Multiple reaction monitoring for the detection of disease-related synaptic proteins

Synaptic dysfunction occurs early in Alzheimer’s disease (AD) and is acknowledged as a primary pathologic target for treatment. Synaptic degeneration is the pathological feature most strongly correlated with loss of cognitive function ante mortem (Terry et al., 1991). Synapses are heavily damaged in hippocampal and neocortical regions of AD brain, whereas motor and occipital cortices are relatively spared (Honer et al., 1992). Despite extensive work, the molecular mechanisms underlying synaptic degeneration are largely unknown.

Proteomics comprises a suite of techniques to identify proteins that show disease-related expression differences in biological tissues, including brain. A number of semi-quantitative approaches, including 1-dimensional and 2-dimensional (2D) gel electrophoresis, Western blot, mass spectrometry (MS) and tandem MS have been applied to such questions. Over the past few years, multiple reaction monitoring (MRM) has emerged as a powerful MS tool for quantifying proteins in complex mixtures including serum, plasma, cerebrospinal fluid and tissue. This paper will focus on using AD human autopsy tissue. Selected proteins of interest are targeted, thereby enabling hypothesis-driven studies that complement discovery-based experiments.

In MRM analysis, analytes that have been ionized in an electrospray source enter the first quadrupole, which identifies and selects peaks in a narrow mass to charge ratio (m/z) range around that of the target precursor ion. These are guided into the second quadrupole for fragmentation by collision-induced dissociation. The third quadrupole is set to a narrow m/z range around that of the target fragment ion. This precursor/fragment ion m/z pair is called a transition. Information-dependent acquisition analysis is conducted during the initial stage of MRM optimization to ensure that the transition truly derives from the target peptide. The two levels of mass selection effectively filter co-eluting ions, making MRM a highly sensitive and selective technique. Several transitions for each protein are monitored and compared in successive analyses for unambiguous quantification.

We found that the synaptosomal expression of peroxiredoxin-1 was significantly higher in AD hippocampus than in AD motor cortex, and significantly lower in normal, non-AD control hippocampus than in non-AD motor cortex. Peroxiredoxin-1 is an antioxidant protein that neutralizes free radicals, which are produced by oxidative phosphorylation and leak from mitochondria: synaptic boutons contain abundant quantities of these organelles (Coyle and Puttfarcken, 1993). It is widely accepted that free radical-induced oxidative stress is implicated in AD pathogenesis, mediated by mitochondrial dysfunction, calcium dysregulation, overload of antioxidant capacity, and protein aggregation (Stadtman and Levine, 2003). Higher peroxiredoxin-1 mRNA and protein expression has been reported in transgenic mouse models of AD and in AD whole-brain brain homogenates, and we detected differences between AD and normal brain synaptosomes with a 2D-gel based proteomic approach (Kim et al., 2001; Lee et al., 2011; Chang et al., 2013); our MRM study confirms the veracity of the last-mentioned finding.

Our MRM study also revealed significantly higher synaptosomal expression of dihydropyrimidase-related protein-1 (DRP1) in AD hippocampus than in AD motor cortex, but no regional difference in non-AD normal subjects. Interestingly, our previous 2D-gel study had shown no significant regional difference in synaptosomal DRP1 expression in AD cases (Chang et al., 2013). Peptide mass fingerprinting identified peptide SIPHTSDR from the DRP1 protein spot on a 2D-gel; this peptide is missing in an isoform of DRP1. We speculate that the lack of expression difference in the earlier study could be that of an isoform. DRP1 protein has not been fully characterized, but the dihydropyrimidase family is involved in neuronal morphogenesis and axonal outgrowth (Charrier et al., 2003), which suggests that the abnormal expression of DRP1 could be a compensatory mechanism for synaptic loss.

MRM showed that synaptosomal creatine kinase B and synaptotagmin-1 expression did not differ between AD hippocampus AD motor cortex; however, our 2D gel-based study showed significantly lower and higher expression (respectively) in AD hippocampal synaptosomes (Chang et al., 2013). Since our MRM assays specifically targeted proteotypic peptides with no known modification sites, the extent of
post-translational modification may be altered in AD hippocampus. Decreased creatine kinase B level correlates not with gene expression but with post-translational oxidative modification (Aksenov et al., 1997; Aksenova et al., 1999). Hence, creatine kinase B oxidative modification may be higher in AD hippocampus, which could lead to neuronal dysfunction (Aksenova et al., 1999; Castegna et al., 2002). Likewise, synaptotagmin-1 functionality might be altered in AD hippocampus, leading to impaired vesicle trafficking and synaptic dysfunction.

Even when samples are separated on a large format 2D-gel, there is a possibility that two or more proteins can migrate to the same protein spot. This inevitably introduces error in quantitation. Although 2D-gels have the advantage that protein modifications can be visualized as trains of spots, distinguishing the variants is cumbersome. On the other hand, MRM assay offers the ability to target peptides of interest. Peptides with modification can be targeted and quantified to assay post-translationally modified proteins, given only that a sufficient number of transitions can be developed for each protein variant.

A major pitfall of MRM is that great care must be taken to avoid false-positive peptides. It is essential to distinguish these from correctly identified peptides because enzyme digestion can produce homologous peptides from different proteins. Our initial list of targets included α-actin; two peptides SYELPDGQVITIGNER and VAPEEHPVLLITEAPLNPK gave strong transition signals. However, BLAST search revealed the former peptide sequence can also be derived from α-actin. Because using only one peptide for protein quantification is unreliable, β-actin was excluded from the study. Each targeted peptides should be BLAST searched to ensure that the peptide is unique to the protein of interest. The Skyline software we used for analysis (downloaded from public sources; see Chang et al., 2014a) has the ability to screen the preselected spectral library to pick out such proteotypic peptides. However, there is no guarantee that all the peptides/proteins of a target proteome will be present in the accessed spectral library. The full-scan tandem mass spectrum is subjected to de novo sequencing to aid the avoidance of false positives.

With the use of an internal standard protein, we achieved linear and reproducible MRM assays with a coefficient of variation of less than 9%. Linear MRM signals were obtained over the concentration range 1–4 μg (half and doubled the amount studied) for the transitions giving the highest peak area (R² = 0.999) and the lowest (R² = 0.997). MRM is an invaluable technique for the detection and reliable quantification of low-abundance proteins; disease-related proteins are often expressed at low levels. This study provides a platform for creating new avenues of investigation of many different neurological disorders.

Financial support was provided by the Alzheimer’s Australia Dementia Research Foundation Scholarship Program (AAR Postgraduate Research Scholarship), Alzheimer’s Association (USA) under grant # RG1-96-005 and the Judith Jane Mason and Harold Stannett Williams Memorial Foundation. The Queensland Brain Bank, part of Australian Brain Bank Network, is supported by an NHMRC (Australia) Enabling Grant No. 605210.

Rachel Yoon Kyung Chang, Peter R. Dodd
School of Chemistry and Molecular Biosciences, University of Queensland, Australia

Corresponding author: Peter R. Dodd, Ph.D.
Email: p.dodd@uq.edu.au.
Accepted: 2014-11-12
doi:10.4103/1673-5374.147926
http://www.nrronline.org/
Chang RYK, Dodd PR. Multiple reaction monitoring for the detection of disease-related synaptic proteins. Neural Regen Res. 2014;9(23):2042-2043.

References
Aksenov MY, Aksenova MV, Payne RM, Smith CD, Markesbery WR, Carney JM (1997) The expression of creatine kinase isoenzymes in neocortex of patients with neurodegenerative disorders: Alzheimer’s and Pick’s disease. Exp Neurol 146:458-465.
Aksenova MV, Aksenov MY, Payne RM, Trojanowski JQ, Schmidt ML, Carney JM, Butterfield DA, Markesbery WR (1999) Oxidation of cytosolic proteins and expression of creatine kinase BB in frontal lobe in different neurodegenerative disorders. Dement Geriatr Cogn Disord 10:158-165.
Castegna A, Aksenov M, Aksenova M, Thongboonkerd V, Klein JB, Pierce WM, Booze R, Markesbery WR, Butterfield DA (2002) Proteomic identification of oxidatively modified proteins in Alzheimer’s disease brain. Part I: creatine kinase BB, glutamine synthase, and ubiquitin carboxy-terminal hydrolase L-1. Free Radic Biol Med 33:562-571.
Chang RYK, Nouwens AS, Dodd PR, Etheridge N (2013) The synaptic proteome in Alzheimer’s disease. Alzheimers Dement 9:499-511.
Chang RYK, Etheridge N, Dodd PR, Nouwens AS (2014a) Targeted quantitative analysis of synaptic proteins in Alzheimer’s disease brain. Neurochem Int 75:66-75.
Chang RYK, Etheridge N, Dodd PR, Nouwens AS (2014b) Quantitative multiple reaction monitoring analysis of synaptic proteins from human brain. J Neurosci Methods 227:189-210.
Charrier E, Reibel S, Rogemond V, Aguera M, Thomasset N, Honnorat J (2003) Collapsin response mediator proteins (CRMPs): involvement in nervous system development and adult neurodegenerative disorders. Mol Neurobiol 28:51-64.
Coyle JT, Puttfarcken P (1993) Oxidative stress, glutamate, and neurodegenerative disorders. Science 262:689-695.
Honer WG, Dickson DW, Gleenon J, Davies P (1992) Regional synaptic pathology in Alzheimer’s disease. Neurobiol Aging 13:375-382.
Kim SH, Fountoulakis M, Cairns N, Lubeck G (2001) Protein levels of human peroxiredoxin subtypes in brains of patients with Alzheimer’s disease and Down syndrome. J Neural Transm Suppl223:235.
Lee YJ, Goo JS, Kim JE, Nam SH, Hwang IS, Choi SI, Lee HR, Lee EP, Choi HW, Kim HS, Lee JH, Jung YJ, Kim HJ, Hwang DY (2011) Peroxiredoxin I regulates the component expression of γ-secretase complex causing the Alzheimer’s disease. Lab Anim Res 27:293-299.
Stadtman ER, Levine RL (2003) Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acids 25:207-218.