Toward Understanding Nrf2 Oxidative Stress Response Pathway with ToxCast

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Introduction and Significance

- Over 30 million chemical substances are currently in commerce worldwide. And 85 percent of the approximately 700 commercial chemicals introduced to the U.S. market for manufacturing each year lack safety data.
- 212 chemicals found in household products, including hazardous chemicals such as polybrominated diphenyl ethers , bisphenol A .
- Testing all commercial chemicals with animal bioassays is not feasible due to the high time and resource costs. Bioassay-based testing under REACH is projected to cost 9.5 billion euros. The U.S. EPA mandate to evaluate toxicity of 100 chemicals per year is estimated to cost up to $1 million per chemical.
- Alternative assessments have been proposed to eliminate the hazardous chemicals currently on the market. To inform alternative assessment, and prevent regrettable substitution, green chemists proposed molecular design guidelines for reduced toxicity.
- The work focuses on developing design guidelines for reduced chemical potential to incur adverse effect via oxidative stress.
- The approach utilize mechanistic understanding of toxicity and incorporates regulatory recommendation to consider adverse outcome pathways (AOPs) during model development.

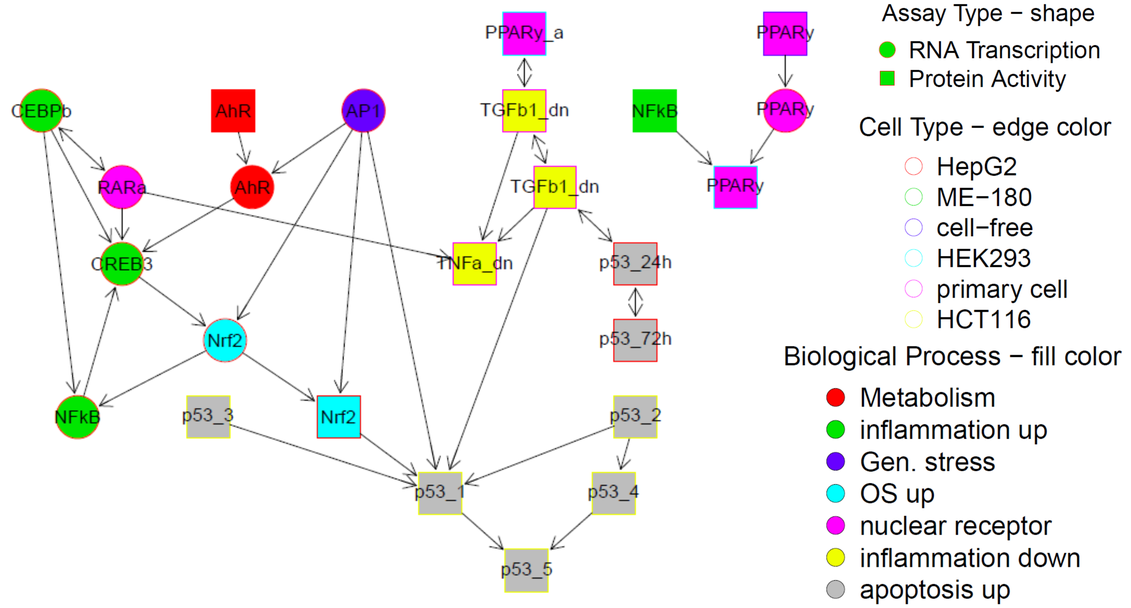
Biological Complexity & System Modeling

- Reactive oxygen species (ROS) and oxidative stress (OS) are associated with a wide range of adverse outcomes through variable AOPs.
- Improved understanding of chemical interactions with Nrf2-Keap1 complex can elucidate chemical parameters important to ROS toxicity. In context of alternative assessment, the insight will help develop *in silico* models to fill experimental data gaps and design chemicals with reduced OS activity.
- Biological systems are complex and single *in vitro* tests can often produce false positive and negative results; thus network analysis is preferred.

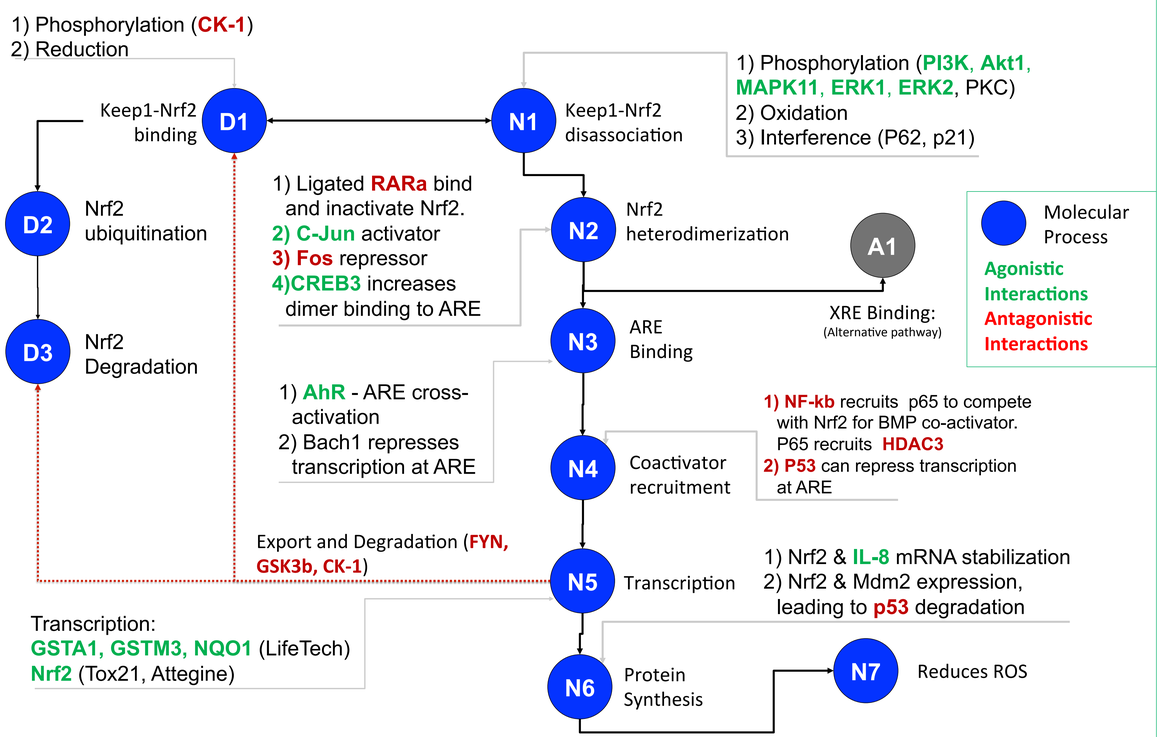
Data and Modeling Methods

- ToxCast and Tox21 initiatives present the largest HTS effort to date. ToxCast phases I & II includes HTS data for over a thousand commercial chemicals, pesticides, and pharmaceuticals. The effects of these compounds on gene and protein expression is assessed in multiple cell lines.
- The high volume HTS creates incredible opportunities for machine learning algorithms and system analyses.
- We identified an exhaustive set of genes and proteins involved in OS regulation, directly or indirectly. We analyzed the relationship between these biological agents when the system is disrupted by chemical exposure. Directed acyclic graph (DAG) models, IVR method, and R software were used.

Graphical Model Inference Results



Nrf2 - OS Response Regulatory System Scheme



Discussion

- Observed a clear difference in relationship between inflammation and apoptosis pathways. AP-1 activity as a general stress responder is evident in most models.
- Anti-inflammatory genes show conditional dependence with apoptosis response pathways. Genes involved in Inflammation and survival show higher dependence on one another in response to xenobiotic exposure and pathway perturbations.
- Nrf2 activity can contribute to both apoptosis and inflammation, depending on the circumstances.
- It is unclear how the measures of transcriptional activity and protein activity relate to each other. In the current analysis the HTS data does not provide a clear picture for the direction of interaction. However, some ambiguity is expected due to the feedback loop in the examined transcription factors.
- Higher dependencies are observed in assays using same cell lines

Research Directions

- Identify chemicals and chemical types that perturb different branches of the Nrf2-related OS-response network.
- Quantify physico-chemical and QM properties responsible for the availability and strength of interactions of xenobiotic with Nrf2 pathway.
- Establish the most likely mechanism of Nrf2 activation or lack of specific activity for major chemical classes through Bayesian inference.

Table 1: Assays and Target Gene

Name	Assay Measures	Biological Relevance
AhR	RNA, protein	Xenobiotic response, Metabolism
CEBPb	RNA	inflammation
AP-1	RNA	Multiple stress responses
NRF2	RNA, protein	Oxidative stress
NF-kb	RNA, protein	inflammation
PPARy	RNA, protein	Nuclear receptor, Nrf2 repression
RARa	RNA	Nuclear receptor, Nrf2 repression
TGFb1	RNA, protein	inflammation
TNF-a	protein	inflammation
p53	protein	survival, apoptosis
CREB3	RNA	inflammation

Key References:

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