Reducing Posterior Capsule Opacification by Eliminating Myo/Nog Cells Through Targeted Drug Delivery Using 3DNA Nanocarriers and the G8 Antibody

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ABSTRACT

Purpose: Posterior Capsule Opacification (PCO) is a vision impairing disease that occurs in some adults and most children following cataract surgery. In fibrotic PCO, myofibroblasts migrate onto the posterior capsule and contract to produce wrinkles that affect visual acuity. Myofibroblasts are derived from Myo/Nog cells that express the skeletal muscle transcription factor MyoD, the BMP inhibitor Noggin and the G8 protein. *Ex vivo* depletion of Myo/Nog cells in human lens tissue had been achieved by incubating explants with the G8 monoclonal antibody (mAb) and complement. The goal of this study was to test the specificity and efficacy of the G8 mAb complexed to 3DNA nanocarriers intercalated with Doxorubicin (G8-3DNA-Dox) in explants of human lens tissue and rabbits undergoing cataract surgery.

Methods: Anterior human lens tissue obtained by capsulorhexis was cultured in serum free medium. Lens explants were incubated with G8-3DNA-Dox, G8-3DNA or 3DNA-Dox. Explants were assayed for apoptosis using TUNEL. Rabbits were injected at the time of cataract surgery with either balanced salt solution (BSS) or G8-3DNA-Dox at doses of 7.0 or 70ug/ml. The rabbits were observed for a period of four weeks for the development of adverse reactions, including PCO.

Results: G8-3DNA-Dox specifically targeted and induced apoptosis in Myo/Nog cells in human lens tissue. Control conjugates did not affect Myo/Nog or lens epithelial cells. G8-3DNA-Dox also targeted Myo/Nog cells in the lens of rabbits that underwent cataract surgery. Slit lamp, gross exam and histopathology analyses revealed that rabbits injected with 70ug/ml of G8-3DNA-Dox had mostly clear posterior capsules with scant areas of PCO without fibrosis. Control rabbits injected with BSS or 7.0ug/ml G8-3DNA-Dox developed extensive PCO and fibrosis

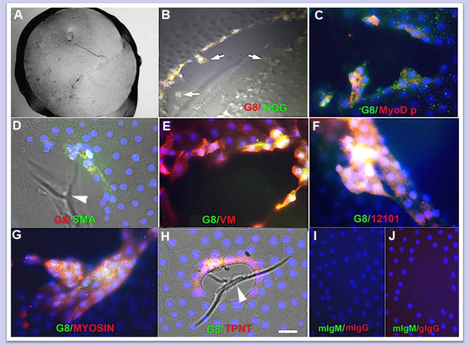
Conclusions: Antibody-3DNA nanocarriers are highly specific reagents for delivering cytotoxic cargo to a subpopulation of cells. The 3DNA nanocarriers themselves are nontoxic. Administration of G8-3DNA-Dox at the time of cataract surgery may reduce the incidence of PCO and maintain vision.

INTRODUCTION

3DNA nanocarriers are branched structures built from interconnected monomers of natural or synthetic DNA. The 3DNA monomers are composed of two DNA strands hybridized together with a double stranded "waist" and four single-stranded "arms". 3DNA is layered by successive annealing of monomer arms. The typical 2-layer 3DNA has a diameter of approximately 70nm and consists of approximately 4,000 bases with 36 single stranded arms. A variety of molecules may be attached to the arms, including fluorescent reporters, siRNAs, peptides and antibodies. Cytotoxic cargo, such as the anthracycline antibiotic Doxorubicin, can be intercalated into the double-stranded regions of the 3DNA.

The purpose of this study was to test an antibody-3DNA-Doxorubicin conjugate for specificity and toxicity *in vitro* and *in vivo*. The targeting monoclonal antibody, called G8, recognizes a cell surface molecule on Myo/Nog cells, so named for their expression of the skeletal muscle specific transcription factor MyoD and the bone morphogenetic protein inhibitor Noggin (Gerhart *et al* 2006). Myo/Nog cells are widely distributed in the embryo and in normal and diseased tissues of the adult (Gerhart *et al* 2001, 2008, 2009, 2012; Walker *et al* 2010). Myo/Nog cells are present in the anterior and equatorial regions of the human lens, the cornea and ciliary body (Gerhart *et al* 2014). In addition to their role as regulators of BMP signaling, Myo/Nog cells develop into myofibroblasts in response to injury (Gerhart *et al* 2008, 2011, 2012, 2014; Walker *et al* 2010).

This work was supported by the Sharpe-Strumia Research Foundation.



Distribution of Myo/Nog Cells in Human Anterior Lens Tissue Removed During Cataract Surgery

Anterior lens tissue fixed directly after capsulorhexis was double labeled with the G8 mAb and antibodies to Noggin (NOG), MyoD protein (MyoDp), alpha smooth muscle actin (α-SMA), Vimentin (VM), the skeletal muscle specific 12101 antigen (12101), sarcomeric myosin heavy chain (MYOSIN), and troponin T (TPNT). Tissue incubated with secondary antibodies alone, lacked fluorescence (I-J).

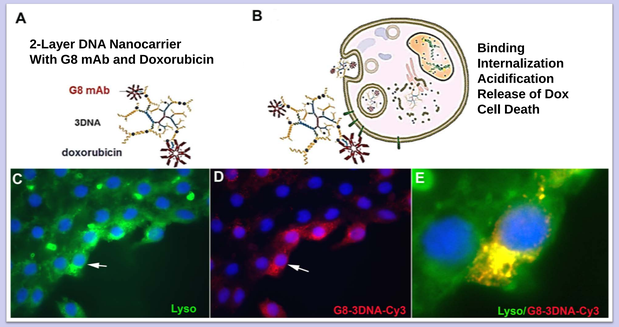
Myo/Nog cells were present in low numbers throughout the lens epithelium.

Myo/Nog cells were concentrated around areas of the capsule denuded of epithelial cells.

Some Myo/Nog cells had migrated onto the capsule (arrows in B).

MyoNog cells express α-sma and skeletal muscle proteins in the anterior lens.

Wrinkles appeared in the capsule in some areas surrounded by Myo/Nog cells (arrowheads in D and H).
(Gerhart *et al* 2014)



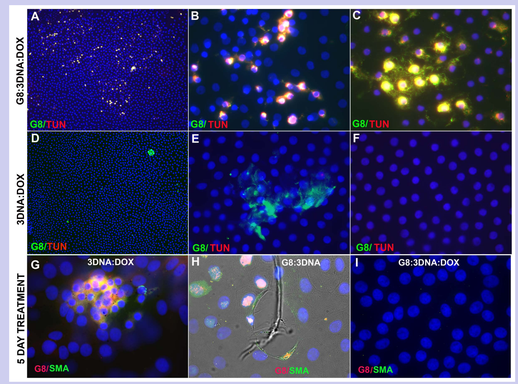
Targeted Delivery of Cytotoxic Cargo

A. Schematic representation of the G8 IgM mAb coupled to a 2-layer 3DNA nanocarrier intercalated with Doxorubicin (G8-3DNA-DOX).

B. Proposed mechanism of targeted cytotoxicity: Doxorubicin diffuses from 3DNA following antibody binding and internalization of the conjugate into acidic compartments of the cell.

C-E. Anterior lens tissue was incubated with Lysosensor dye that fluoresces green at an acidic pH and G8-3DNA-Cy3 (red) for 2 hours. Co-localization of red and green appears yellow in the merged image in E. Nuclei were labeled with Hoechst dye (blue). The cell indicated with the arrows is enlarged in E.

G8 mAb coupled to 3DNA is internalized into acidic compartments of the cell.

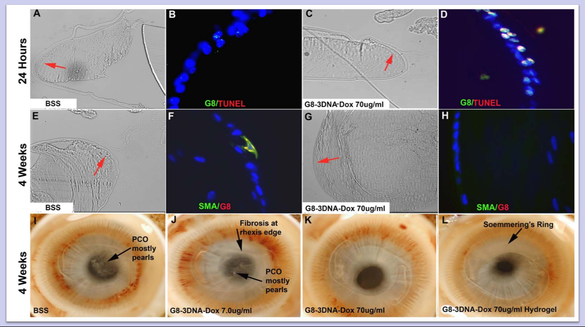


Depleting Myo/Nog Cells in Anterior Lens Tissue with G8-3DNA-DOX

Anterior lenses were incubated with the G8-3DNA-Dox or 3DNA-DOX (Control) for 24 hours after plating. Lenses were fixed and screened for G8 expression and apoptosis using TUNEL reagents. TUNEL positive cells are red and G8 positive cells are green. Overlap of red and green appears yellow (A-F). Some lenses were incubated for 5 days with G8-3DNA-DOX, or with the control reagents 3DNA-DOX and G8-3DNA (G-I). Lenses were fixed and screened for G8 (red) and α–SMA (green).

Treatment with the G8-3DNA-DOX specifically targets and kills Myo/Nog cells.

No G8 positive or α–SMA cells were observed in the lenses treated with G8-3DNA-Dox.



Depleting Myo/Nog Cells *In Vivo*

Rabbits were injected with either balanced salt solution (BSS) or G8-3DNA-Dox at doses of 7.0 or 70ug/ml following capsulorhexis and phacoemulsion. The rabbits were observed for a period of four weeks for the development of adverse reactions, including PCO. A few animals were culled after 24 hours and screened for G8 expression and apoptosis using TUNEL reagents. TUNEL positive cells are red and G8 positive cells are green. Overlap of red and green appears yellow (A-D). After 4 weeks the remaining animals were culled and the globes were examined for the presence of PCO (I-L). Tissue sections were screened for G8 (red) and α–SMA (green), (E-H).

Treatment with the G8-3DNA-DOX at 70ug/ml specifically targets and kills Myo/Nog cells.

The number of α–SMA positive cells is reduced in lenses treated with G8-3DNA-DOX at 70ug/ml.

Treatment with the G8-3DNA-DOX at 70ug/ml reduces PCO.

Treatment	Central PCO	Peripheral PCO	Soemmering's Ring Intensity
Balanced Salt Solution	2.16 +/-1.32	2.66 +/-1.17	5.9 +/-1.42
G8-3DNA-Dox 7.0ug/ml	1.5 +/-0.8	2.16+/-0.76	5.33 +/-1.15
G8-3DNA-Dox 70.0ug/ml	0.62 +/-0.47	1.12 +/-0.62	4 +/-1.41
G8-3DNA-Dox Hydrogel 70.0ug/ml	0.5 +/-0	0.66 +/-0.28	2.66 +/-1.15
G8-3DNA-Dox IOL Presoak 70.0ug/ml	0.5 +/-0.5	1.16 +/-0.76	4.66 +/-2.30

Quantification of PCO Following Injection of G8-3DNA-Dox During Cataract Surgery

Following phacoemulsion, rabbit lenses were injected with BSS, 7ug or 70ug G8-3DNA-Dox, or 70ug G8-3DNA-Dox in a hydrogel. Some animals were injected with 70ug G8-3DNA-Dox plus the intraocular lens was soaked in 70ug G8-3DNA-Dox prior to implantation. Four weeks post surgery the animals were culled and their globes were assessed for severity of PCO and Soemmering's ring formation using methods established in the laboratory at the Intermountain Ocular Research Center (Werner L, Mamalis N, *et al* 2005).

Treatment with the G8-3DNA-DOX at 70ug/ml reduces central and peripheral PCO and the intensity of Soemmering's ring.

SUMMARY AND CONCLUSIONS

Myo/Nog cells are present in the anterior and equatorial regions of the human lens.

In anterior lens tissue removed during cataract surgery, Myo/Nog cells are enriched around cell free areas of the capsule. Some areas surrounded by Myo/Nog cells contain a wrinkle in the capsule, suggesting that Myo/Nog cells may be contractile.

3DNA nanocarriers are novel reagents for delivering cytotoxic cargo to cells.

3DNA nanocarriers can be bound with antibodies or other molecules to specifically target a subpopulation of cells.

Treatment of anterior lens tissue *in vitro* and rabbit lenses *in vivo* with G8-3DNA-DOX specifically targets and kills Myo/Nog cells.

Injection of G8-3DNA-DOX into the rabbit lens at the time of cataract surgery reduced PCO.

Depleting Myo/Nog cells during cataract surgery may decrease the incidence of lens re-opacification and capsular wrinkling caused by myofibroblast-like cells.

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