Mitochondrial Priming of New Targeted Agents in Acute Myeloid Leukemia

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Abstract

As numerous molecularly targeted agents are entering clinical trials, predictive testing is highly desirable.4,5 We investigated if response to certain agents correlates with mitochondrial priming as measured by mitochondrial outer membrane permeabilization (MOMP) following exposure to BH3-mimicking BHS3 domains of BHS3-only proteins. We performed mitochondrial priming by measuring changes in cell mitochondrial permeability following exposure to BH3 peptides (BIM, PUMA, NOXA, BMF, HRK, or PUMA2A). JC-1 was used as a probe for mitochondrial depolarization.22 AML cell lines, including OCI-AML3, MOLM-13 and MV4-11 cells, were exposed to BH3-mimicking peptides. As expected, BFL-2, BCL-2, and BCL-XL protein expression correlated with changes in cell mitochondrial permeability following exposure to BH3-mimicking peptides.22

Methods

1. Reagents and cells: KPT-330 was synthesized at Karyopharm Therapeutics. (Natnick, MA). MDM2 antagonist Nutlin-3a was purchased from Cayman Chemical Company (Ann Arbor, MI). BCL-2 inhibitor ABT-199 was purchased from Selleckchem. Total 22 AML cell lines, including OCI-AML3, MOLM-13 and MV4-11 cells, were exposed to BH3-mimicking peptides (BIM, PUMA, NOXA, BMF, HRK, or PUMA2A). JC-1 was used as a probe for mitochondrial depolarization.22 AML cell lines, including OCI-AML3, MOLM-13 and MV4-11 cells, were exposed to BH3-mimicking peptides.22

Results

BH3 profiling and correlation with drug sensitivity

<table>
<thead>
<tr>
<th>BH3 peptide</th>
<th>Sensitive</th>
<th>Partially sensitive</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIM</td>
<td>69%</td>
<td>21%</td>
<td>10%</td>
</tr>
<tr>
<td>PUMA</td>
<td>73%</td>
<td>24%</td>
<td>3%</td>
</tr>
<tr>
<td>NOXA</td>
<td>80%</td>
<td>16%</td>
<td>4%</td>
</tr>
<tr>
<td>BMF</td>
<td>85%</td>
<td>15%</td>
<td>0%</td>
</tr>
<tr>
<td>HRK</td>
<td>78%</td>
<td>20%</td>
<td>2%</td>
</tr>
<tr>
<td>PUMA2A</td>
<td>82%</td>
<td>16%</td>
<td>2%</td>
</tr>
</tbody>
</table>

Conclusion

1. BH3 profiling (%[BAD]-%[HRK]) is highly predictive for cell sensitivity to BCL-2 dependent apoptosis ABT-199.
2. Ara-C-induced apoptosis unexpectedly revealed BCL-2 dependency, similar to ABT-199.
3. BH3 profiling does not predict for Nutlin-induced p53-mediated apoptosis or KPT-induced apoptosis.
4. BCL-2 protein levels correlate with BCL-2-dependent mitochondrial priming.
5. BH3 profiling is superior to BCL-2 protein level in terms of predicting cell sensitivity to BCL-2 dependent apoptosis by ABT-199.
6. BCL-1 protein expression predicts resistance to ABT-199 compared to Ara-C.
7. BCL-2 overexpression is associated with Ara-C resistance and BCL-2 protein levels correlate with BCL-2 protein levels of chemotherapeutic agents.

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Conflict of Interest

WP, MC, RL and GD are employees of Karyopharm. SC has research support from Karyopharm and is a member of its scientific advisory board.