Marine Natural Products with Potential as Treatments for Pancreatic Cancer: Manzamine A

Esther A Guzmán, PhD
Marine Biomedical and Biotechnology Research
Harbor Branch Oceanographic Institute at FAU
Cancer/Pancreatic Cancer

Cancer is a general name for a group of more than 100 diseases

- Uncontrolled cell growth in a part of the body
- Damaged DNA

Pancreatic Cancer

- 4th leading cause of cancer death in the US
- Only 6% of patients will survive 5 years after diagnosis
Manzamine A

- An alkaloid first isolated by Tatsuo Higa in 1986 from an Okinawan sponge
- Has been isolated from sponges of the genera *Haliclona* sp., *Xestospongia* sp. and *Pellina* sp.
- Has anti-tumor, insecticidal, antibacterial, anti-malarial, and anti-inflammatory activities
- A microbe that can produce manzamine A through fermentation has been isolated
Manzamine A Is Not Very Cytotoxic

Cell Viability AsPC-1; 48hr

Relative Percent Viability

μM manzamine A

Manzamine A Abrogates Cell Dissociation

NT

MeOH

52.6 μM U0126

2.5 μM HB-071

5 μM HB-071

10 μM HB-071

Sensitivity to Cell Death

Normal cell

TRAIL

Anti-TRAIL Ab

Apoptosis

Cancer cell

Survival

HBOI compound

Cancer cell

Apoptosis
Manzamine A Abrogates Resistance to Apoptosis


Late apoptosis

Early apoptosis

7-Aminoactinomycin D (7-AAD)

Annexin V PE

NT

MeOH

2.5µM Mz A

5µM Mz A

10µM Mz A

NT + TRAIL

MeOH + TRAIL

2.5µM Mz A + TRAIL

5µM Mz A + TRAIL

10µM Mz A + TRAIL

3.4%

3.3%

2.4%

2.2%

20.4%

14.7%

8.5%

8.7%

15%

16%

19.9%

14.7%

8.5%

8.7%

15%

16%

19.9%

14.7%

8.5%

8.7%

15%

16%

19.9%

14.7%

8.5%

8.7%

15%

16%

19.9%

14.7%

8.5%

8.7%

15%

16%

19.9%

14.7%

8.5%

8.7%

15%

16%

19.9%

14.7%

8.5%

8.7%

15%

16%
Cell Invasion Assay

1. Transwell membrane 8μM pore size
2. Collagen matrix
3. Create chemotactic gradient
4. Add Cells ± HBO1 compounds
5. 24-well Plate

- 5% FBS
- 10% FBS

- Stain cells with Diff-Quick
- Count cells
Manzamine A Prevents Cell Invasion

Manzamine A Acts through GSK3β

Graphical representation of the average of three flow cytometry experiments ± standard deviation

Hip-Hop Assay

Haploinsufficiency profiling (HIP)
- Uses yeast strains which have a single copy of a gene knocked out
- Strains most sensitive to a drug become depleted over time
- Identifies an inhibitory compound’s candidate targets.

Homozygous profiling (HOP)
- Uses yeast strains that carry complete deletions of non-essential genes
- Strains most sensitive to a drug become depleted over time
- Identifies genes that are required for growth in the presence of compound.
- This assay can be particularly informative for compounds that lack a direct protein target.
Hip-Hop Profiling of Manzamine A

No significant hits in HIP means no direct binding target
Manzamine A clustered in HOP with known uncouplers of vacuolar ATPases
Accumulation of Acidic Autolysosomes after Treatment with Manzamine A

Kallifatidis et al, Manuscript in Preparation
Manzamine A Blocks the Last Step of the Autophagic Pathway

![Diagram showing the autophagic pathway and the effect of Manzamine A on LC3-II level](image)

Microtubule-associated protein 1A/1B-light chain 3 (LC3)

AsPC-1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (h)</th>
<th>LC3-I</th>
<th>LC3-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veh, 0.5 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veh, 2 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veh, 24 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veh, 48 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μM Manzamine A</td>
<td>0.5 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μM Manzamine A</td>
<td>2 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μM Manzamine A</td>
<td>24 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μM Manzamine A</td>
<td>48 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starvation, 0.5 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starvation, 2 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starvation, 24 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starvation, 48 h</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Kallifatidis et al, Manuscript in Preparation
Effects of Manzamine A in AsPC-1 Pancreatic Cancer Cells

Manzamine A

- Not very cytotoxic on its own
- Increases phosphorylation (inhibition) of GSK3β
- Potential anti-metastatic agent
  - Prevents migration of AsPC-1 cells through a collagen matrix
  - Reduces cell dissociation
- Restores the ability of pancreatic cancer cells to undergo apoptosis
- Uncouples vacuolar ATPases, increasing lysosomal acidity
- Inhibits autophagy
Acknowledgements

Data Presented Generated by HBOI:
Esther Guzmán
Georgios Kallifatidis

Novartis:
Dominic Hoepfner
Tiphaine Jaeg

Funding:
NIH R01CA093455 (Wright)

CMBBR
Amy Wright, PhD
Peter McCarthy, PhD
Esther Guzmán, PhD
John Reed, MS
Susan Sennett, PhD
Georgios Kallifatidis, PhD
Kayanne Cook-Russell, PhD
Floyd Russell, PhD
Brunilda Vera, PhD
Priscilla Winder, PhD
Daniel Feder
Dedra Harmody
Kathleen Janda
Tara Pitts
Lynn Roberts
Jill Roberts
Gail Samples