REVIEW ARTICLE

Application of proteomics in brain's aging research

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Abstract: Proteomics is one of the commonly used techniques to explore the protein composition or protein modification status in various healthy or diseased brain tissues in the past decades. Aging is an extremely complex biological process including physiological function decline with age increasing. To have a better understanding of protein changes along with aging, proteomics has been applied in aging-associated research trying to uncover protein changes or post-translational modification (PTM) occurs in aging with the advantage of screening proteins on a large scale. In this review, we summarized protein expression differences detected by proteomics in human or animal brains at different age stages. Protein differences among species or brain regions are obvious, which reminds us to carefully consider these factors in brain aging research. Important protein changes have been found in multiple brain regions in the aging process and these differentially expressed proteins are mainly involved in cellular components, activities of metabolism, mitochondria changes, oxidative modification and some specific signaling pathways.

Keywords: proteomics; brain tissue; aging.

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INTRODUCTION

Proteomics contains a series of methods such as two-dimensional gel electrophoresis (2-DE), liquid chromatography-mass spectrometry (LC-MS) and time of flight mass spectrometer (TOF-MS) (Martinez-Val *et al.*, 2022) for protein expression analysis. Proteomics provides potent access for large-scale analysis of proteins and gives us an overall insight into the proteomic changes in biological processes or disease initiation and development (Vinaiphat & Sze, 2022; Raffe *et al.*, 2020; Jayathirtha *et al.*, 2023). A large scale of research has proved the suitability of proteomics for investigating proteins in brain tissues either in qualitative or quantitative ways when combined with bioinformatics (Chen *et al.*, 2022, Dai *et al.*, 2022; Rozanova *et al.*, 2023; Park *et al.*, 2023), Plentiful hints would be presented to us by proteomics. Thus, proteomics contributes to our understanding of the sophisticated biological process regulation that occurs in the brain in an unbiased manner. In addition, thousands of identified proteins from brain tissues constitute the foundation for further excavation of molecular mechanisms including metabolism (Kashem *et al.*, 2016; Wesseling *et al.*, 2013; Johnson *et al.*, 2022; Yang *et al.*, 2022), PTM (Ratovitski *et al.*, 2017; Bai *et al.*, 2021) and signaling pathway networks (Saia-Cereda *et al.*, 2015; Bai *et al.*, 2020), which would be of great significance to uncover deeper or new mechanisms of the aging process.

Aging, a naturally processed biological cycle portrayed by time-dependent functional decrease in living organs and leading to

death at last (Yousefzadeh *et al.*, 2021; Tuttle *et al.*, 2020), is one of the most challenging scientific problems making scientists confused. Several common denominators of aging have been summarized by scientists, these characters including precarious genetic, progressive shortening of telomeres, epigenetic modifications, loss of protein homeostasis, mitochondria dysfunction, changes in intercellular signaling and communication, stem cell depletion and cell senescence (Hou *et al.*, 2019; Azam *et al.*, 2021; López-Otín *et al.*, 2023). Among the summarized features of aging, protein changes play a foundational role. The accumulated changes of proteins in cells gradually project to tissues and organs and finally embody the programmed aging paradigm (Libertini & Ferrara, 2016; Lathe & St Clair, 2023), degradation of organs and loss of function disclose the ruthlessness of aging. Some evidence points out that chaotic gene regulation may explain aging in some sense (Huang *et al.*, 2021; Ximerakis *et al.*, 2019; Song *et al.*, 2022), dysregulated gene expression leads to dysregulated protein expression (Chen *et al.*, 2019; Xiao *et al.*, 2020). Protein changes influence aging-related symptoms in various ways. For example, Upregulation of FK506-binding protein 12.6/1b in hippocampus (HIP) improves memory impairment by regulating neuronal Ca^{2+} flow (Gant *et al.*, 2018), protein arginine methyltransferase 8 is a strong neuroprotection factor against age-related cellular stress (DNA impairment, oxidative response and protein misfolding), its low expression causes degeneration in spinal cord motoneurons (Simandi *et al.*, 2018). Abundant protein changes are accompanied by the aging process and to make the relationship clear is necessary. Proteomics gives us a potent tool to screen protein changes on a large scale in the aging process.

To have a better understanding of the brain protein changes along with aging, human and animal brain tissues at different age stages have been adopted to research this spontaneous biological process at the molecular, cellular, or tissue level (Orock *et al.*, 2020; Arrázola *et al.*, 2023; Donega *et al.*, 2019). As a core part of the human body, the brain controls our behaviors and springs out of thoughts or emotions (Tooley *et al.*, 2022; van Hoorn *et al.*, 2019; McDonald *et al.*, 2020). When aging comes, the brain becomes dull and susceptible to dementia (Short *et al.*, 2023; Pike *et al.*, 2022; Toader *et al.*, 2023). How aging affects brain structure, function as well as pathways remains elusive to us. Brain tissues of animal models have provided important information for the discovery of underlying mechanisms behind aging, but access to normal human brain tissues is still severely limited except for donated brain tissues from the human brain bank. One of the obstacles lying in the way of aging research on the human brain is the insufficient supply of human brain samples (Shepherd *et al.*, 2019). The current situation emphasizes the importance of the establishment of a human brain bank and reasonable collection of brain tissues from donors who died from diseases or accidents (Ma *et al.*, 2019; Huitinga *et al.*, 2019; Wang *et al.*, 2019; Mansour L *et al.*, 2023). In this review, we summarize the protein differences at different age stages aiming to discover potential protein targets to minimize brain health problems caused by aging.

DIFFERENT BRAIN REGIONS ARE ABUNDANT IN DIFFERENT PROTEINS

Human brain consists of the cerebrum, brainstem and cerebellum (CBC) and different parts are in charge of different physiological functions (Watson *et al.*, 2019; De Benedictis *et al.*, 2022; Burbaud *et al.*, 2022). The anatomical regions like HIP and cerebral cortex were frequently researched for their key role in cognition, learning and memory formation (Theves *et al.*, 2021; Zheng *et al.*, 2022; Spaak & de Lange *et al.*, 2020; Shine, 2019). However, more standardized (Wang *et al.* 2022) operated subdivided anatomical regions or microstructures of the brain still lack exploration. For example, dentate gyrus, CA1, CA2, CA3, CA4 and entorhinal cortex in HIP were frequently researched by immunohistochemistry or immunofluorescence methods but the proteomics method was less (Gao *et al.*, 2022). The dilemma is how to dissect these regions freshly without staining. Certainly, the microstructures could be dissected by laser capture microdissection (Das *et al.*, 2023) if these microstructures could be distinguished after stained or processed by other methods. Aging research in microstructures of brain tissues using proteomics is limited but worth exploiting. As the HIP is considered to be responsible for learning and memory, the temporal lobe (TL) is critical for long-term memory formation, these two parts were chosen to detect the protein changes in four different age stages (Xu *et al.*, 2016; Xu *et al.*, 2016). Carlyle and his colleagues selected 7 crucial structures

of brain, including cerebellum (CBC), striatum (STR), hippocampus (HIP), dorsolateral prefrontal cortex (DL-PFC), primary visual cortex, amygdala, as well as the mediodorsal thalamic nucleus to detect protein differences with a period from one-year post conception to 42 years using tandem LC-MS. They detected at least 6,529 proteins in all 7 regions. Their clustering analysis revealed that the major differential expression of proteins occurred in a short period after birth, after that, protein abundance changed gently over the developmental period. However, protein differences between brain regions were obvious (Carlyle *et al.*, 2017). The 29 Brodmann area of the human cerebral cortex was detected by proteomics to help us understand the protein composition basis for different brain functions. (Guo *et al.*, 2022). These results remind us that it is necessary to consider brain region differences before conducting aging research on the brain.

PROTEIN CHANGES IN HUMAN BRAIN AGING

Conventional morphological changes in the aging brain contain cerebral sulci deepening and atrophy (Kochunov *et al.*, 2012; Hanseeuw *et al.*, 2023). Decreases in volume and neuronal plasticity were observed in aging HIP (Burke & Barnes, 2006; Griffioen, 2023). In contrast to these obvious morphological changes that occur in aging, the protein changes with aging are relatively poorly understood. One study used non-diseased 16 brains from the Brain Bank of the Chinese Academy of Medical Sciences & Peking Union Medical College and divided them into 4 age groups: A group: 22~49, B group: 50~69, C group: 70~89, and D group: >90. Protein variations were found in D/A, C/A, B/A groups. In HIP, altered proteins could be matched to the respiratory chain and synaptic vesicle fusogenicity pathways and the proteins in the two pathways are downregulated with aging (Xu *et al.*, 2016). In TL, altered proteins are associated with neuronal degeneration activities including neuronal firing change, abnormality of myelin and cellular structure stability (Xu *et al.*, 2016). The orbitofrontal cortex is located in PFC and is in charge of some cognitive tasks, learning and decision-making works. Proteomics identified 65 up-regulated and 62 down-regulated proteins between 15 younger (<45 years) and 18 older (>60 years) postmortem brain samples. These altered proteins are mainly involved in cellular communication, deregulation of nutrient availability and functional decline in proteostasis (Pabba *et al.*, 2017). These biological functions or pathway analyses may provide important clues to exploit deeper molecular mechanisms in aging.

Apart from direct protein changes in number and quantity, protein modification status also plays a crucial part in aging. Samples of the human parietal cortex, frontal cortex, and cingulate gyrus in moderately aged and old-aged were used to identify lipoxidized proteins. The functions of these differently oxidized proteins are engaged in metabolic activity, cytoskeleton, protein homeostasis, neuro-transmission, O2/CO2, as well as the metabolism of hemoglobin (Domínguez *et al.*, 2016).

Different brain regions show different vulnerabilities to aging. Loss of dendritic spines and synapses is more severe in HIP than occipital cortex. The synaptic proteome of rhesus macaque and human brain tissues at different age groups was determined by quantitative proteomics. The authors screened proteins for regional spatiotemporal expression specificity during aging, most of the candidates were located downstream of TGF-β1, which was confirmed to be a conserved upstream pathway to regulate synaptic vulnerability during aging (Graham *et al.*, 2019).

PROTEIN VARIATIONS DURING AGING IN PRIMATE ANIMAL BRAIN

It is better to use longer life span animal models to research aging, which will mimic human aging better. Rhesus monkeys are longer-lived than mice or rats, stratum as well as cortex from rhesus monkeys at 2, 8 and 22 years were collected to isolate synaptosomes before conducting quantitative proteomics. Results showed that aging increased the expression of ubiquitin-conjugating enzyme UBE2N and reduced ubiquitin-proteasomal activity. In combination with in vitro experiments, it indicated that upregulated UBE2N enhanced mutant huntingtin accumulation in synaptosomes during aging (Yin *et al.*, 2015). Another study gathered stratum as well as cortex from rhesus monkeys aged from 8.5 to 32.9 years to assess perineuronal nets (PNNs) and neuronal plasticity in the retrosplenial cortex. Results demonstrated that aging contributed to the increase of microglial activity, which resulted in a reduction in PNNs and excess plasticity (Gray *et al.*, 2023).

PROTEIN VARIATIONS IN RAT BRAIN AGING

Subventricular zone (SVZ) is considered to have the capacity to generate new neurons. Glial fibrillary acidic protein (GFAP), ubiquitin carboxy-terminal hydrolase 1 (UCHL1), glutathione s-transferase omega (GSTO) were found upregulated while collapsing response mediated protein 4 (CRMP-4), CRMP-5 and microsomal protease ER60 decreased with aging. It was supposed that these protein changes were related to the proliferation ability decline of neural stem/progenitor cells in the SVZ during aging (McGinn *et al.,* 2012). Cellular alternations appear in various parts of the brain during normal aging. Considering the strong correlation between aging and neurodegenerative diseases, Yolanda and her colleagues quantifed the expression of proteins from the substantia nigra (SN) of rats associated with idiopathic Parkinson's disease at four stages of life. They proved the pivotal role of GFAP in contributing to differentially expressed proteins. Furthermore, proteins involved in processes of redox reaction, protein aggregation, tau binding and proteins associated with metabolism change could cause age-related neuronal death and loss (Gómez-Gálvez *et al.,* 2020). In the research by Philipp et al, they further proved that aberrant proteostasis is one of the culprits leading to neuronal dysfunction in aged rats and identified three proteins (ARP3, NEB2, BRAG2) that are engaged with age-related devastating impairment of cognitive function. When comparing the proteome of aged (2 years) with adult (3 months), a list of significantly upregulated and downregulated proteins was found, including hyaluronan and proteoglycan link protein, GFAP, myelin-associated oligodendrocyte basic protein (MOBP), etc. (Ottis *et al.,* 2013). The decline of remyelination efficiency related to age may be a primary factor in the progression of disease. Through quantitative MS-proteomics by de la Fuente et al, they identified the proteome of oligodendrocyte progenitor cells (OPCs) at 3 stages of rats, newborn, young and aged and discovered several processes including proteostasis, inflammation and cholesterol biosynthesis that may cause less remyelination with aging. (de la Fuente *et al.,* 2020). These identified proteins will provide new insights into aging.

Finding ways to improve aging damage is one of the goals of brain aging research. Caloric restriction was found to contribute to the decline of protein oxidation in aged rats and the increase in glutamate dysregulation, loss of function in mitochondria and protein synthesis (Poon *et al.,* 2006). Hormone therapy shows neuroprotective effects in postmenopausal women, but the beneficial effects are impacted by age factors (Shumaker *et al.,* 2004; Nerattini *et al.,* 2023), estrogen receptor β (ERβ) is the key factor in mediating hormone efficacy. Natasha and her workmates determined proteins co-precipitated with ERβ in the region from ventral HIP, they found 19 proteins significantly altered under 17 β-estradiol treatment in young groups, but only 5 of 19 proteins were changed in old groups. This result may explain why hormone treatment is influenced by aging (Mott *et al.,* 2014). A further study conducted by Zhang and his group showed that the effects of this kind of hormone therapy are related to age. The PTM state of ERβ may partially account for age-related changes. They proposed that receptor function may vary due to exogenous hormone therapy through absolute quantification of four putative ERβ phosphorylation sites, and the natural fluctuations of hormone were responsible for regulating certain pathways required for the phosphorylation of ERβ under physiological conditions (Zhang *et al.,* 2021). Aging and brain injury have effects on both motor coordination and cognitive deficits, to identify its underlying protein basis, a traumatic brain injury model of rats in three age groups was used and fifteen differentially changed isoforms were found associated with both age and injury (Mehan & Strauss, 2012). However, plasma from the youth may become a new way to reverse the injury. The changes in plasma proteins of different age groups were screened by proteomics, and some functional circulating factors that may have a protective role were found, like IGF-1(Yuan *et al.,* 2019).

Protein oxidation is reported to be tightly associated with brain aging and neurodegenerative diseases. Through comparing proteomic results between 1-month and 24-month-old rat temporal cortex, protein oxidation levels of SOD1, SOD2, peroxiredoxin1, peptidylprolyl isomerase A, cofilin 1, and adenylate kinase 1 were upregulated in the old rat (Wang *et al.,* 2010). Another set of data from the dentate gyrus of rats identified 6514 protein entries in total and confirmed that the quantitative variation occurred in inflammation, synaptic plasticity pathways and the process of generating energy. The results showed a remarkable reduction in proteins with antioxidant capacity in the dentate gyrus region, like PRDX6 (Lubec *et al.,* 2019). Increased oxidative stress is considered a significant

signature in the aging process. The proteins modified by oxidative processes in the brain regions of the cortex, HIP, STR and CBC were identified between aged (12 months) and senescent (28 months) male rats. Some energy-related proteins like pyruvate kinase, ATP synthase, aldolase, creatine kinase, and a-enolase were found in an increased oxidation state in the senescent rats (Perluigi *et al.,* 2010). However, the redox processes during aging have a gender bias. By assessing the redox state in the cerebral cortex and hippocampus, the authors found a higher superoxide dismutase (SOD) activity of older females in both regions than older males, though still lower than both of the young groups, as well as more stable redox homeostasis because of better mitochondrial functionality in older female (Santín-Márquez *et al.,* 2021).

To get more precise knowledge of synaptosomes, the ''large'' and the ''small'' synaptosomes were separated from the cerebellar cortex of Wistar rats at the ages of 2, 6, 12, 18, and 24 months. Energy metabolism states were evaluated by functional proteomics. The results indicated that the energy metabolism of the ''large'' and the ''small'' synaptosomes were specific and independent. Aging exerts influences on cerebellar energy metabolism and neurotransmitter synthesis at the subcellular level in a selective way (Ferrari *et al.,* 2015).

MICE BRAIN PROVIDES ABUNDANT INFORMATION FOR THE DISCOVERY OF AGING MECHANISMS

Brain development goes through from the embryonic stage to the adult stage. Brains from mice embryos at day 16 (Ed16), one week (1W) and eight weeks (8W) after birth were selected to detect protein changes in pre-and postnatal stages of the mice brain by 2-DE technique. A total number of 63 spots were found different between Ed16 and 1W, and 74 spots were different between 1W and 8W. Among the upregulated and downregulated proteins, 12 identified proteins were statistically different between Ed16 and 1W, and there were 13 significantly changed proteins between 1W and 8W (Seefeldt *et al.,* 2006). Neocortical postsynaptic density (PSD) from mice at five different stages of life was used to detect to track the change of protein. PSD is stage-specific and can be subdivided into four components which perform functions depending on the stage and cell type. They found Rho GTPase signaling and Rac1 pathway in the perinatal stage were important for PSD development (Wang *et al.,* 2023). In another study conducted by Kai Stühler, etc., they took two different 2-DE systems to detect protein differences in three developmental stages (Ed16, 1W, 8W) of mice. Through contrast of the two systems, they found that procedures of sample preparation and resolution of the two dimensions need to be advanced, and the repeats of the 2-DE method should be increased (Stühler *et al.,* 2006). Deep quantitative proteomics from MS technique has high sensitivity and wider detection range compared with 2-DE. Przemysław Duda and his colleagues quantitatively identified more than 5,200 proteins from HIP, cortex and CBC in 1 or 12-month-old mice. They found that proteins that make up the brain structures were rarely changed by aging, however, some receptors and signaling pathways associated with proteins in charge of learning and memory were found significantly altered between young and middle-aged mice. Proteins affecting neuronal plasticity including SynDIG4, Grik, Gabra, Camk2, Camk4, Mapk were statistically changed in HIP. Similarly, neuronal plasticity-associated proteins also changed in the cortex (Gabbr, Camk2, Camk4, Mapk) and CBC (Grik, Grin, Gabbr, Camk4, Prak). Significantly changed protein kinases participate in glutamate-induced neuronal plasticity found by quantitative proteomics proving the weakening of neuronal plasticity by aging. Glutamatergic transmission-associated proteins were also found to be affected by aging. Gria1, SynDIG4 and Grik2 were downregulated while Grin2a was upregulated in HIP of the adult group than the young group. In CBC, Gria4, Grin2b, Grm4 and Grm5 were downregulated in adult mice. In Cortex, Grin1, Grin2b and Grm7 were decreased in adult mice. In addition, some proteins involved in GABAergic transmission were also changed by aging. In combination with immunofluorescent experiments, this research indicated that the structure components were relatively stable during aging, while less neuroplasticity and neurotransmitter activities than younger, which might be a normal phenomenon caused by aging in brain (Dudaz *et al.,* 2018). The study led by Gostomska-Pampuch *et al.* also proved that the total proteins in brain were rarely changed with age. The in-depth quantitative of energy metabolism proteomes in hippocampal, cortical and cerebellar was achieved to analyze the differences between mice at young and old age. In the aged animals, the capacity of glucose oxidation improved, but the proteins of fatty acid metabolism declined. However,

the trends of each studied structure are inconsistent due to their unique potential for adaption (Gostomska-Pampuch *et al.*, 2021). Dominika Drulis-Fajdasz and her colleagues used an MS-based technique to determine the levels of proteins associated with synaptic plasticity. From three brain structures of the 1 or 22-month-old mice, HIP, CBC and cerebral cortex were analyzed and an excess of 7000 proteins were found, based on which they suggested that the cerebellar proteome was more stable than the hippocampal and cortical proteomes. Besides, they concluded a general description of how synaptic plasticity changes under the influence of normal aging. (Drulis-Fajdasz *et al.*, 2021). Proteins have properties of multiple functions. The proteins affecting aging may have an impact on other physiological activities. Aaron and his mates identified 43 unique proteins from the cerebral cortex of mice relevant to sleep and senescence. These proteins could be grouped according to their functions: signaling transduction, cytoskeletal, energy metabolism, exocytosis, heat shock proteins, mRNA processing/trafficking, and serum proteins (Pawlyk *et al.*, 2006). Considering the close link between sleep and cognitive ability, another group focused on research on the influence of aging on circadian expression patterns of proteins, which greatly affect hippocampal-dependent memory. The results demonstrated a notable downregulation of these proteins in HIP, 15% circadian rhythms in the young versus 1.6% in the old group. The impairment concerned energy metabolism, neurotransmission, synaptic vesicle cycling (Adler *et al.*, 2020). In one research conducted by Lei Mao, C57BL/6 mice ranging from Ed10 to 100 weeks were used to determine the protein changes caused by aging. They found that nonfunctional protein accumulation and loss of proteasome components may contribute to aging (Mao *et al.*, 2010). In one research conducted by Kazuya Tsumagari, they identified more than 7000 proteins in the cortex and hippocampus of C57BL/6 male mice at three age points (3, 15, and 24 months old) to determine the protein changes caused by aging. They found that extracellular proteins appeared to account for most of the significantly upregulated proteins, while in the cortex the synaptic functions made up the largest part of the remarkably upregulated proteins (Tsumagari *et al.*, 2023). Interestingly, other studies found a decrease of proteasome α subunits 3/6 and age-dependent changes of Pax6 involving cell metabolism and survival signaling pathways in the aging process. The decline of olfactory ability was accompanied by aging (Yang *et al.*, 2008; Srivastava & Mishra, 2023). Different proteins of the olfactory epithelium and olfactory bulb between old and young mice were associated with metabolism, transport/motility, stress response and lipid transport (Poon *et al.*, 2005; Tzeng *et al.*, 2021). To get more promising drug targets in GSK-3 signaling pathway for age-associated degenerative disease. HIP, cortex and CBC of young and old mice were used to conduct quantitative proteomics, and several protein changes in the upstream and downstream of GSK-3 signaling pathway were identified. (Drulis-Fajdasz *et al.*, 2018). Through proteomic comparison in HIP between 1month and 12-month-old mice, one lab discovered enhanced glycogen metabolism and altered mitochondrial energy metabolism in aged mice brain, in combination with the immunofluorescent staining, they found enzymes related to glycogen metabolism process relocated from astrocytes to neurons in young HIP (Drulis-Fajdasz *et al.*, 2018).

Gene knock-out mice and other kinds of animal models provide pivotal research tools for brain aging research. Joan M. Jasien and her colleagues adopted HIP and cortex from the autism spectrum disorder (ASD) model (BTBRT+tf/j mouse) to detect relationships between age and this disease using quantitative proteomics. They discovered an interesting phenomenon that the social behavioral phenotype, as well as protein characteristics of the ASD model, were age-resistant. They concluded that upregulated synaptic proteins such as Picalm, Itsn1 and Mobp could protect aged ASD mice against cognition declines (Jasien *et al.*, 2014). Kisaretova and her colleagues screened the papers concerning transcriptome and proteomics in the hippocampus and corticostriatal area of ASD model (BTBRT+tf/j mouse) to detect relationships between age and this disease. They concluded that there is a unidirection of protein expression of Macf1, Bsn, Psd3, and Chchd3 between the cortex and hippocampus, the proteins were related to cellular morphology and synaptic function (Kisaretova *et al.*, 2023). Ercc1 is reported to be in charge of DNA repair (Wang *et al.*, 2012; Birkisdóttir *et al.*, 2023), a kind of Purkinje cell-specific Ercc1 knockout mice was used to determine protein variations in different age stages. The significantly changed proteins indicated that DNA damage in Purkinje cells mainly affects plasticity. Results of quantitative proteomics and immunohistochemistry staining proved the downregulation of synaptic-associated proteins with aging.

These proteins were involved in many aspects of neuronal plasticity like neurotransmitter receptors (i.e. GluRδ2 and mGluR1), and downstream signaling molecules (i.e. PKCy and cGK1). The DNA damage mice model plus quantitative proteomics of CBC give us an insight into temporal and spatial protein dynamics in aging (de Graaf *et al.*, 2013; Drulis-Fajdasz *et al.*, 2021). SAMP10 mouse, representative of a strain of age-associated cerebral deterioration, was identified by MS in the limbic forebrain to detect the underlying brain aging mechanisms. Results showed that the expression level of pyridoxal phosphate phosphatase was upregulated in 3-month-old SAMP10 mice, while collapsin response mediator protein 2 and phosphorylated α-internexin were increased in 8-month-old SAMP10 mice than its control (Furukawa *et al.*, 2010).

Research objects of quantitative proteomics are not restricted to cells or tissues, subcellular organelles are suitable for proteomics if the isolated quantity is enough. In a study conducted by Kelly L Stauch and his mates, they isolated synaptic mitochondria of mice at three age points (5, 12, 24 months) and examined the proteomic alterations during aging. Proteins detected successfully predicted dynamic changes in mitochondrial transcriptional regulation during aging, Immunoblot of TFAM expression further verified the correctness of the prediction. Protein subunits in the electron transport chain were found altered, in combination with results of immunoblot, these subunits including ATP5H, NDUFB8, MTCO1, UQCRC2 and ATP5A1 were proved to be in a dynamic process during aging. Oxygen consumption rate (OCR) is impacted by mitochondria function, OCR actually showed differences in different aging stages. In addition, proteins involved in mitochondrial dynamics (DRP1 and MFN1) and mitophagy (SQSTM1) also changed during aging. Interestingly, despite mitochondrial impairment occurring in old mice, mitochondrial function was still maintained. They inferred that proteomic alternations in synaptic mitochondria during aging should be involved in the maintenance of synaptic mitochondrial function (Stauch *et al.*, 2014). In research by Sen Yang and his mates, they summarized that mitochondrial morphology and distribution changes induced by aging occurred in CA1 and dentate gyrus regions. Apart from reduced numbers of mitochondria, it also exhibited malfunction in the aged brain, involving decreased Ca^{2+} buffering capacity, ATP production fall and oxidative injury. Furthermore, aging can lead to deletions of mitochondrial DNA (mtDNA), activation of nuclear respiratory factor 1 (NRF1) and peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1A) mediated mitochondrial fitness pathways, which occur to reduce the impairments and preserve the function of mitochondrial respiration, such as the up-regulation of proteins controlling oxidative phosphorylation (OXPHO) activity, antioxidant activity, mitochondrial fusion and mitophagy. (Yang *et al.*, 2023). Further analysis of the proteomics, proteins including glycolysis and TCA cycle were found to be changed with aging. Changed biological processes like OXPHO, mitochondrial electron transport chain and production of precursor metabolites and energy (Schrötter *et al.*, 2017). Take the cortical microvessels (MVs) for example, from brain tissues of young to old mice, the glycolytic proteins were found to be decreased with aging. Other biological activities like aerobic glycolysis, mitochondrial function, oxidation response, mRNA or protein stability, basement membrane (BM) composition were also changed (Chandra *et al.*, 2022). Mitochondria were isolated from mouse brain tissues to analyze mitochondrial proteins. Respiratory chain complex I subunits, complex II and one complex IV subunits were found to be downregulated during aging while one subunit of complex III and complex V were both found upregulated. The results indicated that there should be feedback regulation in the respiratory chain to maintain mitochondrial function during aging (Mao *et al.*, 2006; Adlimoghaddam *et al.*, 2022).

To explore oxidative stress caused by aging, 4-month-old and 12-month-old brains of SAMP8 mice were chosen to conduct 2-DE experiments. Apart from the changed proteins such as TPI, LDH-2, α-Spectrin 2, NF-L, and hsp86 were detected, brain proteins including α-Enolase, LDH-2, CK, DRP-2 and α-Spectrin were found oxidatively modified. These expression-changed or oxidatively modified proteins were involved in biological processes such as energy utilization and interneuronal communications of the brain (Poon *et al.*, 2004). Another group of authors chose 16-week-old and 80-week-old brains of C57BL/6J mice to analyze through proteomics. They recognized the significant role of several selective protein changes in the oxidation state. The changes in cysteine oxidation during aging are involved in biological processes such as energy utilization and proteasomal-mediated protein degradation of the brain (Urrutia & Bórquez, 2023). Similarly, protein differences between 80W old and 6W old C57BL/6

male mice were found, expression of dihydropyrimidinase-like 2, α-enolase, dynamin-1, and lactate dehydrogenase 2 were upregulated in old mice, oxidation level of β-actin, glutamine synthase and neurofilament 66 were increased, which were involved in neurodegenerative diseases (Poon *et al.*, 2006). Furthermore, protein differences between 5-month-old and 21-month-old C57BL/6 male mice in the cortex, cerebellum, as well as their synaptic fractions were found, which revealed an alteration in the stability of proteins. The alteration in proteostasis was reported to participate in neurodegenerative diseases, suggesting that aging is an important factor in neurodegeneration (Kluever *et al.*, 2022).

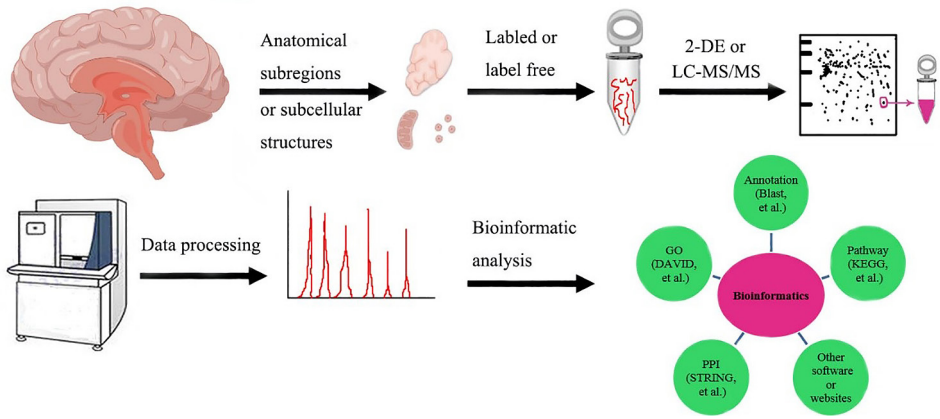

Figure 1. Outline of the proteomics application in brain aging research. Species selection at first step, brain region dissection and subcellular structures or organelles separation can be conducted according to research aims. Abundant protein expression information from MS or 2DE+MS provides raw data for bioinformatic analysis.

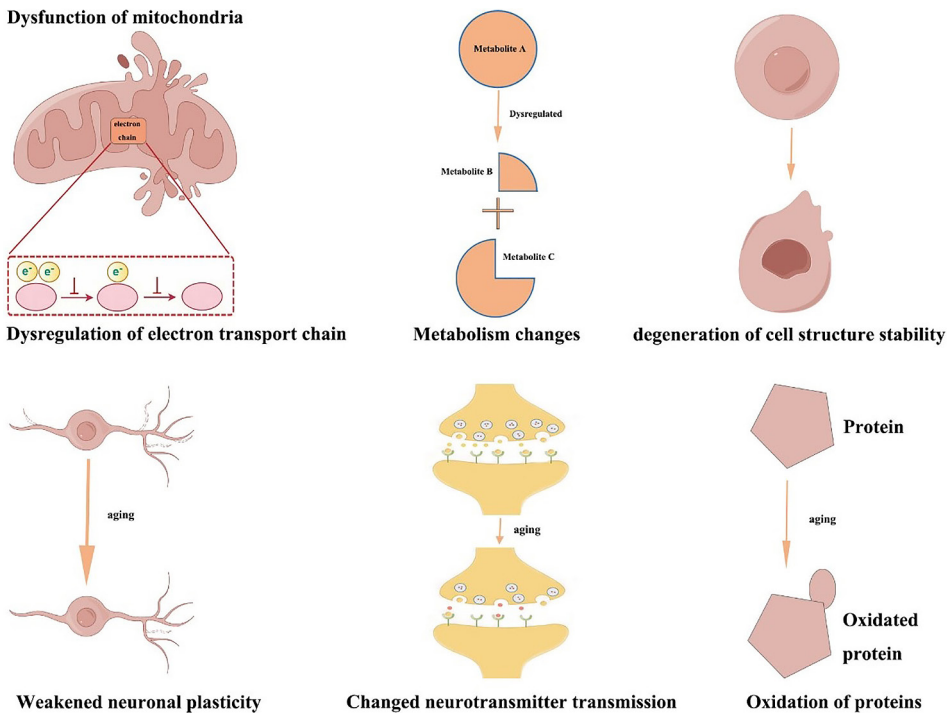

Figure 2. Biological activities involved in brain aging. Aging-related protein changes lead to dysfunction of mitochondria, dysregulation of electron chain, metabolism changes, degeneration of cell structure stability, weakened neuronal plasticity, changed neurotransmiter transmission, oxidation of proteins. This graph summarizes the biological activity changes during brain aging founded by proteomics.

CONCLUSION

In summary, we discuss the present application of proteomic technologies in aging research using brain samples. Different age stages as well as species chosen to research brain aging by proteomics are not sufficient and the time points are limited. Mice brains or rat brains are more frequently used than human brains. The anatomical regions chosen to be detected by proteomics are far from enough to reveal the exquisite differences in the human brain. The outline of how to use proteomics to research brain aging has been summarized in Figure 1. According to the research aim, brain region selection should be first considered. Subcellular structures (such as mitochondria and synaptosomes) isolation can be considered as the technologies are feasible. 2DE technology plus MS technology screen out protein differences and provide clues for further mechanisms discovery. Figure 2 shows the current founded mechanisms of aging concluded from proteomics. We can conclude that a vast network of biological process dysregulation caused by dysregulated proteins occurs during the aging process. The specific mechanisms during aging include mitochondria dysfunction, metabolism changes, degeneration of cell structure stability, changed electron transport chain, weakened neuronal plasticity, changed neurotransmitter transmission, oxidation of specific proteins, etc. In addition, the pivotal role of TGF-β1 signaling and GSK-3 signaling pathways during brain aging was identified by proteomics. The advantage of proteomics is its ability to screen proteins and detect the relative fold changes. We can infer biological process changes and pathway changes during brain aging by comparing the protein changes with existing databases, but the most valuable information should be the hints provided to excavate deeper or new mechanisms.

PROSPECTIVE

The mechanisms for aging need further exploration, different brain regions have different work divisions. However, which brain region is affected firstly or mostly by aging is still elusive. A comprehensive detection of brain regions is beneficial for aging research. As shown in this review, exquisite divided brain regions used for proteomics in aging research are limited. There is still a long way for the application of proteomics in different anatomical regions of the brain. The human brain has a bigger volume and more complex functions than mice or rats. The mechanisms of aging found in animal models may be not completely suitable for human beings. When researching the aging mechanisms in the human brain, the human brain bank plays a key role in supplying enough and high-quality brain samples (Liu *et al.,* 2022). The combination of the human brain bank with various rapidly developed techniques seems a promising way for human brain aging research.

The improvement of proteomic technologies has made it easy to detect thousands of proteins at one test, not only oxidative modification occurs in aging, acetylation, alkylation, methylation, phosphorylation, glycation and other kinds of PTM may play crucial roles in aging, these PTMs could be investigated through MS analysis (Zhang *et al.,* 2023; Ying & Li, 2022; Yu *et al.,* 2021; Creecy *et al.,* 2021), these PTM research would extremely enrich our knowledge about aging in an epigenetic view. Thus, expanding the research ranges of brain aging by proteomics is extremely promising. Proteomics enables us to look for biomarkers or possible dysregulated pathways that occur in aging via a high-throughput screening way. We hope the combination of proteomics with other techniques such as metabonomics (Liu *et al.,* 2021) and transcriptomics (Jia *et al.,*2021; Luo *et al.,* 2023) of brain tissues would bring more useful clues for aging research.

List of abbreviations

Full name	Abbreviation
two-dimensional gel electrophoresis	2-DE
mass spectrometry	MS
liquid chromatography-mass spectrometry	LC-MS
time of flight mass spectrometer	TOF-MS
hippocampus	HIP
post translational modification	PTM
cerebellum	CBC
temporal lobe	TL
dorsolateral prefrontal cortex	DL-PFC
striatum	STR
subventricular zone	SVZ
glial fibrillary acidic protein	GFAP
myelin-associated oligodendrocyte basic protein	MOBP
ubiquitin carboxy terminal hydrolase 1	UCHL1
glutathione s-transferase omega	GSTO
collapsing response mediated protein 4	CRMP-4
estrogen receptor β	ERβ

embryos at day 16 . . . . . .Ed16
eight weeks 8W
one week 1W
autism spectrum disorder . . . . . .ASD
oxygen consumption rate . . . . . .OCR
tacrolimus FK506
prefrontal cortex . . . . . .PFC
Advanced lipoxidation end-products . . . . . .ALEs
transforming growth factor-β1. . . . . .TGF-β1
ubiquitin conjugating enzyme E2N UBE2N
perineuronal nets . . . . . .PNNs
substantia nigra. . . . . .SN
multiple sclerosis . . . . . .MS
oligodendrocyte progenitor cells . . . . . .OPCs
apolipoprotein E . . . . . .ApoE
huntingtin . . . . . .Htt
macrophage migration inhibitory factor . . . . . .Mif
α-Synuclein. . . . . .Snca
protein translational modifications. . . . . .PTM
superoxide dismutase . . . . . .SOD
peroxiredoxin 6 . . . . . .PRDX6
postsynaptic density . . . . . .PSD
ras-related C3 botulinum toxin substrate 1 . . .Rac1
synapse differentiation-induced gene4 . . SynDIG4
glutamate ionotropic receptor
kainate type subunit α . . . . . .Grik
gamma-aminobutyric acid type A
receptor subunit alpha . . . . . .Gabra
calcium-calmodulin dependent
protein kinase II Camk2
mitogen-activated protein kinases . . . . . .Mapk
glutamate ionotropic receptor
AMPA type subunit 4 . . . . . .Gria4
glutamate [NMDA] receptor
subunit epsilon 2 . . . . . .Grin2b
glutamate metabotropic receptor 4. . . . . .Grm4
glycogen synthase kinase 3 . . . . . .GSK-3
toll-like receptor 4 . . . . . .TLR4
microtubule actin crosslinking factor 1. . . . Macf1
protein bassoon. . . . . .Bsn
excision repair cross-complementation
group1. . . . . .Ercc1
glycine N-methyltransferase . . . . . .GNMT
mitochondrial transcription factor A . . . . . .TFAM
mitochondrial DNA . . . . . .mtDNA
nuclear respiratory factor 1. . . . . .NRF1
peroxisome proliferator-activated
receptor gamma coactivator 1 α. PGC1A
oxidative phosphorylation . . . . . .OXPHO
tricarboxylic acid . . . . . .TCA
microvessels . . . . . .MVs
basement membrane . . . . . .BM

Conflict of Interest

All the authors have no conflicts of interest.

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