Thermally targeted delivery of anticancer therapeutic peptides using elastin-like biopolymers

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Overview

- Localized tumors - current treatment
- Background of the ELP system
- Applications in cancer drug delivery
- Targeting c-Myc in breast and brain cancer
Localized Tumors - Current Treatment

- Surgical resection, followed by chemo- and/or radiotherapy
- Limited by normal tissue tolerance and/or inherent tumor radio or chemo-resistance
- Only a small fraction of the administered dose of drug reaches the tumor site, while the rest of the drug is distributed throughout the body
- To make chemotherapy more effective and less toxic, site-specific drug delivery vehicles would increase the amount of drug that reaches the intended target and would simultaneously reduce nonselective cytotoxicity.
Targeted Drug Delivery Systems

Passive Targeting

- uses the physicochemical properties (particle diameter, hydrophilic properties, etc.) of a carrier (transporter of the drug) to control behavior inside the body

Active Targeting

- adds special mechanisms to the passive type to tightly control the directionality toward the target tissue.

- "missile drugs" that use carriers consisting of combinations of ligands, e.g. antibodies, peptides, sugar chains, etc., that have specific molecular recognition features that can find target molecules of certain cells that make up the target tissue
Elastin-like Polypeptide (ELP)

- Synthetic protein consisting of VPGxG repeats
- Thermally responsive
- Biologically inert
- Expressed and purified from *E. coli*
- Can be fused to therapeutic peptides or small molecule drugs
ELP Expression and Purification

1. Cold Centrifuge
2. Discard pellet
3. T>Tₜ
4. Warm Centrifuge
5. Decant Supernatant
6. T<Tₜ
7. Start new cycle

Marker | Lysate | Cell debris | PEI sup. | PEI pellet | 1 cycle | 2 cycles | 3 cycles | 4 cycles

20 | 25 | 37 | 50 | 75 | 100 | 150 | 250
Advantages of Polymeric Drug Carriers:
- Passive targeting: Enhanced permeability and retention effect.
- Increased solubility and plasma half-life.
- Systemic toxicity is reduced.

Further advantages of ELP:
- Active Targeting: Thermally responsive.
- Genetically engineered, easy to manipulate sequence, express in *E. coli*, and purify.

Hypothesis: Systemically injected ELP will accumulate at locally heated regions, but will circulate and eventually be cleared in non-heated tissues.

Hyperthermia can be applied in the clinical setting using high intensity focused ultrasound or radio-frequency radiation.
Thermal Targeting

\[ T_{\text{body}} (37-38 \, ^{\circ}\text{C}) < T_t (~41 \, ^{\circ}\text{C}) < T_{\text{hyperthermia}} (42-43 \, ^{\circ}\text{C}) \]

Complimentary with established advantages of:

**Macromolecular Carriers**
- Increased solubility
- Increased plasma half-life
- High drug loading capacity

**Hyperthermia**
- Increased chemo- and radiosensitivity
- Increased macromolecular extravasation
Clinical Application of Hyperthermia

- **HIFU**: High Intensity Focused Ultrasound
  - HIFU technology uses a high-intensity convergent ultrasound beam generated by high power transducers to produce heat.
  - As an acoustic wave propagates through the tissue, part of it is absorbed and converted to heat. With focused beams, a very small focus can be achieved deep in tissues.

![MRI-guided HIFU](image)
Therapeutic Peptides

• Advantages:
  • Easy to design using “rational” strategies
  • Can target nearly any protein or pathway
  • Highly specific for their targets

• Disadvantages:
  • Short plasma half-life
  • Easily degraded in vivo
  • Don’t penetrate biological membranes
  • Can be immunogenic
Design of the ELP Drug Delivery Vector

Cell Penetrating Peptide | ELP | Drug or inhibitory peptide
---|---|---
Penetratin | RQIKIWFQNRRMKWKK | 
Tat | YGRKKRRQRRR | 
Bac | RRIRPRPPRLPRPRPLPFPRPG | 
SynB1 | RGGRLSYSRRRFSTSTGR |
Previous Applications of ELP for Drug Delivery in Cancer

### Therapeutic Peptides

<table>
<thead>
<tr>
<th>Protein Target</th>
<th>Peptide Name</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>c-Myc</td>
<td>H1-S6A, F8A</td>
<td>NELKRAFAALRDQI</td>
</tr>
<tr>
<td>p21</td>
<td>W10</td>
<td>GRKRRQTSMTDFYHSKRLIFSKRKP</td>
</tr>
<tr>
<td>IKKβ</td>
<td>NBD</td>
<td>TALDWSWLQTE</td>
</tr>
<tr>
<td>p53</td>
<td>Peptide 46</td>
<td>GSRAHSSHLKSKKGQSTSRHKK</td>
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<td>PRMT5</td>
<td>GRG</td>
<td>GRGGRGGRGGRGGRGGRGGRGGRGGRG</td>
</tr>
<tr>
<td>Bad</td>
<td>BH3 Bad</td>
<td>NLWAAQRYGRELRRMSDEFVD</td>
</tr>
<tr>
<td>(mitochondrial membrane)</td>
<td>KLAK</td>
<td>KLAKLAKKLAKLAK</td>
</tr>
</tbody>
</table>

### Small Molecule Drugs

- **Doxorubicin**
- **Paclitaxel**
- **Methotrexate**
A c-Myc Inhibitory Peptide

Mitogen receptor + ligand → Ras → MAP kinase → myc gene → myc transcript → Max-Myc → Odc → Cyclin D → SCF subunit → Max-Myc → E2F → ENTRY INTO S PHASE

plasma membrane

nuclear membrane
In vivo Evaluation - E0771 Breast Cancer Model

• Medullary adenocarcinoma
• Isolated from a spontaneous tumor in C57BL/6 mice
• Very aggressive, and will invade the peritoneal cavity and metastasize to the lungs
Bac-ELP-H1 Tumor Uptake

Reduction of E0771 Breast Tumors

The C6 Glioma Model

- C6 is a rat glioma model
- C6 cells were derived from methylNitrosourea-induced gliomas in Wistar rats
- C6 cells generate rapidly growing tumors when injected orthotopically in SD rats
- They mimic human glioblastoma in histology, rapid proliferation, and intracranial dissemination.
Delivery to Intracranial C6 Tumors

CPP-ELP-Rho  FITC-dextran  Merge

ELP1

SynB1-ELP1

Bac-ELP1
Delivery to Intracranial C6 Tumors

A. Saline

B. Bac-ELP1-H1

C. Bac-ELP2-H1

Mean RFU

Unheated Tumor

Heated Tumor

*
Inhibition of Intracranial C6 Tumor Growth
Inhibition of Intracranial C6 Tumor Growth

Days After Implantation

% Survival

Saline
Saline + Hyperthermia
Bac-ELP1
Bac-ELP1-H1
Bac-ELP1-H1 + Hyperthermia
Delivery of Doxorubicin (Dox) with ELP

- Doxorubicin is commonly used in the treatment of a wide range of cancers, including hematological malignancies, many types of carcinoma, and soft tissue sarcomas.

- Intercalates into DNA and induces double strand breaks by stabilizing topoisomerase II cleavage complexes.

- Acute adverse effects of doxorubicin can include nausea, vomiting, and heart arrhythmias.

- Doxorubicin's most serious adverse effect is life-threatening heart damage.
Delivery of Doxorubicin (Dox) with ELP

Dr. Kratz, CytRx Corporation, Freiburg, Germany
Figure 1. Schematics of the ELP-based drug delivery vector. The delivery system consists of the SynB1 cell penetrating peptide at the N-terminus, followed by the thermally responsive elastin-like polypeptide with three terminal cysteine residues, and the thiol reactive prodrugs of doxorubicin, paclitaxel or methotrexate.
Plasma Clearance and Heart Toxicity

Delivery of Dox with ELP

Free Dox

Time post-injection (min)

Dox plasma level (µg/ml)

No hyperthermia
With hyperthermia

CPP-ELP1-Dox
Free Dox

Time post-injection (min)

Heart distribution (% ID/g)

CPP-ELP1-Dox
Free Dox

Tumor distribution (%ID/g)

CPP-ELP1-Dox₃
Free Dox

ND
E0771 Tumor Reduction with SynB1-ELP1-ggc3-Dox injected on Day 0, Day 2, and Day 4
Thermally targeted delivery of chemotherapeutics (Dr. Kratz)

Doxorubicin-EMC

Methotrexate-EMC

Paclitaxel-EMC

SUMMARY

Molecular Biology → Protein on Drug → Tissue Culture → Animal Treatment

Human Treatment

[Diagram showing the flow from molecular biology to protein on drug, then to tissue culture, and finally to animal treatment, with a human treatment illustration at the bottom.]
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Future Studies and Collaborations

- Delivery of chemotherapeutics
- Targeting Ras Signaling Cascade
- Thermally targeted therapy for prostate cancer
- Targeting mutant p53 protein to increase selectivity for chemotherapeutics
- Targeting Notch signaling pathway
- Targeting splicing machinery components with therapeutic peptides
- Biophysical characterization of an Elastin-Like-Polypeptide
- Application of ELP in treatment of SCA-1
Tumor Heating with IR Light

Bac-ELP-H1 Tumor Uptake

Bac-ELP-H1 Tumor Uptake

Inhibition of GBM Cell Proliferation

![Graph showing inhibition of GBM cell proliferation](image-url)
Heating Intracerebral GBM Tumors

![Graph showing temperature changes over time for tumor and body temperature.](image-url)
Delivery to Intracranial C6 Tumors

![Images of brain sections with different treatments]

![Graph showing relative fluorescence units (RFU/Co) for different treatments]

- Autofl.
- ELP1
- SynB1-ELP1
- Tat-ELP1
- Bac-ELP1

* indicates significant difference compared to ELP1.
Delivery to Intracranial C6 Tumors

Hoescht  FITC-dextran  CPP-ELP-Rho  Merge

SynB1-ELP1-Rho

Bac-ELP1-Rho
Inhibition of Intracranial C6 Tumor Growth

Polypeptide treatment

Day 0 8 9 10 11 12 15 18 22

Imaging

Day 0

Saline  Saline + Hyperthermia  Bac-ELP1  Bac-ELP1-H1  Bac-ELP1-H1 + Hyperthermia

Day 10 15 18 22

* * *
Plasma Clearance

Free Dox

Delivery of Dox with ELP

Dox plasma level (µg/ml) vs. Time post-injection (min)

- Free Dox
- Delivery of Dox with ELP

No hyperthermia
With hyperthermia

Plasma Clearance

Free Dox

Delivery of Dox with ELP

Dox plasma level (µg/ml) vs. Time post-injection (min)

- Free Dox
- Delivery of Dox with ELP

No hyperthermia
With hyperthermia
Saline
Saline with hyperthermia
SynB1-ELP1-Dox
SynB1-ELP1-Dox with hyperthermia
Dox
Dox with hyperthermia

Tumor volume (mm$^3$)

Day 0 500 1000 1500 2000 2500

0 2 4 6 8 10 12 14 16

% of pretreatment wt

Tumor weight (g)

0.0 0.4 0.8 1.2 1.6 2.0 2.4

0 2 4 6 8 10 12 14 16

No Hyperthermia
With Hyperthermia

Saline CPP-ELP1-Dox Dox

% of pretreatment wt

0 20 40 60 80 100 120

0 20 40 60 80 100 120

1 6

Hyperthermia

No Hyperthermia
With Hyperthermia

Saline CPP-ELP1-Dox Dox

% of pretreatment wt

0 20 40 60 80 100 120

0 20 40 60 80 100 120

1 6