

Bioinformatics analysis of potential essential genes that response to the high intraocular pressure on astrocyte due to glaucoma

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Abstract

• **AIM:** To study the gene expression response and predict the network in cell due to pressure effects on optic nerve injury of glaucoma.

• **METHODS:** We used glaucoma related microarray data in public database [Gene Expression Omnibus (GEO)] to explore the potential gene expression changes as well as correspondent biological process alterations due to increased pressure in astrocytes during glaucoma development.

• **RESULTS:** A total of six genes were identified to be related with pressure increasing. Through the annotation and network analysis, we found these genes might be involved in cell morphological remodeling, angiogenesis, mismatch repair.

• **CONCLUSION:** Increasing pressure in glaucoma on astrocytes might cause gene expression alterations, which might induce some cellular responses changes.

• **KEYWORDS:** high intraocular pressure; microarray; glaucoma; astrocytes

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INTRODUCTION

High intraocular pressure in glaucoma is a high risk for blindness through the damages the retinal ganglion cell

axons^[1,2]. Astrocytes are supporting non-neuronal cells that close contact the nerve fibers and increase reactivity in glaucoma animal models and in the human disease^[3,4]. The astrocytes are arranged as a fan-like radial array, attached ventrally to the optic nerve head (ONH). The current study found that the astrocytes of ONH might be the target of raised intraocular pressure. The researches proposed that the attachments of the astrocytes are the site of initial damage in glaucoma and that the damage to the axons might be not mechanical, but a consequence loss of metabolic support from the astrocytes^[5]. Therefore, analyzing the effects of high intraocular pressure on astrocytes is an effective method to understand the role of astrocyte during the glaucoma. Currently, several astrocyte microarray studies were published, which include gene expression profiles of astrocytes during glaucoma development as well as the systematically detection about the time-response of pressure on normal astrocytes^[6-8]. These microarray studies provide amount of available data and information about the gene expression profiles changes in astrocyte under various diseases status and differentially stresses. In this study, we integrated several microarray data from Gene Expression Omnibus (GEO) to analyze the gene expression changes in astrocyte of glaucoma as well as the genes that response to the high intraocular pressure. Based on the gene expression information, we predict the molecular changes in astrocyte due to high intraocular pressure of glaucoma.

MATERIALS AND METHODS

Microarray Data Collection Bioinformatics analysis of potential essential genes that response to the high intraocular pressure on astrocyte due to glaucoma. Microarray data was collected from GEO database^[9]. GDS1112 and GSE758 microarray data set were selected for microarray data mining. GDS1112 is a study of Expression profiling of astrocytes taken from ONH of patients with glaucoma. Studies suggest that damage in glaucoma is mediated by reactive astrocytes. GSE758 is a study of gene expression in human ONH astrocytes exposed to either 60 mm Hg hydrostatic pressure (HP) or control ambient pressure (CP) compared using Affymetrix GeneChip microarrays to identify HP-responsive genes.

Microarray Data Analysis Tools dChip is used for

Table 1 Six pressure-related genes in astrocytes of glaucoma

Gene Symbol	Gene expression changes in glaucoma		Expression changes over times with pressure effects				
			<i>P</i> (<i>t</i> -test)			ANOVA	
	Fold change	<i>P</i>	6h vs 24h	24h vs 48h	6h vs 48h	<i>P</i>	F
IFIT1	1.77	0.001135	0.760868	0.003738	0.028333	0.0049	5.0019
CTSL1	1.68	0.005214	0.38811	0.031976	0.02125	0.0037	5.2730
TRIM2	1.98	0.005448	0.690579	0.039655	0.012941	0.0617	2.6519
MSH2	1.43	0.004341	0.00728	0.063102	0.00549	0.0048	5.0225
ANGPTL2	2.44	0.000004	0.023812	0.236652	0.016047	0.0040	5.1859
SERPINE1	-2.8	0.014081	0.012939	0.061344	0.000168	0.0003	7.5236

Table 2 Annotation analysis result of glaucoma pressure-related genes

Symbol	Name	UniProt	UGRepAcc	Cytoband	SPLocal
IFIT1	Interferon-induced protein with tetraatricopeptide repeats 1	P09914	AK095515	10q25-q26	Cytoplasm
CTSL1	Cathepsin L-like 3	P07711	AK055599	9q21-q22	Lysosome
TRIM2	Tripartite motif-containing 2	Q9C040	AF220018	4q31.3	Cytoplasm
ANGPTL2	Angiopoietin-like 2		NM_012098	9q34	Secreted
SERPINE1	Serpin peptidase inhibitor, clade E	P05121	NM_000602	7q21.3-q22	Secreted
MSH2	MutS homolog 2, colon cancer, nonpolyposis type 1	P43246	AK223284	2p22-p21	Nucleus

statistics analysis of differentially expressed genes [10]. The selection criterion of *P*-values is <0.05. Annotation analysis softwares include Gominer[11], SOURCE[12]. PubGene[13] were used for biofunction, network and pathway analysis. dChip was used on following steps: selected menu "View/CEL image", opened the cel files directory to load the files, then used the default values in the dialog to perform normalization, and used the default settings to filter genes. During comparing samples, highlighted the normal samples in the "baseline" listbox and the disease or treatment samples in the "experiment" listbox, reopened the dialog to adjust the comparison parameters until the number of genes is below 1000. Finally selected menu "analysis/hierarchical clustering", to review the data picture to confirm these genes have differential expression values between the specified two groups of samples. Other tools were all web terminal tools and used followed web site guidance.

RESULTS

Differentially Expressed Genes Due to High Pressure in Glaucoma For GDS1112 microarray data taken from GEO, the gene expression comparison was performed between glaucoma and normal astrocytes. A total of 865 differentially expressed genes were identified between glaucoma and normal astrocytes using dChip. The selection criterion of *P* -values is <0.05. For GSE758 microarray data, time-dependent analysis was performed to identify the gene expression alterations related with the pressure changes among 0, 6, 24 and 48h. A total of 24 genes were identified as the earlier pressure-respondent genes, the gene expressions were changed with 24h. While the number of differentially expressed genes increased dramatically with

the time lengthened. In order to identify the pressure-related genes in glaucoma, we tracked the gene expression status in glaucoma samples in GDS1112 microarray experiment. If the pressure related genes also changed in the same way in astrocytes of glaucoma, it will be defined as the pressure-responded genes in astrocytes of glaucoma. A total of 5 up-regulated genes and 1 down-regulated genes were identified as pressure-responded genes in astrocytes of glaucoma through integrating the microarray experiment information.

Table 1 showed 6 pressure-responded genes in astrocytes of glaucoma, including 5 up-regulated genes and 1 down-regulated gene.

Biofunction Analysis of Six Glaucoma Pressure-related Genes in Astrocytes Firstly, annotation analysis was performed by DAVID, Gominer and SOURCE. Integration of these three software's results, we got the general information of the glaucoma pressured related genes (Table 2). Secondly, using pathway and annotation tool PubGene, we obtained the component description and functional description about glaucoma pressure-related genes. Component description result indicated the protein location and protein-protein action about these six glaucoma pressure-related genes (Figure 1). While function analysis results showed that these genes were involved in cell communication, wound healing, mismatch repair and molecular modification (Figure 2). TRIM2 has already been confirmed to be a biomarker for neural cellular alteration. These result showed that pressure in glaucoma might cause multiple molecular processed alterations in astrocytes.

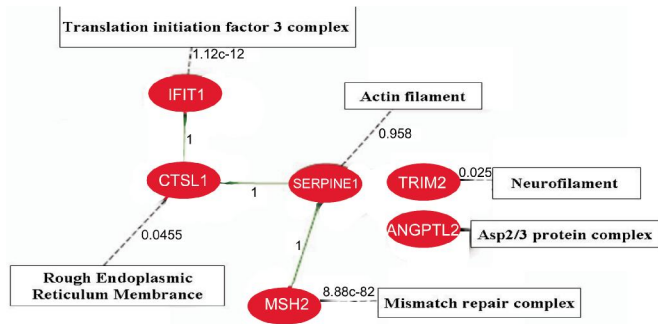


Figure 1 Component descriptions of six glaucoma pressure-related genes analyzed by PubGene.

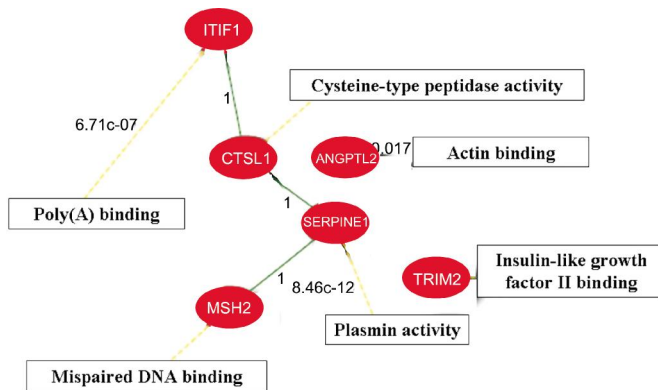


Figure 2 Function descriptions of six glaucoma pressure-related genes analyzed by PubGene.

DISCUSSION

Glaucoma is the leading cause of preventable blindness high intraocular pressure is a sign of glaucoma, which can damage the eye's optic nerve that transmits visual information to the brain^[14-16]. Astrocyte is kind of support cell for optic nerve, in this study, we examined the gene expression changes in astrocyte under glaucoma status to find the pressure related genes in astrocyte during glaucoma development and then analyzed the potential biofunction of these genes to predict the biological process changes in astrocyte with increased-pressure. Our study showed that six genes might be associated with pressure and glaucoma. Since the data used in this study was integrated of two microarray data sets from GEO, these six pressure-related genes in astrocytes of glaucoma (IFIT1, CTSL1, TRIM2, MSH2, ANGPTL2, SERPINE1) demonstrated in this study were definitely also found in the two original studies. However, in the published articles of original studies, they didn't show all the differentially expressed genes, we can't find the detailed descriptions about these six genes in original articles. However, some of these genes have been reported to be related with nerve injury or glaucoma. For example, SERPINE1 is down-regulated gene with increased pressure in our study. Some studies have demonstrated that it was up-regulated in several cell wounds and required for injury repair^[17,18]. These results indicated down-regulation of SERPINE1 might significantly impair wound closure and

cause reverse effects in glaucoma. TRIMRING finger protein TRIM2, highly expressed in the nervous system, is an UbcH5a-dependent ubiquitin ligase and plays a neuroprotective function, if deregulated, triggers neurodegeneration^[19]. ANGPTL2 is another up-regulated gene. Recently, it is found to be induced by mechanical stress and accelerates degeneration and hypertrophy^[20]. The function analysis of up-regulated gene MSH2 showed that this gene seems to act as a scaffold for the other MutS homologs, providing substrate-binding and substrate specificity and participates in mismatch repair process, which indicated that DNA mismatch might be occurred in astrocyte during glaucoma because of increased intraocular pressure and are supposed to play a negative role in cellular process in glaucoma^[21,22]. These evidences are consistent with our study. IFIT1 and is systemic lupus related gene and CTSL1 is involved in antigen processing and presentation and is Important for the overall degradation of proteins in lysosomes^[23,24]. The relationship between CTSL1 and glaucoma is unknown. By bioinformatics analysis, we identified six genes related with increasing pressure in glaucoma on astrocytes, which might be involved in cell morphological remodeling, angiogenesis, mismatch repair and other uncertain processes. These results were consensus with recent findings that astrocytes might play a role in the remodeling of the extracellular matrix of ONH, present unstable cellular processes that may affect the axons of the retinal ganglion cells, which might be related with the axonal loss and retinal ganglion cell degeneration^[25]. Further experimental investigations are needed to explore the effects of these molecules on the progression of glaucoma.

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Conflicts of Interest: Yang Y, None; Duan JZ, None; Di Y, None; Gui DM, None; Gao DW, None.

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