Genome-wide Transcriptome Profiling of Human Trabecular Meshwork Cells treated with Dexamethasone

Abstract

Purpose : The purpose of the present study was to profile genome-wide transcriptional alterations in cultured normal human trabecular meshwork (TM) cells treated with dexamethasone and also to identify the genes and gene-gene networks dysregulated by a known glaucoma stimulus.

Methods : Primary normal human TM cells (n=6) were grown to confluence and treated with 100nM dexamethasone for 16 hours. Total RNA was extracted after treatment and RNA-Seq was performed with 50 bp paired end reads (60 million reads/sample) using the Illumina NextSeq500 by Exiqon. Bioinformatics analysis used a combination of analysis components (Tuxedo, Bowtie2, Tophat, Cufflinks and Bioconductor). Gene ontology enrichment analysis was performed with a standard Fisher's test and the 'Elim' method. Ingenuity Pathway Analysis (IPA) was used for further functional analyses. Nine candidate genes were selected and validated by real time-qPCR.

Results : The read quality was high: 99.9% of reads had a Q score >30. 40-57 million reads were obtained from each sample and the average genome mapping was 82%. Differentially expressed genes (DEGs) were ranked by logFC and p values. 4793 genes were differentially expressed. Zinc Finger and BTB Domain Containing 16 (ZBTB16) and FK506 binding protein 5 (FKBP5) were the most significantly up-regulated (ZBTB16: logFC=8.3; FKBP5: logFC= 2.68) genes. Plasminogen Activator Inhibitor Type 2 (PAI) and Matrix metallopeptidase 1 (MMP-1) were the most significantly down-regulated genes (PAI: logFC=-3.59; MMP1: logFC=-1.81). IPA analysis highlighted angiogenesis, axonal guidance signalling and p38 MAPK signalling as key enriched pathways.

Conclusions : RNA-Seq is a powerful tool to investigate genome-wide alterations in gene expression in TM treated with a known glaucoma stimulus (steroid) as a hypothesis-independent approach. A number of key DEGs and pathways were identified. RNA-Seq can identify therapeutic targets for future molecular therapies to treat the TM in steroid induced glaucoma

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