Phosphoproteomics

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Abstract

Proteins from the spinal ligament cells of wild-type and null mice were separated using two-dimensional gel electrophoresis and stained with Pro-Q Diamond phosphoprotein gel stain (Thermo Fisher Scientific, Waltham, MA, USA) followed by SYPRO Ruby protein gel stain (Thermo Fisher Scientific). The gel was visualized by using a Molecular Imager FX Pro Plus multi imager system (Bio-Rad, Hercules, CA, USA), and the images were acquired using PDQuest software, version 8.0 (Bio-Rad). The composite images were digitally pseudo-colored and overlaid. The gels were treated with a phosphorylation reagent, and the phosphorylation spots were verified using LC–MS/MS after in-gel digestion using trypsin and peptide extraction, which were performed according to a previously published protocol. Purified peptides (20–30 pmol) were analyzed by using the UltiMate 3000 RSLCnano system (Thermo Fisher Scientific) coupled to an Orbitrap Elite linear ion trap mass spectrometer (Thermo Fisher Scientific) with an in-house manufactured nano-electrospray ionization interface. For micro reversed-phase LC-MS/MS analysis, the samples were injected into a trap column (nano; 75 × 280 µm inner × outer diameter; packed with 15 cm Acclaim Pep Map C18). Buffer A (0.1 % formamide) and Buffer B (80 % acetonitrile and 0.0 8% formic acid) were used to elute the bound peptides with a split flow system (flow rate: 300 nL/min) for 60 min on a linear gradient. In positive ion mode, spectra were acquired with cycles of one full MS scan in the linear trap quadrupole (m/z 350–2000) followed by 20 data-dependent MS/MS scans with a normalized collision energy of 35 %.

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