Spatial Mapping of the Neurite and Soma Proteomes Reveals a Functional Cdc42/Rac Regulatory Network

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Overview

• Neurons morphologically polarize by extending neurites directionally in response to soluble chemokine gradients and adhesive cues present in the extracellular environment.
  
• To understand the complex signaling mechanisms that mediate neuritogenesis, global proteome profiling was applied in combination with a novel neurite purification methodology for comparative analysis of the soma and neurite proteomes of neuroblastoma cells.
  
• Bioinformatics and functional analyses of 4855 identified proteins revealed a spatially compartmentalized Rac/Cdc42 signaling network that operates in conjunction with novel GEFs and GAPs to control neurite formation.
  
• Our findings provide:
    - Insight into the spatial organization of signaling networks that enable neuritogenesis.
    - Comprehensive system-wide profile of proteins that mediate neurogenesis, including those that control Rac and Cdc42 signaling.

Methods

Extending neurite and soma purification from neuroblastoma cells

1. Proteomics analysis: SCX-C18 2D-LC-MS to reveal relative protein abundances in neurite and soma fractions.
   - Equivalent amounts of protein digests from neurite and soma fractions were separately analyzed by 2D-LC-MS/MS using the LTQ linear ion trap.
   - For protein identification, peptides were searched in the database search approach in conjunction with SEQUEST.
   - The ratio of total peptide spectrum counts (neurite/soma) for a given protein in each fraction was used to quantify the relative abundance of the protein.

2. A protein was considered to be increased or decreased in a particular fraction if its neurite/soma ratio change was greater than 2 fold or if it was detected in only one fraction (unique). All other proteins were considered to be equally abundant in both the fractions.

Biological validation and characterization

1. Quantitative Western blotting/densitometry to confirm the relative changes in protein abundance measured by LC-MS/MS.

2. Ingenuity Pathway Analysis program and Knowledge Base (IPA) to identify spatial organization of signaling networks.

3. RNA interference analysis, immunofluorescence and time lapse imaging for functional characterization of Rac, Cdc42, GEFs and GAPs.

Results

Global proteomics analysis of neurite and soma

Fig. 1: (A) 3D reconstruction and volume rendering of a confocal series of α-tubulin stained neurons on filter. (B) Validation of proteomics data by quantitative Western blot/densitometry.

Fig. 2: Gpi4e ontology analysis of the most significant canonical pathways present in the neurite (blue, 10 out of 39 shown), soma (yellow) or equally distributed proteins (red). Green dotted line represents significance threshold as measured by Fisher’s test (p<0.05).

Fig. 3: Rac and Cdc42 GTPase activity assay activation. Erk2 is equimolar in neurite and soma fractions and serves as a control. Collapse was induced by incubation of 1µg/mL LPA in the lower chamber for 10 min.

Fig. 4: The interactome and spatial localization of Rac and Cdc42 GTase. Protein enrichment in different fractions is color coded as in Figure 2. Green arrows point to candidate proteins for RNAi analysis.

Fig. 5: Morphodynamics and cytoskeletal changes of cells transfected with the indicated GEF and GAP siRNAs. (A) Time lapse series of control and ArhGAP30 siRNA transfected neurons. Neurite by trajectory is shown in red. Scale bar = 50 µm. (B) Representative neurites tip tracks of control, ArhGAP30, Dock10 and Dock10 siRNA transfected cells. Scale bar = 100 µm. (C) Control, SiGAP2 and Trio siRNA transfected cells were allowed to spread for 8 h on laminin-coated coverslips and immunostained for actin (green), tubulin (red), nucleus (blue). (D) Time lapse analysis of neurite formation of SiGAP2 and Trio knockdown cells. Red arrows indicate regions on non-collapsing phase glia-like structures indicative of retracting neurites. Scale bar = 20 µm.

Conclusions

• Proteomics analysis identified 4855 proteins and provided reliable quantitation data on relative protein abundances in purified neurite and soma fractions from neuroblastoma cells.

• The neurite compartment is highly enriched with proteins that control integrin function, the actin cytoskeleton, and axonal guidance, whereas the soma is enriched with proteins that control DNA/RNA metabolism and ubiquilinification.

• Bioinformatics and functional testing of Rho family GEFs and GAPs revealed a complex network of regulatory proteins that control different aspects of the neurite including neurite extension/retraction kinetics, length, and shape.

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References


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