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"

Andrew Z. Fire

Stanford University

Nobel Prize: physiology or medicine, reverse side. [Photograph]. Retrieved November 12, 2006, from Encyclopedia Britannica Online: http://www.britannica.com/eb/art-18048

Craig C. Mello

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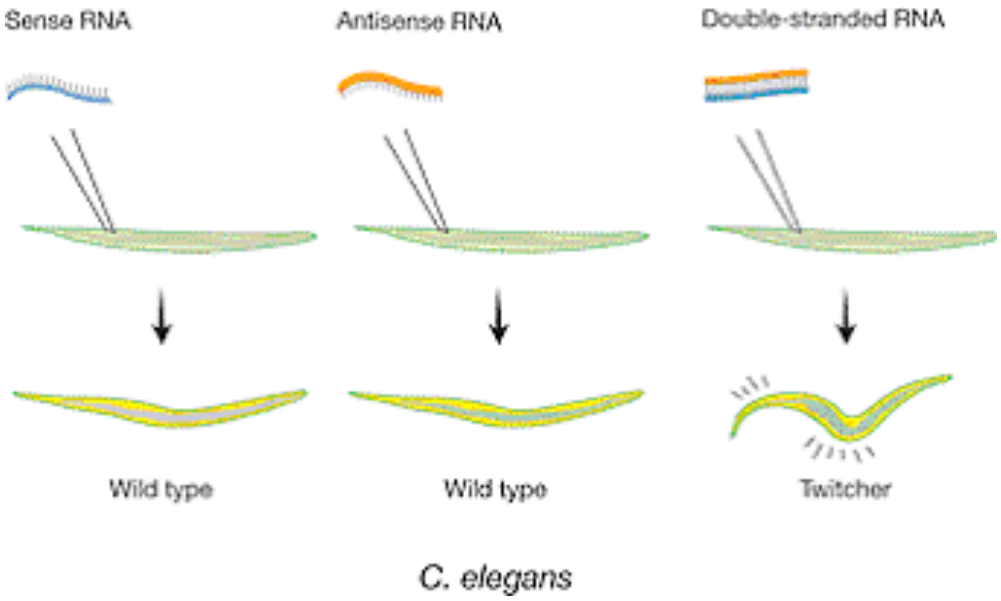
Discovery of RNAi

❖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

http://nobelprize.org/nobel_prizes/medicine/laureates/2006/adv.html

RNA interference

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

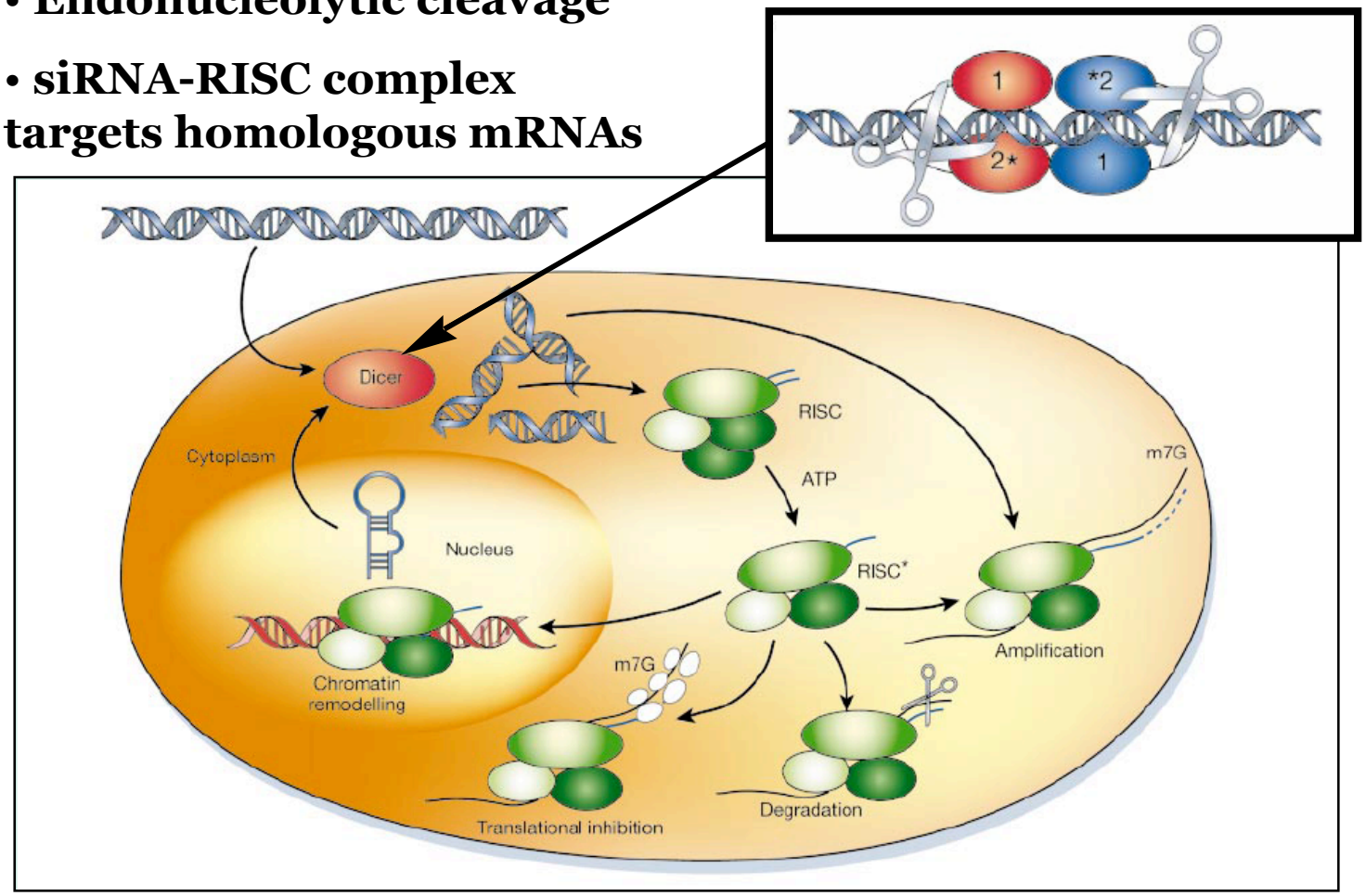
Structural Features:

- 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**

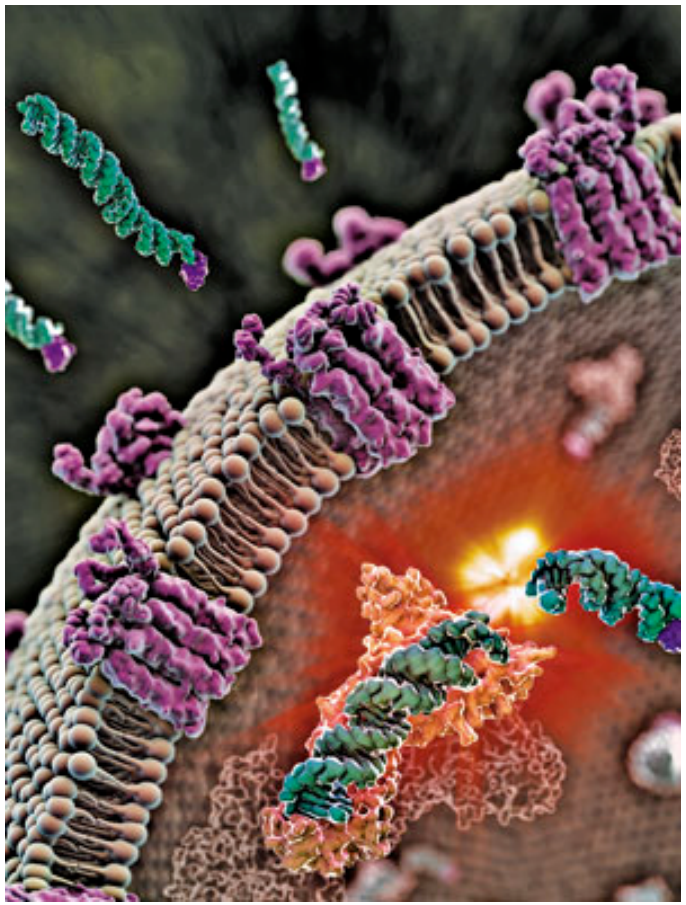


Nature. **418,** 244 (2002)

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



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

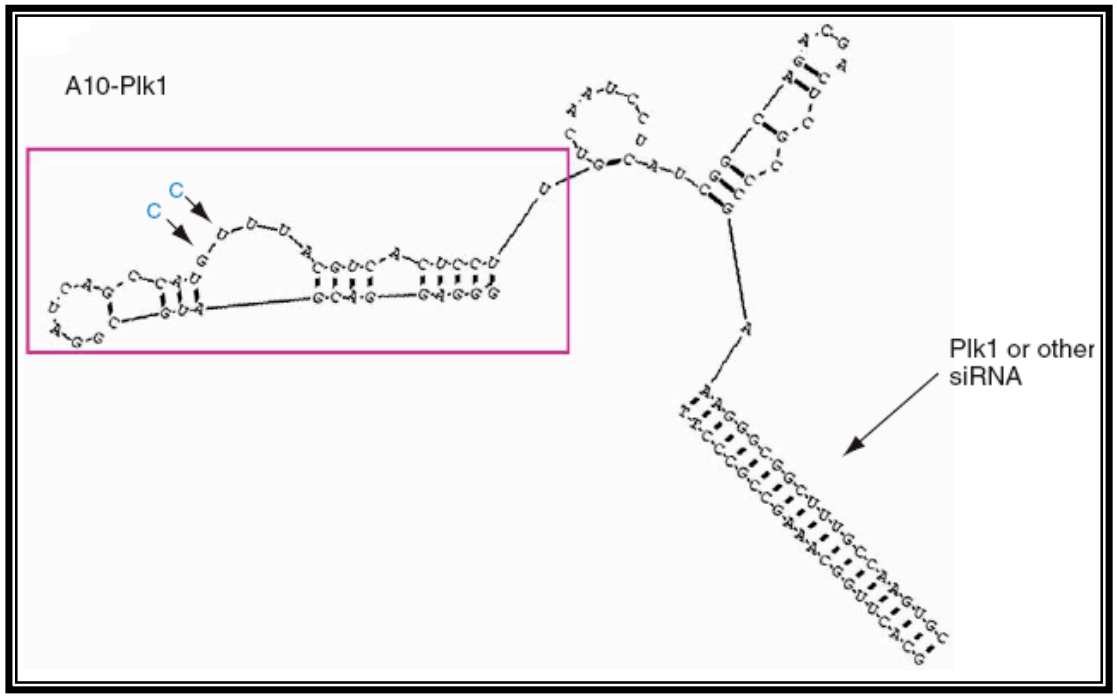
- Tested aptamer-siRNA chimeric RNAs ability to:

  - Specifically bind prostate cancer cells expressing PSMA

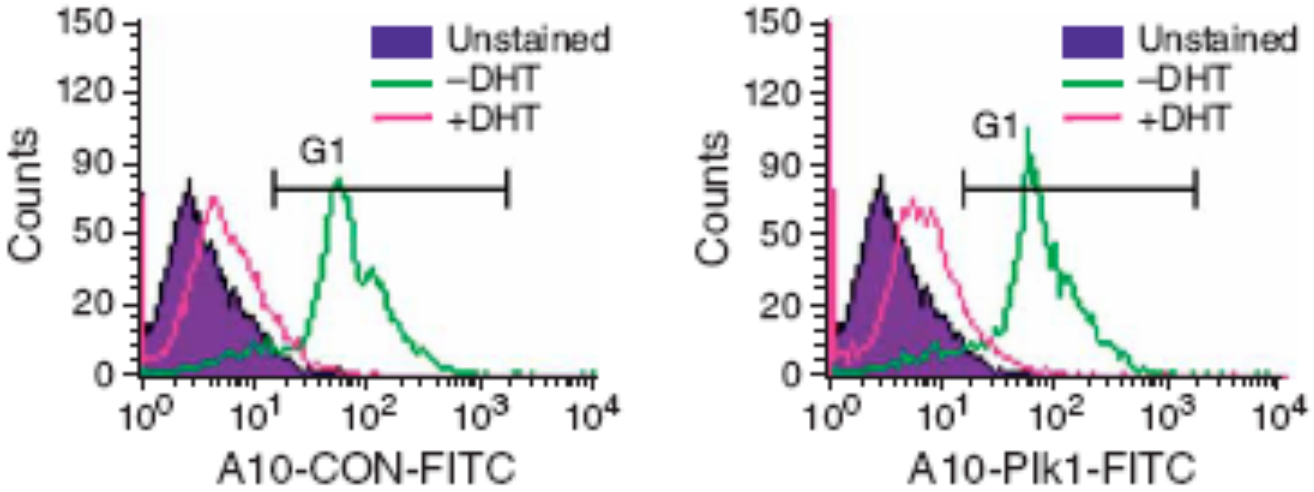
  - Deliver therapeutic siRNAs that target PLK1 and BCL2

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

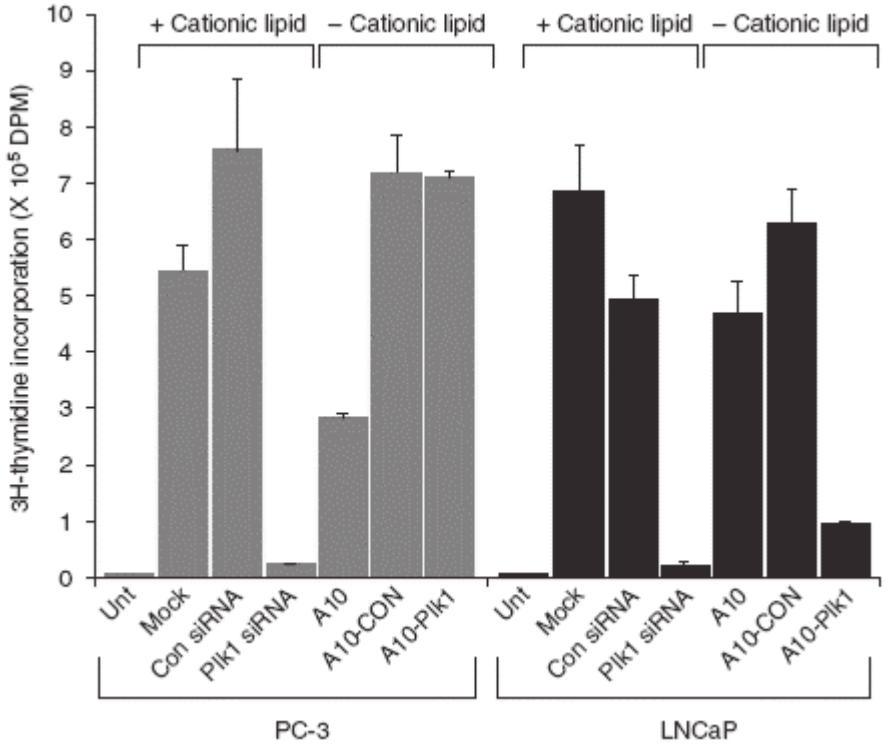
- Observed cell surface reduction of PSMA

- 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

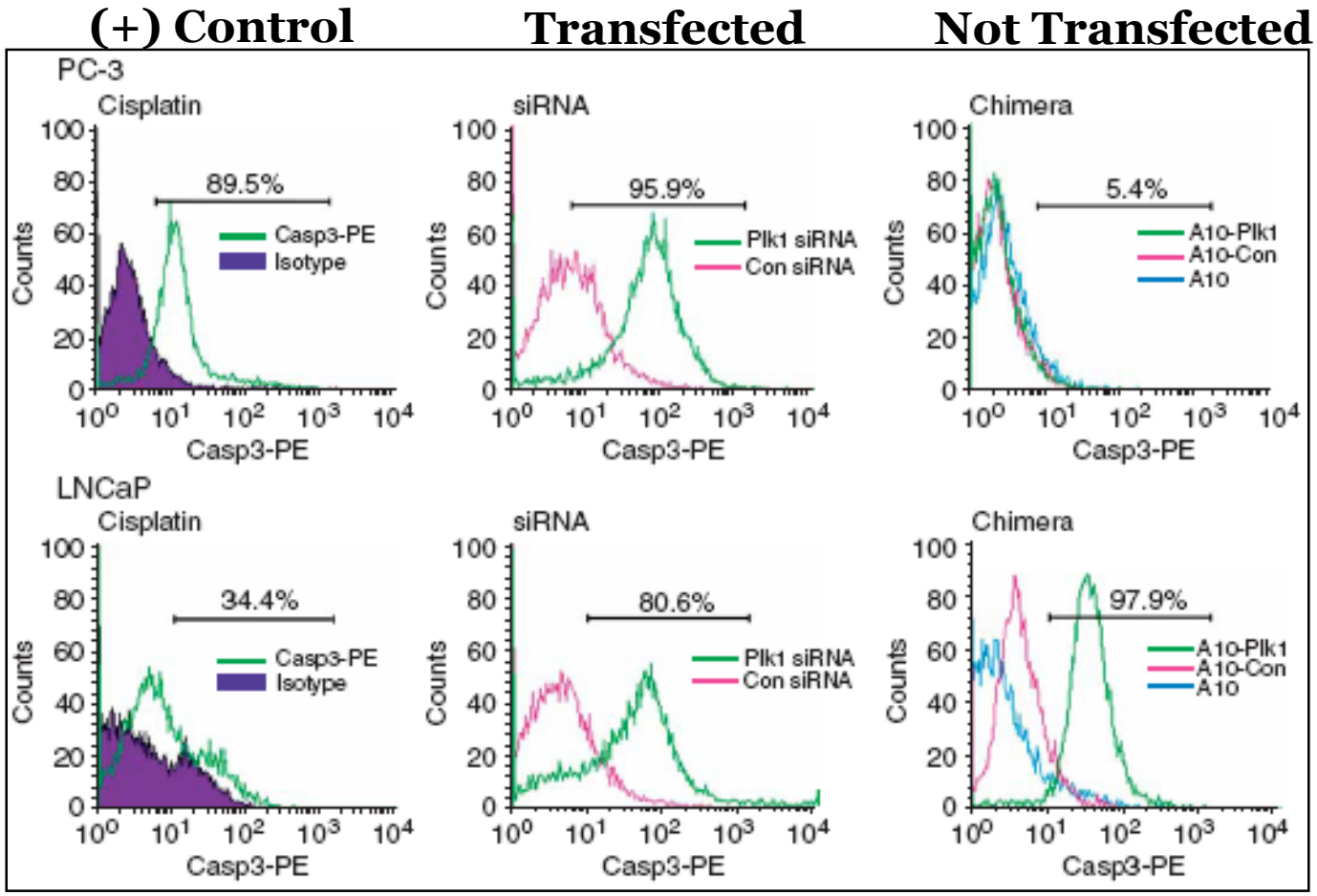
Functional Characterization

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

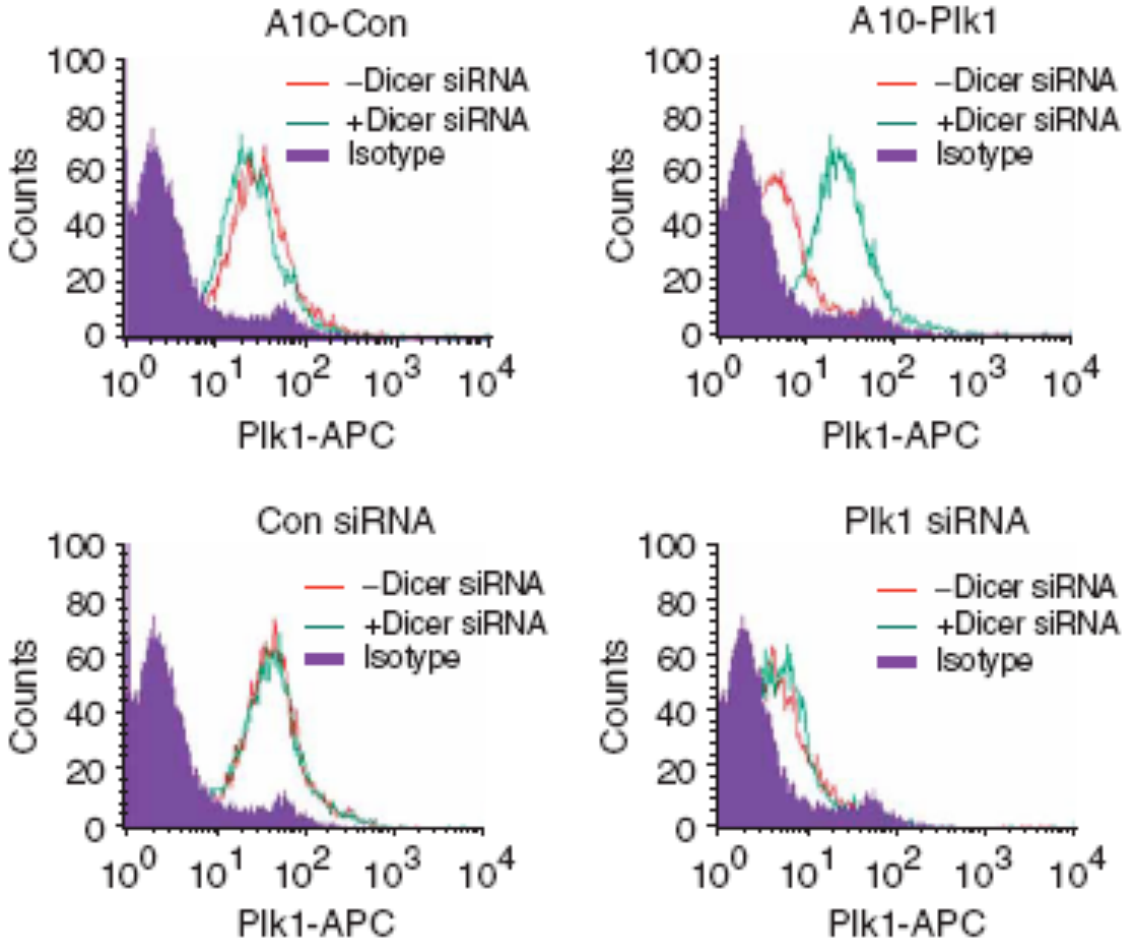


- Cell proliferation assessed by ^{3}H-thymidine incorporation
- Reduced proliferation observed for A10-PLK1 but not A10-CON
  - Specific for PSMA expressing cells
  - Same result observed in presence and absence of transfection reagent

- 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



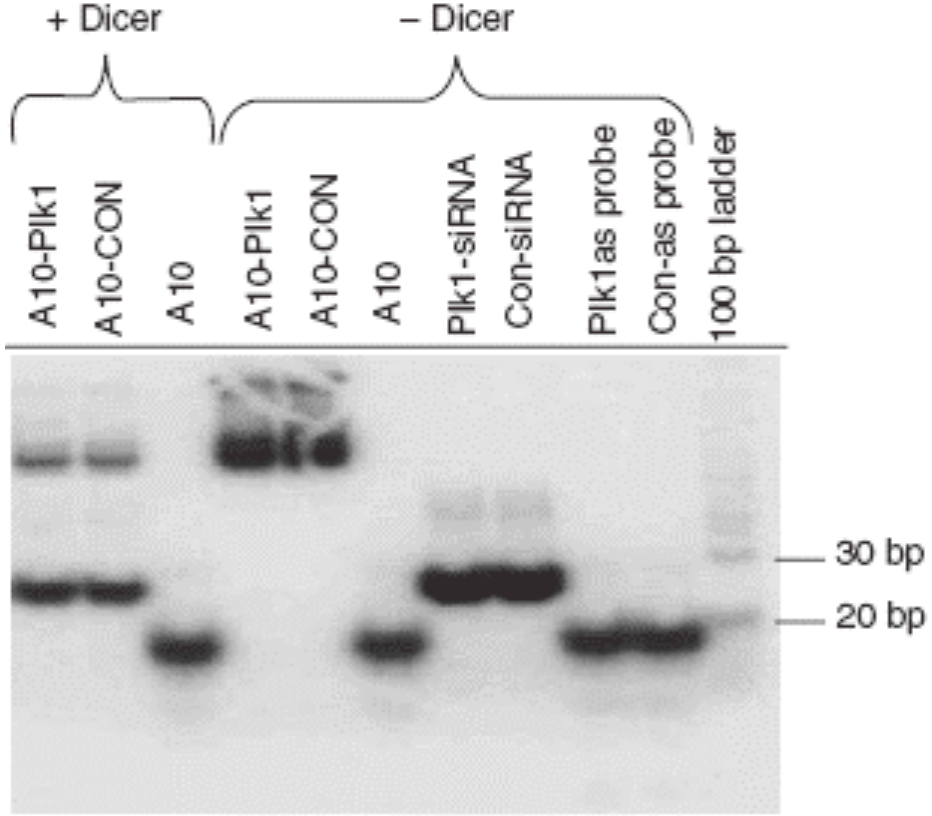
Mechanism: Dependent on Dicer?



- 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

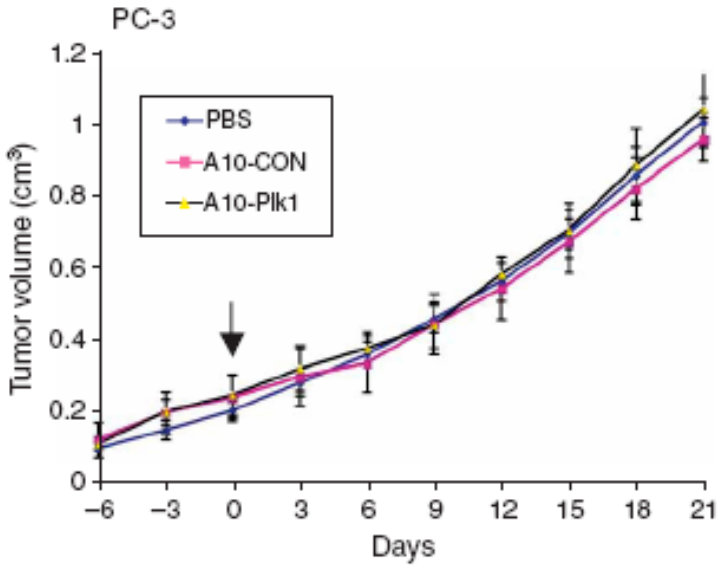
Mechanism: Dependent on Dicer?

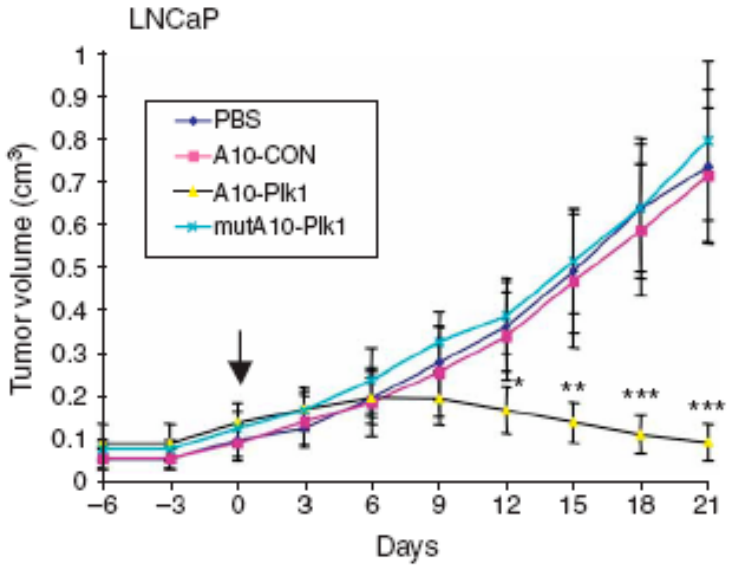
- Incubated RNAs with recombinant Dicer enzyme in vitro, resulting fragments resolved with non-denaturing PAGE
  - Aptamer-siRNAs chimeras (A10-CON and A10-Plk1) cleaved by dicer 21- 23 nt lengths
  - Not A10 or the longer single stranded sense strand of the aptamer-siRNA chimeras



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

A10- Plk1 Treated:

- Chimeric RNAs non-toxic
- Vacuolated
- Extensive granulation
- Evidence of Necrosis
- Less epithelium present in tumor area

Control Tumors:

- Dense
- Composed of epithelium
- Little to no necrosis observed
  - No substantial change in tumor volume

Conclusions

- 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