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THE HONG KONG UNIVERSITY OF SCIENCE & TECHNOLOGY
Division of Life Science

Quantitative N-Terminomics and Phosphoproteomics Reveal Distinct Signalling Networks Governing Regulated Necrosis of Neurons

by

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Abstract

Excitotoxicity, initiated by over-stimulation of ionotropic glutamate receptors (iGluRs), is a major pathological process directing regulated necrosis of adult neurons in both acute and chronic neurological disorders. Upon over-stimulation, iGluRs allow massive influx of calcium ions into the affected neurons, leading to over-activation of two groups of neurotoxic calcium-dependent enzymes: (i) the cysteine proteases calpains, which catalyse limited proteolysis of specific neuronal proteins to modulate their functions and (ii) neuronal nitric oxide synthase (nNOS), which generates excessive NO to induce oxidative damages. The calpain-proteolysed proteins and the NO-induced oxidative damages in turn modulate the activities of proteases, protein kinases and phosphatases to perturb the expression and phosphorylation of specific neuronal proteins. Presumably, these perturbed proteins form the signalling networks that direct neuronal necrosis. To define these neurotoxic signalling networks, we performed quantitative proteomics and biochemical analyses to identify the calpain substrates and the perturbed proteins in neurons undergoing excitotoxic cell death. Using the Terminal Amine Isotopic Labelling of Substrates (TAILS) proteomics method, we identified the exact sites of cleavage in ~300 neuronal proteins proteolytically processed by calpains and other proteases activated in neurons undergoing necrotic death. Additionally, using the stable isotope dimethyl labelling method and neuronal lysates with 50-100 µg of proteins, we definitively identified ~1300 neuronal proteins and ~1000 phosphosites. Among them, around 150 neuronal proteins were found to exhibit dynamic changes in abundance and/or phosphorylation levels at different time points after glutamate over-stimulation. Based upon the consensus phosphorylation sequences of known protein kinases defined by arrayed peptide library screening and the literature-curated knowledge, we were able to predict the upstream protein kinases targeting a subset of these identified phosphosites. Bioinformatic analysis predicted that these identified proteins and their predicted upstream kinases form distinct signalling networks. Using biochemical approaches, we found that some components of the predicted signalling networks induce neuronal death by aberrant regulation of key protein kinases such as Erk1/2 and Akt, which are protein kinases critical to neuronal survival. Taken together, our findings illustrate how results of quantitative proteomic analyses can form the conceptual framework for investigation to define the molecular mechanism governing regulated necrosis of adult neurons.

Date: 23 Nov 2017 (Thur)
Time: 11:00 am – 12:30 pm
Venue: Room 1504

(Host faculty: Prof. Robert QI)
All Are Welcome!