Applying Interdisciplinary Nanotechnology for the Treatment of Cancer

Esther H. Chang, Ph.D.
Professor, Oncology and Otolaryngology
Lombardi Comprehensive Cancer Center
Georgetown University Medical Center
Washington, DC

Email = change@georgetown.edu
Seeking Synergy in Cancer Treatment:
The Whole is Greater than the Sum of the Parts

Cancer Treatment Strategy:

Molecular Therapeutics + Conventional Cancer Treatments

Improved Efficacy
Lower Side Effects
Less Recurrence
Less Resistance
SynerGene Therapeutics, Inc.
Synergy in Cancer Treatment by Bringing the Pieces Together

Nanotechnology
- Nanocapsular
- Tumor-Targeted
- Systemic Delivery

Molecular Therapeutics
- Gene Therapy
- siRNA Therapy
- Antisense Therapy
- Small Molecule Therapeutics

Conventional Therapeutics
- Chemotherapy
- Radiotherapy

The whole can be greater than the sum of the parts!
Characteristics of an Ideal Cancer Therapeutic

- Is selectively delivered to tumor cells thereby minimizing side effects on normal cells

- Is capable of reaching not only the primary tumor but also distant metastases (that end up killing patients)

- Is effective at killing the tumor cells it reaches
Tumor-targeted Nanocapsules

Molecules used for targeting include ligands & antibodies.

Targeting moiety causes “homing” to tumor cells.

- Lipid Shell
- Molecular Therapeutic or Contrast Agent
- Small Molecule Antigen for Vaccine
Selective Targeting of Tumors by Nanocapsules

Targeted Liposomes Can Distinguish Cancer Cells

Cancer cells have surface receptors or antigens that bind targeted liposomes.

Targeted liposomes can deliver genes selectively to cancer cells.

Normal cells lack such surface molecules.
## Ligand Targeting

<table>
<thead>
<tr>
<th>Folate</th>
<th>Transferrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Receptor elevated in many tumor types</td>
<td>- Receptor elevated in many tumor types</td>
</tr>
<tr>
<td>- Internalized by receptor-mediated endocytosis</td>
<td>- Internalized by receptor-mediated endocytosis</td>
</tr>
<tr>
<td></td>
<td>- Receptor levels correlate with proliferative ability</td>
</tr>
</tbody>
</table>
TfR Staining:

Tumor

Normal Tissue
Features Required for a Gene Therapy Approach to Killing Tumor Cells \textit{in vivo}

1. Selective delivery of the plasmid encoding the gene to tumor cells

2. Expression of the gene delivered in the tumor cells (the more the merrier or efficacious “bystander effect”)

3. Gene product either kills cells in which it is expressed or makes these cells more susceptible to killing by another agent
If a cell of 10µm were as wide as this slide, this bar would represents a 100nm nanoparticle:
Cell Surrounded by 100nm Nanocapsules
Tumor-Targeted Nanocapsules

Higher magnification of “virus-like” nanocapsules

100 nm
Topographical and Phase SPM Images of Nanoimmunocomplexed siRNA

The inserts represent the magnified Phase contrast image. The scales are the same in each panel.
ANIMAL TUMOR MODELS

Mouse Xenograft (Subcutaneous, Orthotopic, Intracranial, or Metastatic)
- Head and Neck (Targeting/Efficacy) Sub, Ortho
- Prostate (Targeting/Efficacy) Sub, Ortho
- Breast (Targeting/Efficacy) Sub, Metas
- Pancreatic (Targeting/Efficacy) Sub, Ortho
- Glioblastoma (Targeting) Sub, IntraC
- Hepatic (Targeting) Sub
- Bladder (Targeting/Efficacy) Sub, Ortho
- Cervical (Efficacy) Sub

Mouse Syngeneic
- Melanoma (Targeting/Efficacy) Metas
- Breast (Targeting/Efficacy) Metas

Rat Syngeneic
- Glioblastoma (Targeting/Efficacy) IntraC
Systemic Delivery of siRNA via Nanocapsules
Lac-Z Gene Targeted to Primary and Metastatic Pancreatic Cancer Cells
Targeted Delivery of the Lac-Z Gene to Primary and Metastatic Breast Cancer Cells
Demonstration of Tumor Specific Transfection Efficiency of a Systemically Delivered LacZ Gene into Bone Mets in a Prostate Cancer Mouse Model

Mouse Femurs
With Prostate Cancer Mets

Met
Penetrating the Blood Brain Barrier

Demonstration of Brain Tumor Specific Transfection Efficiency of a Systemically Delivered Complex Carrying a Gene Encoding for Green Fluorescence Protein (GFP) in a Rat Intracranial Glioma (C-6)

Normal Brain Tumor
Systemic Delivery of siRNA via Nanocapsules
Tumor Targeted Systemic Delivery of Fluorescent Labeled siRNA (nonsense 21 mer) in an Orthotopic Human Prostate Xenograft Mouse Model

TriLink Biotechnologies
Delivery of FL-siRNA to Metastatic Breast Cancer Cells

Brightfield

Fluorescence

Met
Synergy in Cancer Treatment by Bringing the Pieces Together

Nanotechnology
- Nanocapsular Tumor-Targeted Systemic Delivery

Molecular Therapeutics
- Gene Therapy
- siRNA Therapy
- Antisense Therapy
- Small Molecule Therapeutics

Conventional Therapeutics
- Chemotherapy
- Radiotherapy

The whole can be greater than the sum of the parts!
SynerGene’s First Gene Therapy Target: The Tumor Suppressor p53

P53 protein (on the left) is shown interacting with both the major and minor grooves of the DNA helix (on the right).
p53 Gene Therapy Restores Sensitivity to DNA Damage

DNA Damage

Damaged Cells Commit Suicide (Apoptosis)

Cells After p53 Gene Therapy

wt-p53
Loss of Functional p53 Confers Resistance to DNA Damage

DNA Damage (Radiation or Chemical)

Damaged Cells Commit Suicide (Apoptosis)

Cells With Functional p53

Cells Lacking Functional p53

Damaged Cells Continue to Grow
Radiosensitization
Systemic P53 Gene Delivery by Nanocapsules to Prostate Cancer Cells

Combination therapy results in long-term “cure” for mice.
Mice Bearing Human Prostate Tumors Treated with Nanocapsules Carrying P53

<table>
<thead>
<tr>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
</tr>
<tr>
<td>Radiation Alone</td>
</tr>
<tr>
<td>Targeted P53 Gene Alone</td>
</tr>
<tr>
<td>Targeted P53 Gene + Radiation</td>
</tr>
</tbody>
</table>

![Mice image with different conditions]
The Importance of Targeting the Nanocapsules
Chemosensitization
Mouse Melanoma Lung Metastasis Model

Untreated

CDDP only

LipAe-p53 plus CDDP

Tf-LipAe-p53 plus CDDP
Lack of Tumor Growth After the Combination of Systemic Normal Tumor Suppressor (p53) Gene Therapy and Chemotherapy in a Melanoma Lung Cancer Metastasis Model
Clinical Relevance

- Combination treatment with the systemic, tumor targeted nanocomplex carrying a molecular therapeutic agent and a conventional therapy would be effective for primary and metastatic tumors.

- Sensitization of tumors to radiation and chemotherapy could result in lowering the effective dose, thereby lessening the severe side effects or decreasing the probability of recurrence.

- Improved delivery and uptake of contrast agents to tumors could lead to earlier detection and treatment, resulting in better outcome.
Applications of Nanocapsule Delivery

◆ Reporter Genes (e.g., Lac-Z; GFP)
◆ Therapeutic Genes (e.g., P53; RB94)
◆ siRNAs (e.g., Fl-siRNA; anti-HER2)
◆ Antisense Oligonucleotides (e.g., anti-HER2)
◆ Contrast Agents (e.g., Magnevist)
◆ Small Molecules (several under study)
Systemic Delivery of Imaging Agent (Magnevist) to Tumors via Nanocapsules
Enhanced Tumor Imaging via Tumor-targeted Nanocapsules
Clinical Trial

- A Phase I clinical trial will commence in 1Q 2006 at the Lombardi Comprehensive Cancer Center
- Safety study in patients with advanced solid tumors
- John Marshall, M.D. will serve as P.I.
- GMP reagents partially produced via NCI’s RAID mechanism
- Partially funded by a RO1 Grant from NIDCR for inclusion of oral cancer patients
- Sponsored by SynerGene Therapeutics, Inc.
- Technology development partially funded by a number of STTR/SBIR grants
Conclusions

- The ligand targeted cationic liposome complex is a nanosized virus-like particle with a condensed DNA core and ligand decorating the surface.

- The presence of the ligand bestows exquisite tumor specificity when systemically administered targeting both primary and metastatic disease.

- The small size permits efficient and deep tumor penetration.

- This “Platform Technology” not restricted to delivering genes.

- In addition to genes, this nanocomplex has successfully and efficiently encapsulated and delivered Antisense ODNs, siRNA, Imaging agents and Small molecules.

- This approach is now entering a Phase I clinical trial at the Lombardi Comprehensive Cancer Center.
NanoBioTechnology in Cancer

NBT can be used as a means to achieve the following useful ends:

- Earlier and/or more accurate diagnoses
- More efficacious and/or less toxic treatments
- Accelerated development of novel interventions
## Contributors

<table>
<thead>
<tr>
<th>Georgetown University, Lombardi Comprehensive Cancer Center</th>
<th>SynerGene Therapeutics, Inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kathleen Pirollo</td>
<td>Leanne Sleer</td>
</tr>
<tr>
<td>Qi Zhou</td>
<td>Malena Ruiz</td>
</tr>
<tr>
<td>Antonina Rait</td>
<td>Bill Alexander</td>
</tr>
<tr>
<td>Weiqun Huang</td>
<td></td>
</tr>
<tr>
<td>Gozen Ertem</td>
<td></td>
</tr>
<tr>
<td>Laiman Xiang</td>
<td></td>
</tr>
<tr>
<td>Ann Na</td>
<td></td>
</tr>
<tr>
<td>Liang Xu</td>
<td></td>
</tr>
<tr>
<td>Yuzhi Yin</td>
<td></td>
</tr>
<tr>
<td>Debra Watt</td>
<td></td>
</tr>
<tr>
<td>Idalia Cruz</td>
<td></td>
</tr>
<tr>
<td>Wen Hwa Tang</td>
<td></td>
</tr>
<tr>
<td>SungHee Hwang</td>
<td></td>
</tr>
<tr>
<td>Wei Yu</td>
<td></td>
</tr>
<tr>
<td>Mahi Yenugonda</td>
<td></td>
</tr>
</tbody>
</table>

**Funding Support**
- NFCR
- NIH
- DOD