A Penotypic Screening Toolbox Permits the Identification of Novel Compounds with Anti-Cancer Properties Derived from Marine Fungi

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INTRODUCTION

The ‘Marine Fungi’ project, an international FP7 program, aims at identification of novel compounds with anticancer properties from marine fungi. The projects span from the isolation and characterisation of the marine fungi to fermentation, activity guided purification and screening of extracts and compounds. The 3 most interesting natural products are now being analysed in vivo.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>No. of cell lines</th>
<th>Source</th>
<th>Extracts</th>
<th>Organisms</th>
<th>No. hits</th>
<th>Hit Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia</td>
<td>6</td>
<td>Mediterranean sponge fungi</td>
<td>754</td>
<td>206</td>
<td>78</td>
<td>10.3</td>
</tr>
<tr>
<td>Non-Small Cell Lung</td>
<td>9</td>
<td>Chiloean macro-algal fungi</td>
<td>125</td>
<td>125</td>
<td>48</td>
<td>38.4</td>
</tr>
<tr>
<td>CNS</td>
<td>6</td>
<td>Indonesian coral fungi</td>
<td>331</td>
<td>105</td>
<td>47</td>
<td>16.5</td>
</tr>
<tr>
<td>Melanoma</td>
<td>9</td>
<td>Totals</td>
<td>1210</td>
<td>436</td>
<td>173</td>
<td>14.3</td>
</tr>
<tr>
<td>Ovarian</td>
<td>7</td>
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<tr>
<td>Prostate</td>
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</tr>
<tr>
<td>Breast</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Structure of the Marine Fungi consortium

ASSAY PRINCIPLE

The principle of the NCi60 human tumour cell line anticancer drug screen was developed 15 years ago. We adapted the assay for HTS purposes by changing the read out, from dye based to Luminescence, miniaturising it, from 96 to 384 well plates, and reducing the volume per well from 200 µl to 20 µl. The wealth of data available for the cell lines from the NCi60 panel, like COMPARE or the COSMIC database, is an invaluable source of information for the screening process. Parallel to the NIH procedure assays for improved automation, using the BioLevitator™ (Hamilton Bonaduz AG), or alternative read outs, using the CellMetric™ (Solenim Ltd), were evaluated.

RESULTS OF THE CYTOTOXICITY PROFILING AND FURTHER ASSAYS

The screening process is based on a triage of assays. Starting with 4 very sensitive cell lines from the NCi60 to identify also weak anticancer activity, the compounds then undergo a full screening in the NCi60 panel to obtain tissue specific data and an understanding of the mode of action via COMPARE. Most of the marine natural products exhibit an activity in the low micromolar range.

Further assays include apoptosis and necrosis assays as well as pathway analysis using PhosphoFlow analysis. ADMET assays like plasma protein binding or Cytochrome P450 inhibition help to select the most interesting compounds for in-vivo studies.

A research article describing the identification of the first 37 identified natural products has been submitted and will appear in the journal for ASSAY and Drug Development Technologies.

ACKNOWLEDGEMENTS

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