Modeling Zebrafish Developmental Toxicity using a Concurrent In vitro Assay Battery

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Introduction

Goal: determine molecular mechanisms of embryos being exposed to chemicals during an early stage event (48H) for chemicals causing zebrafish developmental toxicity.

Approach:
- Used data from zebrafish developmental assay on 1060 chemicals
- Combined zebrafish data with data from in vitro assays from hundreds of thousand screens
- Added information from the literature on known molecular targets or MOA of chemicals
- Identified candidate MOA where a large fraction of MOA-associated chemicals are active in the zebrafish embryotoxicity assay
- Determined which chemicals may be ZF+ solely because of generalized cell stress or cytotoxicity

Methods

Screening assay:
- AC50 values were determined for each chemical against the individual endpoints (embryonic lethality, hatching delay, yolk sac, heart, head, heart tube). For chemicals screening negative at concentrations below the maximum concentration tested (6mM), otherwise chemicals were scored negative (no AC50 determined).

Validation data:
- Toxicologists (Judson and Houck, 2016) were used to identify a subset.
  - This includes a set of positives and a select subset of negatives. ACTC were determined for both the embryotox assay and the zebrafish test to (1) validate the zebrafish test for chemicals with known embryotoxicity in the zebrafish assay, and (2) to identify others for the zebrafish embryotoxicity assay.

Screening assay positives:
- Confirmed as positive for embryotoxicity, the 20% of chemicals in each of the three stress assay groups ( generalized cell stress, cell stress, and embryo lethal stress) that had at least 50% of chemical responses in the top 20% were selected for the zebrafish developmental assay.

Screening assay stresses:
- The stress classes were chosen based on prior knowledge of known relationships between chemicals in the screening assay.

Stress observations:
- The data were analyzed with a two-stage model.
  - Stage 1 involved determining which chemicals were ZF+ solely because of generalized cell stress or cytotoxicity
  - Stage 2 involved determining which chemicals were ZF+ because of targeted stress pathways.

Candidate MOA:
- Identified candidate MOA where a large fraction of MOA-associated chemicals are active in the zebrafish embryotoxicity assay (≥75%).
- Identified which chemicals in each MOA class are given in parentheses. UCI=90% upper confidence interval.

Sodium ion channels 33 27 0.82 [***]
General ion channels 33 27 0.82 [***]

Conclusions:
- Identified candidate MOA where a large fraction of MOA-associated chemicals are active in the embryotoxicity zebrafish test.
- Identified which chemicals may be ZF+ solely because of generalized cell stress or cytotoxicity.
- Determined which chemicals may be ZF+ solely because of targeted cellular mechanisms.

Frequent MOAs are:
- Generalized cell stress
- Specific cell stress (microtubule, mitochondria)
- Lipid synthesis disruption that can affect signaling, cytoskeletal organization, and hormone synthesis
- Endocrine pathways (estrogen, androgen, thyroid, progesterone)

Next Steps:
- Review literature for support linking these MOA to developmental toxicity
- Manually review other targets related to development, e.g. sonic hedgehog
- Use CRISPR-Cas9 to knockdown candidate genes (chemical agnostic) to test if loss of gene function causes embryotoxicity

References

1. Pacific, Colorado 2012. Inhibitors of Topoisomerase II. 110-119
3. Deck, Wambaugh et al. 2014. Applied Toxicology. 121-122
5. EPA Open Targets. Targets identified with ZF+ in ≥50% of chemicals.