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

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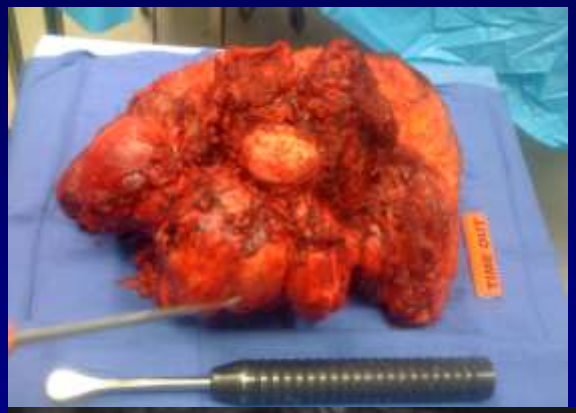
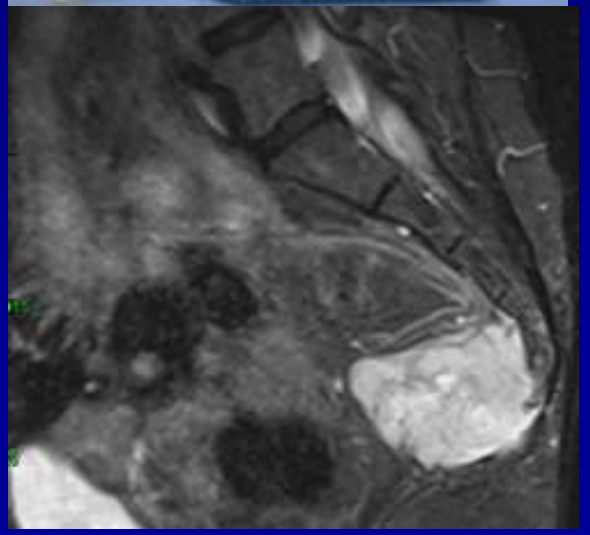
Disclosures

The authors have no conflicts of interest to report

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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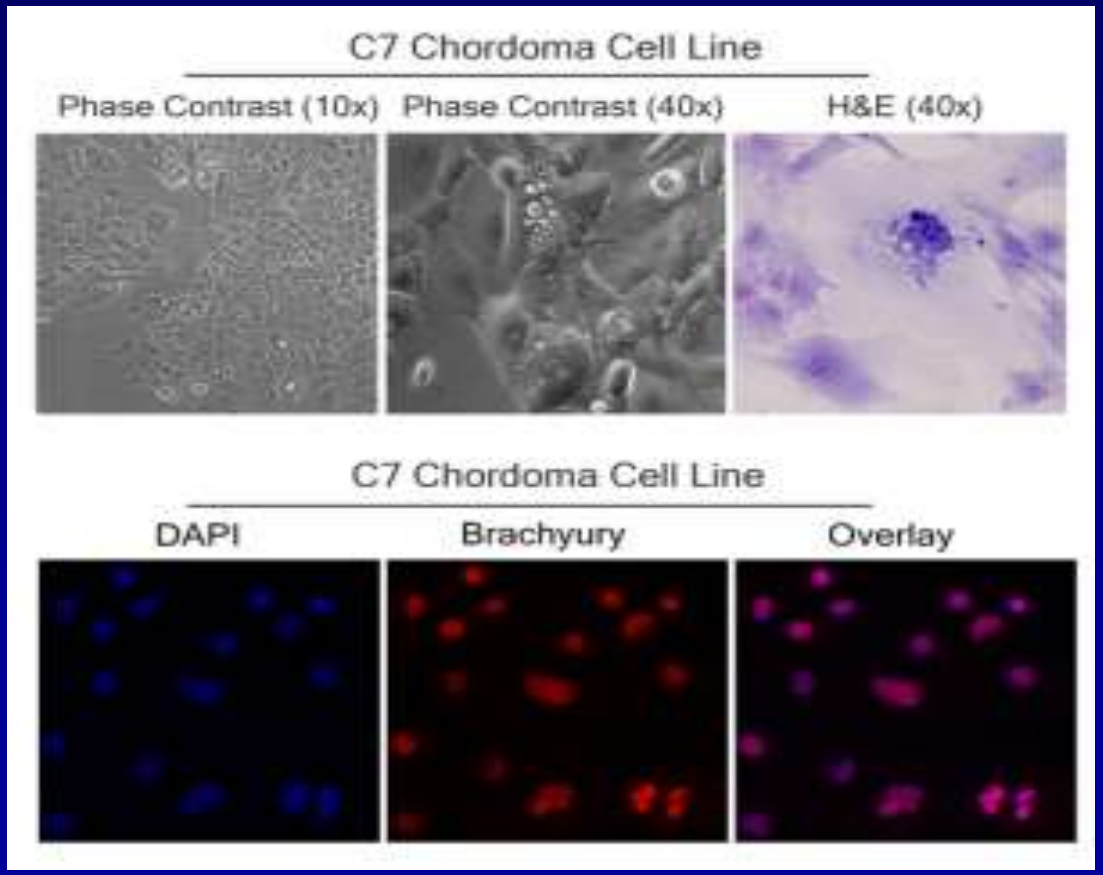
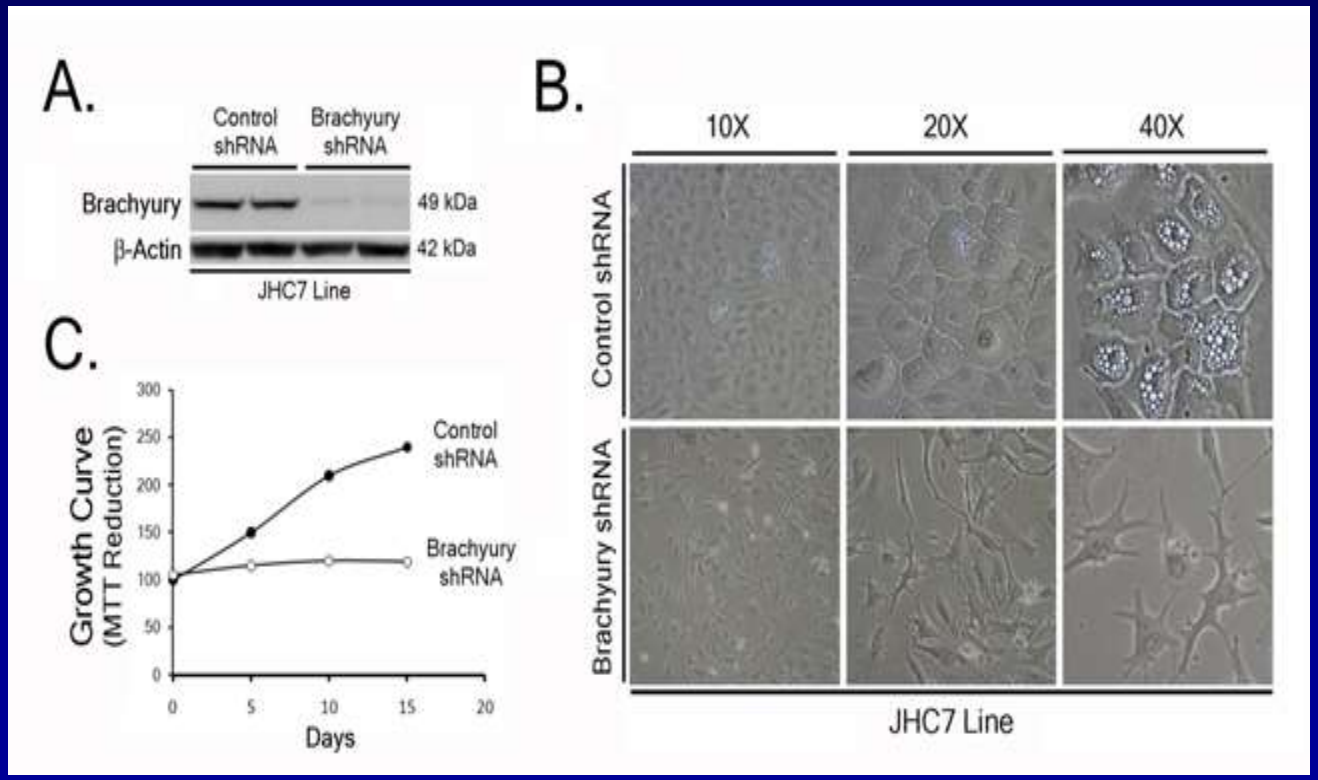


Blocking Brachyury Stops Chordoma Growth

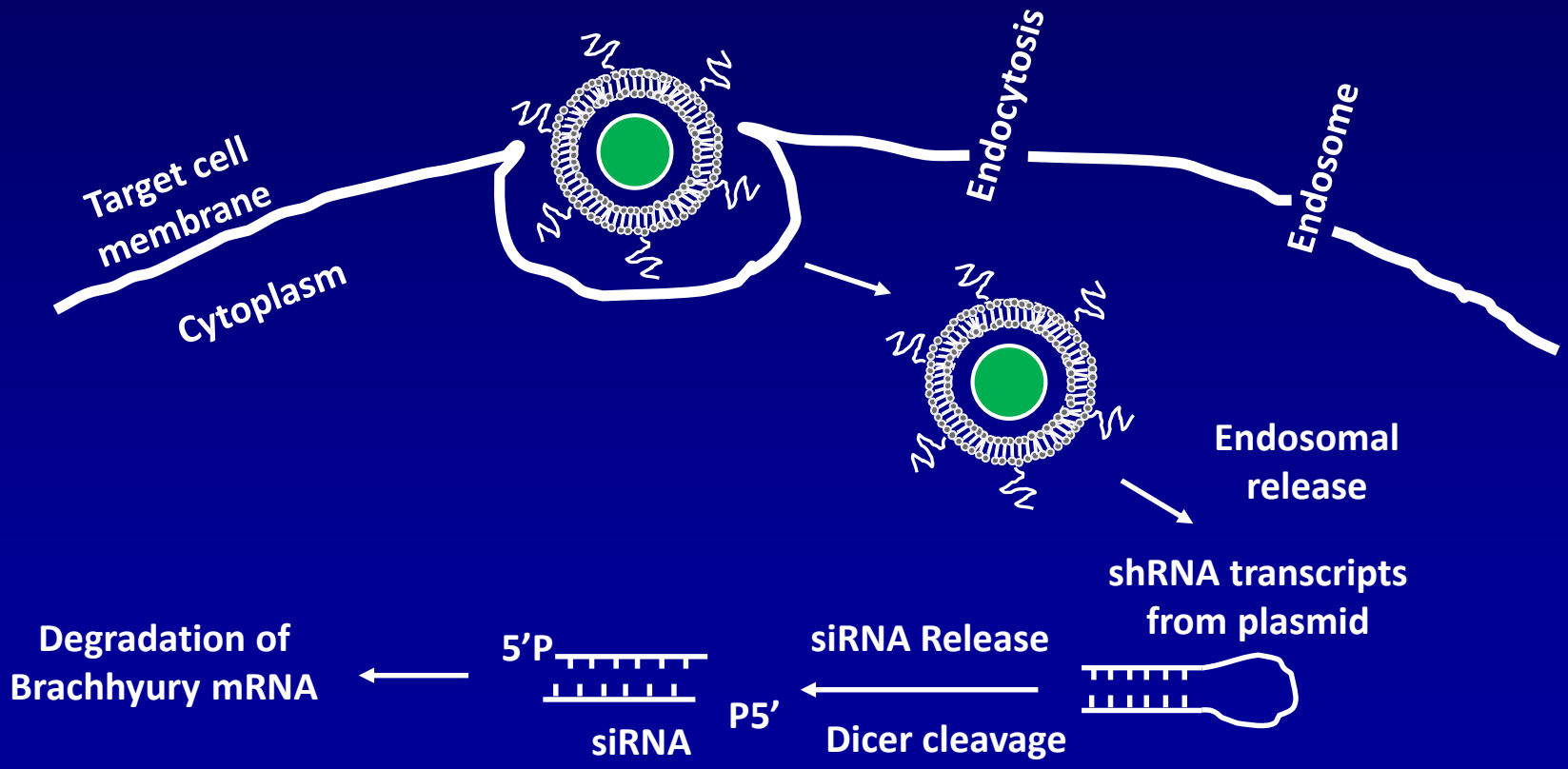


Delivery of brachyury shRNA by lipid-based nanoparticles

- 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.

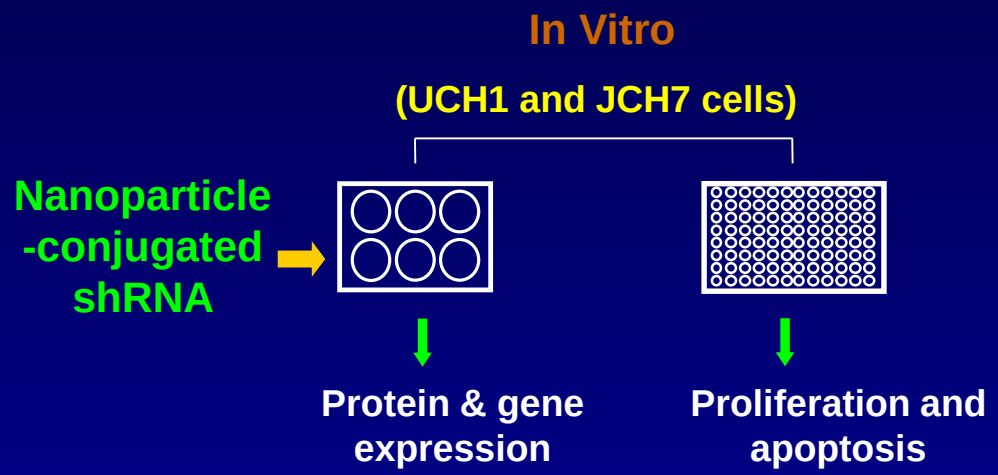
Delivery of brachyury shRNA by lipid-based nanoparticles

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



Experimental Design

In vitro study



In vivo study

(NOD-SCID mouse)

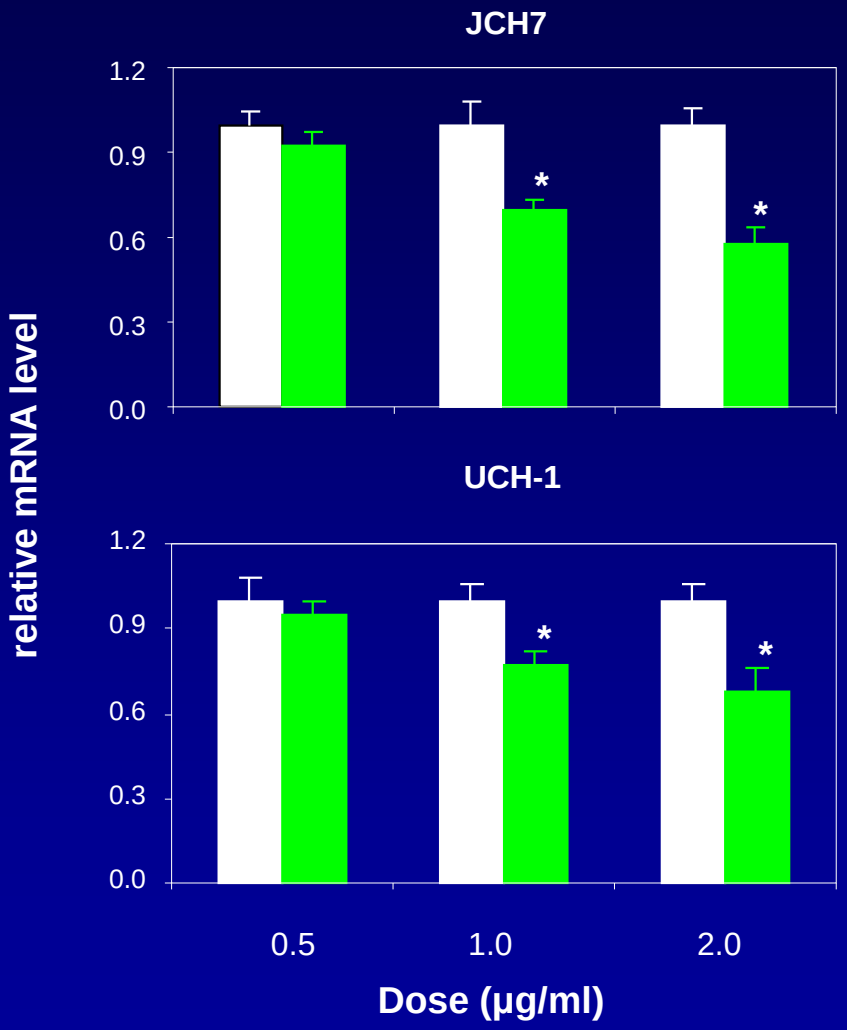
Tumor growth

JHC7 1 cells: subcutaneously injected

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

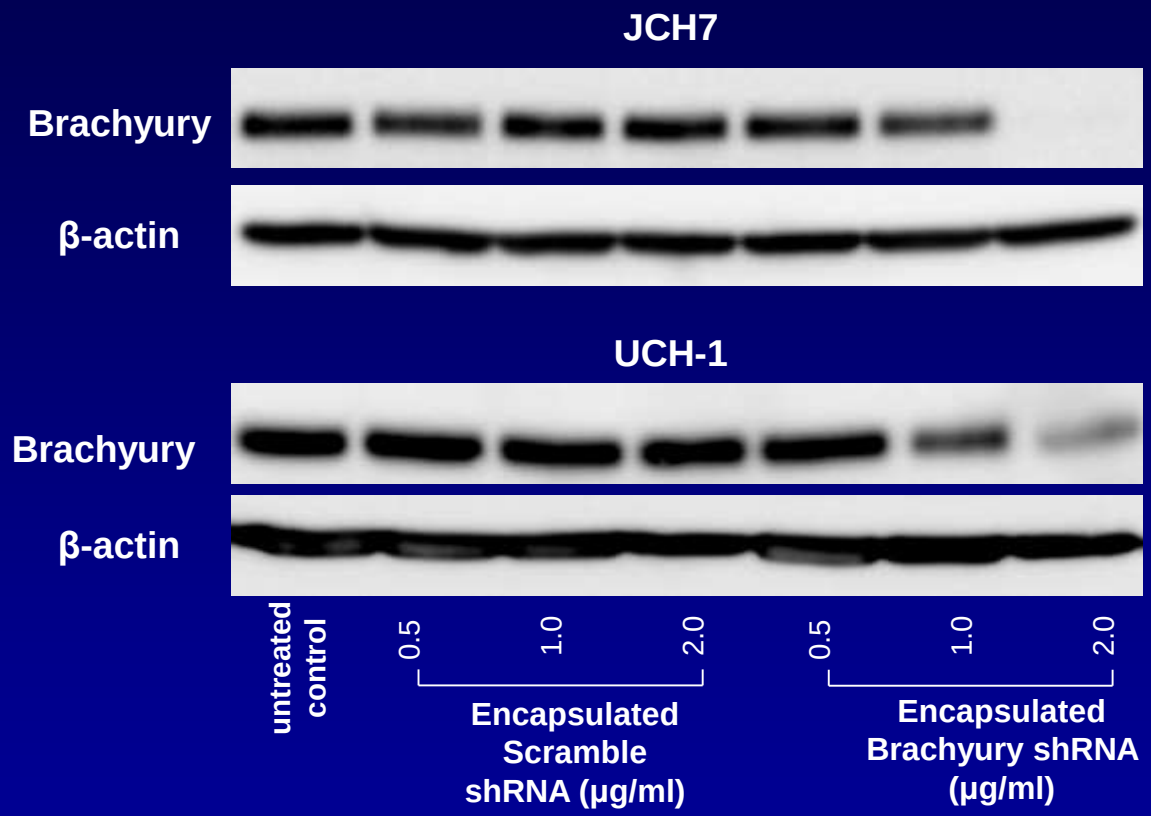
3 groups: PEGylated nanoparticles, PEGylated scramble shRNA and PEGylated Brachyury shRNA

Liposome nanoparticles delivered Brachyury shRNA and resulted in a decreased Brachyury gene expression in chordoma cells*



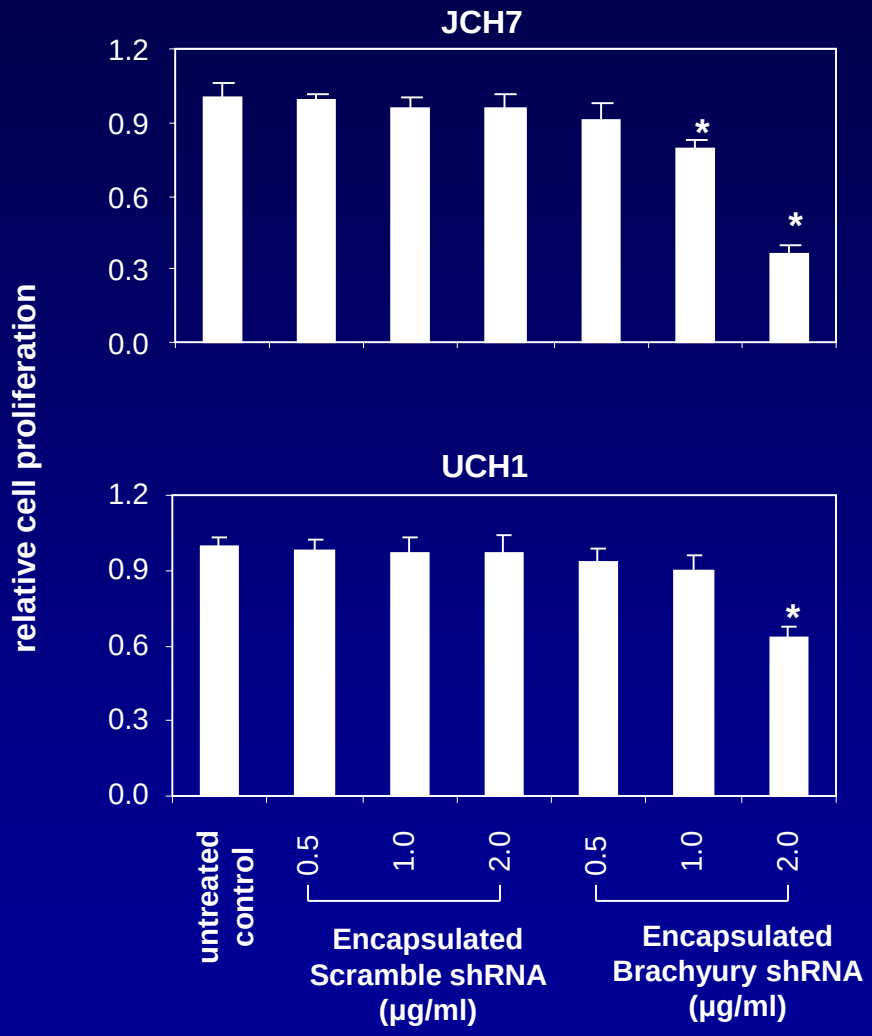
* Cells were treated for 24hrs

Liposome nanoparticles delivered Brachyury shRNA and resulted in decreased Brachyury protein expression in chordoma cells*



* Cells were treated for 72hrs

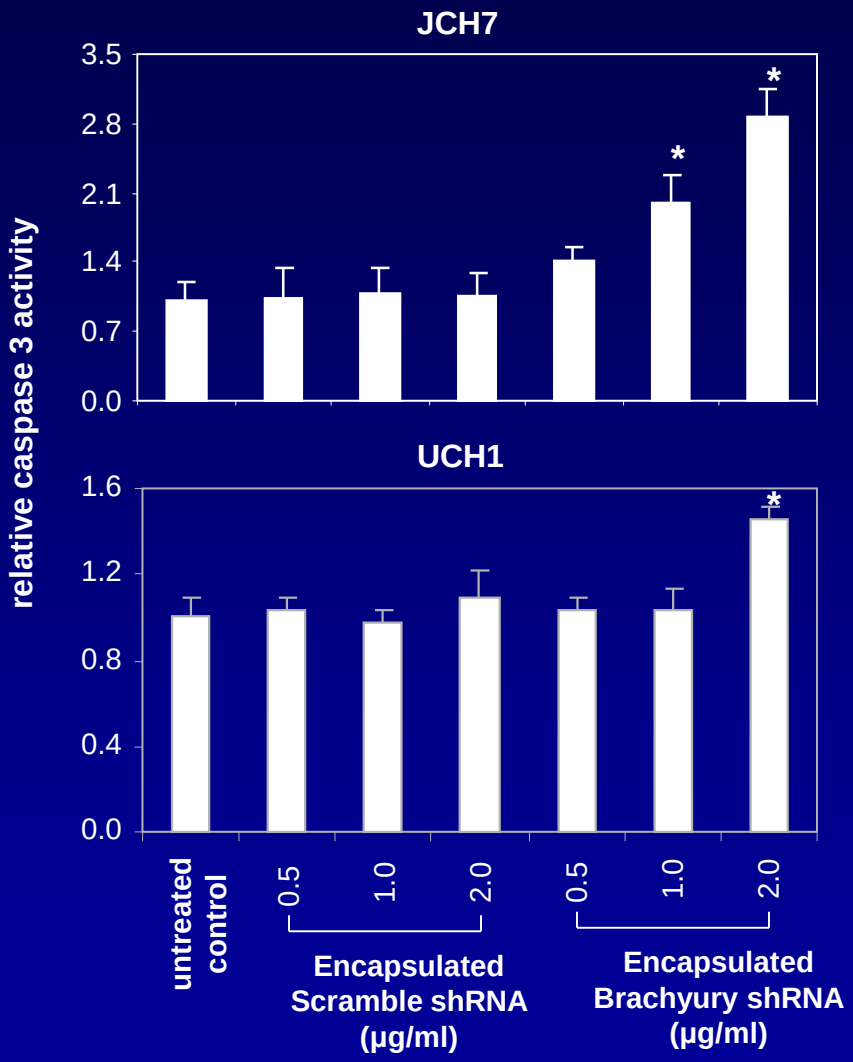
Liposome-encapsulated Brachyury shRNA inhibited cell proliferation



*** p<0.05, compared with untreated control**

Cells proliferation were measured after treated for 72hrs

Liposome-encapsulated Brachyury shRNA induced apoptosis in chordoma cells



* p<0.05, compared with untreated control

Caspase 3 activity was measured after treated for 72hrs

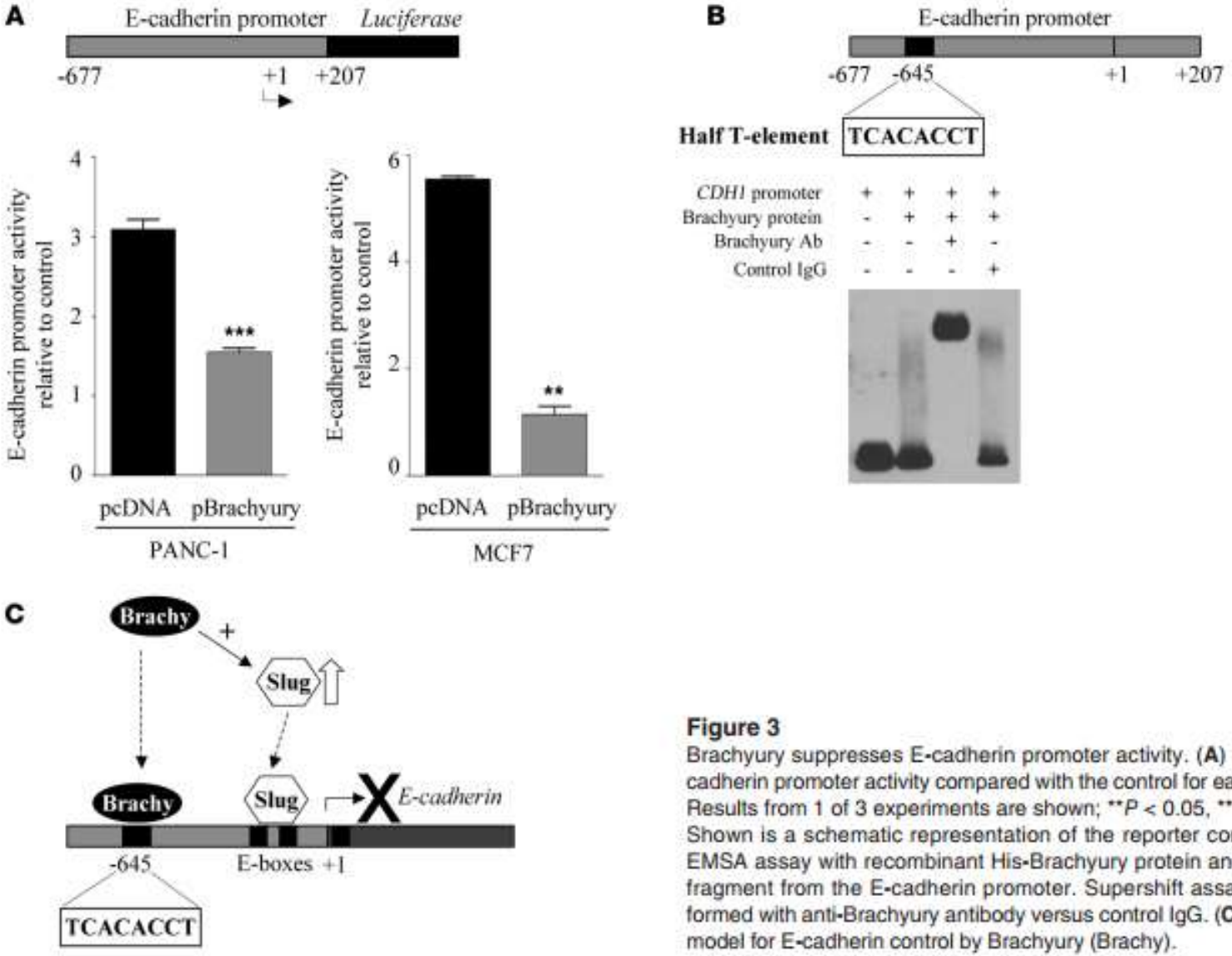


Figure 3

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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