that we see as senescence (Fridovich 2004). The production of o_2^- and H_2O_2 in mitochondria is large and the defensive enzymes, such as superoxide dismutase and glutathione peroxidase, act against those oxidative damage. The activity of glutathione peroxidase (GPx), the essential enzyme for reduction of hydrogen and lipid peroxides, was significantly lower in aged mice than in younger ones (Kishido *et al.* 2007) and this decline in glutathione peroxidase activity is a reason for brain senescence. But the positive point is that there are evidences that consumption of green tea catechin prevents the decline in GPx activity and protein oxidative damage in aging mouse brain (Kishido *et al.* 2007) as well as put favorable effects on reduction of cognitive deficits and brain morphological changes in senescence accelerated-prone mice P8 (SAMP8) (Chan *et al.* 2006).

The brain undergoes many structural and functional changes during aging. Some of these changes are regulated by estrogens, which act mainly through their intracellular receptors, estrogen receptor ER_α and ER_β. The expression of these receptors is regulated by several factors including their own ligand estrogen, and others such as growth hormone and thyroid hormone. The levels of these factors decrease during aging which in turn influence estrogen signaling leading to alterations in brain functions (Thakur and Sharma 2006). Estrogen treatment in the gonadectomized aged rats has been shown to be responsible for the reversal of hippocampus related memory impairment, blocking of long-term depression, decreased cytosolic calcineurin activity, increased level of growth associated protein-43 and choline acetyltransferase. Estrogen is also involved in the modulation of expression of amyloid precursor protein (APP) associated with AD in old brain. Of the various APP isoforms (APP770, APP751 and APP695), the APP695 is predominantly found in the brain and its level

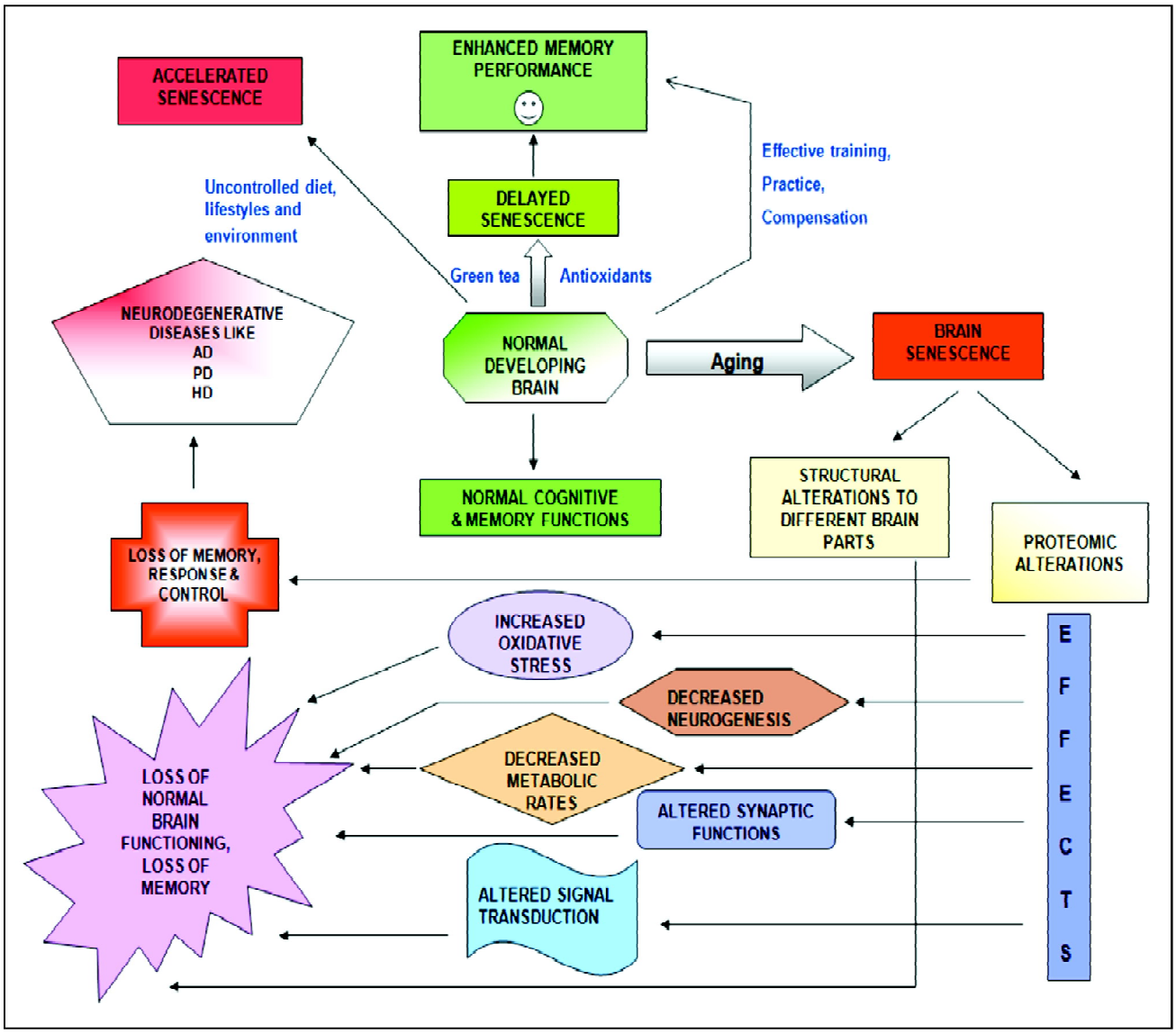

Figure 1: **Model for brain senescence and its effects on various physiological pathways**

Onset of brain senescence and its implications. Structural and proteomic alterations together can result in increased oxidative stress, decreased neurogenesis, metabolism and altered synaptic functions along with altered signal transduction; ultimately leading to loss of normal brain functioning and in some instances, neurodegeneration. Brain senescence can also be delayed depending upon the nature of lifestyles and diet and if these are uncontrolled, can accelerate the process of senescence.

remains high under non-pathological conditions (Thakur and Sharma 2006).

Neurogenesis continues to occur throughout life but dramatically decreases with increasing age. This decrease is mostly related to a decline in proliferative activity as a result of an impoverishment of the microenvironment of the aged brain, including a reduction in trophic factors and increased inflammation. Peripheral injection of human umbilical cord blood in aged rats can increase the proliferative activity of neural stem cells as well as increase neurogenesis (Bachstetter *et al.* 2008). The injection of umbilical cord blood can significantly improve the microenvironment of the aged hippocampus. This evidence raises the possibility of a peripherally administered cell therapy as an effective approach to improve the microenvironment of the aged brain.

Another factor often used nowadays is some anti-aging drugs, but use of those has to overcome many ethical challenges. Some kind of medicinal herbs and plants are believed to increase the

memory performance. Another factor, which can improve the memory and cognitive functions of the brain, is compensation. Although the age related memory decline is very much obvious, there are still some high-performing older adults who show over-activation of some functional regions of the brain (Cabeza *et al.* 2002, Reuter-Lorenz and Lustig 2005). This over-activation may be correlated with the compensatory brain activity, which is required to attain the similar functionality of the brain. There is always a chance of training-related memory improvement in adulthood and aging (Nyberg *et al.* 2003). Cognitive training programmes and active involvement into a particular matter obviously can increase memory performance (Reuter-Lorenz and Lustig 2005). Recent studies suggest that consumption of diets rich in antioxidants and anti-inflammatory components such as those found in fruits, nuts, vegetables, and spices, or even reduced caloric intake, may lower age-related cognitive declines and the risk of developing neurodegenerative disease (Joseph *et al.* 2009).

Concluding remarks

Thus senescence in the brain and differential expression of the important proteins of different brain regions due to normal aging can well be correlated with the neurodegenerative diseases. The neurodegenerative diseases may be an integral but alternative part of the aging process. The proteomic identification of the proteins helps to develop targets for normal aging with or without any neurodegeneration. An important issue is that even when there are no signs of neurodegenerative disease, normal aging can also deteriorate important brain functions such as memory and cognition. This kind of functional decline upon aging may occur due to the structural and morphologic alterations in several functional domains of the brain. Much more region-specific studies of the brain are required to understand the mechanisms and consequences of brain senescence, an area where the so-called "omics" research can help a lot. The correlation between the changes in brain morphology, difference in the gene expression in different age groups due to aging matched with the proteome level and the functional alterations as a consequence of these changes has to be determined. More studies of the neuronal loss of functionality and synaptic alterations upon aging also have to be done. Proteomic analysis in combination with the recently developed functional imaging techniques can help these kinds of study of brain senescence.

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