Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras

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*Nature Biotechnology, 2006, 24, 1005*

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November 25th, 2006
Discovery of RNAi

- 2006 Nobel Prize in Physiology or Medicine

“for their discovery of RNA interference – gene silencing by double-stranded RNA”

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Stanford University

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Discovery of RNAi

- Regulation of gene expression in *C. Elegans*
  - dsRNA induces highly specific gene silencing
  - Silencing triggers:
    - exogenously induced dsRNA, RNA viruses, transposons, endogenous short dsRNAs
  - Posttranscriptional cytoplasmic mechanism
  - Systemic Silencing
  - Effects first generation progeny

RNA interference

- dsRNA when injected into cell is cleaved by Dicer
  - A Class 3 RNase III endonuclease

Structural Features:

- 21-25 nt sequence, composed of duplex (19-23 bp)
- 3’ overhang, 2-nt segment
- 5’-phosphate and 3’-hydroxyl termini

*Nat. Struct. Biol. 3, 214 (2004).*
Mechanistic Model

- dsRNA recognized by Dicer
- Endonucleolytic cleavage
- siRNA-RISC complex targets homologous mRNAs

*Nature. 418, 244 (2002)*
**RNAi Therapeutics**

**Advantages:**
- Target-gene specificity
- Low immunogenicity
- Simplicity of design/testing
  - easily synthesized, large scale
  - low cost
- Amenable to chemical modifications
- Size: aptamer vs antibody
  - <15 kDa
  - 150 kDa

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CEN. 2006, 84(46), 16-23
Cell type-specific delivery of siRNAs

- RNA-based approach for specific delivery of siRNA
- Exploit structural potential of RNA to target siRNAs to a cell surface receptor

- Tested aptamer-siRNA chimeric RNAs ability to:
  - Specifically bind prostate cancer cells expressing PSMA
  - Deliver therapeutic siRNAs that target PLK1 and BCL2
**A10 Aptamer- siRNA Chimeras**

- RNA structure program used to predict secondary structure of A10-aptamer
- Compared predicted structure to a homologous A10 aptamer that also binds to PSMA
Binding PSMA-expressing Cells

• Tested binding of chimeras on LNCaP cells with and without DHT treatment

- Verified DHT-mediated inhibition of PSMA gene expression

- Observed cell surface reduction of PSMA

- Correlated with reduced binding of the A10-CON and A10-plk1 aptamers with LNCaP cells

Binding of aptamers was dependent on the predicted PSMA binding region
Functional Characterization

• To determine whether the aptamer-siRNA chimeras targeting oncogenes and anti-apoptotic genes reduce cell proliferation and induce apoptosis

- Cell proliferation assessed by $^3$H-thymidine incorporation
- Reduced proliferation observed for A10-PLK1 but not A10-CON
  - Specific for PSMA expressing cells
  - Same result observed in presence and absence of transfection reagent
• Assessed ability of A10-Plk1 and A10-Bcl2 chimeras to induce apoptosis of PSMA-expressing cells
• PC-3 and LNCaP cells treated by addition of A10, A10-CON, A10-Plk1 or A10-Bcl2 to the media
• Measured apoptosis by measuring production of active caspase3

(+ ) Control     Transfected     Not Transfected

![Graphs showing apoptosis induction](image)
Mechanism: Dependent on Dicer?

- A10-Plk1 chimera-mediated gene silencing tested for dependence on Dicer expression
- Silencing of Plk1 expression by A10-Plk1 chimera inhibited by co-transfection
- Aptamer-siRNA chimera mediated gene-silencing dependent on dicer
- Occurs via RNAi pathway
- Inhibition of Dicer had no effect on transfected Plk1 siRNA-mediated silencing
Mechanism: Dependent on Dicer?

- Incubated RNAs with recombinant Dicer enzyme in vitro, resulting fragments resolved with non-denaturing PAGE
  - Aptamer-siRNAs chimeras (A10-CON and A10-Plk1) cleaved by dicer 21-23 nt lengths
  - Not A10 or the longer single stranded sense strand of the aptamer-siRNA chimeras
A10-Plk1 Promotes Tumor Regression

• Assessed efficiency and specificity of A10-Plk1 chimera for its ability to limit tumor growth in athymic mice bearing tumors derived from both PSMA (+) and (-) -human prostate cancer cells

• No difference on tumor volume with any treatment

• Indicates chimeric RNAs did not have any nonspecific cell killing effect

• Pronounced tumor reduction observed for tumors treated with A10-Plk1 chimera

• Regression of LNCaP tumor volume was specific to A10-Plk1 group only
## A10-Plk1 Treated Tumors vs Controls

<table>
<thead>
<tr>
<th>A10- Plk1 Treated:</th>
<th>Control Tumors:</th>
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<tbody>
<tr>
<td>• Chimeric RNAs non-toxic</td>
<td>• Dense</td>
</tr>
<tr>
<td>• Vacuolated</td>
<td>• Composed of epithelium</td>
</tr>
<tr>
<td>• Extensive granulation</td>
<td>• Little to no necrosis observed</td>
</tr>
<tr>
<td>• Evidence of Necrosis</td>
<td>- No substantial change in tumor volume</td>
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<tr>
<td>• Less epithelium present in tumor area</td>
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</table>
• Developed and characterized aptamer-siRNA chimeras that target specific cell types → triggering cell type-specific gene silencing

• Targeted anti-apoptotic genes with RNAi specifically in cancer cells expressing cell surface receptor PSMA

• Gene silencing by chimeric RNAs is dependent on the RNAi pathway

• Developed RNA chimeras suitable for targeting tumors in mice in vivo which could prove to be useful therapeutics for treating human prostate cancer as well as other diseases in the future