High-Content Analysis
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High-Content Analysis of Neurite Outgrowth in Primary Neurons Treated with Amyloid Beta: An In Vitro Model of Alzheimer's Disease to Evaluate Small Organic Molecules

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Increased production of amyloid beta (Abeta) peptides is associated with the pathology of Alzheimer's disease and often recognized as a major causative factor of the disease. One of the early defects associated with AD and impairment of cognitive functions is a reduction in synaptic structural integrity and function which is followed by the formation of dystrophic neurites. Exploiting these characteristics with the aim of studying potentially neuroprotective small organic molecules, we developed a neurite outgrowth (NOG) assay in primary cortical neurons treated with Abeta peptides. The neuronal cultures were treated with either amyloid beta 1-42 or its active fragment encompassing residues 25-35 to induce neurite degeneration, while their respective reverse peptides were used as a non-toxic control. The effect of Abeta peptides and the compounds was quantified by monitoring the changes of 20 features, including 10 NOG measurements and 10 additional measures derived from nuclear labeling. These parameters were analyzed by making use of two different multivariate approaches: MANOVA analysis and Neural Network based activity classifier. MANOVA analysis is performed on the 20 features; the first canonical component is then normalized with respect to negative and positive control and One-Way ANOVA analysis with post hoc pair wise comparison is performed. In the Neural Network based approach, a Self Organising Map has been trained to topologically map the multivariate input space by making use of positive and negative controls present in each assay plate. The resulting map has been added to the final multilayer neural network classifier as hidden layer with radial transfer function, while the second hidden layer was randomly initialized with the log-sigmoid transfer function. In the production phase, all the multivariate data of an assay plate, including controls data, are used as input for the classifier. The network output maps each multivariate well data in the unit interval whose boundaries correspond to negative and positive control; the output is then analyzed with One-Way-ANOVA analysis followed by multiple comparison test. Compounds with a mean significantly different from the negative control are considered to be active. The assay showed robustness and allowed us to evaluate a collection of potentially

neuroprotective small molecules for their ability to revert amyloid-induced toxicity. For instance, a peptide containing residues 16-20 of Abeta (D-KLVFFA), described as an Abeta1-42 antiaggregant (Chalifour et al, 2003), was successfully classified as a neuroprotectant against Abeta1-42. In summary, a high-content-analysis based assay was developed for the identification of small molecules neuroprotective against Abeta toxicity, and can therefore prove useful in drug discovery screening for Alzheimer's disease therapeutics.

Chalifour et al. (2003) JBC, 278, 34874-34881.