Eliminating Myogenic Cells in the Human Lens Through Targeted Drug Delivery Using 3DNA Nanocarriers and the G8 Antibody

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ABSTRACT

INTRODUCTION

3DNA nanocarriers are branched nanoparticles built from interconected monomers of natural or synthetic DNA. The 3DNA nanoparticles are composed of a double stranded ‘stem’ and single-stranded ‘arm’ that is designed to be a flexible arm allowing for growth of monomer arms. The typical 2 stranded “waist” and four single stranded arms that affect visual acuity. We have discovered a subpopulation of cells in the human lens that express the skeletal muscle specific transcription factor MyoD and bone morphogenetic protein (BMP) inhibitor Noggin. These Myo/Nog cells can be found throughout the adult lens and surround wounds in the lens epithelium. Myo/Nog cells have myofibroblastic characteristics and are contractile. They stain positive for MyoD, Noggin, and other muscle specific markers and appear to undergo partial differentiation to skeletal muscle.

Myo/Nog Cells in the Anterior Lens are Immunoreactive for Proteins Found in Skeletal Muscle

Anterior lens tissue fixed after capsulotomy was double labeled with the G8 mAb and antibodies to skeletal muscle specific proteins such as myosin, myogenin, and sarcomeric myosin heavy chain (MYOSH), the skeletal muscle specific 12101 antigen (12101) and troponin I (TNP). Some Myo/Nog cells had incorporated the capase specific siRNA (G8-Dox), G8 mAb coupled to a 2 layer 3DNA dendrimer (G8:3DNA:DOX) that fluoresces green at acid pH and the G8 mAb coupled to Cy3 labeled 3DNA (red) or Cy2 labeled 3DNA (green). We have also shown that 3DNA nanoparticles are non-toxic reagents that have multiple applications for basic, translational and clinical research.

Myo/Nog cells are present in the anterior and equatorial regions of the human lens and surround wounds in the lens epithelium. These cells have undergone a partial differentiation to skeletal muscle. Myo/Nog cells in the anterior lens have undergone a partial differentiation to skeletal muscle. Myo/Nog cells express sarcomeric proteins and are immunoreactive for 12101 antigen and muscle specific proteins in the anterior lens. These Myo/Nog cells have stained positive for MyoD and Noggin, the skeletal muscle transcription factor MyoD and BMP inhibitor Noggin.

Results:

Myo/Nog cells were enriched around cell free areas of the capsule.

Methods:

Control conjugates of G8 mAb coupled to 3DNA intercalated with the cytotoxin Doxorubicin (G8:3DNA:Dox) or control complexes were assayed for apoptosis by labeling with TUNEL reagents.

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The targeting monoclonal antibody, called G8, recognizes a specificity and toxicity. The targeting monoclonal antibody, called G8, recognizes a cell surface molecule on Myo/Nog cells named that expression of the anterior muscle specific transcription factor MyoD and BMP inhibitor Noggin. In addition to their role as regulators of BMP signaling, Myo/Nog cells develop into myofibroblasts in response to injury. The targeting monoclonal antibody, called G8, recognizes a cell surface molecule on Myo/Nog cells named that expression of the anterior muscle specific transcription factor MyoD and BMP inhibitor Noggin. In addition to their role as regulators of BMP signaling, Myo/Nog cells develop into myofibroblasts in response to injury.

Distribution of Myo/Nog Cells in Anterior Lens Tissue

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Methods:

All lenses were then fixed and stained for G8 positive cells and SMA (green). No G8 positive or SMA positive cells were observed in the lenses treated with G8:3DNA:DOX.

Results:

With G8:3DNA:DOX, G8 mAb coupled to 3DNA and green appears yellow in merged images. A, anterior lens; EL, equatorial lens; BR, back region; CP, ciliary process; CE, corneal epithelium; S, stroma.

Myo/Nog cells expressing G8, MyoD, Noggin and myogenin are in the anterior, ciliary processes, and anterior, equatorial and free areas of the lens.

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Anterior lens tissue was incubated with the G8 3DNA:DOX (control) for 24 hours after phacoemulsification. Lenses were fixed and stained for G8 and SMA. Lenses were then fixed and stained for G8 positive cells and SMA (green). No G8 positive or SMA positive cells were observed in the lenses treated with G8:3DNA:DOX.

SUMMARY AND CONCLUSIONS

Myo/Nog cells are present in the anterior and equatorial regions of the human lens and surround wounds in the lens epithelium. Myo/Nog cells in the anterior lens have undergone a partial differentiation to skeletal muscle. Myo/Nog cells surround wrinkles in the capsule, suggesting that they are contractile.

3DNA nanoparticles are versatile, non-toxic reagents that have multiple applications for basic, translational and clinical research. An antibody to a cell surface molecule facilitates intracellular delivery of 3DNA’s cargo to the targeted population. The specificity of drug delivery minimizes off-target effects and permits dosing well below the LD50 for Doxorubicin.

Depleting Myo/Nog cells during cataract surgery may decrease the incidence of visual impairment and PCO caused by skeletal muscle-like cells.

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