Methods of detection and delivery of therapeutics to malignant tumors.

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Outline

• Direct placement methods
  • wafers, particles, thermogels...

• Targeted delivery
  • antibody-drug conjugates
  • penetrating peptides
    • pHLIP

• Conclusions
Anticancer Drugs and Side Effects

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

![Drug Side Effects Table]

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<thead>
<tr>
<th>Site of Toxicity</th>
<th>Cyclophosphamide</th>
<th>Methotrexate</th>
<th>Fluorouracil</th>
<th>Vincristine</th>
<th>Doxorubicin</th>
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- Clear needs for targeted therapies
One way to circumvent side effects: place drugs locally

A and B are in clinical use, others are in different stages of pre-clinical and clinical trials

Gliadel Wafer

- Most well-studied and successful drug delivery implant for treatment of recurrent brain cancer (glioma).
- 14mm in diameter, 1mm thick (size of a dime).
- Contains chemotherapeutic *carmustine*
- Placed in the tumor resection cavity (up to 8 wafers)
- Biodegradable polymer (gone after 6-8 weeks)
- Releases the drug over time (5 days)
Paclimer Microspheres

- Intended for intraperitoneal administration and prevention of recurrent ovarian cancer.
- Contains drug *Paclitaxel*
- Average diameter: 53 µm
- Range: 20-200 µm
- Rate of drug release: 1-2%/day over 90 days.
- In Phase I clinical trial.
Nanoparticles

- When taken up by the cells, end up in the lysosome (where pH ~ 5) Under acidic pH conditions, swell 10x and release their drug.

- Their utility has been demonstrated in two cancer models in rodents: lung and mesothelioma.

- Have not been tested in humans.
Thermogels

- Eg: Chitosan hydrogel
- Made from chitin (found in the shells of crustaceans - eg. crabs and shrimp)
- Biodegradable and biocompatible
- Can deliver chemotherapy or radiation
- Liquid at room temperature - gels at body temperature
- Pre-clinical studies demonstrate efficacy against solid tumors in mice.
What about “long distance” targeting of tumors?
Some tumor hallmarks

- Increased permeation
- Decreased pH
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:

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

- However, this effect is small for certain tumors.

- Other more specific strategies have been developed
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
Disadvantages of targeting biomarkers (like cell surface receptors)

1. Healthy cells also have the same biomarkers.  
   * In lower number than in cancer cells. 
   ○ Targeting system also interact with healthy cells.

2. Different cancers have different biomarkers. 
   ○ Design of specific ligand for each type of cancer.

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

4. These systems still need to be transferred into the cells. 
   ○ Need for a delivering “device”
Delivering Devices: Cell Penetrating Peptides

- Most are small positively charged piece of protein (peptide).
- Bind to the negative charges present on the surface of cells.
- Mechanisms of entry are not well understood.
Existing Targeting and Delivering Systems

They are often big and made up of many components:

1. Carrier
2. Drug
3. Specific ligand
4. Penetrating peptide
Another example of biomarker targeting: Antibody-drug conjugates (ADCs)

Delivery Device: Antibody

Requirements:

- A target antigen, which is cancer specific

- An antibody that displays a high binding affinity to the antigen

- A cytotoxic agent that is highly potent (sometimes more than 1)

- A linker designed to allow ADCs to remain inactive when in the blood

[Link](http://www.spirogen.com/technologies/technology.php?id=161)
How do ADCs work?

• The ADC is delivered intravenously and localizes to target tumor cells by binding to tumor specific antigens.
• Internalization of the ADC.
• Proteases and hydrolases digest the antibody and linker to release free drug.
• The drug then binds to its molecular target, leading to cell death.
Difficulties with ADCs

- Many ADCs are in clinical trials (most in Phase I and II)
- Few did not pass Phase III trials
- One ADC was recently approved by FDA (February, 2013)
  - trastuzumab emtansine - ADC against HER2-positive advanced breast cancer - improves survival by 6 months.
- The idea behind ADCs is great, but because each component has to be carefully selected, these often fail before getting to Phase III trials.
- final ADCs are often heterogeneous: mixtures rather than clean products.

Need for a simpler and more general targeting system.
A peptide that senses changes in pH

state I
in solution

state II
+ cell membrane

state III
inserted

pH ~ 6


Reshetnyak et al. PNAS (2008)
• Bacteriorhodopsin = Light-driven proton pump found in the membrane of halobacteria.

• Helix C: only one to insert spontaneously across lipid bilayers at low pH.

○ New name = pH(Low) Insertion Peptide or pHLIP
Why is low pH interesting?

- Cancer cells have a micro-environment different from surrounding normal tissues. Tumors are characterized by a lower extracellular pH (acidic) when compared to healthy tissues:

- Normal cell: $\text{pH}_i = 7.2$, $\text{pH}_e = 7.5$
- Cancer cell: $\text{pH}_i = 7.5$, $\text{pH}_e = 6.9$

- Acidosis = general biomarker of tumors.
Opportunities

Imaging

Drug delivery

“low” pH

C

N

HS
Imaging tumors in vivo: pHLIP finds and accumulates in tumors

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

pHLIP can help detecting sub-millimeter tumors after tumor removal

Andreev et al. PNAS (2007)

Delivery of cell-impermeable molecules into cells

Conjugation to pHLIP

variable positions

fluorescent probe

X = Ser
X = Asp
X = Asn
X = Arg

Conclusions

• Some of the drug delivery methods are in successful clinical use.
• Many are still in pre-clinical or clinical trials.
• Use of these methods (likely in combination with other approaches (surgery, radiation, chemotherapy) is making patient outcomes more positive.
• But, there is still a lot more work to do!