A BIOMARKER-BASED HUMAN STEM CELL ASSAY APPLIED FOR RANKING A RETINOID SERIES BASED ON RELATIVE DEVELOPMENTAL TOXICITY POTENTIAL

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Introduction

We previously developed an in vitro biomarker-based human induced pluripotent stem (iPS) cell-based assay to screen compounds for developmental toxicity. The assay measures changes in two amino acids (cysteine and proline) involved in cell proliferation and differentiation. This assay (devTOX™) is currently being applied as an alternative model to in vivo ongoing worldwide efforts to reduce animal testing.

In this work we demonstrate the use of the assay to rank order the relative developmental toxicity potential of a characterized set of retinoids. Results were compared to published data for both in vivo and in vitro models. iPS responses to retinoids is variable for both human and animal models due to the developmental toxicity potency of these compounds in early development. Because retinoic acid signaling plays a key role in embryogenesis we also tested the mechanistic relevance of cis- and trans-retinoic acid using iPS models using the retinoic acid receptor (RAR) antagonist Ro 41-5253. Overall, rankings in the absence of the antagonist were consistent with published values for either in vitro or in vivo studies, but did not directly correspond with in vivo potency rankings. The lack of in vivo kinetics and metabolic processes and species-specific differences in metabolism could explain these differences. Observations of the presence of Ro 41-5253 were consistent with the developmental toxicity response being mediated through RAR and suggest iPS cells do not have the ability to metabolize retinoids to its active metabolic processes and species.

Methods

Assay Workflow

Results

6 μM Ro 41-5253 Inhibits 10 nM All-trans Retinoic Acid-Induced Response in the c/o Ratio in iPSC Cells

Graphical representation of devTOX™ results.

IPS cells Exhibit a Biphasic Response to All-trans Retinoic Acid

Table: Test Compound Structures and Relationship

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Synonym</th>
<th>Description</th>
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<tbody>
<tr>
<td>All-trans Retinoic Acid</td>
<td>TPA</td>
<td>Retinoic Acid</td>
</tr>
<tr>
<td>9-cis Retinoic Acid</td>
<td>All-trans</td>
<td>Retinoic Acid</td>
</tr>
<tr>
<td>Retinol</td>
<td>Isotretinoin</td>
<td>Retinoic Acid</td>
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Developmental Toxicity Response is Mediated through the Retinoic Acid Receptor

devTOX™ Results Match Published in Vivo Results but Differ Slightly from Published in Vivo Results

Test Compound | Live Cell Viability Ranking | iPS Cell Ranking | In Vivo Ranking
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Conclusions

The current study demonstrates the utility of this human cell-based assay toward ranking compound series.

- Compound ranking trends are concordant with live cell and in vivo models.
- Use of the antagonist demonstrates that the developmental toxicity response is mediated via the RAR pathway.
- Changes in the metabolic response are independent of cell viability, with changes in cell viability observed at all high exposures.
- iPSC cells do not have the ability to metabolize etretinate to its active form.

References