Extension of the ToxTracker reporter assay for classification of compounds with a clastogenic or aneugenic mode of action

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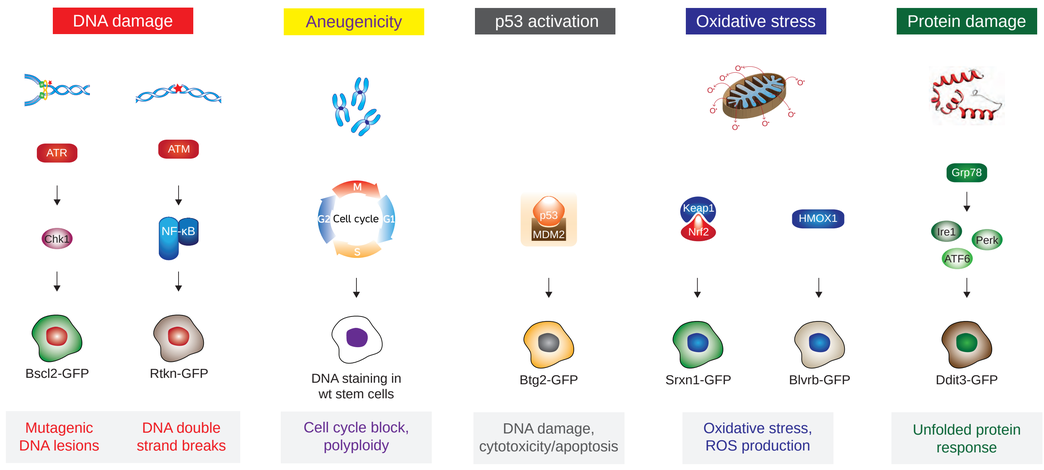
Introduction

Understanding the mode of action (MOA) and the classification of clastogens and aneugens is important for regulatory considerations. ToxTracker is a mammalian stem cell-based reporter assay that detects the activation of specific cellular signalling pathways upon exposure to compounds to provide insight into the MOA. ToxTracker contains six different GFP-tagged reporters that allow for the discrimination between the induction of DNA damage, oxidative stress and protein damage in a single test. Here we extended ToxTracker to include cell cycle and polyploidy analysis to allow for discrimination between clastogenic/mutagenic and aneugenic modes of action.

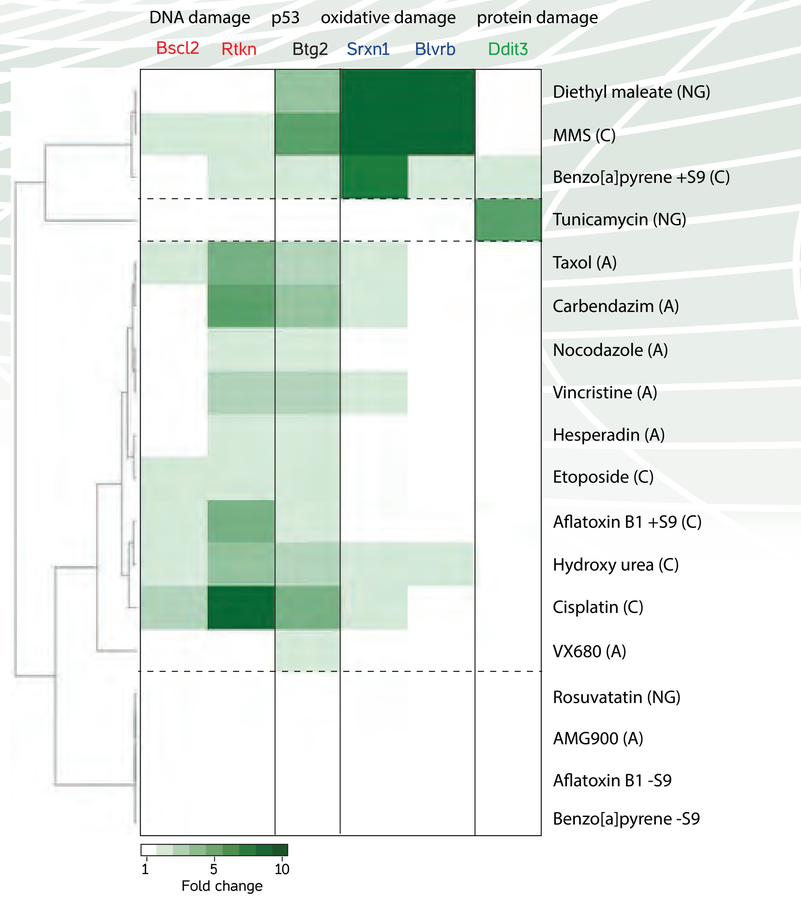
In conclusion, the selective activation of the different DNA damage reporters in ToxTracker ACE (Aneugen Clastogen Evaluation) together with a DNA stain enables the rapid and quantitative evaluation of the clastogenic and/or aneugenic potential of compounds in a single assay. Furthermore, test materials causing oxidative stress or protein damage can readily be identified within the same assay.

The ToxTracker ACE assay

- The ToxTracker assay is a stem cell-based reporter assay consisting of 6 GFP reporter cell lines.
- GFP-tagged biomarkers are activated upon specific cellular responses to DNA damage or other stress.
- Activation of the biomarkers is analysed using flow cytometry.
- ToxTracker ACE includes cell cycle analysis and polyploidy detection for aneugen vs clastogen evaluation.



ToxTracker analysis of genotoxic agents

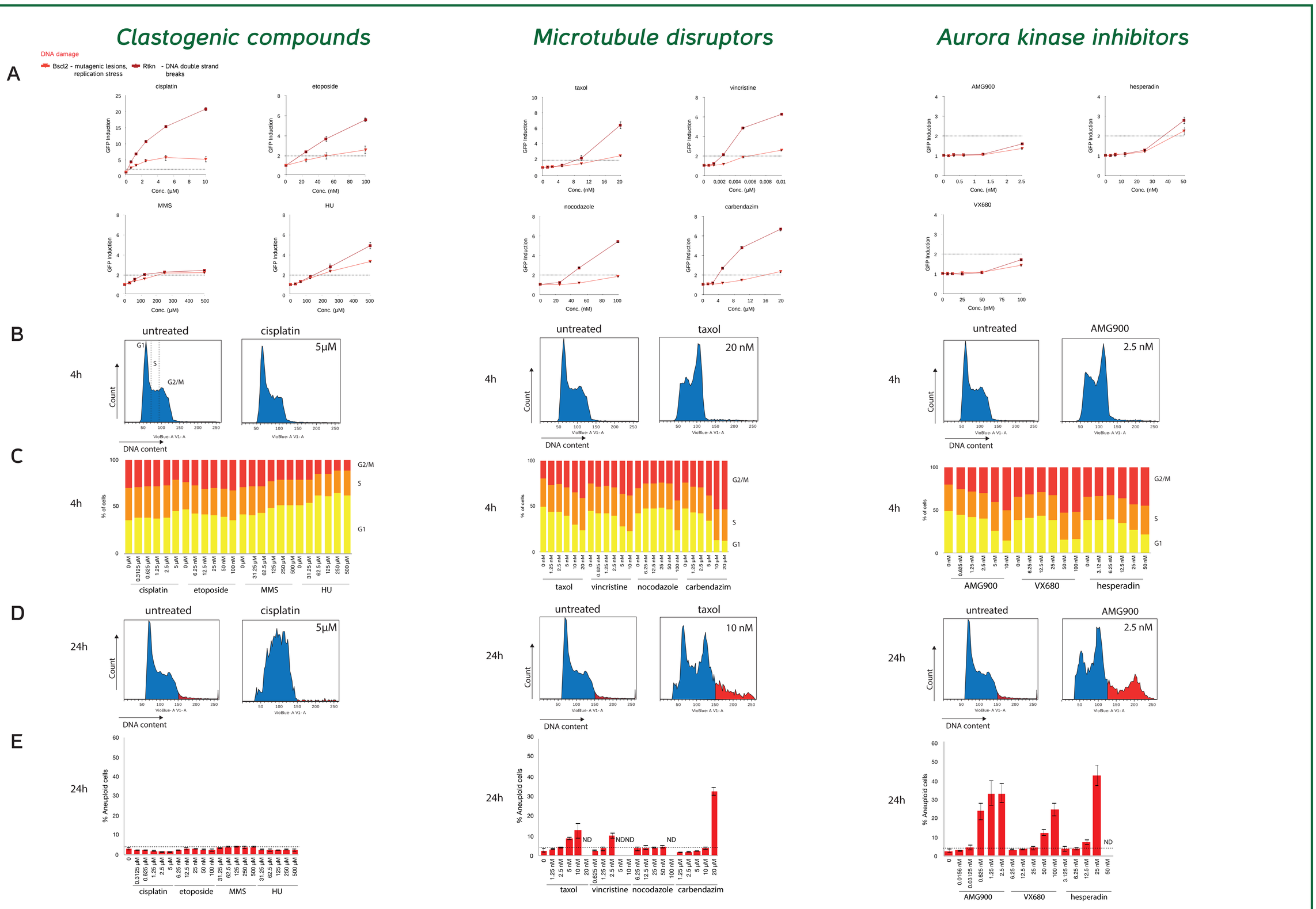


Most aneugenic and clastogenic compounds activate the Rtkn-GFP and/or Bscl2-GFP reporters in ToxTracker. Based only on the activation of these reporters, aneugens can not be separated from clastogens. The ToxTracker assay also contains reporters for protein damage and ER-stress (Ddit3-GFP) and oxidative stress (Srxn1-GFP and Blvrb-GFP). Heatmap shows the activation of the ToxTracker reporters at 50% cytotoxicity. The clustering is based on agglomerative hierarchical clustering. (A) Aneugen, (C) Clastogen or mutagen, (NG) non-genotoxic compound.

Clastogenic compounds

Microtubule disruptors

Aurora kinase inhibitors



ToxTracker ACE analysis of clastogenic compounds and aneugenic compounds (microtubule disruptors and aurora kinase inhibitors). A) Graphs showing activation of DNA damage reporters in ToxTracker. Assay performed after 24 hours of exposure and GFP intensity measured in intact cells. The dashed line indicates the threshold for a positive test (2-fold induction). B) Examples of cell cycle profiles after 4 hours of treatment. C) Quantification of cell cycle distributions after 4 hours of treatment. D) Examples of cell cycle profiles after 24 hours of treatment. Aneuploid cells (>4n) are shown in red. E) Quantification of the percentage of aneuploid cells after 24 hours of treatment. A threshold for aneuploidy (4%, dashed line) was calculated based on vehicle control data (average + 2xSD). ND: not determined due to cytotoxicity.