In vitro validation of putative GRP78 inhibitors previously found in silico

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1 Background

The glucose-regulated protein 78 kDa (GRP78) plays a major role in cancer, contributing to tumorigenesis and chemoresistance [1]. Under endoplasmic reticulum (ER) stress, common in cancer and in some viral infections, GRP78 can be translocated to the cell-surface (cs) membrane and activate classic oncogenic pathways [2]. Since cancer cells intrinsically exhibit higher ER stress levels, cs-GRP78 inhibitors are likely to be selective against them, thus, cs-GRP78 appears as a promising molecular target for cancer treatment. Preliminary results from a structure-based virtual screening study conducted by our collaborators, identified the FDA-approved drug imatinib and other bioactive molecules, such as selonsertib, as being putative GRP78 inhibitors [3].

2 Aim

To validate the in silico results and to confirm the antitumor potential and chemosensitizing effect of some of the identified compounds in GRP78-overexpressing cancer cell lines, such as A549 (non-small cell lung cancer), MDA-MB-231 (triple negative breast cancer) and BxPC3 and Panc-1 (pancreatic adenocarcinoma) cell lines.

3 Methods

Cell culture of GRP78-overexpressing cancer cell lines

Subcellular fractionation for isolation of plasma membrane and cytoplasmic fractions

Confirmation of GRP78 expression levels by Western Blot in the membrane and cytoplasmic fractions

Assessment of the cytotoxic effect of the putative GRP78 inhibitors by the Sulforhodamine B (SRB) Assay

Analysis of the effect of the putative GRP78 inhibitors on GRP78 expression levels by Western Blot

4 Results

Table 1. Growth inhibition concentration (GI50) of imatinib and selonsertib in A549, MDA-MB-231, BxPC3 and Panc-1 cancer cell lines treated for 48 h, determined with the SRB assay. GI50 values correspond to the mean ± S.E.M. of at least three independent experiments performed in duplicate.

<table>
<thead>
<tr>
<th>Cancer cell line</th>
<th>GI50 concentration of Imatinib (µM)</th>
<th>GI50 concentration of Selonsertib (µM)</th>
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<tbody>
<tr>
<td>A549</td>
<td>10.56 ± 1.58</td>
<td>5.68 ± 0.66</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>17.38 ± 1.12</td>
<td>5.31 ± 0.64</td>
</tr>
<tr>
<td>BxPC3</td>
<td>14.54 ± 2.41</td>
<td>10.73 ± 2.10</td>
</tr>
<tr>
<td>Panc-1</td>
<td>43.77 ± 4.17</td>
<td>5.74 ± 0.33</td>
</tr>
</tbody>
</table>

✓ MDA-MB-231 and BxPC3 cell lines express GRP78 in both cytoplasmic and plasma membrane fractions

✓ The GI50 concentrations of imatinib and selonsertib were determined in the four cancer cell lines

5 Conclusions

- MDA-MB-231 and BxPC3 cells present GRP78 both in the cytoplasmic and plasma membrane;
- The GI50 concentrations of imatinib and selonsertib were determined in the four cancer cell lines tested;
- Growth inhibition curves for the effect of imatinib and selonsertib were determined in the four cancer cell lines tested;
- Imatinib did not alter the plasma membrane GRP78 levels in MDA-MB-231 cells.

6 Future work

- Complete these experiments in the other cancer cell lines;
- Investigate the effect of other GRP78 inhibitors previously identified in silico [3] on the cell-surface GRP78 activity and on its downstream pathways;
- Evaluate the potential of these inhibitors to sensitize cancer cell lines to first-line chemotherapeutic drugs through drug combinations.

7 References