’Omic Biomarkers for Assessing Cellular Toxicity: Integration of In Vivo and In Vitro Data—Why It Is Important

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Introduction

- There is a pressing need for new toxicity test systems which can provide useful risk assessment information in a rapid, cost effective manner with minimal use of animal tests.

- “Omic” based tests offer one mechanism–based set of approaches which are capable of addressing the complex issue of sensitive sub-populations.

- In order for “omic” biomarkers to be fully accepted as valuable risk assessment tools, they must be validated by ancillary data and identification of modifying factors which affect dose response relationships.
Integration of *in vivo* and *in vitro* data via computational techniques is an essential central component in both the development and validation of such biomarker tests.

This presentation will attempt to briefly review some *in vivo* and *in vitro* studies focused on identification of gender differences in responses to binary mixtures of metallic semiconductor elements in target cell populations.

The overall goal of these studies is to provide new “mode of action”-based tests for identification of sensitive subpopulations at special risk for toxicity.
Biomarkers

- Need for objective measures (biomarkers) of cell injury from toxic chemicals that more precisely link exposure (dose) and biochemical effects that occur prior to clinical disease

- Need for improved risk assessments for multiple chemical exposures (e.g., Mixtures) and sensitive sub-populations

- To be of maximal value, it is essential to establish mechanistic linkages between biomarkers and other parameters of toxicity

- Ideally biomarker responses should be conserved or consistent across species
Predictability of Biomarker Responses (Biomarker Validation Needs)
Biomarkers

- **GENOMICS:** Chemical-specific alterations in gene expression patterns in target cell populations

- **PROTEOMICS:** Chemical-specific alterations in the actual expression of gene products (proteins)

- **METABOLOMICS/METABONOMICS:** Chemical-induced alterations in essential biological pathways with measurement of metabolic products/precursors in accessible body fluids (e.g., urine)
Biomarker Modifying Factors

- **Dose Response/Time Course**

- **Exposed Population**
  (Age, Gender, Nutritional Status, Genetic Susceptibility)

- **Compensatory Mechanisms**
  (Inducible Enzyme Systems, Metal-Binding Proteins, Stress Proteins)

- **Antioxidant Systems**
  (e.g., Glutathion)
Mitochondrial Functions

Cellular energy production (ATP)

Urea cycle → Heme synthesis

Carbohydrate metabolism

fatty acids
Experimental Design

Syrian Golden Hamsters

Injected s.c. with 100 mg/kg InAs or GaAs Particles

Urine collection 24 hr prior to sacrifice

Sacrifice after 10 or 30 days

SDS gel electrophoresis of liver, kidney, urine proteins
Fig. 9. Metabolism of indium arsenide in vivo.

(), excretion route
Urinary Porphyrrins
Cellular toxicity assay using LDH release or Alamar Blue for hamster kidney epithelial cells exposed to In, Ga, or As at 100 μM concentrations, In + As or Ga + As at 100 μM or 50 μM concentrations for each element, showing that the combination of In + As and Ga + As at 100 μM concentrations were the most toxic. The data also indicate a correlation between LDH release and inhibition of Alamar Blue metabolism.
Conclusions

- InAs and GaAse each produce compound-specific proteinuria patterns as determined by 2-D gel electrophoresis and silver staining. The intensity of these patterns appeared to increase with duration of exposure.

- Western blot studies of the urine showed no evidence of the 32, 70, or 90 kD stress proteins despite evidence of their induction in the liver and kidney indicating that these proteins are not translocated or appear in the urine as a result or cell death.

- Overall, the results of these studies indicate that the observed proteinuria patterns maybe of potential use as biomarkers for assessing nephrotoxicity from these agents.
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1) Spot number denoted on gel  
2) Spot intensity (ratio of tx/control)  
* ≥ 2 fold increase/decrease in polypeptides
# Changes in Polypeptides at 30 Days After Exposure to InAs or GaAs in Female Hamster Kidney Cells

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Conclusions

- There are a number of established and evolving biomarkers for assessing the effects of toxic agents, such as Ga, In, or As alone or in mixtures and delineating the “biologically active” fraction of these agents prior to the onset of clinical disease.

- Validation of biomarkers for risk assessment studies on human populations requires a validation process that includes consideration of modifying factors such as GENDER that may alter relationships between exposure and effect.

- Integration of data from in vivo and in vitro studies and extrapolation to human populations via in silico approaches is essential for improving risk assessments and identifying sensitive sub-populations.
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