Specific Targeting and Delivery of Therapeutics to Cancer Cells Based on the Tumor Microenvironment

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Bioscience in the 21st century (Sep. 14, 2018)
Anticancer Drugs and Side Effects

- Most anticancer drugs have off-target side effects.
- Severely limit the efficacy of chemotherapy.

➡ Clear needs for targeted therapies
Drug Carrier Systems

• Can improve the therapeutic index by reducing:
  * Side effects in healthy tissues.
  * The overall dose by concentrating the drug in the targeted tissue.

• Carrier systems include:
Drug Carrier Systems

- Can improve the therapeutic index by reducing:
  - Side effects in healthy tissues.
  - The overall dose by concentrating the drug in the targeted tissue.

- **Passively** target tumors due to the increased permeation of many solid tumors.

- However, this effect is small for certain tumors.

⇒ Specific targeting strategies have been developed.
Current Targeting Strategies

• Most take aim at specific cancer cell surface biomarkers.
  ✗ Example: over-expressed cell surface receptors.
  ✗ Involve the addition of ligands to the carrier system.

➡ Allows specific interaction with cancer cells

How do they get into cells?
Current Targeting Strategies: Relying on Endocytosis

• Surface receptors are recycled through endocytosis:
• Antibodies can be raised against any cell membrane receptors.
Current Targeting Strategies: Monoclonal Antibodies

- Mechanism of action of two FDA-approved antibody-drug conjugates:

- Approved for: Hodgkin lymphoma and systemic anaplastic large cell lymphoma (Adcetris)

- Approved for: HER2-positive metastatic breast cancer (Kadcyla)

1. Healthy cells also have the same biomarkers.

2. Different cancers have different biomarkers.

3. Even in the same tumor, cancer cells can have different biomarkers.

   - uptake into normal tissues
   - unacceptable toxicity profiles
   - therapy resistance and disease progression

➡ Needs for a more general biomarker
• **Tumors**: characterized by a *lower extracellular pH* when compared to healthy tissues.

- **Cancer cell**
  - $pH_e = 5.5 - 6.5$
  - $pH_i = 7.5$

- **Normal cell**
  - $pH_e = 7.5$
  - $pH_i = 7.2$

$⇒$ **Acidosis** = **General** biomarker of tumors.

**How can we target acidosis?**
pHLIP: pH(Low) Insertion Peptide

pHLIP: AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTG

state I
in solution

state II
with lipids

pH50 ~ 6

state III
inserted

Bacteriorhodopsin
(from Halobacterium salinarum)

Reshetnyak et al. PNAS (2008)
pHLIP: Imaging Tumors in vivo

Nude mouse with cancer cells expressing the Green Fluorescent Protein (GFP)

Andreev et al. PNAS (2007)
pHLIP: A Targeting and Delivery Agent

\[ pHLIP: \text{NH}_2\text{-GGQNP} \]

\[ \text{IYWARYA} \text{D W LFTTPLLLL D L ALLV} \]

\[ \text{DADEGT - CO}_2\text{H} \]

Cancer Cell
low pH

\[ \text{S-S} \]

\[ \text{SH} \]
pHLIP: Therapeutic Strategies


Strategy #1

Specific Delivery of Auristatin Derivatives
Monomethyl Auristatins: Potent Cytotoxics

- **Monomethyl Auristatins**
  - Family of antimitotic agents.
  - Derived from Dolastatin 10.
  - Inhibits tubulin polymerization.
  - Extremely toxic.
  - **Must be delivered specifically.**

### Table

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>R’</th>
<th>Log P&lt;sub&gt;o/w&lt;/sub&gt;</th>
<th>IC50 (nM)</th>
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<tbody>
<tr>
<td>Dolastatin 10</td>
<td>H</td>
<td>C&lt;sub&gt;3&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;NS</td>
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<td>0.1</td>
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<tr>
<td>MMAE</td>
<td>OH</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
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<td>0.1 - 2</td>
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<tr>
<td>MMAF</td>
<td>H</td>
<td>COOH</td>
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<td>105 - 250</td>
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<tr>
<td>MMAF-OMe</td>
<td>H</td>
<td>COOMe</td>
<td>2.8</td>
<td>0.001</td>
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</tbody>
</table>

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pHLIP-MMAE: Inhibition of Cancer Cell Proliferation

2 hour treatments
Measure cell viability after 72h
HeLa = Cervical cancer cells
MDA-MB-231 = Triple negative breast cancer cells

➡ pH- and concentration-dependent cytotoxicity

**pHLIP-MMAE: Inhibition of Cancer Cell Proliferation**

- pHLIP variant (D25E) with a pH$_{50}$ = 6.5
  
  AAEQNPIYWARYADWLFTTPLLLELALLVDADegtG

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**pHLIP-MMAE:** In vivo Targeting

Alexa750-pHLIP-MMAE
NCr nu/nu mice
MDA-MB-231 xenograft
Intravenous injection

Next Generation Conjugate: MMAF and pHLIP Variants

MMAE
(Log Po/w = 2.2)
crosses cell membrane readily

MMAF
(Log Po/w = 0.7)
more polar than MMAE —> more difficult to cross membranes

<table>
<thead>
<tr>
<th>pHLIP Variant</th>
<th>Sequence</th>
<th>pH50</th>
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<tbody>
<tr>
<td>WT</td>
<td>AAEQNP IYWARYADWLFTTPLLDSLALLVDADEGTCG</td>
<td>6.1</td>
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<tr>
<td>D25E</td>
<td>AAEQNP IYWARYADWLFTTPLLLELALLVDADEGTCG</td>
<td>6.5</td>
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<td>P20G</td>
<td>AAEQNP IYWARYADWLFTTGLLLDLALLVDADEGTCG</td>
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<td>R11Q</td>
<td>AAEQNP IYWARYAQYADWLFTTPLLLDLALLVDADEGTCG</td>
<td>5.8</td>
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<tr>
<td>R11Q + D14Up</td>
<td>AAEQNP IYWARYAQYDAWLFTTPLLDDLALLVDADEGTCG</td>
<td>5.6</td>
</tr>
<tr>
<td>D14Gla + D25Aad</td>
<td>AAEQNP IYWARYAGlaWLFTTPLLLLAdLALLVDADEGTCG</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Gla
(Y-carboxyglutamic acid)

Aad
(α-aminoadipic acid)

Onyango et al. (2015) Angewandte Chemie
Fendos et al. (2013) Biochemistry
Barrera et al. (2013) PNAS
Next Generation Conjugates: Cytotoxicity in HeLa Cells

- **pHLIP(WT)-MMAF** over 100-fold more potent than MMAE conjugate!
- Lead agent for further in vivo studies

**pHLIP-MMAF: In vivo Therapy Study**

- NCr nu/nu mice bearing HeLa tumors (injection of with $5 \times 10^6$ cells)
- Injection of 1 mg/kg i.v. (Days 1, 3, 5 and 8)
- 10 mice per cohort

- 2 treated mice were excluded from calculations because initial tumors were too small:
  - did not end up growing tumors - 1 is certainly cured!!

- Histopathology of tumors for Ki-67 (a marker of cellular proliferation)
  - number of cells undergoing cell division is significantly lower in the treated vs non-treated tumors.

**pHLIP-MMAF:** Studies in immuno-competent mice

**Toxicology**
in BALB/c mice
1 mg/kg i.v. (Days 1, 3, 5 and 8)
37 ‘markers’

**Therapeutic Efficacy**
4T1 murine breast cancer cells
in BALB/c mice
2 mg/kg i.v. (Days 1, 3, 5 and 8)

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**Markers**:
- Alanine aminotransferase (ALT) (U/L)
- Urea Nitrogen (mg/dL)
- Monocytes absolute (per µL)
- Red Blood Cells (10^3/µL)
- White blood cell count (cells/µL)
- Urea Nitrogen (mg/dL)
- White blood cell count (cells/µL)
- Red Blood Cells (10^3/µL)

**Cell Viability**
- % cell viability at pH 5.0 and pH 7.4 for 4T1 cells

**Tumor Volume**
- Tumor volume (mm³) over time for pHLIP-MMAF, MMAF-linker, and vehicle groups.

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