Mechanistic toxicity profiling of chemicals and nanomaterials by combining the ToxTracker genotoxicity assay and MiToxView mitochondrial toxicity assay

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Introduction

The ToxTracker assay uses a panel of mouse embryonic stem (mES) cells that contain different GFP reporter genes for induction of DNA damage, oxidative stress, protein damage and general cytotoxicity. Extensive validation using different reference compound libraries as well as a collection of nanomaterials showed that the integrative approach of the ToxTracker assay is a powerful tool for in vitro carcinogenic hazard identification. The MiToxView platform on mouse liver mitochondria allows identification of compounds inducing direct mitochondrial damages by assessing global membrane permeabilization (swelling), transmembrane potential (ΔΨm) loss, cytochrome c release and oxygen consumption through respiratory chain complex I and complex II.

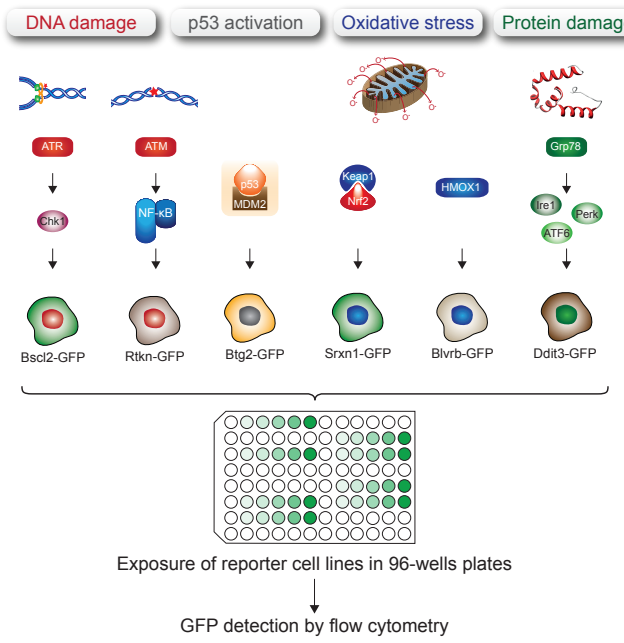
Results

We have evaluated the improved mechanistic toxicity profiling by combining the ToxTracker and MiToxView in vitro toxicity platforms. For this we tested a selection of 20 primarily DILI compounds and various metal or metal-oxide nanoparticles. ToxTracker reliably identified the genotoxic properties of the tested chemicals and nanoparticles. These substances generally also affected mitochondrial function. Various tested DILI compounds that induced mitochondriotoxicity in MiToxView activated the oxidative stress or unfolded protein response in the ToxTracker platform. However, there we're several established DILI compounds that did not activate the ToxTracker reporters but clearly affected mitochondrial integrity.

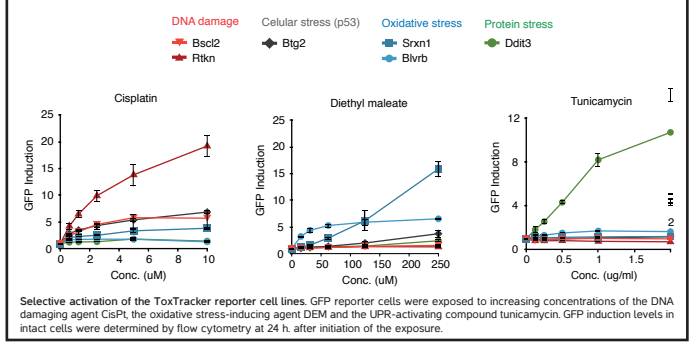
Conclusion

Together, our data indicate that integration of a cellular signalling reporter assay with a functional mitochondriotoxicity system can significantly improve reliable identification of the hazardous properties and the mechanism of action of new compounds and nanomaterials

The ToxTracker reporter assay

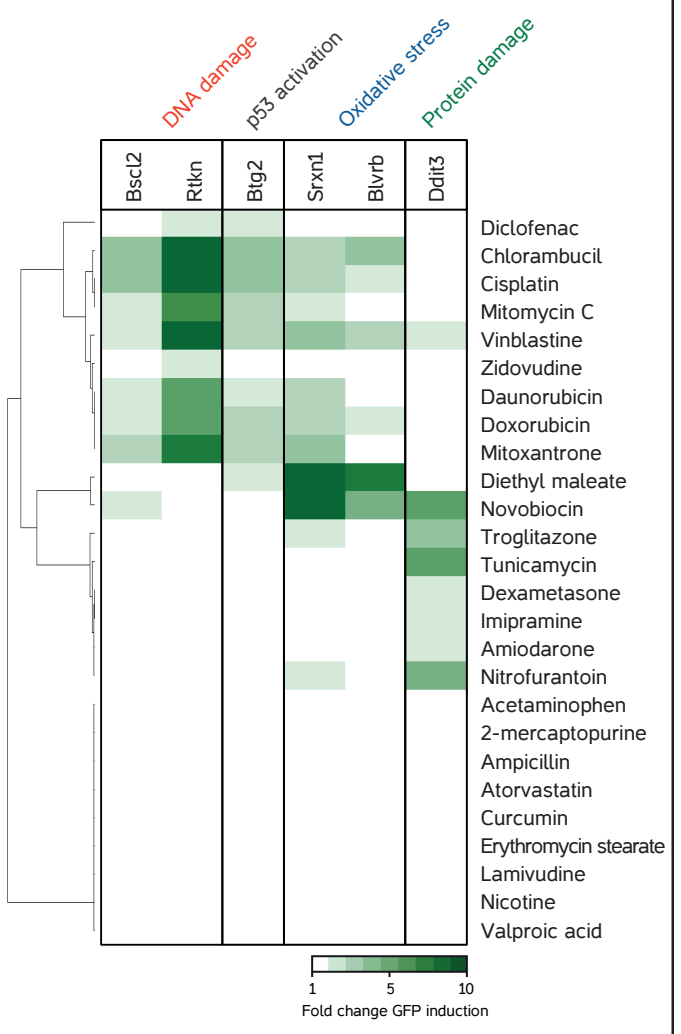


Specificity of the ToxTracker reporters



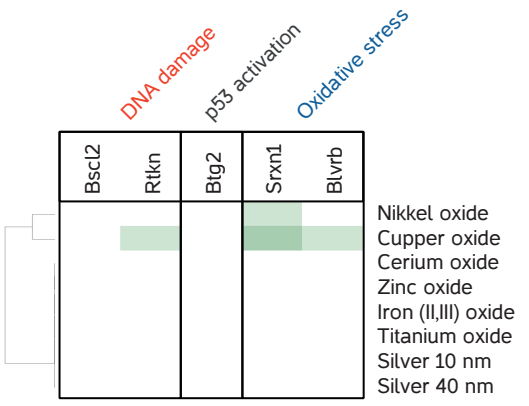
Selective activation of the ToxTracker reporter cell lines. GFP reporter cells were exposed to increasing concentrations of the DNA damaging agent CisPt, the oxidative stress-inducing agent DEM and the UPR-activating compound tunicamycin. GFP induction levels in intact cells were determined by flow cytometry at 24 h. after initiation of the exposure.

Multiple toxic properties of DILI compounds

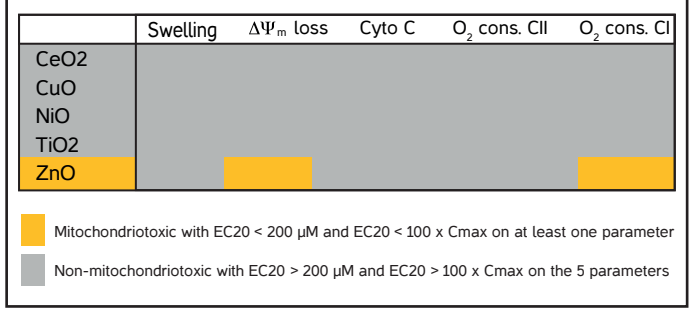


Selective induction of the ToxTracker reporters indicate different toxic properties of the tested DILI compounds. Induction levels of the different GFP reporters was calculated for all compounds at an equitoxic concentration that induced 50% cytotoxicity. The ToxTracker reporter cells were exposed for 24 h. to the compounds prior to GFP detection and cytotoxicity assessment by flow cytometry. GFP levels were determined by linear regression of the GFP induction data points of the two doses encompassing 50% cytotoxicity.

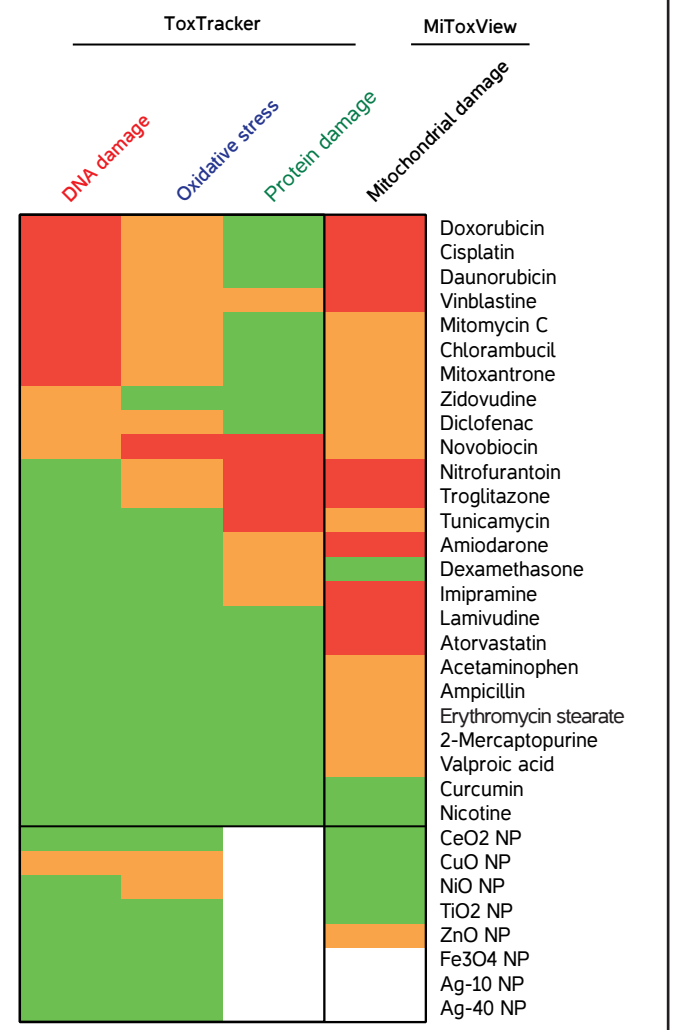
Genotoxic properties of nanoparticles



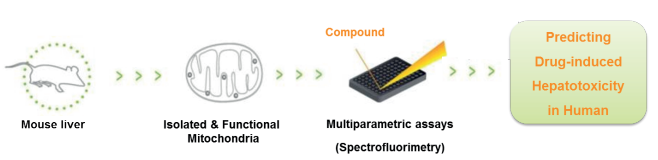
Mitochondrial damage by nanoparticles



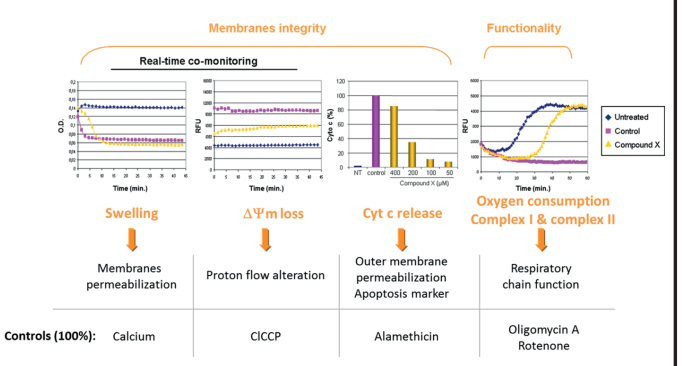
Extended mechanistic toxicity profiling



The MiToxView mitochandrial toxicity assay

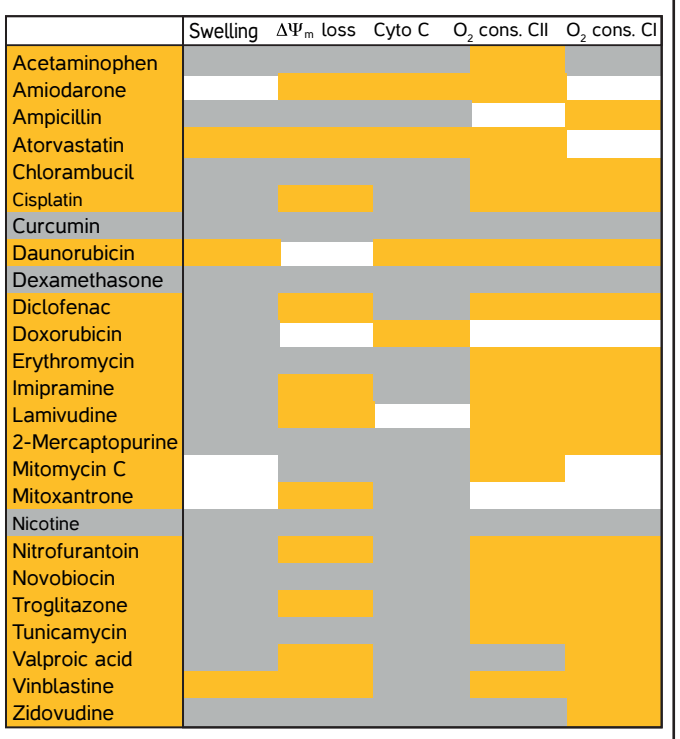


MiToxView screening platform allows assessment of the mitochondrial membrane permeabilization (swelling), mitochondrial transmembrane potential (rhodamine 123), cytochrome c release and O2 consumption driven by Complex I and Complex II (MitoXpress, Luxcel Biosciences) and ROS production (MitoSox) on isolated mitochondria.



Screening of more than 120 compounds on MiToxView has shown a high correlation between mitochondrial toxicity and DILI with Positive predictive value >82% (p<0.001) and Sensitivity = 92% (Porceddu et al., Toxicol Sci. 2012).

Mitochondrial damage by DILI compounds



Mitochondriotoxic with EC20 < 200 µM and EC20 < 100 x Cmax on at least one parameter

Non-mitochondriotoxic with EC20 > 200 µM and EC20 > 100 x Cmax on the 5 parameters