Development of a reporter cell line for detection of *Brachyury* expression in human nucleus pulposus cells

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Introduction

- The intervertebral disc (IVD) is composed of two distinct regions: the inner gelatinous nucleus pulposus (NP) and the outer annulus fibrosus (AF)[1]
- The IVD degrades with age: tissue engineering is a promising therapy

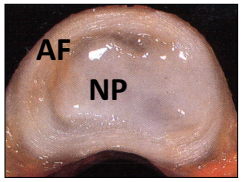


Figure 1: 7 year-old IVD labeled with nucleus pulposus (NP) and annulus fibrosus (AF)

- Healthy IVD tissue is associated with immature NP cells, but they are poorly characterized and difficult to differentiate from chondrocytes and degenerate NP cells
- *Brachyury* (T) is a transcription factor necessary for differentiation of the notochord[2]
- *Brachyury* has been identified as a marker for immature NP phenotype[3]

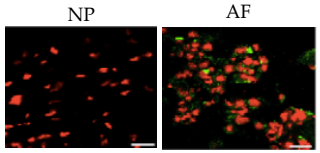


Figure 2: *Brachyury* expression in 1 month rat NP and AF cells[3]

Objective

- Generate a reporter cell line to track *Brachyury* expression in human NP cells
- Use reporter cells to identify cell culture conditions promoting immature cell phenotype

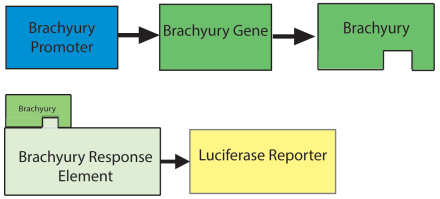


Figure 3: Schematic of *Brachyury* reporter system

Preliminary Results

Cell Imaging

- Cells grown on Matrigel exhibited greater clustering

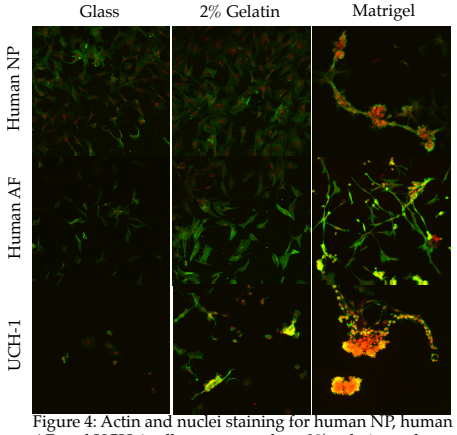


Figure 4: Actin and nuclei staining for human NP, human AF, and UCH-1 cells grown on glass, 2% gelatin, and Matrigel.

Real-time PCR

- Human NP *Brachyury* expression greater than that of human AF
- Both UCH-1 and Dox overexpression vectors exhibit a 1,000,000 fold increase in expression

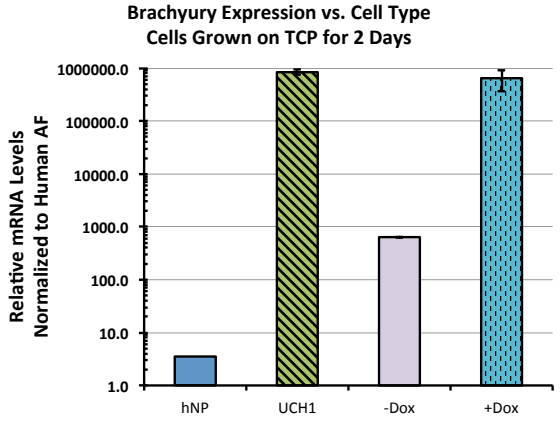
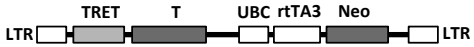


Figure 5: Preliminary PCR data for *Brachyury* expression in human NP and AF tissue compared to positive controls UCH-1 and + Dox

Materials and Methods

Cell Types

- Human NP and AF cells were acquired from discarded surgical tissue
- UCH-1 cells, derived from a chordoma tumor, are known to highly express *Brachyury*
- Chordoma cells with doxycycline-inducible *Brachyury* overexpression (+/- Dox)



Cell Culture Conditions

- Cells cultured on either tissue culture plastic, 2% gelatin, or soft, laminin-rich Matrigel
- Hypothesize that the Matrigel condition will promote greatest *Brachyury* expression

Real-time PCR

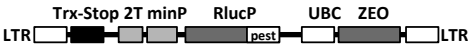
- Quantified *Brachyury* expression using an ABI StepOnePlus RT PCR system
- GAPDH housekeeping gene

Cell Imaging

- Human NP cells grown on substrates for 2 days
- Stained for actin with Phalloidin, counterstained nucleus with propidium iodide
- Cells imaged using confocal laser scanning microscopy

Future Direction

- Infect NP cells with *Brachyury* reporter lentivirus



- Monitor *Brachyury* expression, via luminescence, over time
- Evaluate the effects of substrate, including ligand and stiffness, on *Brachyury* expression

References

1. Setton et al. Prin of Tissue Eng, 2006; 2. Kispert et al. EMBO Journal, 1995; 3. Tang et al. PLOS One, 2012

Acknowledgements

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