Application of a Novel Human Reporter-Based Platform for Detection of Mitochondrial Toxicity

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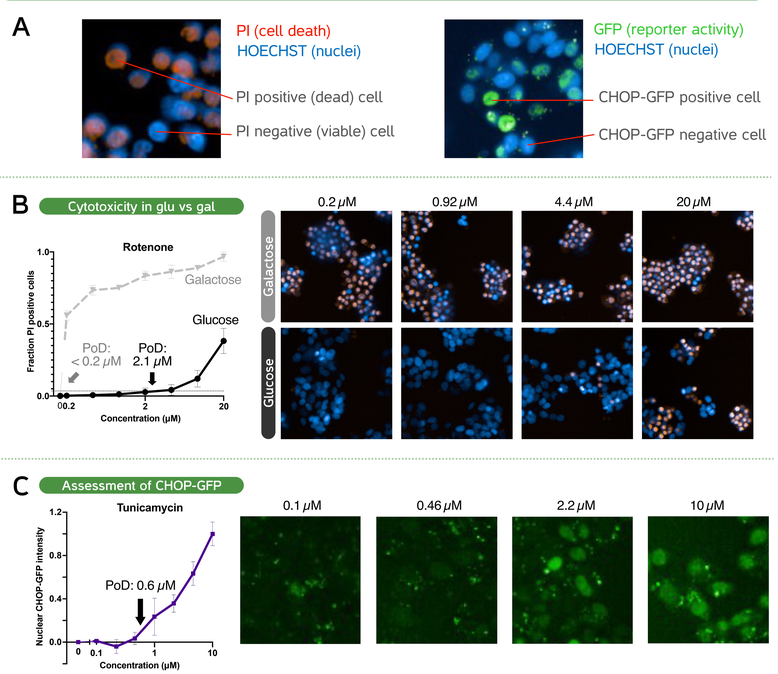
Introduction

- Impairment of mitochondrial function is a major mechanism underlying drug- and chemical-induced toxicity and a common cause of late-stage drug development failure. Early detection of mitochondrial liabilities is therefore crucial for strengthening chemical safety evaluation.
- Current screening strategies lack sensitivity and specificity, limiting their predictive value for chemical safety and human health risk assessment.
- We developed a human cell-based reporter assay that integrates the glucose/galactose (glu/gal) metabolic shift with endoplasmic reticulum (ER) stress detection and mitochondrial morphology analysis to accurately predict mitochondrial toxicity.

Integration of glu/gal medium switch and ER stress detection



(A) Interpretation of confocal images showing propidium iodide (PI) and Hoechst staining as well as induction of CHOP-GFP. (B) Increased cytotoxicity under galactose (gal) compared to glucose (glu) conditions following exposure to the mitochondrial toxicant rotenone, measured by the fraction of PI-positive cells. (C) Concentration-dependent activation of CHOP following exposure to the ER stress inducer tunicamycin.

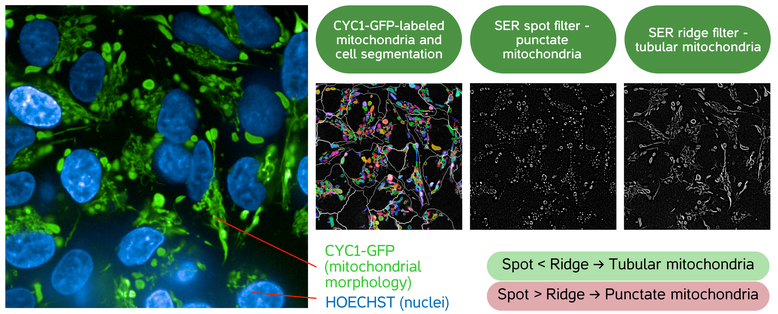
CHOP–glu/gal assay performance

Mitochondrial toxicant *	Compound name	Anticipated mitochondrial MoA	CHOP	Glu/gal	Mitotox call
yes	Rotenone	Inhibition of electron transport chain (ETC) complex I	yes	yes	yes
yes	Thifluzamide	Inhibition of ETC complex II	yes	yes	yes
yes	Antimycin A	Inhibition of ETC complex III	yes	yes	yes
yes	Oligomycin	Inhibition of ETC complex V	yes	yes	yes
yes	Carbonyl cyanide m-chlorophenylhydrazone (CCCP)	Mitochondrial uncoupler	yes	yes	yes
yes	Sodium fluoroacetate	Inhibition of citric acid cycle	no	no	no
yes	Zidovudine	Inhibition of mitochondrial DNA replication	no	no	no
yes	Perhexiline	Inhibition of fatty acid oxidation	no	no	no
yes	Troglitazone	Mitochondrial permeability transition (MPT) pore opening	yes	yes	yes
yes	Fenpyroximate	Inhibition of ETC complex I	yes	yes	yes
no	Tunicamycin	–	yes	no	no
no	Streptomycin sulfate	–	no	no	no
no	Mannitol	–	no	no	no
no	Thapsigargin	–	yes	no	no
no	Chloroquine	–	no	no	no
no	Brefeldin A	–	yes	no	no
no	Ampicillin	–	no	no	no
no	Amisulpride	–	no	no	no
no	Cadmium chloride	–	no	no	no
no	Sucrose	–	no	no	no
no	Vildagliptin	–	no	yes	no

* based on mitotox.org database and literature

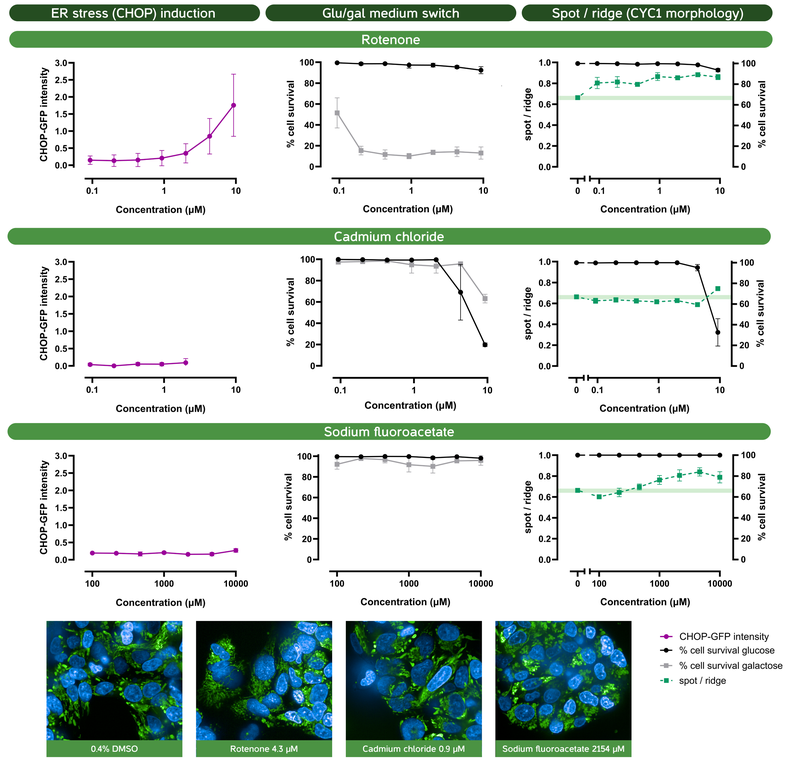
The glu/gal metabolic switch combined with CHOP-GFP detection was used to assess mitochondrial and non-mitochondrial toxicants. A mitochondrial toxicity call was assigned when CHOP was activated and the glu/gal cytotoxicity ratio exceeded two-fold. 18/21 compounds were correctly identified. The assay predicted mitochondrial toxicity with a sensitivity of 70% and a specificity of 100%.

Quantification of tubular and punctate mitochondria



Mitochondrial morphology is quantified using CYC1 (Cytochrome C1)-GFP live-cell imaging. Image segmentation pipelines are applied to detect mitochondrial structures within individual cells. Spot-Edge-Ridge (SER) ridge filters identify elongated tubular mitochondria, while spot filters detect punctate or fragmented mitochondria, enabling quantitative assessment of mitochondrial morphology.

Improvement of mitochondrial toxicity detection



Three representative compounds are shown: a correctly classified mitochondrial toxicant (rotenone), a correctly classified non-mitochondrial toxicant (cadmium chloride), and a previously classified false negative compound (sodium fluoroacetate). While glu/gal and CHOP responses did not indicate mitochondrial toxicity for sodium fluoroacetate, mitochondrial morphology analysis (spot/ridge features) revealed clear mitochondrial morphology changes with an increase in punctate mitochondria.

Conclusions

- Combining the glu/gal metabolic switch assay with ER stress detection using the CHOP-GFP reporter assay provides a sensitive platform for detecting mitochondrial dysfunction.
- Mitochondrial morphology analysis using the human HepG2 CYC1-GFP reporter improves assay performance by identifying mitochondrial morphology changes.