Supplemental Materials

Probing for chemotherapy-induced peripheral neuropathy in live dorsal root ganglion neurons with atomic force microscopy

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Additional Materials and Methods

**DRG explant culture**

DRG explant and dissociated cultures were prepared from adult C57BL/6 mice (8-12 weeks old).\(^1\) DRGs were dissected out and cleaned of spinal and peripheral roots and were then plated onto poly-D-lysine-coated (Sigma-Aldrich) 8-well chambers (Millipore) with Matrigel (BD Biosciences, San Diego, CA). DRG neurons were cultured in Full Neurobasal (NB) (Invitrogen) media supplemented with B27, 200 mM L-glutamine, penicillin/streptomycin, 50 ng/ml NGF (Invitrogen), 2 ng/ml GDNF (Sigma-Aldrich) and 10 µM Ara-C. Vincristine sulfate (Sigma-Aldrich) was dissolved in PBS and added to the cultures at 2.5 ng/ml, 10 ng/ml or 50 ng/ml, as indicated. After 48 h, DRG explants were fixed and immunostained, as described in the Immunocytochemistry section. For each explant, non-overlapping quadrant images were taken at low magnification (4×) using a Nikon Eclipse 90i fluorescence microscope. Total neurite length and the longest neurite length for each DRG explant were measured using automated WIS-NeuroMath software (Weizmann Institute of Science).\(^1\) Data were obtained from at least three separate experiments repeated in triplicate.

**Primary dissociated DRG culture**

DRGs were digested in mild collagenase and dispase II solution (Roche Diagnostics, Mannheim, Germany), trypsinized and mechanically dissociated using flame-polished Pasteur pipettes.\(^2\) A total of 5000 DRG neurons were plated onto poly-D-lysine/laminin (Invitrogen) coated 20-mm coverslips and grown in Full NB media with supplements and vincristine as described above. Paclitaxel (Taxol\(^®\)) (Bristol-Myers-Squibb, Princeton, NJ) in Cremophor EL/ethanol (50:50) was added to the cultures at 500 ng/ml; Cremophor EL/ethanol (50:50) (Sigma-Aldrich) was used as a vehicle control for all paclitaxel experiments. After 17 h of incubation, primary DRG neurons were used for live AFM imaging and analysis.

**AFM imaging and analysis**

AFM imaging and analysis were conducted on untreated and vincristine-treated DRG neurons. Each DRG sample per condition was immersed in Hank’s Solution (Invitrogen) and directly observed under AFM equipped with an inverted optical microscope. Before obtaining AFM images, AFM probe and targeted DRG neurons were first located using the inverted optical microscope connected to a charge-coupled device camera. AFM imaging on each DRG neuron per condition was then
performed. The corresponding three-dimensional (3-D) AFM height image and peak force error image were obtained. A high-magnification AFM image was obtained by zooming into the selected region (10 µm × 10 µm) of DRG cell surface from the 3-D height image. Neurons for AFM imaging were prepared from at least three separate experiments. At least 12 DRG neurons per condition were characterized, and a representative image was selected.

**Confocal scanning parameters**

All confocal images were obtained using the same scanning parameters, including the scan speed (200 Hz), line averaging (2), frame averaging (2), z-stack (1 µm each stack), pinhole (151.6 µm), laser power and gain levels (900 V).

**Statistics**

Data are presented as the mean ± SEM. Data were analyzed by one-way ANOVA with post hoc Newman-Keuls test (>2 groups) and Student's t-test (two groups) where appropriate.

**References**


Figure S1. Confocal laser scanning microscopy revealed the polymerization of cytoskeletal microtubules in DRG neurons. (A) Microtubules (shown by immunostaining of anti-β-tubulin III) were well-organized to form the cytoskeletal scaffold. (B) Polymerization of microtubule was observed after treating with 500ng/ml of paclitaxel, as indicated by white arrows. (C) Quantification of β-tubulin III fluorescence intensity showed the increase in fluorescence intensity after treating with 500ng/ml of paclitaxel. Values represent mean ± SEM of triplicates; **p<0.01, Student’s t-test.