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Proteomic Biomarkers in Alzheimer's disease

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Alzheimer's Disease: Proteomic Biomarkers

Alzheimer's disease (AD) is a major problem of health in developed countries. From a pathogenic perspective, this prevalent form of dementia is a polygenic/complex disorder in which over 600 defective genes distributed across the human genome may be involved, in conjunction with epigenetic aberrations and diverse environmental risk factors [1]. The abnormal expression of genes and subsequent post-transcriptional modifications may lead to changes in proteins, either conformational, mutant forms or quantitative variants. Pathognomonic hallmarks are represented by the accumulation of extracellular β-amyloid protein (Aβ) in senile plaques and intracellular hyperphosphorylated tau protein in neurofibrillary tangles, which have been used as potential biomarkers for AD in brain tissue and body fluids (plasma, cerebrospinal fluid) [2-4]. These and other still undefined neuropathological hallmarks may be expressed in brain tissue and body fluids 2-3 decades prior to the onset of the disease. Consequently, the identification of predictive biomarkers is of paramount importance for the implementation of preventive programs in the population at risk [1,5,6]. Furthermore, at present, the clinical diagnosis of AD is not an easy task, and there is a great interest in developing specific, sensitive, and practical tools to differentially diagnose and discriminate the different types of dementia [7].

During the past decade, a great effort has been made to identify proteomic biomarkers for AD with still poor results. Over 1,000 proteins have been identified to be differentially expressed in proteomic analyses of AD cases; however, interpretation of results is difficult, and a direct connection between specific proteomic profiles and AD pathogenesis is still undefined [8]. Similarly, several epigenetic signatures (DNA methylation, histone modifications, miRNA dysregulation) have been reported as diagnostic aids with still unclear consequences [9,10]. Therefore, AD biomarkers are urgently needed for both early and accurate diagnosis and prediction of disease progression [11], as well as for monitoring treatment [1,12].

The abnormal expression of proteins in AD brains is a frequent finding. Some of these proteins might represent novel markers for AD neuropathology; however, in most studies, similar anomalies in protein expression are currently seen in different neurodegenerative disorders [13,14]. In AD, Aβ interacts with a variety of Aβ-associated proteins, some of which can form complexes with Aβ and influence its clearance, aggregation or toxicity. The secreted Wnt pathway protein Dickkopf-related protein 3 (Dkk-3) is a potential Aβ-associated protein. Dkk-3 co-localizes with Aβ in the brain, is expressed in neurons and in blood vessel walls, is secreted by leptomeningeal smooth muscle cells in vitro, and is abundantly present in both cerebrospinal fluid and serum, but its levels are similar in non-demented controls and patients with AD, Lewy body dementia, and frontotemporal dementia [15]. In a study of the human brain-insoluble proteome in AD by mass spectrometry, 4,216 proteins have been identified, among which 36 proteins accumulate in the disease, including U1-70K and other U1 small nuclear ribonucleoprotein (U1 snRNP) spliceosome components. Similar accumulations in mild cognitive impairment cases indicate that spliceosome changes occur in early stages of AD. A dysregulated RNA processing with accumulation of unspliced RNA species, including myc box-dependent-interacting protein 1, clusterin, and presenilin-1, occurs in AD. U1-70K knockdown or antisense oligonucleotide inhibition of U1 snRNP increases the protein level of amyloid precursor protein (APP), indicating U1 snRNP pathology and abnormal RNA splicing in AD pathogenesis [16]. Brain tissue from diabetic patients with cerebrovascular dementia or AD contains significant deposits of oligomerized amylin [17]. Visinin-like protein 1, RUFY3 protein, and Copine 6 are abnormally expressed in the olfactory bulb of autopsy cases with AD, Lewy body disease, frontotemporal lobar degeneration, and mixed dementia; and only dipeptidyl aminopeptidase-like protein 6 shows a specific down-regulation in AD [18].

Changes in phosphorylation levels were found in 19 proteins involved in energy metabolism, neuronal plasticity, signal transduction, and oxidative stress response in the parietal cortex of AD patients at different stages of dementia [19]. Histone post-translational modifications have been found in the frontal cortex of AD patients, affecting methylation of H2B K108, methylation of H4 R55, and ubiquitination of H2B K120 [20]. Novel techniques have been developed for proteomic bioanalysis of microdissected cells; 400 proteins have been identified in microdissected neurons, 50% of which were associated with AD [21].

The expression levels of DCC-interacting protein 13-beta, serum albumin, creatine kinase B-type, heat shock 70 kDa protein 1A, T-complex protein 1 subunit beta, adenylate kinase isoenzyme 1, pyruvate dehydrogenase E1 component subunit beta mitochondrial, and V-type proton ATPase catalytic subunit A have been found to be 1.5 times higher in transgenic AβPPswe/PS1dE9 mice which express a chimeric mouse/human amyloid-β protein precursor (Mo/HuAβPP695swe) and mutant human presenilin 1 (PS1-dE9) associated with early-onset AD; in contrast, the expression levels of dihydropyrimidinase-related protein 2, actin cytoplasmic 2, isoform 1 of V-type proton ATPase catalytic subunit, tubulin alpha-1C chain, F-actin-capping protein subunit alpha-2, ubiquitin carboxyl-terminal hydrolase isozyme L1, and actin cytoplasmic 1 were lower in the transgenic model. These proteins are involved in cytoskeletal structure, energy metabolism, synaptic components, and protein degradation [22]. Using a redox-proteomic approach, 12 proteins involved in energy metabolism, protein folding, cell structure, signal transduction and excitotoxicity were found to be significantly altered in the levels of protein carbonyls in the hippocampus of 3xTg-AD mice before the appearance of Aβ plaques and neurofibrillary tangles. Alpha-enolase

and glutamine synthetase were identified as the common targets of oxidation in the brains of 3 × Tg-AD mice, mild cognitive impairment (MCI) sufferers and AD patients. The oxidation of t-complex protein 1 subunit epsilon and protein disulfide-isomerase A3 was reported to be associated with AD [23].

Abnormal processing of tau protein is central to the etiology of tauopathies (AD, frontotemporal dementia, progressive supranuclear palsy, post-traumatic dementia) [13]. Tau protein associates with the ribonucleoproteome, including major protein complexes involved in RNA processing and translation, and binds to several heat shock proteins, the proteasome- and microtubule- associated proteins. Expression of P301L mutant tau disrupts interactions of the C-terminal half of tau with heat shock proteins and the proteasome. The higher propensity of P301L mutant tau to aggregate may reflect a perturbation of its chaperone-assisted stabilization and proteasome-dependent degradation [24]. MAP2c prevents arachidonic acid-induced in vitro aggregation of tau. MAP2c possesses chaperone-like activity while tau does not. Phosphorylation impairs the chaperone activity of MAP2c, implying a crucial role of chaperone in preventing tau fibrillation. MAP2c/MAP2 might be one of the regulators maintaining tau homeostasis in the cell [25]. The tau N-terminal domain may play a direct role in the regulation of microtubule stabilization, since the Gln124-tau fragment displays a strong ability to bind and stabilize microtubules [26].

Novel markers associated with major AD neuropathological hallmarks have been reported, including CSF oxidative stress signatures [27,28], heat-shock proteins [29], markers of the ubiquitin-proteasome system (UPS) [30], and neuroimmune biomarkers [31].

The measurement of plasma Aβ levels has been used by researchers for years, with contradictory results. This failure might be attributed to the heterogeneity of the disease, deficient patient recruitment, non-specificity of peripheral Aβ levels as an AD biomarker, and the non-recognition that Aβ levels are genotype-dependent [32,33]. For the past 5 years some new biomarkers have been postulated as potential diagnostic and/or prognostic candidates for AD. Among peripheral candidate proteins, most AD-related proteins reported in the literature were not specific and were found to be affected by other brain disorders [34,35]. Several proteins associated with brain atrophy and cognitive decline have been identified in plasma, including the following: A1AT, alpha 1 antitrypsin; A1M, α-1-microglobulin; ApoA1, apolipoprotein A1; ApoA2, apolipoprotein A-2; ApoB, apolipoprotein B; ApoC3, apolipoprotein C3; ApoC4, apolipoprotein C4; ApoE, apolipoprotein E; ApoH, apolipoprotein-H; Aβ40, amyloid beta 1-40; BDNF, brain-derived neurotrophic factor; C3, complement C3; C3a, complement C3a; C4a, complement component C4a; C8, complement C8; CF1, complement factor-I; CgA, chromogranin A; Chk2, serine/threonine-protein kinase Chk2; FGG, γ-fibrinogen; G-CSF, granulocyte-colonystimulating factor; ICAM, intercellular adhesion molecule; IFN-γ, interferon-gamma; IGFBP2, insulin-like growth factor binding protein 2; IL-10, interleukin-10; IL-13, interleukin-13; IL-1ra, interleukin 1 receptor antagonist; IL-2, interleukin-2; IL-4, interleukin-4; IL-6, interleukin-6; MIP1α, macrophage inhibitory protein 1α; NAP2, nucleosome assembly protein 2; NCAM, neural cell adhesion molecule; NSE, neuron-specific enolase; PDGF, platelet-derived growth factor; PPY, pancreatic polypeptide; PSA-ACT, prostate-specific antigen complexed to α1-antichymotrypsin; RANTES, regulated on activation normal T cell expressed and secreted; SAP, serum amyloid-P; sRAGE, soluble receptor for advanced glycation end products; TNF-α, tumor necrosis factor alpha; and TTR, transthyretin [35].

A novel serum proteomic approach to interrogate the low-molecular weight proteome for serum AD found 59 novel potential AD biomarkers, 4 of which showed diagnostic replicability. These biomarkers were characterized as 602.3 (amino acid sequence L/IAENR), 804.55 (a phosphatidylcholine with two fatty-acid side chains), 874.6 (a peroxidated phosphatidylcholine), and biomarker 804.53 (an oxidized glycerophosphatidylcholine ($C_{42}H_{78}NO_{11}P+H^+$) with 3 primary possible structures: 1-palmitoyl-2-(14,15-di-hydroxy9-keto-10, 12-octadecadienoyl) glycerophosphatidyl choline; 1-palmitoyl-2-(13,14-di-hydroxy-10-keto-8, 11-octadecadienoyl) glycerophosphatidyl choline; or 1-palmitoyl-2-(13-hydroperoxy-8-keto-9, 11octadecadienoyl)glycerophosphatidylcholine) [36].

Panel-based proteomics on plasma samples from Twins-UK subjects revealed that genetic factors explain ~26% of the variability in blood protein levels on average. The plasma level of the mitogen-activated protein kinase (MAPK) MAPKAPK5 protein was found to positively associate with the 10-year change, and MAP2K4 was found to associate negatively with the volume of the left entorhinal cortex [37].

Multiple markers were identified to be differentially expressed in the cerebrospinal fluid (CSF) of AD patients as compared with control subjects. The currently available cerebrospinal fluid (CSF) biomarkers for AD (Aβ, total Tau (t-Tau), phosphorylated Tau (p-Tau)) have a high sensitivity and specificity for AD, but there is still no test to effectively predict the development of AD in a pre-symptomatic stage [2,3,38,39]; however, measurement of Aβ1-42 and tau in CSF is a valuable marker in AD research, where low levels of Aβ and high levels of tau indicate AD [2,3,35]. In addition, CSF and plasma levels of Aβ are closely associated with APOE genotypes, with APOE-4/4 carriers exhibiting the lowest Aβ levels [1,12,32,33].

Neuronal secretory protein VGF and neuronal pentraxin receptor-1 (NPTXR) are decreased in AD (at baseline, 21% and 17%, respectively), with a decreased rate/year of 10.9% and 6.9%, respectively [40]. Thirty-eight proteins show abnormal expression in Lewy body dementia CSF by proteomic profiling; 4 proteins (ectonucleotide pyrophosphatase/phosphodiesterase 2, lysosome-associated membrane protein 1, pro-orexin, transthyretin) are specific for the identification of AD from LBD; 9 proteins (4 novel: malate dehydrogenase 1, serum amyloid A4, GM2-activator protein, and prosaposin) are elevated in both AD and LBD versus controls [41]. Patients with mild cognitive impairment (MCI) who are converted into AD cases have an abnormal CSF glycosylation profile. CSF glycosylation changes may occur before the onset of the disease. Glycosyltransferase GnT-III might be involved in AD inducing specific sugar modifications in the BACE-1 glycoprotein [42].

From the vast majority of the studies reported so far, some conclusions can be drawn:

(i) Less than 30% of AD cases can be accurately diagnosed with genomic and/or proteomic biomarkers; (ii) a major problem with most proteomic analyses is the coexistence of similar findings in different forms of neurodegenerative disorders; (iii) most proteomic analyses with positive results as potential AD biomarkers need further validation and replicability; (iv) although the most accepted proteomic biomarker for AD is the analysis of CSF Aβ and tau, with decreased levels of Aβ and increased levels of tau reflecting AD, its clinical utility in elderly subjects is limited by the invasive nature of lumbar puncture; (v) attention should be paid to the standardization of measurement of

AD biomarkers due to large inter-laboratory variation; (vi) CSF and/or plasma Aβ are APOE-dependent; (vii) from a practical perspective, blood-based biomarkers for AD represent a very attractive option; however, the complex composition of blood may pose technical challenges for an efficient identification of reliable biomarkers; (viii) the identification of a universal biomarker for AD is highly unlikely; and (ix) since protein expression depends on the genomic background of each patient, proteomic signatures should be correlated with the genomic profiles of the patients.

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