Prediction of *in vivo* hepatotoxicity effects using *in vitro* transcriptomics data

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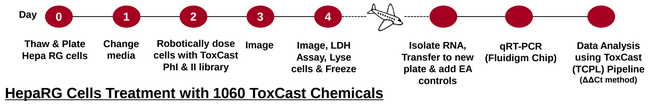
Introduction

- *In vitro* high-throughput screening (HTS) data is used to build predictive models of toxicity.
- A criticism of many *in vitro*-based toxicity testing strategies is the lack of *in vivo*-relevant biotransformation capacity of cells used in bioassays.
- To address this we used an *in vitro* liver toxicogenomics approach in metabolically competent HepaRG cells to explore gene-specific perturbations elicited by 1060 environmental chemicals from the US EPA ToxCast program.
- The expression of 96 genes, including numerous Ph I and II metabolizing enzymes, transporters and known nuclear receptor target genes was evaluated by qPCR.
- The empirical relationship between the transcriptomics data and rat liver endpoints from the Toxicity Reference Database (ToxRefDB) was evaluated using machine learning techniques.

Objective

Utilize *in vitro* transcriptomics data to predict adverse hepatic outcomes *in vivo* using machine learning techniques

Methods for Transcriptomics Analysis



HepaRG Cells Treatment with 1060 ToxCast Chemicals

- HepaRG cells differentiated & treated at Thermo Fisher Scientific (formerly Life Technologies)
- 8-point concentration response (EPA EP-D-11-083)
- LDH (cytotoxicity) assay at 48 h
- Cells lysed and frozen at 48 hours
- Positive control plates with NR activators & cytotoxic agent (Aflatoxin B1) requiring metabolic activation
- Phenobarbital (3 replicates) and Aflatoxin B1 (3 replicates) on each plate

Gene Expression Data Collection and Analysis

- Gene expression conducted by Expression Analysis/Quintiles (EPA EP-D-12-046)
- Real-time polymerase chain reaction using Fluidigm 96.96 microfluidic technology
- ΔΔCt (fold-change relative to DMSO and housekeeping genes)
- 93 genes covering biotransformation enzymes, transporters, cell-cycle, and disease states
- Universal human reference RNA added on each plate

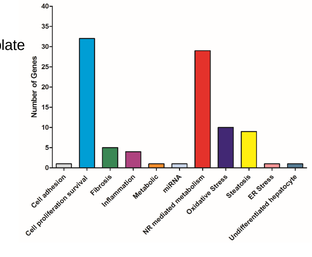


Figure 1. Summary of Gene Categorization. Number of genes associated with each biological processes or disease state on Fluidigm 96.96 microfluidic array. Abbreviations: miRNA = miRNA (miR-122), ER Stress = Enodplasmic Reticulum Stress

Methods for Machine Learning

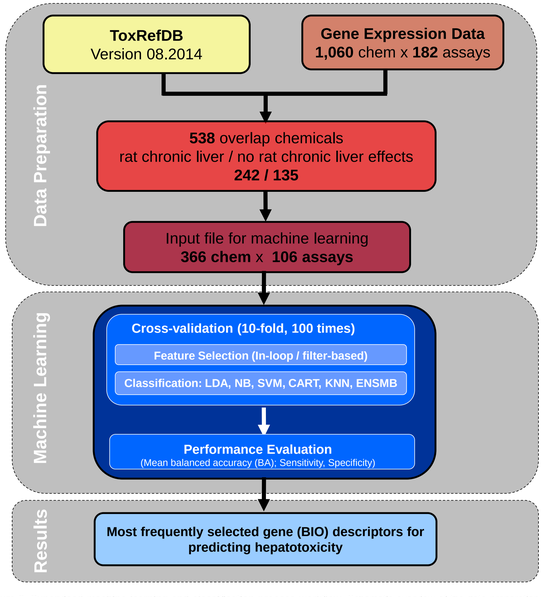


Figure 2. Supervised machine learning and classification process workflow. Schematic overview of the data preparation and machine learning procedure. Half-maximal activity (AC50) values for 1060 chemicals and 90 genes (assays/BIO descriptors) were fit in both the _up (increasing) and _down (decreasing) direction. Chemicals with rat chronic liver effects (n = 242) and non-hepatotoxicants (n = 135) were identified from ToxRefDB (V 08.2014) and grouped to three lesion categories: hypertrophy (hyp), injury (inj) and proliferative lesions (pro) (Liu et al). Ten-fold cross validation testing was used for evaluation and repeated 100 times across six machine learning algorithm, including linear discriminant analysis (LDA), support vector machines (SVCL0, SVCR0), Naïve Bayes (NB), classification and regression trees (CART0), k-nearest neighbor (KNN) and an ensemble of classifiers (ENSMB). For each step in the cross-validation loop, the subset of best descriptors was filtered using a t-test to measure the univariate association between hepatotoxicity class and gene (BIO) descriptor.

Data Set of Chemicals Used for Classification				
Total Chemicals	**Hypertrophy**	**Injury**	**Proliferative Lesions**	**Negative Set**
366	**183**	**-**	**-**	**135**
	-	**112**	**-**	**135**
	-	**-**	**101**	**135**

Table 1. Description of Data Set. Number of chemicals in data set with gene expression data and rat chronic toxicity endpoints based on 145 histopathological endpoints observed after chronic oral administration of the test chemicals.

Results of Classification Performance

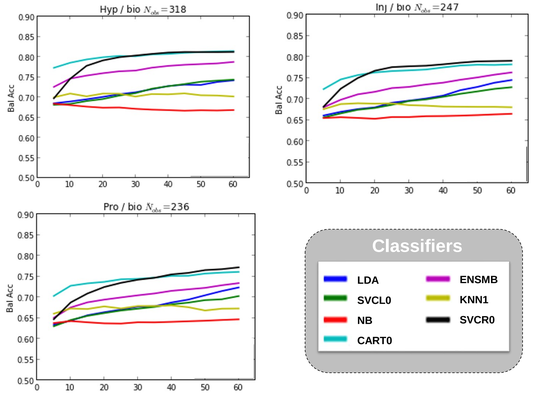


Figure 3. Cross-validation performance results of classifiers for data sets. The 10-fold cross-validation performance for the data set described in Figure 2. The x-axis shows the number of descriptors (Nd = genes up or down regulated) and the y-axis shows the relationship between the mean balanced accuracy (Bal Acc). Each curve shows the relationship between the mean balanced accuracy and the number of descriptors for each different classification algorithm including: linear discriminant analysis (LDA), support vector machines (SVCL0, SVCR0), Naïve Bayes (NB), classification and regression trees (CART0), k-nearest neighbor (KNN) and an ensemble of classifiers (ENSMB).

Maximum Predictive Performance						
Toxicity Category	**Bioactivity Descriptor**	**Classifier**	**# BIO Descriptors**	**BA**	**Sensitivity**	**Specificity**
Hypertrophy	**ToxCast HTS***	**SVCR0**	**60**	**0.76 (0.07)**	**0.52 (0.14)**	**0.99 (0.04)**
	HepaRG GE	**SVCR0**	**60**	**0.81 (0.07)**	**0.85 (0.09)**	**0.75 (0.12)**
Injury	**ToxCast HTS***	**SVCR0**	**60**	**0.75 (0.08)**	**0.51 (0.15)**	**1.00 (0.02)**
	HepaRG GE	**SVCR0**	**60**	**0.79 (0.08)**	**0.77 (0.13)**	**0.82 (0.11)**
Proliferative Lesions	**ToxCast HTS***	**SVCR0**	**60**	**0.75 (0.08)**	**0.50 (0.17)**	**1.00 (0.02)**
	HepaRG GE	**SVCR0**	**60**	**0.77 (0.09)**	**0.73 (0.17)**	**0.82 (0.02)**

Table 2. Maximum predictive performance by classification method. Table describes results of 10-fold cross-validation testing using the described HepaRG gene expression (HepaRG GE) as bioactivity descriptors comparison to results obtained using in vitro bioactivity descriptors from 711 ToxCast high-throughput screening (ToxCast HTS*) assays from *Liu et al 2015. Shown are the top two performing classification algorithms (classifiers). The results are summarized by the mean balanced accuracy (BA), the number of descriptors that produced that mean (# BIO Descriptors), sensitivity (true positive rate or % positive chemicals predicted correctly) and specificity (true negative rate). The standard deviation is given in parentheses.

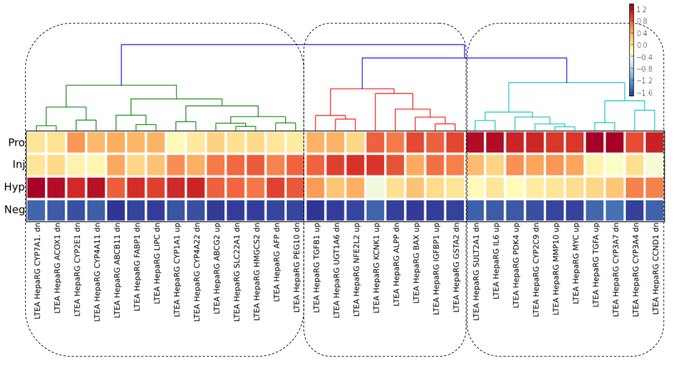


Figure 4. Bioactivity Descriptors most frequently selected in classifying hepatotoxicity. (A) Heatmap summarizing the relationship between toxicity categories (row) including: Negative for liver toxicity (Neg), hypertrophy (Hyp), injury (Inj) and proliferative lesions (Pro) with bioactivity descriptors (AC50 values for HepaRG GE from LTEA) in columns. The standardized values of the descriptors (and colors) are interpreted as follows: close to the mean (yellow), greater than the mean (reds), or less than the mean (blues). The hierarchical clustering further organizes the descriptors into groups. LTEA = Life Technologies-Expression Analysis

Summary

- Predictive accuracy of CART0, SVM, ENSMB and LDA classifiers improved with the number of gene expression (BIO) descriptors used.
- The new ToxCast gene expression (GE) data improved predictivity compared to the previous high-throughput screening (HTS) assays for predicting rat chronic liver hypertrophy and injury.
- The GE data produced a higher sensitivity (true positive rate) and lower specificity (true negative rate) compared to HTS data likely due to the differences in metabolic competency in the *in vitro* assays.
- Hierarchical clustering of GE descriptors identified bioactivity signatures representative of hypertrophy, injury and proliferative lesions.
- Machine learning provides linkages between *in vitro* bioactivity and adverse hepatic outcomes *in vivo.*
- Future investigations will include assessing the predictive performance of combined use of GE and chemical structure descriptors.

References

- Liu et al. 2015 Predicting hepatotoxicity using ToxCast in vitro bioactivity and chemical structure. *Chemical Research in Toxicology*. 28: 738-751
- iCSS ToxCast Dashboard and Toxicity Reference Database (ToxRef): http://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data

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