Brain site-specific protein deamidation in Alzheimer's disease

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Research question and background

Alzheimer's disease (AD) is the common age related neurodegenerative disorder characterized by memory loss. It has been hypothesized that the accumulation of the beta-amyloid protein as a major cause of the pathogenesis of AD. Several researchers emphasized neurofibrillary tangles (NTFs) containing phosphorylated *tau,* synaptic and neuronal loss as a cause of pathogenesis of AD. Although plaques and NFTs are pathogenic but it would be misleading to craft the impression that these are the only main causes of AD. Since our group developed proteomic method to study post translational modification (deamidation, glycosylation, phosphorylation etc.), we would like to apply this novel technique to find global brain protein deamidation and investigate its role in neurodegenerative disease. Further to understand the molecular pathways by which the different pathological changes compromise neuronal function, their integrity, and related clinical symptoms, we applied quantitative proteomics technology to profile quantitative expression of proteins in AD patients and their age matched control.

Methods and tissues used

Protein quantification through the incorporation of stable isotopes has become a fundamental technique in modern proteomics research that made it possible to explore global relative quantitation of proteins across various biological samples in a single experiment. Taking an advantage of this technique and to elucidate the perturbed pathways contributing to pathophysiology of AD, iTRAQ-based quantitative proteomics methodology was applied on brain samples to profile relative protein expression in young and old female AD brain samples. Thus, in this experiment, we used brain samples from young AD and old AD patients and their age matched controls. Frozen brain samples of the medial frontal gyrus and medial temporalis gyrus of AD subjects and age matched control samples were available (40 samples), hence, in another experiment, we used Tandem Mass Tag (TMT) 10-plex reagent that allowed us to analyze and compare ten different AD samples in single LC-MS/MS experiment. While in our third experiment we applied our optimized protocol to study post translational modifications of the proteins from all AD brain samples.

Results and conclusion

The preliminary iTRAQ quantitation data analysis revealed quantification of 4600 proteins with 95% confidence level or about 3800 proteins with 99% confidence. Further analysis suggested association of 248 proteins with the presynaptic boutons while 1255 proteins were involved in postsynaptic functions. Bioinformatics analysis revealed altered expression of proteins of Alzheimer's disease, oxidative phosphorylation, Parkinson's disease, Pyruvate metabolism, Propanoate metabolism, Huntington's disease, citrate cycle (TCA cycle) and many more pathways. As Apolipoprotein play major role in AD e.g. APOE help to maintain amyloid aggregates concentration to normal. However, defects such as post translational modifications in APOE leads to accumulation of amyloid aggregates. Our study quantified APOA, APOB, APOOD, APOE and their abundances were significantly different in young and old AD subjects. We found deamidation of APOE and APOD. This work also found dysfunction of mitochondrial function in AD.