Affinity purification of ubiquitinated proteins and peptides from rat brain extracts

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RESULTS

Post-translational modifications allow dynamic regulation of proteins in the nerve terminal that can respond quickly to physiological and non-physiological stimuli.

Multiple sites of ubiquitination identified in...

1. Enrichment of ubiquitinated proteins using UBD’s from rat brain tissue

2. Summary of ubiquitinated proteins identified from rat brain

Over 1,500 proteins were identified in study. Selected proteins identified are listed below:

3. Summary of ubiquitinated peptides identified by mass spectrometry from rat brain tissue using KGG antibodies

METHODS

Rats were sacrificed either by rapid decapitation or by Focused Microwave Irradiation (FMI) according to Yale University IACUC approved methods. Biochemical subcellular fractionation of rat brain tissue was performed as previously described (Hallett et al. 2008). Primary cortical cultures were prepared from cortices at E18 and cultured using standard methods for DIV14-21.

Enrichment of ubiquitinated proteins from protein extracts. Protein extracts were incubated with Ubiquitin Binding Domains (UBD’s) to purify ubiquitinated proteins. GST-S5a, GST-Ataxin-3-UBA (Biomol), Tubes 1 and 2 (LifeSciences) were used in the study. The bound proteins were washed and eluted using SDS-PAGE. Proteins were transferred to nitrocellulose membrane for western blotting, incubated with primary antibodies and scanned using the LICOR system. The remainder of the eluted protein was analyzed by SDS-PAGE, proteins detected using Coomassie blue protein gel stain and gel bands cut out and subjected to trypsin digestion and LC-MSMS.

Enrichment of ubiquitinated peptides from protein extracts. Protein Extracts were digested with Lys-C (3h, RT), diluted and then digested with trypsin (16h, 37°C). Peptides were purified using a Sep-Pak cartridge and freeze-dried overnight. The Ubiquitin Branch Motif (K-ε-GG) Immunoaffinity Beads (Cell Signaling Technology cat#1990) was used to immunoprecipitate ubiquitinated peptides from the peptide mixture. Beads were washed and bound peptides eluted using 0.15% TFA.

Mass spectrometry. Tryptic peptides were separated by the nanoACQUITY Ultra Performance LC and analyzed on the Thermo linear ion trap (LTQ)-Orbitrap XL mass spectrometer (Thermo-Electron Corp) at the Keck laboratories. The MS data was processed and files searched against the SWISSPROT (rat) database using the Mascot server. The search was performed using the search parameters choosing trypsin with 3 miss-cleavages with variable modifications Propionanin (C), Oxidation (M), Deamidated (NQ) and GlyGly (K). Ubiquitinated peptides were identified by mass spectrometry by 114 Da mass increase (GlyGly (K) left over after trypsin digestion).

REFERENCES


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