Mechanisms of Steroid-Induced Glaucoma

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INTRODUCTION

Steroid-induced ocular hypertension/glaucoma is a secondary form caused by treatment with pharmaceutical forms of glucocorticoids (GCs) in the eye. Treatment with these drugs results in the development of ocular hypertension in up to 50% of adults and almost all children, leading to glaucoma if unchecked. This limits use of a very effective class of drugs.

SIOH is characterized by changes in the trabecular meshwork of the conventional aqueous outflow pathway, including fibrotic changes such as deposition of extracellular matrix rich in fibronectin (FN1), cytoskeletal rearrangements within TBM cells, and the appearance of ACTA2-positive "myofibroblasts".

We hypothesized:

1. That a protein called ELAVL1 stabilizes FN1 mRNA in trabecular meshwork cells of patients treated with GCs, thus contributing to pathology.
2. That we could identify new gene products contributing to SIOH by taking a genome wide association study (GWAS) approach.

DESIGN & METHODS

FN1 Stability in SIOH.

GCs bind to and activate the GC receptor, NR3C1, a member of the steroid hormone receptor superfamily. In the resting state, NR3C1 is complexed with molecular chaperones in the cytoplasm. GC binding leads to nuclear translocation. Once in the nucleus, activated NR3C1 can bind to consensus GC response elements (GREs) on the DNA. The typical result is "transactivation" of nearby genes to upregulate gene transcription. Profiling of the transcriptome in TBM cells has revealed that GCs modify expression of hundreds to thousands of genes. Significantly however, transactivation does NOT appear to be the mechanism regulating the GC-induced increase in FN1. The promoter of the FN1 gene does not have a GRE and studies have implicated regulation at the level of RNA stability.

Stability of many mRNAs, including FN1, are regulated via AU-rich elements (AREs) located within the 3' untranslated region (3'-UTR). These mRNAs are normally rapidly degraded, but they can be stabilized by RNA-binding proteins that bind to the ARE. The protein ELAVL1 (also known as HuR) binds to AREs in many mRNAs to inhibit their rapid degradation. We used cell culture and biochemical methods to investigate the role of ELAVL1.

SIOH GWAS

The Cornea Research Foundation of America (CRFA, Indianapolis, IN) maintains a patient registry from a large surgical practice; all are Fuch's Endothelial Corneal Dystrophy (FECD) patients treated uniformly with steroids following corneal surgery, with detailed pre-surgical characterization and post-surgical IOP follow-up [69]. I partnered with CRFA Director Dr. Marianne Price to enroll a new larger SIOH cohort from this registry. A total of 522 subjects were consented and provided with saliva kits to collect their DNA. A total of 484 subjects (93%) sent their completed saliva kits to my lab in Boston.

After careful review of the clinical data to confirm inclusion/exclusion criteria, 471 subjects were genotyped at the University of Miami Miller School of Medicine's Center for Genome Technology (CGT). As our genotyping platform, we chose the Illumina Infinium Global Screening Array-24 BeadChip.

Dr. Paola Sebastiani and her team from the Tufts CTSI performed GWAS association analysis on the 460 subjects that passed quality control (QC) measures, using a similar quantitative trait as the published study.

DIAGRAM OR EXAMPLE

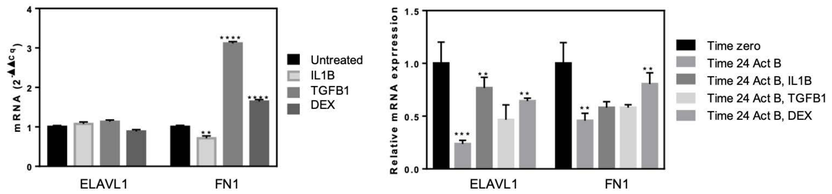

Fig 1 (left). Expression Studies. TM-1 cells were left untreated or treated for 72 hrs with IL1B (10 ng/mL), TGFB1 (10 ng/mL) or dexamethasone (DEX, 250 uM). RNA was then extracted and ELAVL1 and FN1 mRNA quantified by qPCR. ANOVA, n=3, **p=0.01, ****p=0.0001.

Fig 2 (right). Stability Studies. TM-1 cells were left untreated or treated for 24 hrs with Actinomycin B (Act B) alone, or with IL1B (10 ng/mL), TGFB1 (10 ng/mL) or dexamethasone (DEX, 250 uM). RNA was then extracted and ELAVL1 and FN1 mRNA quantified by qPCR. ANOVA, n=3, **p=0.01, ***p=0.001.

RESULTS

FN1 Stability in SIOH

The goal of our first set of experiments, was to confirm that GCs and TGF-beta upregulate FN1 mRNA levels and to learn of any effect on ELAVL1 expression. TM-1 cells were treated with the GC dexamethasone (DEX), TGFB1, or the inflammatory cytokine IL1B. After 72 hours, FN1 and ELAVL1 mRNA levels were quantified by qPCR. Results are shown in Figure 1. We found that dexamethasone or TGFB1 treatment increased FN1 mRNA levels, and IL1B treatment decreased FN mRNA levels, in agreement with results of published studies. However, the level of ELAVL1 mRNA was not affected by any of the treatments.

We next investigated the effects of GCs and TGF-beta on FN1 and ELAVL1 mRNA stability. Cell cultures was treated with DEX, IL1B or TGFB1, and co-treated with Actinomycin D to block transcription. After 24 hours, FN1 and ELAVL1 mRNA levels were quantified by qPCR. Representative results are shown in Figure 2. Actinomycin D alone significantly reduced FN and ELAVL1 mRNA levels, consistent with inhibition of transcription. We found that dexamethasone stabilized FN1 mRNA, and dexamethasone or IL1B stabilized ELAVL1 mRNA.
We confirmed these results on the protein level by western blotting. We tested three different antibodies from AbCam, Cell Signaling, and Invitrogen at 1:1000 dilution. The Cell Signaling antibody detected the 36kDa ELAVL1 protein by western blotting in both HEK293T cells and TM-1 cells.

SIOH GWAS

We identified 43 SNPs of genome-wide significance ($p<5e-8$) associated with 29 different genes. We also identified 377 SNPs of suggestive significance ($p<5e-6$) associated with 284 different genes. Several of the genes appear in POAG GWAS lists (COL11A1, GPR158, PLXDC2, SGCG, THSD7A), but the specific SNPs are different. A quick inspection suggests that the SIOH genes overlap with pathways identified by POAG GWAS, but new pathways are also suggested that are specific for SIOH.

CONCLUSIONS

FN1 Stability in SIOH

Elucidation of the mechanism by which FN1 mRNA is stabilized may provide important new insight into the pathophysiology of SIOH.

SIOH GWAS

We are the first, and still the only team to successfully apply the powerful pharmacogenomics approach to OH. Significantly, while most of the genes we identified in this larger screen are different than those associated with POAG, they are similarly linked to some of the same pathways. We expect our new findings will provide important new knowledge about SIOH, and they will also add innovative dimension to current understanding of other forms of OH. Knowledge of genomic variants might be used predictively to make steroid use safer.

NEXT STEPS

FN1 Stability in SIOH

Once the planned studies are completed, we expect to have sufficient data for a full NIH R01 application. We will move to a mouse model of SIOH to validate our findings in vivo. ELAVL1 is embryonic lethal, thus we cannot employ knockout mice for these studies. However, we can knockdown endogenous ELAVL1 by transfecting the TBM with siRNA, and we can over-express ELAVL1 in the TBM using our lentiviral constructs. We will also conduct experiments to learn how ELAVL1 expression and translation are controlled by GCs and TGFB1. The ultimate goal is to learn whether GCs directly stimulate expression of a gene, via its GRE, that is responsible for ELAVL1 activation.

SIOH GWAS

Results will be compared to GWASs results for POAG and the IOP endophenotype. Our findings in this larger study will provide preliminary data for a new R01 grant from NIH to query UK Biobank, FinnGenn and/or Million Veterans Program for study expansion and to investigate mechanisms whereby selected genes that appear most relevant, interesting and approachable lead to elevated IOP in response to steroids. This will include comparisons with RNA-seq data, transcriptional promoter studies, and functional studies on genes using cell culture and mouse models.

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