Future Applications of Oxidative and Other Stress Pathway Assays to Mode of Action Studies

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Contributing Organizations: NHEERL/ORD

LTG 1 Poster 10

Science Questions

- Can analysis of stress pathways, including oxidative stress, be adapted to high-throughput screening for rapid toxicity characterization of a great number of uncharacterized environmental toxicants?
- Can proteomic and genomic signatures based on oxidative stress mode of action be used to model toxicity patterns and screen untested chemicals?
- Can a virtual lung model be developed which will predict dose of reactive gases to deep lung targets?

Research Goals

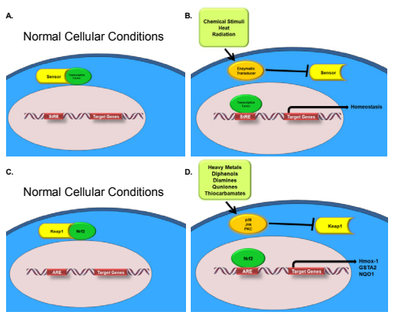
Oxidative stress has been implicated as a 'key event' in the toxicity of many environmental agents. Cells respond to oxidative stress by up-regulating a set of homeostatic genes that can be monitored by molecular techniques.

It is now well recognized that other stress pathways are also activated by the same chemical in concert with oxidative stress. NHEERL investigators have successfully explored the use of 'reporter genes,' as well as genomic and proteomic technologies, to assess oxidative stress as well as other types of cellular stresses such as 'protein denaturation,' 'DNA damage' and 'heavy metal response. Stress reporter gene assays are currently being adapted to ultra high-throughput format in collaboration with the NIH Center for Chemical Genomics Center (NCGC), which has the capability to screen thousands of compounds in a short time.

In addition, it is anticipated that study of the activation patterns (such as order and extent of activation) across the pathway ensemble over a dose-response range of toxicants will be useful in providing insights about the modes of actions (MOA) of toxicants.

Stress Pathway Assay Ensemble: High-Throughput Methodology for Screening and Prioritization

Stress Response Pathways



Cellular stress response pathways share a common architecture. (A) Under normal cellular conditions, the pathway transcription factor is negatively regulated by the pathway sensor. (B) Environmental insults, including toxicant exposure, trigger the activation of enzymatic transducers. The transducers modify the sensor and/or transcription factor which allows the transcription factor to translocate to the nucleus and activate the expression of target genes that work to re-establish homeostasis. (C) In the oxidative stress response pathway, Keap1 (sensor) sequesters Nrf2 (transcription factor) under normal conditions. (D) Compounds that produce reactive oxygen species activate a number of kinases (transducers) that liberate Nrf2 from Keap1. Nrf2 then translocates to the nucleus and up-regulates anti-oxidant gene expression.

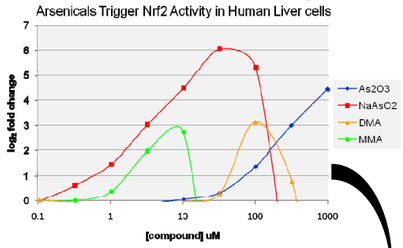
Turning Pathways into Assays

Stress pathway reporter genes are engineered by cloning a stress-responsive gene promoter element upstream of the coding region for a reporter protein such as luciferase. Reporter genes are delivered to cultured human cells using lentiviral vectors. These stable transgenic cell lines are then seeded into mutli-well plates, treated for a pre-determined time period, then assayed to measure stress pathway activity.

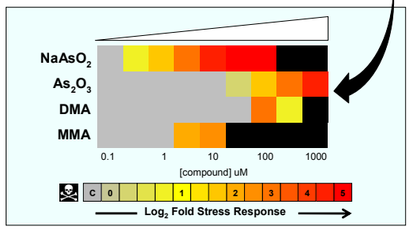
Key Stress Response Pathways

- Oxidative Stress
- DNA-damage
- Metal Stress
- Inflammation
- Hypoxia
- ER Stress
- Osmotic Stress
- Heat Shock

Cellular Oxidative Stress Response

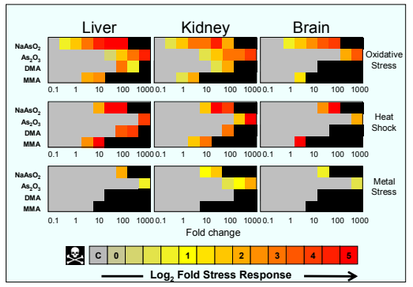


Conversion to Stress Signatures



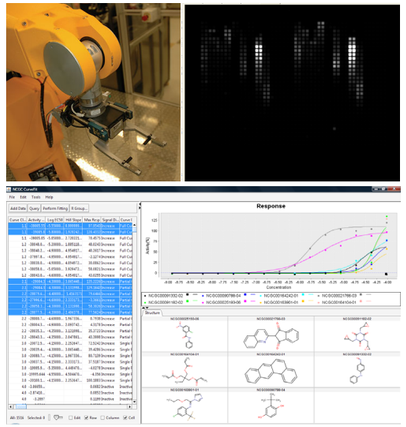
A typical dose-response result from the Nrf2 oxidative stress response assay. Human liver cells harboring a Nrf2-responsive reporter transgene were treated 24 hours with 10nM-1mM $NaAsO_2$, As_2O_3, MMA, or DMA. Nrf2 activation (Y-axis) is represented as log_2-fold change. Results can be graphically depicted in a 'heat-map' format to produce stress signatures that compare compounds for a single assay (as shown above) or across a panel assays for a single chemical (not shown).

Stress Signatures of Arsenicals



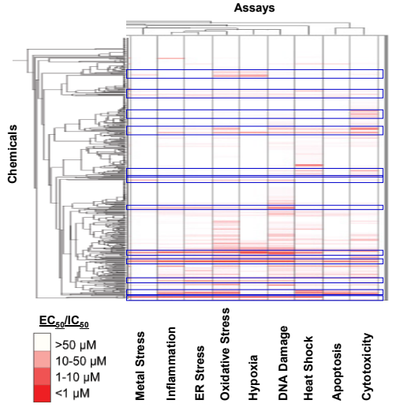
Stress signatures from 24 hour treatment of human liver, kidney and brain transgenic cells with 10nM-1mM $NaAsO_2$, As_2O_3, MMA, or DMA. Stress signatures were generated for oxidative stress, heat shock, and metal stress pathways.

NCGC: Ultra high-throughput Screening



Through a tripartite agreement between EPA, the National Toxicology Program and the NIH Chemical Genomics Center (NCGC), we are currently implementing a limited number of assays from the stress pathway assay ensemble into a 1536-well quantitative high-throughput screening (qHTS) approach. This qHTS format employs state-of-the-art robotics (top, left) and quantitative CCD imaging (top, right) and allows for the rapid assessment of thousands of chemicals in dose-response manner (bottom).

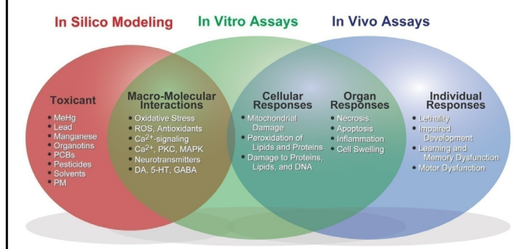
Derivation of Mode(s) of Action
(hypothetical simulation)



Results from parallel high-throughput assays, including those from the stress pathway assay ensemble as well as commercially available assays for cytotoxicity and apoptosis can be used to form compound response patterns that can be grouped by activity profile (blue boxes) using unsupervised clustering algorithms. Compound mode of action can then be derived by comparison to chemicals of known mode(s) of action that co-cluster with the compound of interest. Note that the clustering is based strictly on biological activity in toxicity assays and not on prior assumptions about a compound's mode of action. (Note: this figure is strictly hypothetical and does not reflect actual results.)

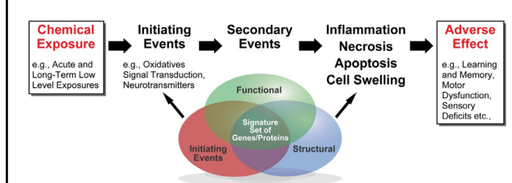
Predictive Models for Screening

Known Neurotoxicity Pathway(s) at All Levels of Biological Organization



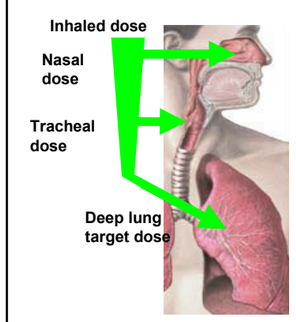
Predictive In Vitro Model Based on Systems Biology for Neurotoxicity Testing

Understand Toxicity Pathways at All Levels of Biological Organization



Products:
(1) Identification of neurotoxicity pathways *in vitro* and *in vivo*.
(2) Identification of "Signature set of genes/proteins" linked to toxicity pathways.
(3) Development of *in vitro* predictive model based on the signature gene/protein set for screening untested chemicals.

Validate a Virtual Lung Mathematical Model for Dose Extrapolation



A 'virtual lung model' will use empirical data and fluid dynamics to calculate the dose of inhaled chemically reactive gases to sensitive target tissues in the deep lung.

These models will link whole body exposure data with 'toxicity pathway' information obtained *in vitro* (see LTG 1-11)

Impact and Outcomes

Reporter gene assays and identification of genomic/proteomic signatures that can be applied to high throughput screening will assist the Agency with screening the large number of chemicals (about 100,000) used in commerce that have not been evaluated toxicologically. Development of these methods should also lead to cost saving and decreased use of laboratory animals in research.

The methods described above will also result in scientifically defensible characterization of 'toxicity pathways' that will assist in risk assessments. Well-studied pollutants will be used as prototypes to validate the modeling tools, one of which calculates the dose of inhaled reactive gases in deep lung targets. Assays and biomarkers will be streamlined and their interpretation clarified with increased use such that a greater practical significance can be ascribed to them. The resulting key events will enable a more complete identification of the nature and extent of hazard of a wide array of chemical toxicants.

Future Directions

In addition to continued development of reporter gene assays, efforts will be focused to extend molecular approaches to the following areas: 1) further investigations of the mechanism of iron disregulation and disruption of cellular signaling pathways, 2) work to enhance the understanding of the linkage between inflammatory and pollutant-induced oxidative stress in the cardiopulmonary system and for vascular effects such as formation of atherosclerotic plaques, arrhythmias and hypertension and 3) modeling of ozone-induced DNA and protein adduct data already collected from humans, primates and rodents to supplement the virtual lung efforts by the National Center for Computational Toxicology (NCCT).

Researchers Involved

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