A Novel Immunoassay (ELISA) for Quantitative γH2AX Detection and Pharmacodynamic Monitoring of DNA Damage Induced by Chemotherapeutic Agents and PARP Inhibitors

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Methods

The human colon cancer cell line HCT 116 was used for ELISA development and comparisons to Western blot and IFA. The human melanoma cell line A375 was used for xenograft mouse studies. Both cell lines were acquired from ATCC and cultured with 10% fetal bovine serum at 37°C in 5% CO₂ incubator. In vitro treatments were topotecan, TRAIL, and ionizing radiation. Cells were extracted in 100 µL (per million cells) of Cell Extraction Buffer (Invitrogen) with protease inhibitors (Roche) and 1% PMSF (Sigma-Aldrich) on ice for 30 min for ELISA and Western blot analysis. In parallel, cell aliquots were spotted on slides by cytospin and were stained by IFA. γH2AX detection was performed using a mouse monoclonal antibody (Millipore). The assay has a lower limit of detection of < 4 pg/mL, upper limit of quantitation of 2000 pg/mL, and a coefficient of variation of 20%.

Results

γH2AX ELISA will be useful in situations when higher throughput and accurate quantitation are needed and γH2AX analysis does not need to be restricted to nuclear foci.

The ELISA can quantify γH2AX concentrations as low as 16 pg/mL in crude extracts from cancer cells and solid tumors.

The assay has a lower limit of detection of < 4 pg/mL, upper limit of quantitation of 2000 pg/mL, and a coefficient of variation of 20%.

The assay shows utility for drug discovery screening, molecular pharmacology studies, and pharmacodynamic monitoring.

Summary

- The γH2AX ELISA will be useful in situations when higher throughput and accurate quantitation are needed and γH2AX analysis does not need to be restricted to nuclear foci.
- The ELISA can quantify γH2AX concentrations as low as 16 pg/mL in crude extracts from cancer cells and solid tumors.
- The assay has a lower limit of detection of < 4 pg/mL, upper limit of quantitation of 2000 pg/mL, and a coefficient of variation of 20%.
- The assay shows changes in γH2AX induced by a variety of treatments in vitro and was overall highly correlated to both IFA and Western blot following treatment with irinotecan or TRAIL. With the less active PARP inhibitor, there was modest correlation between ELISA and Western blot, but the high correlation with IFA remained.
- In a mouse xenograft model, the assay detected apparent potentiation of DNA damage with combined irinotecan and PARP inhibitor treatment, an effect that reached statistical significance versus irinotecan alone for the lower dose of MK-4827 with irinotecan and both doses of A22881 with irinotecan at the 6-h post-dose time point.

The assay shows utility for drug discovery screening, molecular pharmacology studies, and pharmacodynamic monitoring.

Table: Comparison of γH2AX quantitation using ELISA, immunofluorescence assay, and Western blot in HCT 116 cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>γH2AX ELISA</th>
<th>IFA</th>
<th>Western blot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>238 2.5 2.0</td>
<td>237 1.9 1.8</td>
<td>237 1.9 1.8</td>
</tr>
<tr>
<td>0.5 Gy</td>
<td>287 4.7 4.6</td>
<td>286 4.6 4.5</td>
<td>286 4.6 4.5</td>
</tr>
<tr>
<td>1.0 Gy</td>
<td>287 4.7 4.6</td>
<td>286 4.6 4.5</td>
<td>286 4.6 4.5</td>
</tr>
<tr>
<td>2.0 Gy</td>
<td>287 4.7 4.6</td>
<td>286 4.6 4.5</td>
<td>286 4.6 4.5</td>
</tr>
</tbody>
</table>

References


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