Application of a Multiplexed Flow Cytometric Assay and Machine Learning to Provide Genotoxic Mode of Action Information

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In an effort to more easily and efficiently generate genotoxic mode of action data, several biomarkers associated with cellular responses to DNA damage or overt cytotoxicity were multiplexed into a homogenous flow cytometric assay. Reagents included a detergent to liberate nuclei, a nucleic acid dye, fluorescent antibodies against γH2AX, phospho-histone H3, and p53, and fluorescent particles to serve as counting beads. The assay was applied to TK6 cells and 67 diverse reference chemicals that served as a training set. Exposure was for 24 continuous hrs in 96-well plates, and unless precipitation or foreknowledge about cytotoxicity suggested otherwise, the highest concentration was 1 mM. At 4- and 24-hrs aliquots were removed and added to microtiter plates containing the reagent mix, and robotic sampling facilitated walk-away data acquisition. Univariate analyses identified biomarkers and time points that were valuable for classifying agents into one of three groups: clastogenic, aneugenic, or non-genotoxic. A particularly high performing multinomial logistic regression model was comprised of four factors: 4 hr γH2AX and phospho-histone H3 values, and 24 hr p53 and polyploidy values. For the training set chemicals, the four-factor model resulted in 91% concordance with our a priori classifications. A test set of 17 chemicals that were not used to construct the model were evaluated, some of which utilized a short-term treatment in the presence of a metabolic activation system, and in 16 cases mode of action was correctly predicted. These initial results are encouraging as they suggest a machine learning strategy can be used to rapidly and reliably predict new chemicals' genotoxic mode of action based on data from an efficient and highly scalable multiplexed assay.