A Gene Expression Biomarker Identifies Activation of XBP1 by Chemical Inducers of the Unfolded Protein Response (UPR) in a Gene Expression Compendium

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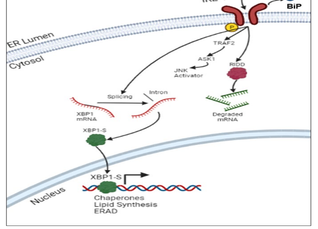
Abstract

The US EPA has developed a tiered testing strategy, including high-throughput transcriptomics (HTTr) testing, to identify molecular targets of thousands of environmental chemicals. These findings can be linked to adverse outcomes and can be used in Integrated Approaches to Testing and Assessment (IATA). Exposure of human cell lines to environmental chemicals can lead to stress responses including the unfolded protein response (UPR). One of the three major arms of the UPR is controlled through activation of the endoplasmic reticulum (ER)-resident protein kinase/endoribonuclease inositol-requiring enzyme 1 (IRE1), leading to activation of X-box binding protein 1 (XBP1). Here, we describe a method that uses a gene expression biomarker to identify UPR inducers that activate XBP1 in a microarray compendium after chemical exposure.

The IRE1α to XBP1 Pathway induced by the UPR



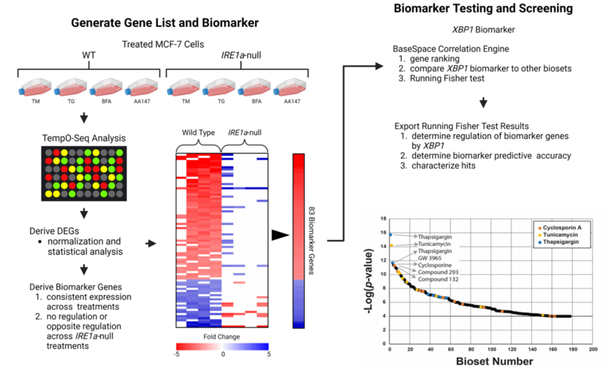
Under conditions of the UPR, un- or mis-folded proteins bind to IRE1α located in the membrane of the endoplasmic reticulum. Once bound, IRE1α dimerizes, autophosphorylates, and initiates a cascade that leads to the splicing of XBP1 to XBP1s (spliced), which travels into the nucleus and regulates gene expression.

Methods

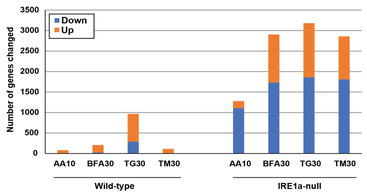
Transcript profiles were generated using full-transcriptome TempO-Seq with MCF-7 cells

- WT and *IRE1*-null cells were treated for 6 hrs with 4 UPR inducers (AA147, brefeldin A, thapsigargin, tunicamycin) or with DMSO
- Genes were identified that were similarly regulated in wild-type cells across the 4 treatments but not in the nullizygous cells
- The resultant gene list containing 83 genes and associated fold-changes averaged across the treatments in wild-type cells were imported into the Illumina BaseSpace Correlation Engine (BSCE) for comparison to other gene lists using the Running Fisher test
- Gene function was determined using Ingenuity Pathways Analysis and by ChIP-Seq analysis of datasets examining target genes of XBP1
- Biomarker accuracy in identifying UPR inducers was determined using known positive chemicals and chemically treated profiles from ≥ 72 hrs
- Examined the effects of exposure to 16 bisphenol A derivatives using gene expression biomarkers with known accuracy that predict modulation of estrogen receptor (ER) NRF2, HIF1a, HSF1, MTF1, NFKB as well as histone deacetylase inhibition (TGx-HDACi) and DNA damage (TGx-DDI).

Strategy to build and characterize the XBP1 biomarker



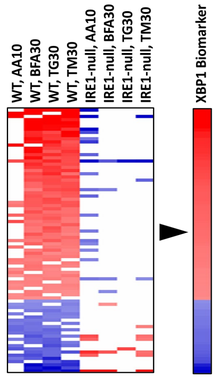
Generation of the XBP1 biomarker



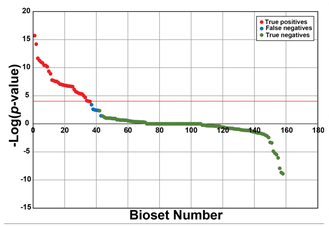
A. Number of genes altered after exposure to Tunicamycin (TM), Thapsigargin (TG), Brefeldin A (BFA), and AA147 (AA). The numbers indicate the concentrations (in µM, DMEM media, 0.2% DMSO) without cytotoxicity (as determined through MTS assays).

B. Construction of the XBP1 biomarker. The heatmap shows the consistent expression of the genes in the wild-type cells and opposite or lack of expression in the null cells. The biomarker consists of 83 genes and associated fold-changes after averaging expression across the treatments in the wild-type cells.

C. XBP1-regulated genes. IPA and ChIP-Seq analysis indicates that a) the main factor regulating the identified genes is XBP1 and most of the genes are directly regulated by XBP1 (data not shown)

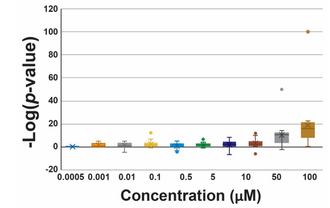


Determination of accuracy



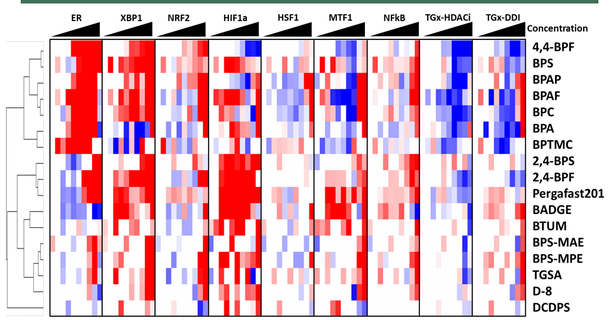
The biomarker accurately predicts XBP1 chemical activation.
The XBP1 biomarker was compared to 149 biosets representing exposure to 46 chemicals using the Running Fisher test. There were 36 true positives, 106 true negatives and 7 false negatives resulting in 84% sensitivity, 100% specificity, 100% positive predictive value, 94% negative predictive value, and 92% balanced accuracy.

XBP1 is induced by high chemical concentrations



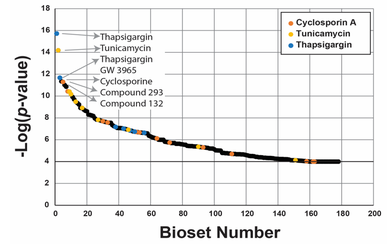
MCF-7 cells were treated with BPA or one of 16 BPA alternatives at the indicated concentrations. The transcript profiles were compared to the XBP1 biomarker. Boxplots show the increases in –Log(p-value)s with increasing concentration for the collective exposures.

Relationships between XBP1 activation and other stress factors



The transcript profiles from MCF-7 cells treated with BPA or one of 16 BPA alternatives were compared to the indicated biomarkers consisting of multiple genes. The chemicals were clustered using hierarchical clustering. The colors represent the –Log(p-value)s of the correlation of each biomarker to each of the profiles with red representing activation and blue representing suppression. XBP1 is activated at higher concentrations for most of the chemicals.

A Computational high-throughput screen for XBP1 activators



The XBP1 biomarker identifies novel chemicals that activate XBP1.
The XBP1 biomarker was compared to a list of 7,823 chemical treatment biosets using the Running Fisher test. The black dots represent additional chemicals, some of which are novel activators.

Summary

- We have constructed and characterized a gene expression biomarker that accurately identifies UPR inducers that can be used to interpret HTTr data streams.
- We implemented a novel approach using wild-type and IRE1-null comparisons to identify the XBP1-regulated genes.
- Pathway analysis and post-hoc ChIP-Seq showed that most of the genes were directly regulated by XBP1.
- Microarray datasets from cells treated with known UPR inducers identified a 6-24 hr window in which XBP1 was maximally induced (data not shown).
- Predictive accuracy was ~92%.
- A screen of a microarray compendium consisting of 12,092 comparisons from human cells exposed to 2,670 individual chemicals found a number of novel regulators of the UPR. It remains to be determined whether the induction is specific or due to high chemical concentrations. The approach using the XBP1 biomarker described here along with other stress biomarkers could be used to identify environmentally-relevant stress activators in HTTr datasets that could be integrated into AOP frameworks as part of IATA.

Acknowledgements

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Disclaimer

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