In vitro Toxicology

γH2AX Double Strand DNA Damage Response Assay

Background Information

- Histone H2A variant H2A.X, a component of the nucleosome core structure has a special role in DNA repair. Phosphorylation of H2A.X at residue Ser-139 (Anti-γH2A.X) by PI3K-like kinases, including ATM, ATR and DNA-PK, is an early cellular response to the generation of DNA double-strand breaks (DSBs).

- Detecting the formation of DSBs using the γH2A.X (Ser-139) has emerged as a highly specific and sensitive molecular marker for monitoring DNA damage.

- DSBs form when both strands of the DNA double helix are broken, irrespective of how they are formed they are found to be highly toxic and can ultimately be fatal.

- Cyprotex's DNA Damage assay uses High Content Screening (HCS) to identify both DNA Damage and Cytotoxicity.

Protocol

- Cell Line
  HepG2 (other cell types available on request)

- Multiplexing
  Combination with other mechanistic endpoints available on request
  Compatible with the in vitro HCS Micronucleus Test (MNT)

- Analysis Platform
  Cellomics ArrayScan® VTI or XTI (Thermo Scientific)

- Test Article Concentrations
  8 point dose response curve with highest concentration based on cell loss or solubility limit (3 replicates per concentration)

- Test Article Requirements
  50 μL solution at 200x highest concentration or equivalent amount in solid

- Time Points
  In absence or presence of aroclor 1254 induced rat liver S9; 24 hr exposure time

- Quality Controls
  Negative control: 0.5% DMSO (vehicle)
  Positive controls: Cyclophosphamide (S9 positive control) and chlorambucil (positive control)

- Data Delivery
  Minimum effective concentration (MEC) and AC₅₀ value for each measured parameter (cell loss, nuclear morphology, DNA fragmentation and DNA damage)
‘The γH2AX assay is based on the total phosphorylation of H2AX histone in response to DNA damage by induction of double-strand breaks (DSBs).’

Figure 1
Representative HCS images for cells treated with (A) 200µM chlorambucil (positive control) (B) vehicle control in the absence of S9 fraction (C) 200µM cyclophosphamide (metabolising system positive control) and (D) vehicle control in the presence of S9 fraction over a 24 hr period. Cell nuclei are stained blue (Hoechst) with pink staining observed in the nucleus of cells positive for γH2AX (indicated by white triangles). Cellular filamentous actin is stained green (phalloidin).

Figure 2
Graphical representation of γH2AX for chlorambucil (positive control) and cyclophosphamide (positive control for metabolising system). A: is in the absence of arcorol 1254 induced rat liver S9. B: is in the presence of arcorol 1254 induced rat liver S9. Red dashed line represents the vehicle control limits.

Chlorambucil causes a concentration dependent increase in γH2AX compared to vehicle control treated cells in both the absence and the presence of metabolising system (arcorol 1254 induced rat liver S9). No response was observed for cyclophosphamide in the absence of metabolising system, however in the presence of the metabolising system 1254 induced rat liver S9). No response was observed for cyclophosphamide in the absence and presence of aroclor 1254 induced rat liver S9. B: is in the presence of aroclor 1254 induced rat liver S9. Chlorambucil causes a concentration dependent increase in γH2AX for chlorambucil (positive control) and vehicle control (negative control) (A) vehicle control (B) 200µM chlorambucil (positive control) (C) 200µM cyclophosphamide (metabolising system positive control) and (D) vehicle control in the presence of S9 fraction over a 24 hr period. Cell nuclei are stained blue (Hoechst) with pink staining observed in the nucleus of cells positive for γH2AX (indicated by white triangles). Cellular filamentous actin is stained green (phalloidin).

Table 1
γH2AX data for 24 validation compounds categorised according to literature data.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Minus Rat Liver S9</th>
<th>Plus Rat Liver S9</th>
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<tbody>
<tr>
<td></td>
<td>MEC, AC50, +/-ve</td>
<td>MEC, AC50, +/-ve</td>
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<td></td>
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<tr>
<td>Benzo[a]pyrene</td>
<td>0.133, 29.8</td>
<td>+, 1.93, 103</td>
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<tr>
<td>Chlorambucil</td>
<td>6.69, 55.2</td>
<td>+, 7.53, 66.2</td>
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<tr>
<td>Cisplatin</td>
<td>0.356, 8.44</td>
<td>+, 0.589, 11.7</td>
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<tr>
<td>Colchicine</td>
<td>0.03, &gt;0.2</td>
<td>+, 0.0029, 0.194</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>NR, NR</td>
<td>-</td>
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<tr>
<td>Cyanamide</td>
<td>8.175, 0.964</td>
<td>+, 0.0776, 10.8</td>
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<tr>
<td>Etoposide</td>
<td>0.346, 5.23</td>
<td>+, 0.703, &gt;10</td>
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<tr>
<td>Formaldehyde</td>
<td>145, &gt;100</td>
<td>+, 97.6, 825</td>
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<tr>
<td>Griseofulvin</td>
<td>8.56, &gt;300</td>
<td>+, 12.5, &gt;300</td>
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<tr>
<td>Hydroxyurea</td>
<td>274, 442</td>
<td>+, 301, 506</td>
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<tr>
<td>Mitomycin</td>
<td>0.0116, 1.12</td>
<td>+, 0.0788, 2.35</td>
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<tr>
<td>Vinblastine</td>
<td>0.0054, &gt;0.02</td>
<td>+, 0.0091, &gt;0.35</td>
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</table>

NR = no response NS = not significant

HepG2 cells were treated for 24 hours with test compound in the absence and presence of arcorol 1254 induced rat liver S9. The compounds were analysed using Cellomics ArrayScan® VTI or XTI (Thermo Scientific). Cyprotex data correlates well with literature in vivo data.1

References