Application of the ToxTracker reporter assay in a mode of action approach for genetic toxicology assessment

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Introduction

ToxTracker is a mammalian stem cell-based reporter assay that detects activation of specific cellular signaling pathways upon exposure to proinflammatory compounds. ToxTracker contains two alfalfarGFP-tagged reporters that allow discrimination between clastogenic damage, oxidative stress and protein damage. In a single test (Tanaka et al. Tox Sci 2016), ToxTracker can be particularly useful in an Adverse Outcome Pathway (AOP) approach for both genotoxic and non-genotoxic carcinogens.

Results

- Aa representative validation study using 300 reference chemicals and 200 proprietary compounds, ToxTracker classified the genotoxic compounds with a sensitivity of 84% and specificity of 88%. By examining the differential induction of the two DNA damage reporters, ToxTracker was able to discriminate between a clastogenic and a mutagenic mode of action. Induction of this reporter was significantly slower (~2h) for the mitotic spindle poisons compared to clastogenic compounds (B). Furthermore, by staining for phosphorylation of histone H3 and including a DNA stain for polyploidy in the reporter cell lines, ToxTracker can identify an aneugenic MOA by inhibition of cell cycle enzymes.

Conclusion

The integrative approach of the ToxTracker assay provides a unique tool for in vitro carcinogenic hazard identification of chemicals by unveiling activation of specific cellular signaling pathways upon exposure and deliver insight into the underlying mechanism of toxicity.

The ToxTracker reporter assay

- Stem cell-based reporter assay
- In vitro carcinogenicity hazard screening
- Insights into mechanisms of genotoxicity

- Six independent GFP reporter cell lines
- High throughput detection by flow cytometry
- Discriminate between induction of DNA damage, oxidative stress and protein damage

Validation of the ToxTracker assay

- Mutagenicity and clastogenicity
- Basal-GFP reporter for promutagenic DNA lesions
- Correlation with bacterial Ames and mammalian (M-M) mutations assays

- Rin-GFP reporter for DNA double strand breaks
- Correlation with in vitro and in vivo micronucleus and chromosome aberration tests

- Clastogenicity and aneugenicity
- - ToxTracker reporter cell lines
- - Antibody staining for phosphorylated histone H3
- - DNA staining for cell cycle analysis and polyploidy
- - Analysis by flow cytometry

Kinase inhibitors

- ToxTracker reporter cell lines
- - Antibody staining for phosphorylated histone H3
- - DNA staining for cell cycle analysis and polyploidy
- - Analysis by flow cytometry

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<th>Oxidative stress</th>
<th>Protein damage</th>
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<td>Colcemid</td>
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