Effects of hsa-miR-9-3p and hsa-miR-9-5p on Topoisomerase IIβ Expression in Human Leukemia K562 Cells with Acquired Resistance to Etoposide

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Methods: For Uncropped Immunoblots with Markers

Western blot analysis was performed as described in manuscript Methods and Materials under the “Immunoassays” heading. All immunoassay images were acquired with the ChemiDoc XRS+ imaging system and analyzed with ImageLab software (Bio-Rad Laboratories) from chemiluminescent signals.

Frequently, membranes were cut prior to antibody addition for separate analysis of GAPDH loading controls (routine attempts were made to cut below 75 kDa, above 50 kDa, and again below the 25 kDa marker) so that membranes would fit in small incubation chambers to keep antibody volumes low. Membranes were also cut for separate antibody incubations with TOP2α/170 or TOP2β/180 depending on the experiment performed. For example, see images from Fig. 5A below.

Markers run in PAGE gels transferred to membranes along with protein bands. Markers were visualized by white light photography on the ChemiDoc XRS+ imager and subsequently merged with the chemiluminescent signals from TOP2α/170, TOP2α/180, and GAPDH using the merge function in Image Lab software. Images shown below from Figs. 1C, 4b, 5A, 6A are labeled for clear visualization of Markers and the protein bands of interest.
Figure 1C

**Markers:**
Membrane Photo

**TOP2β/180**

Markers and Immunoblot
Chemiluminescent: Merged

**GAPDH**
Figure 4B

K562 cells

<table>
<thead>
<tr>
<th>Control mimic (25nM)</th>
<th>3p mimic (25nM)</th>
<th>5p mimic (25nM)</th>
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</thead>
<tbody>
<tr>
<td>250 kDa</td>
<td>TOP2β/180</td>
<td>GAPDH</td>
</tr>
<tr>
<td>150 kDa</td>
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<td></td>
</tr>
<tr>
<td>37 kDa</td>
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</tbody>
</table>

Markers: Membrane Photo

TOP2β Protein

MERGED: Markers & Immunoblot (Chemi-luminescent)

Markers:

GAPDH

37 kDa

25 kDa

36 kDa
Figure 5A

K562 cells

<table>
<thead>
<tr>
<th>XK469 (μM)</th>
<th>0</th>
<th>100</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 kDa</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>150 kDa</td>
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</tr>
<tr>
<td>37 kDa</td>
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</tr>
</tbody>
</table>

TOP2α/170

GAPDH

TOP2β/180

GAPDH

TOP2α/170

MARKERS:
Membrane Photo

TOP2β/180

MERGED:
Markers & Immunoblot (Chemi-luminescent)

MARKERS:
Membrane Photo

180 kDa

36 kDa