Experimental Models for Quantitative Analysis of Hepatocarcinogenesis

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Abstract

Predictive models of chemical-induced liver cancer face the challenge of bridging causative molecular mechanisms to adverse clinical outcomes. The latent sequence of intervening events from chemical insult to toxicity are poorly understood because they span multiple levels of biological organization and timescales. The availability of high-throughput molecular assays provide a global view of epigenetic, transcriptional and pathway level changes that can shed much needed light on the regulatory networks perturbed by xenobiotic stressors. A key challenge in this process is to resolve the role of these networks in the normal homeostatic response of cells as well as irreversible alterations due to persistent stress. To link molecular mechanisms to neoplastic lesions will require quantitative assays on molecular changes and altered cellular phenotypes, as that is the level of biological organization at which tissue damage becomes manifest. This poster outlines the collaboration between NHEERL and NCCT on an integrative experimental strategy aimed at developing a model of nuclear receptor (NR)-mediated hepatocarcinogenesis (The US EPA Virtual Liver v-Liver™). As a proof of concept we are using NR activating chemicals from the EPA ToxCast™ Program to design short-term *in vitro* and *in vivo* studies to generate data on a range of molecular and cellular endpoints.

The Problem

Liver is a common target for ToxCast chemicals

- In mice:
  - 56% have some effect in liver
  - 30% are neoplastic
- Rat:
  - 63% have some effect in liver
  - 9% are neoplastic
- Liver is often the most sensitive target in 2-yr bioassays
- NOAELs/LOAELs established at least partially based on effects in the liver
  - Mouse: 45% of all chemicals
  - Rat: 37% of all chemicals

(In part from Martin et al. (2009) EHP 117:392)

Research Questions:

- What are the modes of action for hepatoxicity in rodents?
- Which modes of action occur in humans?

Vision

- Development of high throughput *in vitro* models of the liver that can be used to assess the potential toxicity of chemicals in humans.
- Prediction of toxicity in humans based on chemical structure and targeted in vitro biochemical, molecular and morphometric assays.
- Use of a virtual liver model that integrates dosimetry and toxicity networks to predict in vivo dosimetry, mode of action and human relevance.

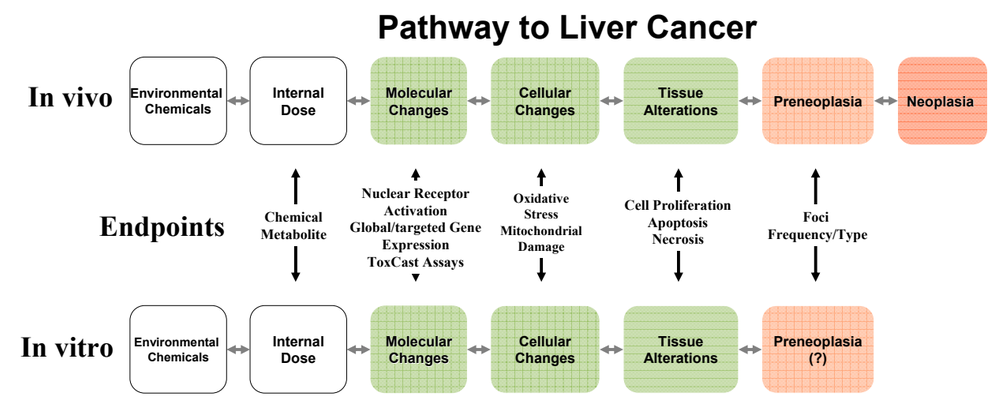
Short-term Goals of the vLiver Project (1-3 yrs)

- Provide NCCT biochemical, molecular and tissue morphometry data to help establish predictive models of liver toxicity.
- Provide guidance for interpreting the toxicological significance of the ToxCast data with a focus on liver cancer.
- Interpret nuclear receptor activation profiles and its impact for categorization of rodent mode of action and human relevance.

Potential Long-Term Goals of the Virtual Liver Project (4-10 yrs)

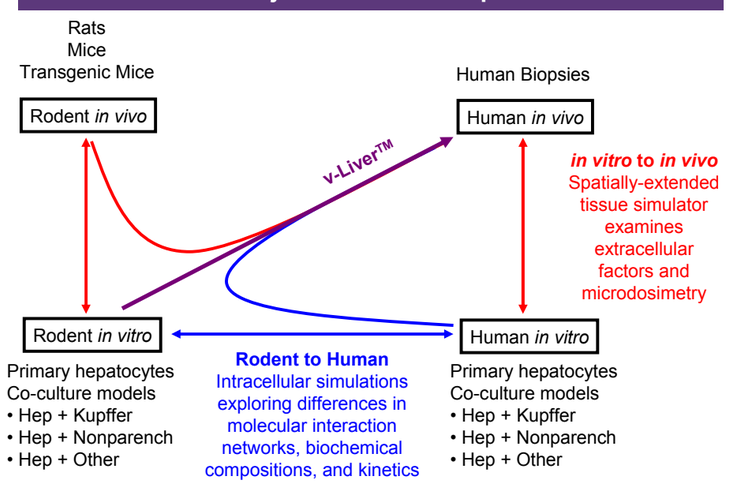
- Characterize the cellular and molecular features of NR-mediated pathways of rodent hepatotoxicity and their human relevance
- Develop *in silico* models for accurate prediction of *in vivo* dose and phenotypic outcomes from in vitro models
- Develop high throughput screening assays for categorization of chemicals in mode of action groups
- Predict extrahepatic toxicity including endocrine disruption
- Characterize differences in the magnitude of chemical sensitivity of susceptible subpopulations

In Vitro to In Vivo Comparisons for Model Building



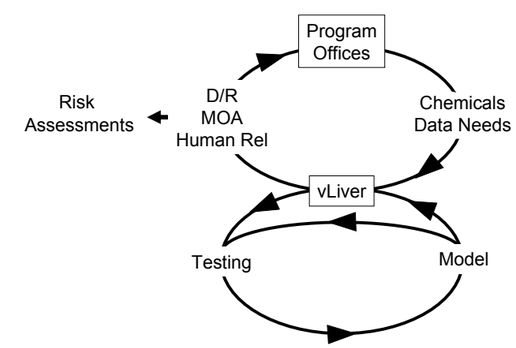
We will measure a number of endpoints in parallel in vivo rodent models and in vitro cell culture models. Experiments will be designed to capture time- and dose-related effects. Data will be used for accurate predictions of in vivo effects from in vitro effects.

Biological and Computational Models to be Used in the vLiver Project to Make Extrapolations



We will establish in vitro methods that recapitulate and predicts in vivo responses. A number of in vitro models exist including co-culture models containing hepatocytes (Hep) and either Kupffer cells, nonparechymal cells (Nonparench) or other cell types.

Communication Strategy



We will:

- Work with our Program Office partners to provide information that fills data needs for targeted groups of chemicals.
- Screen chemicals using *in vitro* models to address dose-response behavior and classify chemicals according to mode of action and determine human relevance.

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