High-throughput immunocytochemical assay to detect adverse effects of substances using cultured hippocampal neurons

Hanamura Kenji[1], Anggraeini Puspitasari[2], Reiko Roppongi[2], Mai Yamamura[1], Yuko Sekino[3], Tomoaki Shirao[1]

[1]Dept. Neurobiol. & Behav., Gunma Univ. Grad. Sch. Med., [2]GIAR, Gunma Univ., [3]Lab. Human Cell-Based Drug Discovery, Grad. Sch. Pharm. Sci., The Univ. Tokyo

To detect adverse effects of toxic substances on neurons, we quantitated neuron number, dendrite length and synaptic status of cultured neurons. An actin-binding protein, drebrin accumulated in the postsynaptic sites of glutamatergic synapses and a tubulin-binding protein, MAP2 were used as markers to detect synaptic changes and to visualize neuronal cell body and dendrites, respectively. We have applied this method for high-throughput analysis and showed that glutamate treatment for 10 min significantly reduced drebrin cluster density of 21-days-in-vitro (DIV) neurons in a dose-dependent manner. In this study, we examined the effects of other toxic substances. Treatment of 0.5-50 μM latrunculin A, which sequesters monomeric actin, for 5 min significantly reduced drebrin cluster density of 21-DIV neurons in a dose-dependent manner. We also confirmed that exposure of 1 Gy X-irradiation to 1-DIV neurons reduces neuron number, dendrite length and drebrin cluster density in the neurons at 21-DIV. In addition, our analysis could efficiently detect staurosporine-induced neuronal cell death in mature neurons. 24 hours exposure of 0.3 and 1.0 μM staurosporine to 21-DIV neurons significantly reduced neuron number. These results suggest that our high-content imaging analysis is useful for analyzing the effects of various toxic substances.