Novel p53-Derived Peptide Induces Rapid Human Pancreatic Cancer Cell Death

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July 25th 2008
Background:

- Molecular modeling studies have identified various potential anti-cancer peptides
- Novel p 53-based synthetic peptides promote anti-cancer activity
Background:

- p 53 peptides from MDM-2-binding domain

Rosal, R. *Biochemistry* 2004; 1854-1861
Background:

- PNC-28

**P 53 Peptide 17-26 - Penetratin**

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ETFSDLWKLL-KKWMRRNQFWVKVQRG
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Kanovsky, M. *PNAS.* 2001; 12438-12443.
**Background:**

- **Tumor cell killing by PNC-28**

<table>
<thead>
<tr>
<th>CELL LINE</th>
<th>CELL TYPE</th>
<th>TIME TO CELL DEATH (1 x 10^6 CELLS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUC-3</td>
<td>Pancreatic Acinar Carcinoma</td>
<td>72 hr</td>
</tr>
<tr>
<td>E-49</td>
<td>Angiosarcoma</td>
<td>72 hr</td>
</tr>
<tr>
<td>SW-1417 *</td>
<td>Colon Cancer</td>
<td>48 hr</td>
</tr>
<tr>
<td>MDA-MB-453 *</td>
<td>Breast Cancer</td>
<td>1 hr</td>
</tr>
<tr>
<td>H1299 *</td>
<td>Lung Cancer</td>
<td>1 hr</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>Breast Cancer</td>
<td>30 min</td>
</tr>
</tbody>
</table>

* Homozygously p53-deleted
Background:

- Anti-cancer observations

  p 53 peptide 17-26 + Penetratin → PNC-28

  Tumor cell membrane penetration (pore formation)

  Rapid tumor cell death

  Preferential induction of tumor cell necrosis

Kanovsky, M. *PNAS.* 2001; 12438-12443.

Do, TN. *Oncogene.* 2003; 1431-1444.
Hypothesis:

- To test if treatment with PNC-28 has similar anti-cancer effect against human pancreatic cancer
Specific Aims:

- Investigate the ability of PNC-28 to inhibit the growth of a human pancreatic cancer cell line in culture

- Elucidate potential anti-cancer mechanism for cell death
Materials and Methods:

- **Cells & treatment**
  - Incubated $2 \times 10^4$ MiaPaCa-2 human pancreatic carcinoma cells with PNC-28 or unrelated PNC-29
  - Peptide administered at predetermined dose
    - (50 – 300 µg/ml)
  - Treatment daily over 4 consecutive days
Materials and Methods:

- Cell morphology & growth inhibition
  - Culture analysis performed daily to measure changes in growth characteristics and cell morphology
  - Growth inhibition determined by counting viable tumor cells
Materials and Methods:

- Cell necrosis & apoptosis
  - Relative cytotoxicity determined by measuring LDH release
  - Initiation of apoptosis by detection of caspase -3 activity was performed
Novel p 53-Derived Peptide Induces Rapid Human Pancreatic Cancer Cell Death

Results:

Anti-cancer observations

- Morphologic studies

![Morphologic studies A](MiaPaCa-2 (No Treat))

![Morphologic studies B](MiaPaCa-2 (PNC-28))
Results:

**Anti-cancer effect**

- **Growth inhibition**

![Graph showing cell number over hours of treatment with different concentrations of PNC-28 peptide](image)

*Triplicate wells

P < 0.001

www.downstatesurgery.org
Results:

Anti-cancer effect

- Absence of growth inhibition
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## Results:

- **Anti-cancer profile**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Response</th>
<th>Peptide (max dose response/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNC-28</td>
<td>+</td>
<td>300 µg</td>
</tr>
<tr>
<td>PNC-29</td>
<td>–</td>
<td>No effect</td>
</tr>
</tbody>
</table>

+ = growth inhibition at 24 hours
Results:

Anti-cancer mechanism

- LDH release at 24 hours

![Graph showing LDH release at 24 hours with PNC Peptide Dose (µg/ml) on the x-axis and % Relative Cytotoxicity on the y-axis. Bars indicate LDH release at different peptide doses, with a significant difference marked at 300-28 with a * symbol and an asterisk indicating triplicate wells with P < .01.]
Results:

Anti-cancer mechanism

- LDH release at 24 hours
Results:

Anti-cancer mechanism

- Absence of caspase -3 activity

![Graph showing Rhodamine Counts over 24 Hours of Treatment]

*Triplicate wells
P < .01
Results:

- Mechanism data implications

- PNC-28 induced release of LDH; anti-cancer activity promotes cell death by necrosis

- Absence of early marker for apoptosis; p 53-derived peptide acts by p53-independent mechanism
PNC-28 is a synthetic peptide derived from the mdm-2-binding domain of p 53 attached to penetratin

- PNC-28 inhibits tumor cell growth
- Anti-cancer mechanism consistent with necrosis
Conclusion:

- PNC-28 inhibits MiaPaCa-2 human pancreatic cancer cell line in culture
- Anti-cancer activity of PNC-28 is dose dependent
- Anti-cancer mechanism appears to be p53-independent necrosis, not apoptosis