Targeting Brachyury Using a Lipid-Based Nanoparticle Delivery System for shRNA inhibits Chordoma Cell Growth In Vitro

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Disclosures

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Chordoma: The Importance of Studying a Rare Tumor

• Spine and Skull Base
  - Malignant transformation of embryologic remnants
• No effective chemotherapy
• Role of radiation therapy unclear
Generation of chordoma cell line JHC7 and the identification of Brachyury as a novel molecular target

Laboratory investigation

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Blocking Brachyury Stops Chordoma Growth
Delivery of brachyury shRNA by lipid-based nanoparticles

• Nanoparticles are an alternative nonviral DNA delivery vector for gene therapy

• Penetrate leaky tumor vasculature but does not transport through tight inter-endothelial junctions in normal issues

• Accumulated nanoparticles tend to be retained in the tumor tissues where no lymphatic drainage system is available for clearing macromolecules.
Delivery of brachyury shRNA by lipid-based nanoparticles

Nanoparticles can bind and trigger endocytosis to cross the cell membrane and enter into their action site—cytoplasm.
Experimental Design

In vitro study

In Vitro

(UCH1 and JCH7 cells)

Nanoparticle-conjugated shRNA

↓

Protein & gene expression

Proliferation and apoptosis

In vivo study

(NOD-SCID mouse)

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Tumor growth

JHC7 1 cells: subcutaneously injected

shRNA: tail-vein injection (1 mg/kg body weight, biweek)

3 groups: PEGylated nanoparticles, PEGylated scramble shRNA and PEGylated Brachyury shRNA
Liposome nanoparticles delivered Brachyury shRNA and resulted in a decreased Brachyury gene expression in chordoma cells*

* Cells were treated for 24hrs
Liposome nanoparticles delivered Brachyury shRNA and resulted in decreased Brachyury protein expression in chordoma cells*

* Cells were treated for 72hrs
Liposome-encapsulated Brachyury shRNA inhibited cell proliferation

Relative cell proliferation

* p<0.05, compared with untreated control

# Cells proliferation were measured after treated for 72hrs
Liposome-encapsulated Brachyury shRNA induced apoptosis in chordoma cells

# Caspase 3 activity was measured after treated for 72hrs
Figure 3
Brachyury suppresses E-cadherin promoter activity. (A) Relative E-cadherin promoter activity compared with the control for each cell line. Results from 1 of 3 experiments are shown; **P < 0.05, ***P < 0.001. Shown is a schematic representation of the reporter construct. (B) EMSA assay with recombinant His-Brachyury protein and a labeled fragment from the E-cadherin promoter. Supershift assay was performed with anti-Brachyury antibody versus control IgG. (C) Proposed model for E-cadherin control by Brachyury (Brachy).
Conclusions

• Nanoparticles can deliver Brachyury shRNA to chordoma cells *in vitro* and inhibit cell growth/promote apoptosis

• This strategy may be a viable alternative to T-cell/viral mediated strategies for Brachyury inhibition *in vivo*
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