Assessment of the developmental toxicity potential of new chemicals is both resource-intensive and time-consuming. Large numbers of laboratory animals are required, and the predictive value of these decades-old tests has been challenged. Availability of more predictive developmental toxicity screens would reduce costs and increase pharmaceutical and chemical safety. A small molecule biomarker-based in-vitro assay was developed using human induced pluripotent stem (iPS) cells and two metabolites (cystine and cysteine), previously identified as biomarkers of teratogenicity in human embryonic stem (hES) cells. The assay uses the ratio of the two metabolites (o/c ratio) to indicate the concentration at which a test compound may perturb cellular metabolism in a manner indicative of teratogenicity.

Our goal was to determine if the assay could be migrated to an iPS cell-based model by testing whether the cells respond to chemical insult in the same manner as hES cells. iPS cells are derived from the genetic manipulation of human somatic cells and are being widely investigated for use in place of hES cells as a less controversial model. While human iPS cells are phenotypically and genetically similar to hES cells in many respects (i.e. morphology, proliferation, gene expression), recent research has revealed that numerous subtle but important molecular differences exist. We tested 31 known compounds (23 training and 8 test set compounds) in both hES and iPS cells. The predictions (teratogen vs. non-teratogen) as well as the concentration at which a compound was predicted teratogenic were compared between the two cell lines. The transition of the targeted biomarker assay to iPS cells harnesses the predictive power of the hES cells without the ethical controversy surrounding them.

Comparison of hES and iPS Cell Culture Conditions

A set of short experiments were performed to determine if the 96-well assay parameters developed for hES cells would apply to iPS cells.

Question 1: Do iPS cells attach with the same efficiency as hES cells?

Question 2: Do iPS cells double at the same rate as hES cells in our 96-well condition?

Question 3: What is the optimum treatment length for iPS cells?

Representative iPS vs. hES cell assay results showing the o/c ratio and cell viability for 4 teratogens and 1 non-teratogen.

The interpolated concentration where the o/c ratio crosses the teratogenicity threshold (i.e., Teratogenicity Potential) for all-trans retinoic acid, busulfan, carbamazepine and doxylamine is within 2-fold.

Hydroxyurea is an example of a compound where the o/c response differs between the two cell types.

Developmental Toxicity Classification

Conclusions and Future Directions

- The current study shows proof of concept that the assay can be transferred from an hES cell-based model to iPS cells.
- 27 of the 31 compounds have the same prediction in iPS and hES cell assays.
- Using a teratogenicity threshold of 0.88 for the o/c ratio (determined with hES cell data), the iPS cell-based assay was shown an accuracy of 91% for classifying potential developmental toxicants.
- Ongoing research will further define the teratogenicity threshold in the iPS cell-based assay to account for subtle differences in response between the two cell lines.

We recently published the hES cell data used for comparison in Birth defects research. Part B. Developmental and reproductive toxicology


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