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Review

Systems biology of Alzheimer's disease: How diverse molecular changes result in memory impairment in AD

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ABSTRACT

Alzheimer's disease (AD) is a protein misfolding-based rapid cognitive impairment in the aging brain. Because of its very widespread molecular background, AD has been approached using genomic and proteomic methods and has accumulated a large body of data during the last 15 years. In this review, we summarize the systems biology data on AD and pay particular attention to the proteomic changes in AD. Applying a systems biology model of the synapse, we attempt to integrate protein changes and provide an explanation of why seemingly diverse molecular changes result in memory impairment. We also summarize the present state of cerebrospinal fluid (CSF) and blood biomarker studies for the diagnosis of AD as well as the results of proteomic studies in tissue cultures and animal models. Finally, we give a systems biology model of AD explaining how AD can develop in an individual manner in each particular subject but always results in a rapidly developing dementia and memory impairment.

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1. Introduction

Memory deficit is both the usual complaint in mild cognitive impairment (MCI) and the cardinal feature of Alzheimer's disease (AD). MCI is considered as a transition state between aging and AD, but it is not a distinct entity (Morris et al., 2001). According to recent studies MCI generally represents early stage AD. The main features of AD have been widely reviewed recently (Mucke, 2009; Castellani et al., 2010). In the case of severe memory impairment, AD patients can be differentiated from subjects with other forms of senile dementia with about 95% accuracy using a combination of diagnostic methods, but the final diagnosis can be done only by *post-mortem* brain pathology. In other words, there is no reliable peripheral biochemical marker for AD and a definitive diagnosis can only be made on histologic examination of the brain at autopsy (Castellani et al., 2010). The actual state of understanding suggests that perhaps there is no single molecular pathological mechanism behind AD, but different molecular mechanisms have been disclosed that lead to AD or an AD-like state. Unquestionably, the first detectable symptom of AD is the memory impairment. The memory trace is based on plastic changes in synaptic proteins tuning synaptic transmission efficiency (Agnati et al., 2004). The synaptic protein assembly, however, reflects a wide range of cellular and molecular events suggested by the recent discoveries on synaptic processes. Systems biology of the synapse and the molecular mechanisms of AD allow the integration of the seemingly diverse molecular findings in AD and animal models of AD by merging genomic, proteomic and bioinformatic data. Thus, we intend to review the available data and ideas about the molecular complexity-based research of AD by applying the principles of systems biology paying particular attention to the changes in protein networks and selected biomarkers relating to memory impairment resulting in synaptic malfunctioning.

2. Molecular mechanisms leading to AD

Evidence suggests that the abnormal production and accumulation of misfolded, toxic proteins initiate and/or maintain AD. In AD, β-amyloid (Aβ) peptides, the microtubule-associated protein tau and the presynaptic protein α-synuclein accumulate.

The most common and distinctive "hallmark" lesions present within the AD-brain are the senile plaques and neurofibrillary tangles (NFTs) (Castellani et al., 2010).

Aβ self-aggregates into assemblies as oligomers, protofibrils and fibrils, forming the core of senile plaques. Tau protein and α-synuclein self-aggregate to oligomers and large inclusions forming NFTs and Lewy bodies, respectively. All patients with AD have plaques and tangles in the brain and most patients also have Lewy

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bodies (Mucke, 2009). Although the pathogenesis of AD is not known and maybe caused by multiple parallel mechanisms, here we summarize some hypotheses about the distinct molecular and cellular mechanisms that can cause AD or AD-like symptoms. Current theories of AD are excellently reviewed (Mucke, 2009; Luque and Jaffe, 2009; Castellani et al., 2010).

There are two main hypotheses for the pathogenesis of AD: the amyloid-cascade and the hyperphosphorylated tau hypothesis.

(1) According to the original amyloid hypothesis (Hardy and Allsop, 1991) fibrillar Aβ peptides of the amyloid plaques are responsible for neurodegeneration in AD. The hypothesis has been radically changed during the past 10 years and soluble Aβ oligomers and protofibrils were found to be the most toxic aggregation species of Aβ peptides (Walsh et al., 2000). The newest amyloid-hypothesis emphasizes the pivotal role of intracellular Aβ species in the pathogenesis of AD (LaFerla et al., 2007). Positron emission tomography (PET) ligands (e.g., ^{11}C-labelled PIB) directly demonstrate the presence of Aβ aggregates in the brain of AD-patients (Svedberg et al., 2009).

(2) The tau-hypothesis focuses on the microtubular protein tau (Iqbal and Grundke-Iqbal, 2005). Brain injuries, hypoxia, hypoglycemia may cause signalization disturbances activating GSK 3β kinase and then tau-protein will be hyperphosphorylated, resulting in collapse of the microtubular system and cell death.

Very recently the modernized amyloid hypotheses for AD pathogenesis (Hardy, 2009) have a wide acceptance. Some new findings support the amyloid hypothesis leaving place also for tau-pathology:

- "Mitochondrial cascade hypothesis": mitochondrial energy production plays an important role in the etiopathology of AD (Manfredi and Beal, 2000; Reddy, 2009; Devi and Anandatheerthavarada, 2010). One of the hallmarks of AD is the very early deterioration of mitochondria in neurons.
- A decrease of Aβ peptides and a simultaneous increase of total tau in the CSF of AD patients indicate that the brain clearance of Aβ has decreased in AD patients. It can cause Aβ accumulation in the brain tissue and the induction of AD (Ward, 2007).
- Aβ oligomers are synaptotoxic. A very early hallmark of the disease is the slow deterioration of synapses and impaired neuronal communication. The Zn ions may contribute to the toxicity of Aβ peptides in the synapses (Zn is co-released with glutamate). (Bush et al., 1994).
- Mutant ApolipoproteinE (ApoE epsilon 4) protein is present in 60% of the Caucasian population of sporadic AD patients. Because ApoE plays an important role in Aβ clearance, it is a clue for the importance of Aβ clearance in AD (Mucke, 2009).
- Biochemical and immunochemical analysis of neurofibrillary tangles (NFTs, the second hallmark of AD) resulted in important information on the proteins of the paired helical filaments. It is widely accepted that the microtubule associated protein tau is the major protein of NFT. However, a wide range of other proteins and carbohydrates were found in NFTs (reviewed by Smith, 1998):
  (1) Cytoskeletal elements, including tau, neurofilaments, high molecular weight microtubule-associated protein MAP2, vimentin, and tropomyosin.
  (2) Protease-related elements, including ubiquitin, α_1-antichymotrypsin, α_1-antitrypsin, cathepsins B and D, trypsin, and elastase.
  (3) Proteoglycans, including heparan, chondroitin, and keratin sulfate proteoglycans.
  (4) Inflammatory molecules, including acute phase proteins, cytokines and complement molecules.
  (5) Amyloidogenic-related molecules, including APP, presenilin, and APOE.
  (6) Serum-related molecules, including P-component.
  (7) Adducts of oxidative stress, including advanced glycation and lipid peroxidation.

On the base of these data we cannot firmly establish one molecular mechanism of AD as of yet. So, recent molecular AD research is facing typical multimolecular, systems biology problems (Pimplikar et al., 2010). Actually, the main objectives of AD research are to determine how the misfolded protein assemblies cause memory impairment, which is the earliest detectable molecular event of AD that has a diagnostic value. Because Aβ modulates cellular functions by binding to a large group of functionally important proteins in the cellular protein network, the classical biochemical approaches have serious limitations in drug discovery and diagnostic development in AD.

3. Expected contribution of systems biology to AD research

The scientific effort studying how complex molecular assemblies work in biological systems is called systems biology. Most proteins have multifunctional properties and thus serve different biological functions in the living organism depending on the surrounding molecular environment. Recently, cell biologists have collected hard evidence that cell functions are performed by complex protein interaction networks linked together by different types of interactions, such as binding, small molecule substrates and gene transcription regulation (Alberghina et al., 2009). The importance of molecular complexity-oriented thinking has been raised due to the fact that Aβ aggregates, even in small oligomers (very probably responsible for the initiation of AD), do not bind one specific target but a large range of intracellular and cell membrane proteins. Because of its interactions, Aβ causes a mistuning of the cellular protein network at several spots at a time rather than attacking a single protein molecule. The main point is to determine how we can disclose a widely scattered and multi-molecular targeting pathological mechanism such as AD.

Recent developments in systems biology clearly demonstrate that if proteins are put together in an organized way, the protein complex can perform specific functions. Such kinds of engineered protein machines resemble the cellular protein networks, but they are actually less sophisticated than a synapse or mitochondria (Bashor et al., 2010). These fascinating protein nano-motors also proved that a little molecular change in the composition of a molecular machine could alter its functioning rapidly, specifically and even reversibly (Bashor et al., 2010). Aβ probably works in a similar way because it is an unspecific "glue" that sticks to functionally important elements of the molecular machines of nerve cells and imposes its rigidity.

Changing the functioning protein network of nerve cells, Aβ triggers signalling system responses regulating gene transcription. Also, Aβ interacts with the mitochondrial metabolic network and almost all functional protein networks of nerve cells and extracellular matrices, as we will discuss later. The intracellular and extracellular forms of Aβ oligomers influence cell functions at many different spots. Also, it has to be noted that diffusible small oligomers can enter the synaptic gap, but large fibrils cannot. This suggests that synaptic action and, in turn, memory impairment induced by Aβ oligomers, are dependent on the size of the oligomers. Therefore, several researchers believe that the multimolecular targets of Aβ and its consequences in the cellular machinery of nerve cells, particularly on the synapse, are the main issues for making progress in the development of the early diagnosis, prevention and better treatment of AD (Shankar and Walsh, 2009). This is the most important expectation from the use of a systems biology approach to AD research.

In the following review, we give a summary of the basic neurobiological discoveries achieved with a molecular complexity-oriented research strategy in AD research, including a genomic, proteomic synthesis of the results using a systems biology model of the synapse. Our aim is to provide a systems biology framework to hopefully enable researchers to understand how such a diverse series of molecular events already disclosed in AD and AD animal models can initiate the major symptom of AD, the rapid memory impairment. We do not summarize the genomic aspects of AD, it is properly reviewed elsewhere (Bertram, 2009; Sleegers et al., 2010).

4. Proteomics of AD

4.1. Critical overview of proteomic research strategies used in AD research

The term "proteome" (Wilkins et al., 1996) is a complement of a genome, and it is used for the total protein content of a cell. In other words, the proteome is the molecular phenotype of the cell including post-translational modifications of proteins, and it is the molecular substrate of biological functions. The cellular proteome is a dynamic network and is directly affected by environmental factors, such as stress, drug treatment and aging (Kim et al., 2004). Changes in the proteome can be measured by proteomics focusing on the analysis of the expression, structure, interactions and post-translational modifications of proteins. On the whole, the proteomic analysis gives information about functional changes in the total protein network and also about functional sub-networks in time. The so-called "omics" era technologies merge the potential of transcriptomics (study of mRNA expression) and proteomics (Götz et al., 2008). Potential protein changes in a biological event are shown by mRNA analysis, but proteome studies reflect which proteins are actually changed. These two sets of molecules, the list of transcriptome and the proteome changes, are not necessarily overlapping because only a fraction of mRNA is translated to proteins, and protein changes are delayed compared to changes in mRNA.

Recent proteomic methods have limitations. Because of alternative splicing in the gene transcription process and the 300 known post-translational protein modifications, the 30,000 human genes encode several hundred thousand different proteins. A particular cell is constructed from 10,000 to 20,000 different proteins in variable abundances. Proteomic methods are limited by the sensitivity of protein detection. Therefore, even the highest resolution technologies can only detect 3000–5000 proteins out of the 10,000–20,000 existing proteins in a cell.

Sampling is very important problem in AD proteomics. Biopsies of AD brain would be highly informative in different stages of the disease but very rare, in practice all samples come from post-mortem brains. Thus the human proteome changes measured usually represent the end-stage of AD. Post-mortem delay of sample preparation, co-morbidities such as pneumonia and the effect of final agonal state also decrease the relevance of the results of proteome changes. Another important technical problem is the application of paraformaldehyde fixed brain samples for proteomic studies. The use of formaldehyde-fixed paraffin-embedded samples for proteome analysis is very limited, although novel methodology allows the cleavage and extraction of some characteristic peptide fragments from brain dissections for identification of fixed proteins (Shi et al., 2006; Azimzadeh et al., 2010). Evaluation of proteome changes in different animal models of AD is also problematic, because these models represent mostly a particular type of pathology (e.g., β-amyloid or tau). However, it is widely accepted that protein alteration in AD animal models may reflect protein changes of the very beginning of human AD.

A further limitation is that tissue samples and biological fluids are dominated by several high abundance proteins (actin, albumin, immunoglobulins, etc.), making it difficult to detect low abundance proteins. The depletion of blood or tissue samples of the most abundant proteins improves the detection of low-abundance proteins. The dynamic range of protein abundance within a cell is estimated to be as high as 10^7. This is the reason why our knowledge about low-abundance proteins is limited (Maurer, 2010). An analysis of a proteome thus often requires the separation to several subproteomes. This analysis is called "focused" or "targeted" proteomics (Zetterberg et al., 2008).

Proteomics is a 15-year-old methodology. Detailed descriptions and many critical reviews of proteomics can be found in the literature and we do not want to deal with methodological issues in this review. However, we give a brief summary of the gel electrophoresis-based and gel-free methods.

Gel electrophoresis is a proteomic method of the highest resolution and sensitivity. Protein separation is done by 2D electrophoresis starting with an isoelectric focusing followed by a separation by size using polyacrylamide gel electrophoresis. After image analysis, spots of different densities are established and selected from the gel and the proteins are digested. The protein digests can be analysed by tandem mass spectrometry. Details of the workflow are thoroughly described by Schulenborg et al. (2006). To improve the sensitivity and linearity of classical post-electrophoresis staining techniques (Coomassie, SyproRuby, etc.), GE Life Sciences introduced the CyDye staining kit that allows an analysis of even 5 μg of total protein (Tonge et al., 2001; Shaw et al., 2003; Miller et al., 2006). There are serious limitations of the technology, including the loss of certain high molecular mass hydrophobic membrane proteins, the loss of extremely acidic and alkaline proteins and some other critical factors. Protein identification also needs an analytical gel of higher protein content and the whole method needs skilled researchers and technicians. However, most of the proteomic data on AD has been obtained using 2D gel-based methods.

Even if the 2D gel-based DIGE methods is the gold standard in proteomics, some gel-free methods have been developed for facilitating a more automated proteome study and decreasing the requirements of labour-intensive skills. Gel-free protein separation is usually done by liquid chromatography, and mass spectrometry is used for the identification of the components altering chromatography peaks. Quantification in gel-free proteomics methods requires labelling with stable isotopes (e.g., isotope-coded affinity tag, ICAT, and isotope-tagged relative and absolute quantitation, iTRAQ). These methods are reviewed elsewhere (Gevaert et al., 2007). There are some quantitative studies on proteome changes in AD blood and cerebrospinal fluid using gel-free methods (reviewed by Montine et al., 2006; Korolainen et al., 2010). The limited number of samples that can be labelled in one experiment is the drawback of the clinical application of gel-free techniques such as ICAT or iTRAQ, and the necessary sample size is larger and the number of proteins separated is much less than 2D gel methods.

Protein separation achieved by binding interactions on a matrix has been performed using the surface-enhanced laser desorption ionization time-of-flight mass spectrometry (SELDI-TOF-MS) technology. The enrichment of protein classes from complex mixtures on chips with special surfaces is achieved (e.g., hydrophobic or antibody-covered) then analysed with TOF-MS. Biomarker studies have used SELDI-TOF-MS (Lewczuk and Wiltfang, 2008). The major disadvantages of the SELDI method are the difficulty of analysing large proteins and the detection of protein profiles only on the basis of molecular weights.

We also list here some promising methodological trials under development. Label-free quantification in gel-free proteomics uses

the MS signal intensities, the HPLC retention times, the numbers of the detected peptides and special software (“Protein Expression”) for the detection of protein changes in a sample. The main advantages of this method are that no labelling is required, and a few samples can be analysed simultaneously. A limitation is the low resolution; only a few hundred (300–500) proteins can be detected from the sample. Proteomics on protein arrays (“chips”) containing 7000–9000 recombinant proteins immobilized on a solid surface is under development. The reproducibility and stability of the chips are not sufficient yet. A new, sophisticated software tool for the processing and quantification of LC/MALDI TOF–TOF proteomics data has recently been introduced (Oh et al., 2010).

Finally, we give some important recent reviews and relevant systems biology articles concerning AD. A review of proteomics in drug discovery and development was published by Soares et al. (2004). The pharmacogenomics of AD was reviewed by Cacabelos (2009). Blood–brain barrier genomics and proteomics have been performed for the elucidation of the phenotype and the identification of disease targets that may enable brain drug delivery for AD treatment (Calabria and Shusta, 2006). Studies on protein biomarkers and co-biomarkers for drug development and diagnostics have also been reviewed (Lee et al., 2008).

4.2. Proteome changes in experimental AD models and AD

Aβ oligomers binding to different components of the neurons step-by-step dysregulate cell functions and, particularly, the synaptic communication, finally resulting in cell death. Brain tissue is a heterogeneous network of neurons and glial cells and the molecular mechanisms of AD are stage- and region-specific (Braak and Braak, 1991). In addition, brain tissue biopsy is rarely possible. Thus, alterations at the cellular and molecular level can be investigated mostly on *post-mortem* autopsy samples that reflect only the final stage of AD. Moreover, after the initial onset, different AD brain areas might become involved in the pathogenesis in each particular case. AD is frequently accompanied with other diseases such as vascular dementia. The occasional presence of Lewy bodies also suggests an overlap with protein changes in other diseases with AD.

The major goals in current AD proteomics research are: (a) the identification of biomarkers for early diagnosis, (b) to follow the progression of the disease and (c) the validation of new targets for the treatment of the disease. Since 2001, there has been an exponential increase in the number of publications reporting 2DE protein analysis in AD. The results have been excellently reviewed by Butterfield et al. (2003) and most recently by Sowell et al. (2009) and Korolainen et al. (2010). Sowell’s work focuses on the key proteins that are altered in animal models of AD, Korolainen’s article summarizes the results of clinical proteomics in AD.

Here we tried to summarize the results of AD proteomics comprehensively from the beginning.

4.2.1. Proteome changes during the progression of AD

There have been several attempts to study proteome changes in AD using different approaches in the last decade. Early AD proteomics was concerned with the analysis of clinical samples using high-throughput methods (Pasinetti and Ho, 2001; Davidsson and Sjogren, 2005; Marcotte et al., 2003; Fountoulakis, 2004). Lymphocytes were challenged as neural probes for AD-related metabolic changes in the brain (Gladkevich et al., 2004). The results of early neuroproteomic and proteomics-driven progress in neurodegeneration research have been reviewed by several authors (Ottens et al., 2006; Johnson et al., 2005; Fountoulakis and Kossida, 2006). The methods of clinical, structural and functional proteomics have been summarized from a mass spectrometric point of view by Drabik et al. (2007). Glycoproteomics, the analysis and quantification of glycoproteins, has been used as a new method for discovering diagnostic and/or prognostic markers of AD (Hwang et al., 2010). Recently, a short summary of the German Human Brain Proteome Project (HBPP) was published by Hamacher et al. (2008) and showed interesting results in AD research. The role of proteomics in gerontology, dementia and AD has been recently reviewed (Zellner et al., 2009; Kovacech et al., 2009; Taurines et al., 2010).

Taking a brief overview of AD-associated protein network processes, it is clear that oxidative stress is an early event in AD. Oxidative modifications of proteins can occur at the side chain of Met, His, Tyr and at a cysteine disulphide bond (Schulenborg et al., 2006). Oxidation catalysed by metal ions involves carbonyl groups in Lys, Arg, Pro and Thr residues in a site-specific manner. The carbonyl groups can be used to detect oxidized proteins by derivatisation with 2,4-dinitrophenylhydrazine (DNPH) prior to separation on a 2D-PAGE. The oxyblot technique, using an anti-DNPH–antibody for the detection of oxidized proteins in AD, has been successfully applied by Butterfield and Castegna (2003) and Butterfield and Sultana (2007). *Post-mortem* oxyblot analyses reveal that the overall level of oxidative damage of proteins, lipids and DNA is elevated in AD brains. In addition, ubiquitin–proteosome dysfunction has been implicated in the pathogenesis of AD, but the interplay between ubiquitination and AD is not fully understood (Oh et al., 2005; Bader et al., 2007).

One of the most important AD-associated protein networks is in the mitochondria. The proteome alterations in the mitochondrial membranes of an AD model rat (particularly the inner membrane with respiratory chain complexes) have been extensively studied (Dencher et al., 2007). Aβ and human amylin aggregate share a common toxic pathway via mitochondrial dysfunction (Lim et al., 2010) that increases the generation of reactive oxygen species (ROS). Sultana and Butterfield (2009) have used redox proteomics and found a number of oxidatively modified brain proteins directly in mitochondria. These results support the hypothesis that oxidatively modified proteins of the mitochondria might be involved in AD progression and pathogenesis (Sultana et al., 2007). The Butterfield group analysed the effect of Aβ-induced oxidative stress on brain protein modifications and Aβ neurotoxicity that led to MCI and AD pathogenesis (Sultana et al., 2009). Ding et al. (2007) revealed that oxidative damage potentially serves as an early event that then initiates the development of MCI and AD pathology. In a novel triple transgenic mouse model of AD (pR5/APP/PS2), a massive deregulation of proteins was found, and one third of these proteins were mitochondrial proteins mainly related to complexes I and IV of the oxidative phosphorylation cascade. The tau hyperphosphorylation deregulates complex I, but complex IV is deregulated by Aβ. This convergent action of Aβ and tau on mitochondria is an interesting molecular interaction in AD pathology (Eckert et al., 2010). Not only ROS, but also reactive nitrogen species (RNS), play a role in the pathogenesis of AD (Polidori et al., 2007). In the inferior parietal lobule of early AD patients, a recent qualitative study identified eight nitrated mitochondrial proteins as peroxiredoxin 2, triosephosphate isomerase, glutamate dehydrogenase, neuropolypeptide h3, phosphoglycerate mutase 1, H^+-transporting ATPase, α-enolase and 1,6-bisphosphate aldolase (Reed et al., 2009). Oxidative stress-induced DNA damage is not completely repaired in neurons and accumulates in the genes of synaptic proteins. This fact explains synaptic dysfunctions in AD (Forero et al., 2006).

Proteome maintenance and protein homeostasis (“taking care of proteins from the cradle to the grave”) is an important cell function. A progressive deterioration of the ability to preserve the stability of the cellular proteome by age can be observed even without any disease in the course of “normal” aging (Morimoto

and Cuervo, 2009). The ubiquitin system, a cellular protein quality control mechanism, preserves protein folding and performs proteolytic degradation of misfolded proteins. The enrichment of misfolded protein aggregations in the AD brain can be the result of a malfunctioning of the neuron-specific ubiquitin-recycling enzyme (Kawaja, 2005).

Most recently, the importance of intracellular Aβ in AD-related synaptic dysfunction and neuron loss was emphasized (Bayer and Wirths, 2010; Friedrich et al., 2010; Gouras et al., 2010). Interestingly, the accumulation of intracellular Aβ in the neurons of human AD brains correlates with the ApoE epsilon 4 genotype (Christensen et al., 2010). Granting that ApoE epsilon 4 is one of the main genetic risk factors of AD, an altered intracellular trafficking of Aβ could increase intraneuronal Aβ aggregation in AD brains. The accumulated intracellular Aβ generates a dysfunction and collapse of the endoplasmic reticulum in the AD brain (Lai et al., 2009). Aβ oligomers disrupt the anchor between the endoplasmic reticulum and microtubules, and thus they decrease the axonal transport of neurotrophins, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). This block of axonal transport contributes to neurodegeneration in AD (Wu et al., 2009). Further proteomic studies should be performed to resolve the controversies in this field by disclosing the protein network of axonal transport and its changes induced by Aβ.

The most complete summary of AD-associated proteome changes reviewed 93 proteins from 13 different brain regions (Korolainen et al., 2010). These proteins showed quantitative differences and/or post-translational modifications in cognitive impairment in early and late AD. Proteins are classified according to the three gene ontology (GO) categories as molecular function, cellular component and biological process. The protein list published by Korolainen contains 56 cytoplasmic, 28 mitochondrial, 20 nuclear and 16 cytosolic proteins. Many of these proteins play a role in oxidation–reduction (12), glycolysis (8), transport (8), metabolic processes (7), protein folding (6), the response to unfolded proteins (5) and cell proliferation (5). In addition, the protein list contains three synaptic proteins with altered expression or modification: synaptosomal-associated protein-25 (SNAP-25), synaptotagmin and syntaxin-binding protein. The general theory of AD suggests that it is a "protein conformational disease", so the relatively long and very diffuse list of AD-related proteins is in accordance with this theory.

4.2.2. Cerebrospinal fluid and plasma biomarkers for the diagnosis of AD

The introduction of novel therapies for AD treatment (Aβ-immunotherapies, secretase inhibitors, Aβ aggregation inhibitors) resulted in drug candidates and treatment strategies to slow down the progression of AD. These new drugs can be effective only in patients with the earliest stages of AD. There is a great need to diagnose AD patients in the pre-dementia stage (prodromal AD or MCI) or at the asymptomatic preclinical phase of AD. This need for early diagnosis and identification of disease-modifying drug candidates led to intensive research for biomarkers that reflect core elements of the disease process. Two biological fluids could be used in this biomarker search, cerebrospinal fluid and peripheral blood.

Plasma and serum biomarkers were first introduced by Irizarry (2004), but the method was not sensitive and specific for AD. Later, many efforts were done to find disease-specific plasma or serum biomarkers (Xiao et al., 2005; Sheta et al., 2006; Song et al., 2009), and several candidate blood biomarkers have been proposed, such as α_1-antitrypsin, complement factor H, α-2-macroglobulin, ApoJ and ApoA-1. However, verification of the results has been difficult by independent studies. The AddNeuroMed collaborative study focused their research on novel plasma biomarkers of AD (Lovestone et al., 2007) and identified AD-associated increases in plasma levels of complement factor H (CFH) and α-2-macroglobulin (A2M). Novel blood biomarkers were introduced by Ray; the application of combined multivariate analysis of 18 signalling and inflammatory proteins of the plasma of AD patients proved to be suitable for predicting the onset of the disease in MCI patients (Ray et al., 2007). Further large-scale testing studies are needed to prove whether this protein array is an optimal plasma biomarker set for diagnosing prodromal AD. Studies on large populations are necessary to introduce plasma biomarkers for AD into the medical practice and there are promising techniques being investigated in several laboratories.

Plasma Aβ was analysed as an AD biomarker in several studies but provided contradictory results. Some studies reported that high levels of plasma Aβ 1–42 or a large Aβ 1–42/Aβ 1–40 ratio were risk factors of AD. Others reported opposing data (Pomara et al., 2005; van Oijen et al., 2006; Graff-Radford et al., 2007). A serious difficulty in Aβ determination is the hydrophobic character of amyloid peptides; Aβ is "sticky" and binds to several plasma proteins resulting in epitope masking and other analytical problems.

4.2.2.1. CSF biomarkers. CSF analysis is a useful diagnostic tool for neurodegenerative diseases mainly because it reflects metabolic processes in the brain in a direct manner due to the free exchange of several molecules between the brain and CSF (Reiber and Peter, 2001). There are standard procedures for obtaining CSF by lumbar puncture and standardised methods for CSF sample handling. CSF biomarkers for AD can be divided into basic and core biomarkers. The core biomarker group has been developed to identify the central pathological processes of AD. CSF biomarker discovery has a long history (D'Ascenzo et al., 2005; Blennow, 2005; Romeo et al., 2005; Davidsson and Sjogren, 2005). According to Galasko (2005), CSF levels of Aβ, tau and phosphorylated tau meet the criteria for biomarkers in AD diagnosis. Further CSF measurements in independent laboratories have validated the importance of the Aβ, tau and phospho-tau (p-tau) levels (Shi et al., 2009; Portelius et al., 2008; Raedler and Wiedemann, 2006; Fonteh et al., 2006; de Jong et al., 2007; D'Aguanno et al., 2007; Lovestone et al., 2007; Roher et al., 2009). CSF Aβ, tau and p-tau biomarker analysis is an advancing tool for the diagnosis of dementia syndromes (Ward, 2007; Lewczuk and Wiltfang, 2008), although these analyses have severe limitations for early diagnosis of AD and differential diagnosis of dementias.

There are other efforts to find new diagnostic tools using CFS proteomics. The quantification of glycoproteins in AD-CSF (Hwang et al., 2009) and several reviews excellently summarize the latest advances in CSF (and plasma) biomarker research (Hampel et al., 2008; Blennow et al., 2010; Cedazo-Minguez and Winblad, 2009). CSF total tau (t-tau) levels reflect the intensity of neuronal and axonal degeneration and the extent of brain damage. AD progression is strictly followed by a CSF t-tau level increase. An increase in CSF t-tau concentration is also associated with a rapid conversion of MCI to AD and correlates with rapid cognitive decline and actual mortality risk in AD patients. The highest known CSF t-tau level was observed in neurodegenerative diseases with rapid neuronal loss (Creutzfeld–Jakob's disease). Thus in general, CFS t-tau concentration is an indicator of neurodegeneration intensity. CSF t-tau also correlates with the neurofibrillary tangle load obtained in *post-mortem* brain samples. CSF levels of phosphorylated tau (p-tau) reflect both the phosphorylation state of tau and the formation of neurofibrillary tangles in the brain. High CSF p-tau associates also with the rapid progression of MCI to AD and the rapid cognitive decline in AD. Data are accumulating about using p-tau level as a biomarker for the differentiation of AD from other forms of dementia (Hampel et al., 2004).

Aβ 1–42, the 42-amino acid form of Aβ, is an established marker of AD (Zetterberg et al., 2008), and can be used to identify AD in the early (MCI) stage with high accuracy. Interestingly, Aβ 1–42 levels in CSF decrease along the progression of AD. Aging and the presence of ApoE epsilon-4 allele accelerate the deposition of Aβ-42 in the brain and decrease the CSF level of Aβ-42 (Vuletic et al., 2008). The connection between high Aβ content in the brain and its low level in CSF is unclear. Perhaps the Aβ distribution is modulated by the oligomerisation of Aβ or the toxic activity of small oligomers. Aβ is not a highly specific biomarker of AD because increased CSF-tau and decreased Aβ concentrations were observed in other neurodegenerative disorders not only in AD (Jellinger et al., 2008).

In conclusion, there is no specific and validated CSF and blood biomarker of AD that sufficiently differentiates AD from other forms of dementia and other neurodegenerative diseases. However, the levels of t-tau, p-tau and Aβ 1–42 are valuable markers for helping the diagnosis of AD. The development of more specific diagnostic tools for AD is still a high need, although recent studies using CSF tau phosphorylated at threonine-181 (P-tau$_{181}$) demonstrate an improved discrimination between autopsy-confirmed AD and non-AD dementias (Koopman et al., 2009).

4.2.3. Proteomics in tissue culture and animal models of AD

Drug discovery research for AD uses cell cultures and animal models, but none of these models is a perfect equivalent of human AD. The animal models of AD reproduce one or more aspects of the human disease and, therefore, they can be used for the study of a certain part of AD pathological mechanism.

Several different cell lines (e.g., SH-SY5Y human neuroblastoma line) have been used in AD research. Proteomic studies were performed on P301L tau overexpressing cells after treatment with Aβ 1–42 fibrils (David et al., 2006) to investigate the mechanisms of neurite disruption induced by Aβ 1–42 in the presence of tau aggregates. This cell model unifies both Aβ and tau-pathology. Aβ-induced alterations of the cellular proteome were observed in metabolic processes-associated proteins, the stress response and cell signalling proteins. Western blot studies validated that 72% of the protein changes in cell cultures were also altered in the human AD brain. Particularly, the stress-related unfolded proteins were validated in the human brain to a large extent. A combined transcriptomic and proteomic analysis was performed to measure the effect of intracellular Aβ peptide accumulation in human neuroblastoma (SH-SY5Y) cells (Uhrig et al., 2008). An increasing Aβ 1–42/Aβ 1–40 ratio elevated the cellular retinoic acid binding protein (CRAB I) level, which reduced the differentiation potential of the SH-SY5Y cell line but increased cell proliferation.

A great variety of transgenic animal models of AD have been developed during the last decade (Götz et al., 2004; Hoerndli et al., 2005). These models show the key histopathological features of human AD (amyloid plaques and NFT) without serious cell loss. Thus, many researchers emphasize that the available animal models of AD are rather models of mild cognitive impairment and early AD.

As for tau-pathology, proteomic studies were done on P301L mutant tau over-expressing transgenic mice showing NFT formation. The altered proteins were clustered into mitochondrial proteins, antioxidant enzymes and synaptic proteins in P301L tau mice (David et al., 2005). In the proteome of P301L mice brain, several metabolic-related proteins, including mitochondrial respiratory chain complex component, antioxidant enzymes and synaptic proteins, are changed. Functional analysis demonstrated mitochondrial dysfunction associated with a reduced NADH-ubiquinone oxidoreductase activity that resulted in impaired mitochondrial respiration and ATP synthesis. Mitochondrial dysfunction induced higher levels of reactive oxygen species. It was concluded that tau pathology in P301L mice involves reduced mitochondrial metabolism and an oxidative stress disorder. Mitochondria also showed an increased vulnerability to Aβ peptide insults (David et al., 2005).

Proteomic analysis was performed on APP, tau and PS-1 over-expressing triple-transgenic mouse (an animal model both of Aβ and tau-pathology) using the iTRAQ method (Martin et al., 2008). Cortex and hippocampus tissue samples of 16 month-old mice were studied. Proteins participating in synaptic plasticity, neurite growth and microtubule dynamics were changed. In another triple transgenic AD mouse model (combining both Aβ and tau-pathology) Aβ and tau synergistically impaired the oxidative phosphorylation system perishing mitochondria (Rhein et al., 2009). Another novel mouse model having mutant ubiquitin (UBB+1, a model of false proteosome activity of AD) was analysed using proteomic methods (Hamacher et al., 2008). Proteomic studies were also conducted on different animal models, including APP/PS1 mouse, human P301L tau and GSKβ tau mouse, ApoE epsilon 4 mouse, senescence-accelerated-prone mouse, Aβ-injected-rat models, transgenic rats expressing Swedish mutant APP and *Caenorhabditis elegans* expressing human Aβ 1–42. The results of these proteomic studies on different animal models of AD are excellently overviewed by Sowell et al. (2009).

The final summary of proteomic studies on animal models of AD clearly demonstrated a common feature of all models; proteins involved in energy metabolism, antioxidant defence and cytoskeletal structural integrity were changed. This suggests that AD has a strong metabolic and cellular reorganization feature that can modulate synaptic functions to cause memory impairment as we have already pointed out.

5. An integrating principle of AD proteomic results: the systems biology model of the synapse

The most striking symptom of AD is memory loss, but memory loss is observed in conjunction with all neurodegenerative disorders making it often difficult to separate AD from other brain diseases in practice. On the other hand, AD has a widespread but fairly characteristic molecular mechanism, as we already summarized, that suggests an AD-specific form of memory loss. The recent understanding of memory formation in the brain tells that the memory trace inscription and recovery is based on molecular rearrangements in synapses that change synaptic efficiency and, in turn, the network properties of neurons that are summarized by Agnati et al. (2004). The first clinically detectable functional change in AD is memory impairment, but it is often retrospectively diagnosed. However, proteomic studies on human AD brain samples and animal models reveal that the earliest change in cell functions in AD is probably a mitochondrial metabolism alteration induced by intracellular Aβ (see above). The recent understanding of synaptic transmission control by the molecular tuning of synaptic protein–lipid assemblies and protein network processes influencing synaptic plasticity provide a novel integrating principle for unifying AD proteomic data synaptic plasticity and the resulting speeding of memory impairment. The systems biology of the synapse can elucidate how mitochondrial, cytoskeletal and other metabolic processes can tune synapses in AD so that they became unable to serve memory encoding and memory recall to a rapidly increasing extent.

The developmental and molecular plasticity of the synapse is a sophisticated and very similar biological process involving protein–protein interactions resulting from functional protein assemblies from more than 1000 different protein components and is nicely described in a review by McAllister (2007). Recent results suggest that the synaptic protein assembly is under the influence of nuclear gene transcription, local gene transcription at the

synapse, mitochondrial metabolism, protein trafficking, changes in the cytoskeleton, protein scaffolds, signalling system, lipid synthesis, lipid protein interactions, inducible proteases, etc. Misfolded proteins in AD interact with such a complex molecular system to induce memory loss.

5.1. Metabolic and structural components of neuronal protein network could change synaptic events

Neurons dedicate a considerably large fraction of energy production to membrane potential maintenance (de Castro et al., 2010). Synaptic activity, as the most dynamic membrane event, is the highest energy consuming process in the brain, which is indicated by the fact that mitochondria are frequently close to the synaptic region. Synaptic mitochondria could control electric events in the synapse by mitochondrial potential and also by ATP production and/or calcium release (Ly and Verstreken, 2006). Neurons, however, cannot process glucose efficiently because of the low concentration of glucose phosphorylation enzymes. So glial cells provide pyruvate and lactate for neuronal energy production (Barros and Deitmer, 2009) and glial and neuronal metabolism are tightly coupled (Benarroch, 2010). It is the molecular basis of applicability of metabolic imaging for the study of brain functions (Dienel and Cruz, 2008). It is evident that synaptic and metabolic activity in the nervous system changes in parallel in many cases. Also, it is clear that metabolic changes in neurons and glial cells can control synaptic events. The mitochondrial control of synaptic function could link mitochondrial effects of AD to the memory impairment process. It suggests that the extensive alteration of mitochondrial metabolism and production of free radicals induce the self-destruction of neurons via the activation of cell death mechanisms on the one hand and, on the other hand, even a mild change in mitochondrial processes close to synapses may alter synaptic function and impair memory. Synaptic mitochondria proved to be more susceptible to Aβ-induced damage than other mitochondria in an AD-mouse model (Du et al., 2010).

The fast neurotransmission-dependent electrical activity in a synapse is reflected by the signalling system terminating on gene transcription regulation. The signalling system activation has a pivotal role in long-term memory trace formation (Kotaleski and Blackwell, 2010), and neuronal development (Denham and Dottori, 2009). Synaptically initiated gene transcription changes influence protein synthesis at the synaptic region and elsewhere in the neurons (Tang and Schuman, 2002; Andreassi and Riccio, 2009). Also, neurotransmitter receptors are present on glial cells and the transmitter spill-over from the synapse can induce gene transcription changes in the glial cells via glial signalling systems (Vijayaraghavan, 2009). Evidently, the protein synthesis is slow and runs on a time scale of minutes and hours. So the rearrangement of the synaptic proteome by *de novo* protein synthesis results in a delayed and sustained molecular tuning of the synapse for the long term. The rapid changes serve as an inscription of an engram of the memory trace and are possibly based on the dislocation of certain proteins and fast post-translational modifications.

The systems biology model of the synapse tells that the modulation of the synaptic protein network correlates with changes in the whole cellular proteome in either neurons or glial cells. Not only altered synaptic proteins can influence memory functions, but also many other cellular processes, like glucose metabolism, lipid and protein synthesis, also control the tuning of the synaptic molecular "engine". This means that the cellular proteome extensively reflects the synaptic activity and vice versa. Small enough aggregates of Aβ, which are able to expose the synaptic molecular machine, can directly influence the synaptic strength and memory. However, those aggregates bind to proteins elsewhere in the neuronal protein networks and may also alter synaptic activity in a secondary manner.

5.2. Systems biology model of the synapse: substrate of interpretation of proteomic data

Functionally, the synapse is an integrated protein–lipid molecular machine performing communication between two nerve cells when it is triggered by an action potential. In a classical sense, the synapse has two separate compartments, the pre- and post-synapse, connected together by cell adhesion molecules in a synaptic gap. The efficiency of influencing the post-synapse by the pre-synapse can be called the actual "weight" of a particular synapse on the synaptic set of a neuron. The generally accepted idea is that the "weight" of a synaptic influence on the postsynaptic cell varies depending on the transmitted action potential frequency (Thomson, 1998). Data accumulating in the past decade supports that the synapse is not a compartmentalized anatomical structure but it is one integrated protein network of the three classical anatomical compartments (Kotaleski and Blackwell, 2010). The synaptic molecular machine is built up from about a thousand different proteins and half of the synaptic proteins are exclusively present in the synapses. The others can also be found in different compartments of nerve cells (Bai and Witzmann, 2007). The synaptic protein network can be divided to sub-networks for a better understanding, but it has to be emphasized that, functionally, the synapse is one molecular machine. One of the well-studied functional synaptic sub-networks is the NMDA receptor-coupled protein network (Husi and Grant, 2001). For practical reasons, we can imagine the synapse as a super-assembly of synaptic proteins composed of several elementary functional building-blocks like vesicles, vesicle trafficking system, signalling systems, synaptic scaffold, synaptic densities, etc. (Pocklington et al., 2006). The elementary functional molecular assemblies are moved by motor proteins like actin (Cai and Sheng, 2009) and anchored by PZD and SH3 domain-containing proteins like postsynaptic density 95 protein (PSD95) and others, such as sin3 associated polypeptide (SAP25), clathrin and coronine (Kim and Sheng, 2004). In turn, the molecular machine of the synapse is a fluently tuned, flexible and rapidly responding 3D protein and lipid based assembly that has certain flexibility but a certain degree of rigidity. The flexibility of a synaptic molecular network is properly demonstrated by synaptic molecular rearrangements under long-term potentiation or other plasticity based events commonly accepted as a molecular substrate of memory trace inscription (Miyamoto, 2006). The synaptic rigidity is shown by the uniform anatomical picture of the synapse and is easily recognizable by electron microscopy, indicating that the binding interactions of synaptic proteins and lipids results in a fairly uniform morphology of synapses and forms the molecular base of recallable long-term memory trace (Bennett, 1999). The molecular kinetic and static processes of the synapse are regarded as the substrate of long-term memory (Kotaleski and Blackwell, 2010) and targets of the early effects of Aβ on memory as well (Nimmrich and Ebert, 2009).

The classical synapse model suggests that the synapse is a converter machine that changes action potential frequency to released transmitter concentrations. Molecular movements in a complex geometry space like the synapse, are controlled by the spatial positioning of interacting molecular assemblies (Tolle and Le Novere, 2010). The migration and the number of released transmitter molecules are limited, and the typical numbers of receptors is less than a hundred (Bevan et al., 1969; Kaatze, 1990; Masugi-Tokita et al., 2007). Thus, it is obvious that the free collision of transmitters and receptors could not happen. In addition, there

is hard evidence and an excellent summary of data supporting the idea that if a receptor faces a release pore, it has very high probability of transmitter capture. Due to these facts, transmitter–receptor interactions can be tuned by cell adhesion molecules and the positioning of the receptors and release pores (Grant, 2003; Gerrow and El-Husseini, 2006; Granseth et al., 2007; Sorensen, 2009). The postsynaptic receptors are positioned by a special part of the post-synapse called postsynaptic density (PSD). PSD anchors receptors, ion channels and scaffold proteins and holds signalling system elements. PSD is organized by the PSD95 protein, which is a 95 kDa PZD domain-containing protein (Feng and Zhang, 2009), and links together several proteins having a PZD or SH3 domain binding site. The distribution and number of PSD95 proteins is changing in many neuro-proteomic studies (Bai and Witzmann, 2007), indicating that it is a flexibly changing and functionally important element of the PSD matrix.

One of the other interesting and rapidly growing fields of research is how inducible extracellular proteases can temporarily modulate synaptic transmission via the degradation of extracellular matrix proteins in the synapse. There is a considerable literature about MMP9, an inducible matrix metalloproteinase synthesized in the vicinity of synapses and activated by strong stimuli-induced synaptic plasticity. Data accumulating about MMP9 function in the synapse suggest that the shaping of the synaptic gap proteome is performed not only by the synthesis but also by the destruction of proteins (Michaluk et al., 2007). Recently, the modulation of synaptic transmission by proteolysis is a cutting edge field of research and the literature about brain proteases is rapidly increasing (Lee et al., 2008).

The systems biology model of the synapse can be summarized so that the synapse is a flexible and rapidly changing protein–lipid assembly of more than a thousand different proteins specialized for communication. The synapse has rigidity to some extent as shown by electron microscopy. Also, the synapse has motility mainly performed by synaptic actin–myosin motors (Cai and Sheng, 2009). Synapses can move from one place to another and synaptic sliding has been proposed in the formation of memory traces (McKinney, 2005). In the synapse, receptor–transmitter binding efficiency is controlled by the relative positioning of release sites and the receptors in the synaptic molecular assembly. Thus, any molecular changes altering the flexibility or changing the actually existing spatial structure of the synaptic molecular machine can modify synaptic function and, in turn, the "weight" of a certain synapse on postsynaptic cell activity. Thus, the synapse is not an automatic converter of action potential frequency to transmitter concentration. The transmission efficacy is adjusted continuously by former synaptic events via the rearrangement of the synaptic molecular machine to reflect previous experiences. This fast and continuous synaptic molecular adjustment is the substrate of memory encoding. As we have already described, Aβ is an unspecific "glue" capable of clamping together the flexible molecular elements in the mitochondria and synapse. Therefore, several molecular processes leading to AD or AD-like symptoms could result in the same final effect: the decrease of molecular plasticity in the synapse resulting in a rapidly developing memory impairment. In fact, one of the earliest effects of Aβ is the decrease in the size of synapses and the disappearance synapses from dendritic spines (Smith et al., 2009).

6. Conclusion: systems biology model of AD

An introduction of a systems biology approach in AD research initiated an essential change in the way of thinking about AD. The "molecular machine" model of the synapse and the "molecular tuning" principle of synaptic plasticity clearly demonstrated that the molecular mechanism of memory trace inscription and recall is embedded into the complete protein interaction network of neurons. The systems biology model of synaptic plasticity could integrate the seemingly diverse molecular processes of AD to explain them or any combination of them, which result in memory impairments at the beginning of the disease without a severe loss of neurons. It can explain how memory is directly controlled by mitochondrial metabolism, movements and the organization of the cytoskeleton and all other biological processes that keep neurons alive (Fig. 1).

The proteomic analysis of the AD brains of humans and rodent models of AD (Table 1) revealed that the molecular mechanism of AD includes several processes: mitochondrial metabolism decrease, elevated production of reactive oxygen species, oxidation of proteins and DNA, changes in cytoskeletal proteins, decreased trafficking of proteins on axons and dendrites, a widespread change in synaptic protein machinery of transmission, elevated production of misfolded proteins and their aggregation, tau hyperphosphorylation, formation of fibrillar protein aggregates and cellular stress induced by aggregates, changes in ubiquitination and the degradation of erroneous proteins. Several combina-

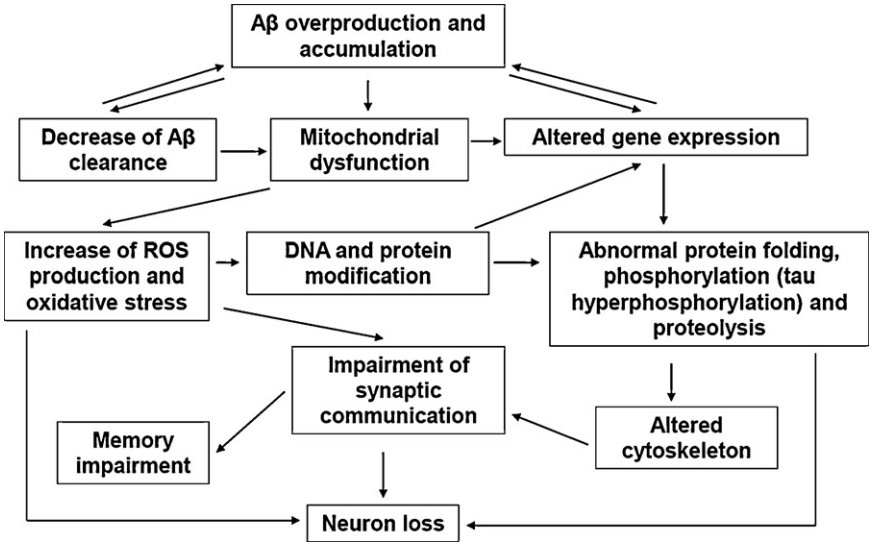

Fig. 1. A scheme of functional interplay of cellular protein networks of neurons underlying pathological changes leading to AD.

Table 1
Overlapping brain proteome changes in human samples and animal models of Alzheimer's diseases. Compilation was based on the protein lists published by Sowell et al. (2009) and Korolainen et al. (2010).

Main biological function/protein name
Cytoskeleton organization
α-Internexin
β-Actin
Fascin
Glial fibrillary acidic protein
Profilin
Tubulin α-chain
Tubulin β-chain
Energy metabolism
α-Enolase
γ-Enolase
Aconitase
Aldolase
ATP synthase α chain
Creatine kinase β chain
Glyceraldehide-3-phosphate dehydrogenase
Isocitrate dehydrogenase
Lactate dehydrogenase
Malate dehydrogenase
NADH ubiquinone oxidoreductase 24-kDa subunit
Phosphoglycerate mutase 1
Pyruvate kinase
Response to oxidative stress/cell redox homeostasis
Glutathione-s-transferase
Peroxiredoxin 2
Peroxiredoxin 3
Peroxiredoxin 6
Chaperones
Heat shock protein 60
Heat shock protein 70
Heat shock protein 90
Synaptic integrity
Dihydropyrimidase like-2
Neurofilament triplet L protein
Synaptotagmin I
Others
Glutamine synthetase
Carbonic anhydrase II
Vesicular fusion protein
β-Synuclein
Hemoglobin α
Nucleoside diphosphate kinase

tions of the above-listed processes might be involved in different neurodegenerative diseases making it difficult to use any of them as specific biomarkers for AD. Oxidative stress is one of the cardinal factors most frequently associated with AD. Recently it was demonstrated that oxidative stress promotes JNK-dependent amyloidogenic processing of normally expressed human APP by differential modification of α-, β- and γ-secretase expression (Qiuroz-Baez et al., 2009). α-Secretase down-regulation and β- and γ-secretase up-regulation results in Aβ generation. Oxidative stress shows good correlation with mitochondrial dysfunction and behavioural changes in a prodromal animal model of AD (Strazielle et al., 2009). Mitochondrial dysfunction proved to be a hallmark of Aβ-induced neuronal toxicity and prevention of mitochondrial dysfunction (e.g., by humanin) attenuates neuronal cell injury (Jin et al., 2010).

Dysfunction of neuronal network may play decisive role in AD progress. Intrinsic neuronal excitability, mainly through profound dysregulation of calcium homeostasis is largely affected (Santos et al., 2010). As a consequence, neuronal communication is disturbed causing cognitive manifestations in cognitive impairment (concept of disconnection syndrome in AD). Observations performed by PET and other imaging studies support this concept (Gulyás et al., 2010).

Overlapping proteome changes of human AD brain samples and animal models of AD shown in Table 1 might be the most important indicators in AD progression. These proteins belong to five important functional groups: (1) cytoskeleton organization; (2) energy production, metabolism; (3) redox homeostasis, antioxidant proteins; (4) chaperones; (5) synaptic integrity. Proteins belonging to these functional groups play a pivotal role in normal function of neurones. Changes in these subsets of the brain proteome may lead dramatic and irreversible destruction of the action of nerve cells and subsequent neurotransmission.

It seems highly possible that a spatio-temporal organized interplay of several disclosed mechanisms results in AD in a particular case. The proteomics of AD confirmed the fact that such a complex interacting network of functional changes in nerve cells impairs memory via the mistuning of synapses and the changing of the input–output properties of neuronal networks by permanently altering synaptic connection strengths. On the other hand, the systems biology of AD revealed that Aβ overproduction and the concomitant conformational change and oligomerisation of Aβ is an essential component of the development of AD from MCI. Aβ induces a complex cellular response and the corresponding decrease in mitochondrial energy production impairs memory first then finally kills the cells. Also, a decreased energy production in mitochondria can trigger Aβ overproduction. This sophisticated, high complexity pathological mechanism has changed the medical treatment strategy for AD. Novel drug discovery efforts for AD are focusing on complex molecular treatment strategies, seeking non-receptor drug sets against AD. As for the early diagnosis of AD, the systems biology of AD clearly demonstrated that combinations of several biomarkers can improve AD diagnosis. Biomarkers in the CSF and blood are not enough. The application of systems biology in AD research resulted in a promising new way of thinking about AD and resulted in a large body of novel evidences about the changing molecular mechanisms and complex interacting protein networks leading to AD.

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