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Applying TT21C to DNA damage: the p53 network, modelling and IVIVE

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The National Academies of Sciences 2007 report, "Toxicity Testing in the 21st Century: A Vision and A Strategy" envisions toxicity testing using human cells *in vitro* to evaluate perturbations of cellular response networks and coupling results from these assays with computational systems biology and in vitro-in vivo extrapolations (IVIVE) to complete human safety assessments. To support this transition in toxicity testing, our laboratory is evaluating key cell response networks to develop proof of concept safety assessments for a series of prototype compounds and networks, including the DNA damage response pathway. The DNA damage toxicity pathway project studies the p53-mediated DNA damage stress response in human cells to determine the underlying response circuitry for the p53 pathway and the dose response behavior for pathway activation after chemical induced DNA damage. This research effort has three overarching goals: (1) map the key determinants of cellular fate following DNA damage induced by chemicals with different mechanisms of action (indirect vs. direct DNA-damage), (2) identify dose-dependent thresholds associated with cellular adaptation and toxicity (mutation) and (3) perform a risk assessment for a prototype chemical based on predicted regions of safety determined from in vitro data. Initial work involved validation of the cell model and collection of a dense data stream comprised of dose response data at the gene, protein (p53, p-p53, p-H2AX, MDM2, p21, WIP1), and cellular (cell cycle arrest, apoptosis, micronucleus; MN) level using prototype chemicals for DNA damage: etoposide (topoisomerase II inhibition), methylmethane sulfonate (DNA alkylation) and quercetin (oxidative damage). 18 point dose-response curves were generated for protein and cell fate endpoints in a p53 competent cell line (HT1080) using flow cytometry and high content imaging. Whole genome transcriptomic analysis was also performed for each prototype chemical at several doses ranging from concentrations with no effect, minimal effect, or maximal effect on protein and micronucleus response. In concert with the data acquisition and pathway inference, a computational systems biology pathway (CSBP) model is being developed to describe p53 response networks that control biological functions in order to calculate dose response behaviors. The goal of the CSBP model is to predict the dose at which the increased level of damage triggers DNA-damage response. Biokinetic models will be then be used to facilitate in vitro-in vivo extrapolation and the determination of safe levels of human exposure from in vitro and CSBP results. This work demonstrates an approach to integrating data acquisition and model building in order to provide an 'in vitro only' risk assessment for prototype genotoxic chemicals as a an initial step in implementing the recommendations of the NAS Toxicity Testing report with the DNA-damage stress network.