

The role of α A-crystallin and lnc-TMPRSS15 when human lens epithelial cells exposed to dexamethosone

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Research Article

Keywords: The molecular chaperone, α A-crystallin, lncRNA, lens, Dexamethasone

Posted Date: February 3rd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1226704/v1>

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Abstract

Objective: To identify the protective and anti-apoptotic role of alpha-crystallin related lncRNAs.

Methods: We established stable overexpression of α A- and α B-crystallin in human lens epithelial cells (HLECs). All cells were treated with 0.1 μ mol, 1 μ mol, 10 μ mol and 100 μ mol Dex for 12 h, 24 h, 36 h, and 48 h then we detected nine related apoptotic proteins and closely related lncRNAs .

Results: Based on western blot results, we determined the most significant exposure factors and cell types and performed long non-coding RNA (lncRNA) and mRNA chip scanning using microchip assay. The results of microchip assay were also verified by real-time PCR. We found that α A- and α B-crystallin had a close relationship with the expression of three anti-apoptotic proteins heat shock protein 60 (HSP60), survivin and P53. However, no significant changes in the expression of cytochrome C, Samc, P65, and Fas were found in HLECs (α A-crystallin overexpressed) and in HLECs (α B-crystallin overexpressed). Following lncRNA and mRNA chip scanning and depth analysis, we found that overexpressed α A-crystallin could increase the expression of lnc-TMPRSS15, TMPRSS15 and related CDH12 and could decrease the expression of HSD11B1, lnc-RNF13-2 and lnc-CDH2-1 which may result in the significantly high expression of anti-apoptotic proteins such as HSP60, survivin and P53.

Conclusion: Our results showed that α A-crystallin promoted the expression of some anti-apoptotic proteins. lnc-TMPRSS15 played a very important role which provides a theoretical basis for the mechanism of glucocorticoid-induced cataract.

Key Messages

1 For studying the pathogenesis of glucocorticoid induced cataract.

2 Our results showed that α A-crystallin promoted the expression of some anti-apoptotic proteins. lnc-TMPRSS15 played a very important role which provides a theoretical basis for the mechanism of glucocorticoid-induced cataract.

Introduction

Glucocorticoids (GCs) have been used in the clinic for more than half a century. Due to their various pharmacological effects and good therapeutic effect, they are still widely used, and are even the first choice of drugs for some diseases. However, long-term application can result in adverse reactions involving many systems causing multi-organ damage. Glucocorticoid-induced cataract (GIC) is one of the adverse reactions observed in eyes. The long-term use of GCs in patients with allergic, autoimmune diseases or organ transplantation can cause posterior subcapsular cataract (PSC), which is known as GIC: however, the mechanism is unclear.

In mammalian lens, there are three major types of lens proteins (α -, β -, and γ -crystallin). The molecular chaperone α -crystallin, belongs to the small heat shock protein (HSP) family, and plays an important role in stress [1–2]. In vertebrate eyes, α -crystallin is the main protein component, and its high density and water solubility play an important role in the transparency of organs. As a member of the small HSP family, α A-crystallin plays a protective role in the prevention of protein misfolding and aggregation in the human lens and other tissues. According to McHaourab et al. [3,4], α A-crystallin can recognize and bind with non-water soluble proteins, and prevent the aggregation of proteins and the formation of amyloid fiber. Thampi and Abraham [5] reported that in the non-aqueous part, the content of destroyed α A-crystallin is very rich, which indicates that in the cataract lens, the destroyed α A-crystallin is more likely to form non-aqueous proteins. In the water-soluble part of the cataract lens, compared with the unchanged α A-crystallin, the selective loss of the phosphorylated form of α A-crystallin indicates that the phosphorylated form will lead to easier hydrolysis of the protein. The study of Pasupuleti, N showed that α A-crystallin inhibits apoptosis by enhancing PI3K activity and inactivating phosphatase tensin homologues, and its anti apoptotic function is directly related to its chaperone activity[6].

The main molecular chaperone function of α -crystallin is to maintain the transparency of the lens [7]. With a decrease in α A-crystallin expression, its protective function is lost. When the rat lens is exposed to high concentrations of Dex, α A-crystallin function in preventing peroxidase aggregation will be reduced, and the expression of superoxide dismutase (SOD) and glutathione (GSH) decreased.

On the basis of previous studies[8], α B-crystallin played an important role in molecular chaperone function and anti-apoptosis effects. The effects of Dex on the expression of α B-crystallin and apoptosis-related protein caspase-3 were studied. We confirmed that α B-crystallin has an anti-apoptosis effect. The results showed that under the high concentration of Dex, the expression of α B-crystallin increased first and then decreased, which may be caused by non-aqueous or structural damage of α B-crystallin.

In addition to, our results showed α A-and α B-crystallins may be more vulnerable to glucocorticoid stress because of diminished important roles, which will in turn provide a mechanism for GIC from a proteomics perspective[9]. High concentrations of Dex in the rat lens resulted in decreased expression of α A-and α B-crystallins, and led to the accumulation of peroxidase and decreased SOD and GSH expression.

Non-coding RNA (ncRNA) is an RNA molecule with functions in the life activities of organisms. NcRNAs can be divided into short ncRNAs and long ncRNAs (lncRNA) [10, 11] according to their length. However, little is known about lncRNAs. The main task is to discover more lncRNAs and their biological functions. At present, studies have shown that lncRNAs can play a role in regulating DNA methylation, histone modification, chromatin reconstruction, mRNA degradation, protein modification and they act as miRNA precursors, mainly by regulating cell proliferation, cell cycle, cell differentiation, cell apoptosis and other biological processes [11]. Recently, lncRNAs has been shown to be differentially expressed in ocular tissues and plays an important role in the pathogenesis of glaucoma, corneal diseases, cataract, diabetic retinopathy (DR) and other vitreous body retinopathy (PVR) and ocular tumors.[12]

There is relatively little known regarding lncRNAs. At present, there is no published study on lncRNAs in steroid-induced cataract. Although several lncRNAs related to cell proliferation or apoptosis have been found, the specific functions of these lncRNAs are not clear, and most of the molecular regulatory mechanisms of lncRNAs are unknown; therefore, it is necessary to identify abnormal expression related to cataracts. Further investigations into the functions of lncRNAs and their molecular biological mechanisms are required to improve the molecular basis of cataract research.

The occurrence of steroid-induced cataract is a complex process, and is the result of the maladjustment of many molecules and links in the cell. However, most of the research on lncRNAs is still at the initial stage, and there are many problems to solve. For example, the current research methods are still at the miRNA stage, the number of lncRNAs with obvious tissue specificity is small, and some of the lncRNAs identified have been found to have unclear specific functions and are extremely large. The regulatory mechanism of several lncRNAs is unknown. Therefore, it is necessary to carry out high-throughput lncRNA differential screening and bioinformatics research.

The *TMPRSS15* gene, formerly known as *PRSS7* or *ENTK*, is located on chromosome 21q21.1, with a total length of 134.54 kb, containing 25 exons and encoding 1019 amino acids. This gene encodes enterokinase (synonym: enteropeptidase), a type II transmembrane serine protease, which is composed of a transmembrane domain, a heavy chain and a light chain. The light chain is a serine protease domain with histidine, aspartic acid and serine active sites. The heavy chain is composed of repeated motifs that are homologous to domains of other proteins[13,14].

A large number of studies on the anti-apoptotic mechanism of different HSPs have revealed that HSPs may act on different links of the apoptotic pathway and block the apoptotic signal transduction pathway. Thus, α A- and α B-crystallins are important. The role of HSPs in cell proliferation and apoptosis and the changes in lncRNA expression caused by HSPs have not been reported.

We previously studied the basic changes in the expression and function of rat lens protein under the action of increasing GCs. We found that in the pathogenesis of GIC, many lens proteins are very fragile under the action of GCs, and can lead to loss of function. These related mechanisms require further study. We carried out two-dimensional electrophoresis and analyzed the protein spots on the gel using TOF-MS/MS. Our detailed and complex research on the lens changes in rats with steroid-induced cataract will provide very important information for the water-insoluble and light scattering properties of lens proteins. These data are particularly important in the analysis of human lens protein related changes [15]. Compared with the normal control group, the expression of α A-crystallin was significantly increased in the presence of 5 μ mol dexamethasone (Dex), but decreased when the lens was exposed to high concentrations of Dex. Under the highest concentration of Dex, the expression of α A-crystallin was lowest, which may be due to the gradual dissolution of α A-crystallin and the destruction of its structure.

In the present study, we established the overexpression of α A- and α B-crystallin in HLECs cells, and observed the effects of the changes in α A- and α B-crystallin on apoptosis, proliferation, the mitochondrial

signal pathway and related lncRNA expression in the presence of different concentrations of Dex, in order to evaluate the role of α A- and α B-crystallins in anti-apoptosis.

Materials And Methods

Reagents

Dex was dissolved in dimethyl sulfoxide (DMSO) as a stock solution (200 mM). All reagents were from Sigma-Aldrich, St. Louis, MO, USA.

HLEC culture

The HLEC line B-3 was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The product number was ATCC CRL-11421. The cells were maintained in culture with Dulbecco's modified Eagle's medium (DMEM; Gibco Co., Gaithersburg, MD, USA) with 20% fetal calf serum, 100 U/mL penicillin, 0.1 mg/mL streptomycin and 2 mM L-glutamine in white six well plates with a transparent bottom (Corning, Sunnyvale, CA, USA) with approximately 10^5 cells per well to yield a confluent monolayer. The treatments were the same for all assays.

Establishment of α A- and α B-crystallin overexpression in cells and detection

The establishment of stable overexpression of α A- and α B-crystallin in HLECs was as follows: The cDNA of α A- and α B-crystallin in the cell was used as a template, and was amplified by primer PCR and cloned to the screen Pcdna3.1-myc-his (abbreviated to pcDNA3.1) blank carrier selected. Digestion and sequencing confirmed whether the construction of α A- and α B-crystallin expression plasmids was successful.

We added 2 μ l plasmid into competent cells of *E. coli*. After successful transformation, observe the growth of bacteria and select monoclonal colonies. A large number of plasmids were successfully extracted from the colonies and transfected into hleb3 cells. After 4–6 weeks, high concentration (1000 μ g/ μ L) G418 was used to screen the monoclonal cell lines expressing foreign genes. Half of the medium was renewed every 24 hours until the cells grew stably. Western blot was used to detect the expression of α A- and α B-crystallins. Purified α A- and α B-crystallin were stored at -80°C . After that, the following experiments were carried out on the obtained stable cell lines: The effect of α A- and α B-crystallin expression on phenotype, and stable transfection of α A- and α B-crystallin and their different domains using EGFP. The expression of EGFP was observed under a fluorescence microscope. Images were obtained by fluorescence microscopy and stored in the computer. 1×10^6 cells were subcultured in a 100 mL culture flask. After culture at four concentrations for four corresponding time periods, the cells were rinsed three times with D-Hank's solution. The cells in the control group were cultured with DMEM without Dex. Other methods were the same as those in the experimental group.

Cell treatment

The HLEC line B-3 obtained from ATCC was cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco) with 20% fetal calf serum, 100 U/mL penicillin, 0.1 mg/mL streptomycin and 2 mM L-glutamine in white six well plates with a transparent bottom (Corning) with approximately 10^5 cells per well to yield a confluent monolayer. The treatments were the same for all assays. Three groups were used for each assay: the pc DNA3.1- HLECs group, the pc DNA3.1- α AC- HLECs group and the pc DNA3.1- α BC- HLECs group. In the Dex +pc DNA3.1- HLECs group, cells were treated with 0.1 μ mol, 1 μ mol, 10 μ mol and 100 μ mol Dex for 12 h, 24 h, 36 h, and 48 h. In the Dex + pc DNA3.1- α AC and α BC-HLECs groups, cells were treated with 0.1 μ mol, 1 μ mol, 10 μ mol, and 100 μ mol Dex for 12 h, 24 h, 36 h, and 48 h.

The expression of nine proteins detected by western blot

Protein samples were collected from cultured cells in the three groups. After treatment with drugs, the cells were treated with 5 SDS PAGE buffer. Protein concentrations were determined using the bicinchoninic acid (BCA) assay (Pierce, Rockford, IL, USA). Samples were electrophoresed on 12% Tris-glycine polyacrylamide gels using commercial apparatus (Bio-Rad Mini-PROTEAN Tetra cell) and then blotted onto a nitrocellulose membrane (Pall-Gelman) using a Transblot cell (Bio-Rad, Hercules, CA, USA). Membranes were blocked with 3% BSA and probed with nine primary antibodies and corresponding secondary antibodies using standard protocols. For HSP60 protein membranes were blocked in 3% BSA and probed with antibody (MA3-013, 1:1000, Invitrogen Antibodies) and secondary antibodies (Goat anti-mouse Ig-G, Beyotime Institute of Biotechnology) ;For Bid protein membranes were blocked in 3% BSA and probed with antibody (MA1-13043, 1:1000, Invitrogen Antibodies) and secondary antibodies (Goat anti-mouse Ig-G, Beyotime Institute of Biotechnology); For Survivin protein, Membranes were blocked in 3% BSA and probed with antibody (MA5-15077, 1:1000, Invitrogen Antibodies) and secondary antibodies (goat anti-rabbit Ig-G, Beyotime Institute of Biotechnology) ;For p53 protein, Membranes were blocked in 3% BSA and probed with antibody (7157-MSM3-P1, 1:1000, enquire BioReagents) and secondary antibodies (Goat anti-mouse Ig-G, Beyotime Institute of Biotechnology).For Smac/DIABLO protein, Membranes were blocked in 3% BSA and probed with antibody (PA1-41007, 1:1000, Invitrogen Antibodies) and secondary antibodies (Goat anti-mouse Ig-G, Beyotime Institute of Biotechnology);For cytochrome C protein, Membranes were blocked in 3% BSA and probed with antibody (710627, 1:1000, Invitrogen Antibodies) and secondary antibodies (Goat anti-mouse Ig-G, Beyotime Institute of Biotechnology);For NF- κ B protein, Membranes were blocked in 3% BSA and probed with antibody (PA5-27617, 1:1000, Invitrogen Antibodies) and secondary antibodies (Goat anti-rabbit Ig-G, Beyotime Institute of Biotechnology);For Fas protein, Membranes were blocked in 3% BSA and probed with antibody (MA5-14882, 1:1000, Invitrogen Antibodies) and secondary antibodies (Goat anti-rabbit Ig-G, Beyotime Institute of Biotechnology).All protein bands were visualized by diaminobenzidine (DAB, Tiangen Biotech Co. LTD) stain using a gel documentation apparatus (FUJIFILM LAS-3000).The membranes visualized by diaminobenzidine (DAB, Tiangen Biotech Co., Ltd) stain using gel documentation apparatus (FUJIFILM LAS-3000).

Blots were reprobbed with anti-actin monoclonal antibody(MA5-11869, 1:1000, Invitrogen Antibodies) with Goat anti-mouse IgM secondary antibody (Beyotime Institute of Biotechnology, China), imaged as

described above, and densitometric analyses were performed using NIH Image (version 163) with values normalized to the actin signal for each sample and expressed as multiples of increases (or decreases) relative to control samples (available at <http://rsb.info.nih.gov/nih-image>; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). Following analysis, we observed the most significantly expressed protein, the exposure factors and cell types.

LncRNA and mRNA chip scanning

Based on the results of western blotting, the RNA samples of HLECs (α A-crystallin overexpressed) were exposed to 0.1, 1, 10, and 100 μ mol Dex for 36 h and 48 h; HLECs (α B-crystallin overexpressed) were exposed to 10 and 100 μ mol Dex for 36 h and 48 h; HLECs were exposed to 0.1, 1, 10, and 100 μ mol Dex for 36 h and 48 h and lncRNA and mRNA chip analysis was performed. After identifying the most significant exposure factors and cell types, total RNA was extracted from the cells and scanned by lncRNA and mRNA chips. RNA was extracted using an RNA extraction kit. The concentration of RNA was detected at 260 nm and 280 nm. The RNA in the experimental sample was matched with an Agilent expression microarray (Agilent, Santa Clara, CA, USA). RNA amplification and a single standard kit were used to amplify and label cRNA, which was purified by the RNeasy Mini Kit. Chip hybridization was performed according to the operating instructions of the Agilent gene chip kit in a rolling hybridization furnace at 65°C. At 10R/min and 17 h after rolling hybridization, the sample size of the hybrid cRNA was 1.65 μ g, and the film was washed in the washing tank. The chips that completed the hybridization were scanned by the Agilent chip scanner, with feature extraction software 10.7 reads data and gene spring software 11.0. The algorithm was used for normalization. Scanning in the Agilent scanner involved resolution of 3 μ m/5 μ m, and the scanner automatically scanned once at 100%.

The Agilent SurePrint G3 Human Gene Expression v3 8x60K Microarray (Design ID: 072363) Chip experiment was used to analyze 20 samples. The total RNA of samples was quantified by Nanodrop nd-2000 (Thermo Fisher Scientific, Scotts Valley, CA, USA) and detected using an Agilent Bioanalyzer 2100 (Agilent). After RNA quality was determined, sample labeling, chip hybridization and elution referring to the chip standard were carried out.

Depth analysis showed that lncRNA and mRNA were highly specific compared with the negative control.

The Pearson's correlation coefficient was obtained by calculating the gene expression correlation analysis, and then the value of expression correlation was determined if it was greater than the pre-set threshold value. The expression correlation was calculated by a statistical method to determine whether it was significantly higher. Pearson's correlation coefficient, with an absolute value of 0.7, was considered relevant. Less than 0.7 represented a negative correlation, and 0.7 or greater represented a positive correlation. Pearson's correlation p-value < 0.05 was considered to be significantly related to expression.

For lncRNA (differential expression), all coding bases in the range of 100 K upstream and downstream were searched. Therefore, there was significant co-expression (Pearson correlation calculation) with lncRNA. The lncRNA is likely to regulate the genes that are close to the genome and co-expressed in the

expression mode. The “Encyclopedia of DNA elements”, the largest international cooperation project after the “human genome project” Encyclopedia of DNA elements (ENCODE for short) was used in this analysis. We plan to release the transcription factors and their target gene datasets in the latest release results. We calculated the intersection of the coding gene set co-expressed by lncRNAs and the target gene set of the transcription factor/chromatin regulatory complex, calculated the enrichment degree of the intersection using hypergeometric distribution, and obtained the transcription factors significantly related to lncRNAs, in order to identify the transcription factor / chromatin regulatory factors that may play a regulatory role jointly with lncRNAs. Thus, we calculated the coding genes co-expressed by lncRNAs and identified the transcription factors of these genes to identify the transcription factors that may play a regulatory role jointly with lncRNAs.

Screening methods and co-expression patterns of differential gene co-expression were obtained by correlation analysis of computational gene expression. The correlation coefficient was obtained, and it was determined whether the value of the expression correlation was greater than the preset threshold. Statistical methods were used to calculate whether the expression was highly correlated.

To determine whether the expression patterns of lncRNAs were significantly different, the expression patterns of co-expressed coding genes was assessed using a Heat map. The differential integration analysis (Venn analysis) was performed to carry out cross and union analysis of different comparisons of the three groups of cells treated with four concentrations for four time periods to evaluate common or unique differentially expressed genes. Two-way cluster analysis was used to investigate the expression patterns of differential gene expression, and the expression patterns of coding genes that were significantly co-expressed. The distance between two pairs of multiple samples was calculated to form a distance matrix. The two categories with the closest distance were a new category. The distance between the new category and the current category was calculated, and then merged until there was only one category. The direct correlation of samples with the selected differential genes or lncRNA expression was calculated. Generally speaking, the same category of samples can appear in the same cluster through clustering, and the genes or lncRNAs in the same cluster may have similar biological functions. Preconditions for the analysis were the samples and genes or lncRNAs for cluster analysis needed to be specified. Each cluster contained at least three samples, and the number of genes should not exceed 1,000 as much as possible.

Comprehensive analysis of specific lncRNAs and mRNAs

For each differentially expressed lncRNA, the co-expressed coding genes were calculated, and the lncRNA-mRNA relationship pairs with significant correlation were screened out. The function of mature mRNA was used to deduce the function of lncRNA, and the mRNA with a significant correlation with abnormal expression of lncRNA was enriched and analyzed. The principle of functional enrichment analysis is to test the significance of a functional class in a group of interested genes with a hypergeometric distribution pattern. The smaller the p value, the more likely the functional module is related to lncRNA, and the biological process of abnormal expression of lncRNA can be obtained. At the same time, in order

to control the situation of significant misjudgment, we introduced the calculation of the false-positive rate (FDR). When $FDR < 0.05$ it is considered that the functional module may be related to lncRNA. We analyzed the different mRNA molecules after comparing the patients with cataracts and normal controls to identify the upregulated and downregulated lncRNA or mRNA.

Then, GO and KEGG enrichment analyses were carried out to determine the biological functions or pathways that were mainly affected by different genes. The GO database provides professional terms to define the properties of gene products. It includes three categories: biological process (BP) which represents the process of a molecular activity event, including the function of cells, tissues, organs and species, it is often the category with the highest degree of relevance to experimental research problems; cell component (CC) represents the cell or its external environment; molecular function (MF) is the active element describing gene products at the molecular level. GO analysis of the differential genes is carried out to describe the function of the genes. The biological processes, molecular functions and cell components of differential genes, up-regulated differential genes and downregulated differential genes were analyzed. A bar chart was drawn of the items in biological process, cell component and molecular function using the results of GO enrichment analysis, which showed the most significant top 10 GO items in the three categories of GO results in the same chart. A bubble chart was drawn for the top 30 items with the lowest p value in the results of the GO enrichment analysis to show the most significant items in this analysis and their corresponding classification (BP, CC, MF). KEGG is a database for systematic analysis of gene functions, the connection of genomic information and functional information. The number of differential genes included in each GO entry was counted, and the significance of differential gene enrichment in each GO entry was calculated by a hypergeometric distribution algorithm. The result of this calculation will provide a p value of enrichment significance, the lower the value, the more statistically significant it is. According to the results of GO analysis and biological significance, we selected the genes for follow-up study. The KEGG data were used to carry out pathway analysis of differential genes, and the hypergeometric distribution algorithm was used to calculate the significance of the enrichment of differential genes in each pathway. The smaller the p value, the higher the correlation between the corresponding pathway and the differential gene. Using pathway analysis of the differential gene, the pathway significantly related to the differential gene was determined, and indicated which pathway changes may be related to the experimental design conditions.

Real-time quantitative PCR to verify the results of lncRNA chip

The qRT-PCR was used to validate some of the differentially expressed lncRNAs and mRNAs. The primer and probe sequences are listed in Table 1. The qPCR was performed using the SYBR Green Premix DimerEraser kit (TaKaRa, Dalian, China) on the Roche LightCycler 480 Instrument II. The relative gene expression was analyzed using the $2(-\Delta\Delta Ct)$ method as previously described [40]. The significance of differences was analyzed using one-way analysis of variance or the Student's *t*-test (unpaired). Multiple comparisons between groups were performed using Tukey's method. SPSS software, version 19.0 (SPSS, Inc., Chicago, IL, USA) was used to perform the statistical analysis. A $p < 0.05$ was considered to indicate a statistically significant difference.

Establishment of alphaA-crystallin overexpression Primary cell and perform Western blot and Real-time quantitative PCR to verify specific anti apoptotic proteins and related lncRNAs

In order to verify that overexpression of alphaA-crystallin will lead to the increase of the expression of related anti apoptotic proteins and further verify the expression changes of related lncRNAs, we carried out the primary culture of lens epithelial cells and established the primary culture cell line of lens epithelial cells with overexpression of alphaA-crystallin. After different concentrations of dexamethasone for different times, Western blot further verified the expression changes of HSP60, bid, survivin and p53 protein in normal primary cultured lens epithelial cells compared with exposure factors, and the expression of lnc-tmprss15 was detected by real-time quantitative PCR.

The donated eyeballs were removed from the eye bank of the eye hospital of the First Affiliated Hospital of Harbin Medical University, and the lens was separated. The anterior capsular membrane of the lens was removed with capsulorhexis forceps and immediately placed in RPMI-1640 medium. The primary cultured cells can be subcultured when they are 80% - 90% full of the bottom of the bottle. The cells with good growth in the second to fourth generations are used in the experiment. The transformation and cell treatment methods, Western blot and Real-time quantitative PCR methods are identified as described above.

Results

The α A- and α B-crystallin overexpression in cell lines was established and stably expressed.

Following the synthesis of α A- and α B crystalline genes, the vector pirespuro was subcloned by $ECOR \text{ } \square$ (gaattc) and $EcoRV$ (gatat C) After the preparation of pIRESpuro- α A and pIRESpuro- α B, HLEB3 cells were transfected. After 24 h of transfection, puro was added for pressure screening, and half of the medium was renewed every 24h until the cells grew stably. Transfection was detected by fluorescence microscopy and the results are shown in Figure 1. After transfection, cell lines overexpressing α A- and α B-crystallin were cultured and then frozen Further detection of α A- and α B-crystallin overexpression in HLECs was determined by Western blot and the results are shown in Figure 2

The expression of nine proteins

The anti-HSP60 antibody, showed positive reactions to HSP60 in western blots of HLECs at the expected molecular size for HSP60 of approximately 58 kD. When HLECs were exposed to the drugs, the expression of HSP60 protein was variable and results from the NIH image (version 163) are shown in Figure 3a. These data indicated that the expression of HSP60-protein significantly increased when HLECs (α A crystallin overexpressed) were exposed to 0.1, 1, and 10 μ mol Dex for 36 h, and 1 μ mol Dex for 48 h.

The anti-Bid antibody, showed a positive reaction to Bid in western blots of HLECs at the expected molecular size for Bid of approximately 21 kD. When HLECs were exposed to the drugs, the expression of

Bid protein was variable and results from the NIH image (version 163) are shown in Figure 3b. These data indicated that the expression of Bid-protein significantly increased when HLECs were exposed to 1 μ mol Dex for 36 h, and 0.1 and 1 μ mol Dex for 48 h; the expression of Bid-protein significantly increased when HLECs (α B-crystallin overexpressed) were exposed to 10 μ mol Dex for 48 h. However, after exposure to Dex, Bid in HLECs (α A-crystallin overexpressed) was not significantly expressed.

The anti-survivin antibody, showed positive reactions to survivin in western blots of HLECs at the expected molecular size for survivin of approximately 16.5 kD. When HLECs were exposed to the drugs, the expression of survivin protein was variable and results from the NIH image (version 163) are shown in Figure 3c. These data indicated that the expression of Survivin-protein significantly increased when HLECs (α A-crystallin overexpressed) were exposed to 1, 10 μ mol, and 100 μ mol Dex for 48 h. However, after exposure to Dex, survivin in HLECs (α B-crystallin overexpressed) was not significantly expressed.

The anti-P53 antibody, showed positive reactions to P53 in western blots of HLECs at the expected molecular size for P53 of approximately 43 kD. When HLECs were exposed to the drugs, the expression of P53 protein was variable and results from the NIH image (version 163) are shown in Figure 3d. These data indicated that the expression of P53-protein significantly increased when HLECs (α A-crystallin overexpressed) were exposed to 1, 10 μ mol and 100 μ mol Dex for 48 h. However, after exposure to Dex, P53 in HLECs (α B-crystallin overexpressed) was not significantly expressed.

Following exposure to Dex, cytochrome C, Samc, P65, Fas and beta actin in HLECs (α A-crystallin overexpressed), and in HLECs (α B-crystallin overexpressed) were not significantly expressed (data not shown).

Principal Component Analysis of lncRNA and mRNA chip

We used the expression of genes to conduct principal component analysis (PCA)[36], observe the distribution of samples, explore the relationship between samples and verify the experimental design. PCA can show the relationship between samples from different dimensions The 2D and 3D PCA images are shown in Figure 4. PCA showed that the samples in the same group were relatively close

Differential expression analysis of lncRNA

The data accession numbers/web links is GSE193629 study at: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE193629>. After deep analysis of the lncRNA and mRNA chip results, we found that when HLECs (α A-crystallin overexpressed) were exposed to 0.1, 1, 10, and 100 μ mol Dex for 36 h and 48 h, the expression of lnc-TMPRSS15 was significantly increased When HLECs (α A-crystallin overexpressed) were exposed to 0.1 and 1 μ mol Dex for 36 h and 48 h, the expression of lnc-CDH2-1 was significantly decreased When HLECs (α A-crystallin overexpressed) were exposed to 10 and 100 μ mol Dex for 36 h and 48 h, the expression of lnc-RNF-13-2 was significantly decreased.

To verify the results of lncRNA microarray, we detected the expression of lnc-TMPRSS15, lnc-CDH2-1 and lnc-RNF-13-2 using qPCR. The results were consistent with the lncRNA array analysis and these results are shown in Figure 5a, b and c.

However, for HLECs (α B-crystallin overexpressed), lncRNAs were not significantly expressed.

GO analysis

The expression of lncRNA-tmprss15 was greatest in the 20 groups. We then analyzed the different genes from GO and KEGG enrichment to determine the biological functions or pathways that the different genes affected. Following analysis, the function of tmprss15 was an integral component of the membrane (CC) which was located in the most significant top 10 GO entries. However, based on the KEGG analysis, we did not find a significant entry for tmprss15. We did not find a significant GO and KEGG entry for RNF-13-2 and CDH2-1, and these results are shown in Figure 6.

Differential expression analysis of related mRNA

Based on the results of GO analysis and the microchip results, we found that the mRNA of HSD11B1 and CDH12 were mostly related to the expression of TMPRSS15. The expression changes in TMPRSS15 are shown in Figure 7a. When HLECs (α A-crystallin overexpressed) were exposed to 0.1, 1, 10, and 100 μ mol Dex for 36 h and 48 h, the expression of TMPRSS15 was significantly increased. When HLECs (α A-crystallin overexpressed) were exposed to 0.1, 1, 10, and 100 μ mol Dex for 36 h and 48 h, the expression of CDH12 was significantly increased. When HLECs (α A-crystallin overexpressed) were exposed to 0.1, 1, 10, and 100 μ mol Dex for 36 h and 48 h, the expression of HSD11B1 was significantly decreased. To verify the results of mRNA microarray, we determined the expression of TMPRSS15, CDH12 and HSD11B1 using qPCR. The results were consistent with the lncRNA array analysis and are shown in Figure 7a, b and c.

Western blot and Real-time quantitative PCR results for verifying specific anti apoptotic proteins and related lncRNAs based on α A-crystallin overexpression Primary cell

Transfection was detected by fluorescence microscopy and the results are shown in Figure 8a. After transfection, cell lines overexpressing α A- protein were cultured and then frozen. Further detection of α A-crystallin overexpression in Primary cell was determined by Western blot and the results are shown in Figure 8b.

The anti-HSP60 antibody, showed positive reactions to HSP60 in western blots of Primary cell at the expected molecular size for HSP60 of approximately 58 kD. When Primary cell were exposed to the drugs, the expression of HSP60 protein was variable and results from the NIH image (version 163) are shown in Figure 8c. These data indicated that the expression of HSP60-protein significantly increased when The primary cultured cells (α A crystallin overexpressed) were exposed to 0.1, 1, and 10 μ mol Dex for 36 h, and 1 μ mol Dex for 48 h.

The anti-Bid antibody, showed a positive reaction to Bid in western blots of Primary cell at the expected molecular size for Bid of approximately 21 kD. When Primary cell were exposed to the drugs, the expression of Bid protein was variable and results from the NIH image (version 163) are shown in Figure 8d. These data indicated that the expression of Bid-protein significantly increased when Primary cell were exposed to 1 μmol Dex for 36 h, and 0.1 and 1 μmol Dex for 48 h; However, after exposure to Dex, Bid in Primary cell ($\alpha\text{A-crystallin}$ overexpressed) was not significantly expressed.

The anti-survivin antibody, showed positive reactions to survivin in western blots of Primary cell at the expected molecular size for survivin of approximately 16.5 kD. When Primary cell were exposed to the drugs, the expression of survivin protein was variable and results from the NIH image (version 163) are shown in Figure 8e. These data indicated that the expression of Survivin-protein significantly increased when Primary cell ($\alpha\text{A-crystallin}$ overexpressed) were exposed to 1, 10 μmol , and 100 μmol Dex for 48 h

The anti-P53 antibody, showed positive reactions to P53 in western blots of Primary cell at the expected molecular size for P53 of approximately 43 kD. When Primary cell were exposed to the drugs, the expression of P53 protein was variable and results from the NIH image (version 163) are shown in Figure 8f. These data indicated that the expression of P53-protein significantly increased when HLECs ($\alpha\text{A-crystallin}$ overexpressed) were exposed to 1, 10 μmol and 100 μmol Dex for 48 h

To verify the results of lncRNA microarray, we detected the expression of lnc-TMPRSS15 using qPCR. We found that when Primary cell ($\alpha\text{A-crystallin}$ overexpressed) were exposed to 0.1, 1, 10, and 100 μmol Dex for 36 h and 48 h, the expression of lnc-TMPRSS15 was significantly increased. The results were consistent with the lncRNA array analysis and these results are shown in Figure 8g

Discussion

At present, due to limited research there is still only a preliminary understanding of the formation mechanism of GIC, and the complexity of its mechanism; however, several hypotheses have been put forward: the theory of damaged crystal protein structure and function, the theory of damaged crystal enzyme function, the theory of abnormal cell adhesion, the theory of abnormal cell differentiation and the theory of out of control apoptosis regulation.

The 60 kDa HSP (HSP60) is a mitochondrial chaperone protein. However, the accumulated data show that it is also limited to the extra mitochondrial septum [16-21]. As a major mitochondrial chaperone, HSP60 is essential for the stability of the mitochondrial protein internal environment [22]. However, it is also associated with cell survival and apoptosis signaling pathways [23]. The human HSP60 gene exists on chromosome 2, and proteins can be induced by various stimuli, including heat, oxidative stress and infection. HSP60 is a highly conserved molecule, which plays a role as a molecular chaperone in the assembly, folding and transport of intracellular proteins [24]. HSP60 can also help denatured, insoluble proteins to restore their natural conformation. HSP60 is an intracellular molecular chaperone, which has been largely described as a warning or injury-related molecular pattern when released extracellularly [25]. The cytoplasmic localization of HSP60 can regulate the activity of the proteasome according to the

needs of cells [26]. A large number of studies have shown that HSP60 plays an important role in anti-infection, autoimmunity, aging and apoptosis. Some results show that the occurrence of cataract is related to apoptosis of lens epithelial cells. HSP60 in cytoplasm can inhibit apoptosis by influencing apoptosis-related factors.

Our data indicate that the expression of HSP60-protein significantly increased when HLECs (α A-crystallin overexpressed) were exposed to 0.1, 1, and 10 μ mol Dex for 36 h, and 1 μ mol Dex for 48 h, which indicated that the overexpressed α A-crystallin influenced the expression of HSP60-protein and could result in the high level of HSP60-protein expression.

Bid protein belongs to BH3 the only subclass of the Bcl-2 superfamily, which only contains the BH3 region in the conservative BH (Bcl-2 Homology) region [27]. Under normal physiological conditions, Bid exists in the form of no inductive activity. When apoptosis is initiated, caspase-8 is activated first, and then the Bid enzyme is decomposed into two fragments, i.e. C-fragment and N-fragment of 15 kD.

Although great progress has been made in the study of Bid-protein, many problems remain unclear [28-31]. Bid-protein can also cooperate with Bax protein to enhance the mitochondrial damage caused by Bax by promoting the binding of Bax to mitochondria and the change in Bax conformation [32-33]. The hypothesis of the synergistic effect of Bid and Bax has been supported by many experiments.

Our data indicated that the expression of Bid-protein significantly increased when HLECs were exposed to 1 μ mol Dex for 36 h; 0.1 and 1 μ mol Dex for 48 h and when HLECs (α B-crystallin overexpressed) were exposed to 10 μ mol Dex for 48 h. However, when HLECs (α A-crystallin overexpressed) were exposed to Dex at four concentrations for four time periods, the expression of Bid-protein did not significantly change. This may indicate that α A-crystallin can depress the expression of Bid-protein.

The cell responses induced by P53 target genes are different, some even have opposite effects, or they are mutually exclusive. By post-translational modification [34] and binding with different CO molecules [35], P53-protein can only activate the target genes that mediate the appropriate cellular response in specific extracellular environments and physiological conditions. P53 plays a role in the zero tolerance of all types of abnormalities that may cause tumors, and can effectively prevent the malignant transformation of cells. Many types of DNA damage can activate P53 through specific post-translational modification [36]. The first step in initiating DNA repair is to upregulate the gene group related to DNA repair.

These data indicated that the expression of P53-protein significantly increased when HLECs (α A-crystallin overexpressed) were exposed to 1, 10, and 100 μ mol Dex for 48 h. However, when HLECs (α B-crystallin overexpressed) and HLECs were exposed to Dex at four concentrations for four time periods, the expression of P53-protein did not significantly change. This may indicate that α A-crystallin can increase the expression of P53-protein.

Survivin is the most powerful inhibitor of apoptosis. Many studies have proved that high expression of survivin can inhibit apoptosis induced by Fas, Bax, caspases, tumor damaging factors and anticancer drugs [37, 38]. Some studies have suggested that survivin can form a stable combination with the end effectors caspase-3 and caspase-7 in the form of a homodimer, in order to inhibit their protein cleavage and block the process of apoptosis [39]. It is now thought that survivin is more likely to be associated with caspase-9 binding which inhibits its activity [40]. Survivin relies on cell proliferation signals to enter the nuclear and CDK4 structure, which leads to CDK2/cyclinE activation. At the same time, p21 is released from CDK4 by the transformation phase. The released p21 translocates to the complex formed between mitochondria and caspase-3, inhibits the activity of caspase-3, blocks the release of cytochrome C from mitochondria, and thus inhibits apoptosis [41].

These data indicated that the expression of survivin-protein significantly increased when HLECs (α A-crystallin overexpressed) were exposed to 1, 10, and 100 μ mol Dex for 48 h. This may indicate that α A-crystallin can increase the expression of the survivin-protein.

In this study, we also detected the expression of the proteins of cytochrome C, Smac, P65 and Fas. However, the results showed that there were no significant relationships between α A- and α B-crystallin and these four proteins (data not shown).

We also studied the relationship between α A- and α B-crystallin and lncRNAs and the related mRNAs. Based on the western blot results, we detected cells (overexpressed α A- and α B-crystallin and negative controls) in which apoptosis-related proteins were significantly expression in microarray analysis.

Following microarray analysis, the expression of lnc-TMPRSS15 was observed when HLECs (α A-crystallin overexpressed) were exposed to 0.1, 1, 10, and 100 μ mol Dex for 36 h and 48 h. However, when HLECs (α A-crystallin overexpressed) were exposed to 0.1 and 1 μ mol Dex for 36 h and 48 h, the expression of lnc-cdh2-1 was decreased. In addition, when HLECs (α A-crystallin overexpressed) were exposed to 10 and 100 μ mol Dex for 36 h and 48 h, the expression of lnc-RNF13-2 was also decreased.

Significant expression of lnc-TMPRSS15 was noted. We further determined the expression of tmpress15 and the expression level was similar to that of lnc-TMPRSS15 which showed that the expression was significantly increased when HLECs (α A-crystallin overexpressed) were exposed to 0.1, 1, 10, and 100 μ mol Dex for 36 h and 48 h. After GO analysis, we found that the term_description of tmpress15 was an integral component of the membrane; thus, we detected other mRNAs which were significantly expressed and belonged to the same term_description as tmpress15. Based on the same term_description, we found that cdh12 (mRNA) was significantly increased when HLECs (α A-crystallin overexpressed) were exposed to 0.1, 1, 10, and 100 μ mol Dex for 36 h and 48 h. However, we found that HSD11B1 (mRNA) was significantly decreased when HLECs (α A-crystallin overexpressed) were exposed to 0.1, 1, 10, and 100 μ mol Dex for 36 h and 48 h.

The TMPRSS15 gene codes for the transmembrane protease serine 15 [42, 43]. We found that the expression was significantly increased when HLECs (α A-crystallin overexpressed) were exposed to 0.1, 1,

10, and 100 μmol Dex for 36 h and 48 h which may indicate that α A-crystallin can increase the expression of TMPRSS15 which is a very important protein and an integral component of the membrane.

We further investigated all the other mRNAs, which belonged to the same term as TMPRSS15 following GO analysis. We found the expression level of CDH12 (mRNA) was the same as the expression of TMPRSS15. CDH12 is a calcium dependent cell adhesion protein. When connecting cells, they preferentially interact with themselves in an affinity manner; therefore, cadherin may contribute to the classification of heterogeneous cell types. CDH12, a member of the cadherin gene family, is a transmembrane glycoprotein with calcium dependent cell-cell adhesion. Each gene exhibits a unique spatiotemporal expression pattern, which is important for morphological development and maintenance of tissue integrity. From our results, we can further speculate that α A-crystallin can increase the expression of CDH12.

In addition, we investigated HSD11B1 which has the same term as TMPRSS15 in GO analysis. Glucocorticoids stimulate the expression of HSD11B1 and the activity of the 11b-HSD1 enzyme through a feed forward loop [44, 45] causing excess GC at the tissue level. We also found that the expression of HSD11B1 was absolutely contrary to the expression of TMPRSS15 in HLECs (α A-crystallin overexpressed) which may indicate that α A-crystallin can depress the expression of HSD11B1.

N-cadherin (cadherin 2, type 1, CDH2-1) is a homotype of the cell adhesion molecule, which regulates the function of fibroblasts [46]. In addition, the increased expression of N-cadherin is related to the progress of various tumor types, especially skin tumors. For example, N-cadherin expression is increased in tumors of patients with highly invasive breast cancer [47], prostate cancer [48, 49], and bladder cancer [50]. In this study, we found that the expression of Inc-CDH2-1 was decreased in HLECs (α A-crystallin overexpressed) at some concentrations and at some time periods which may indicate that α A-crystallin can depress the expression of Inc-CDH2-1.

Previous studies have suggested that RNF13 is involved in a variety of cell activities, and its expression is increased in precancerous cells, pancreatic ductal adenocarcinoma and neurite cell growth [51-53]. RNF13, is a newly discovered gene involved in apoptosis, and it is helpful to study this gene to understand the apoptosis pathway more clearly. Research on RNF13 will help us to understand the physiological mechanism of apoptosis-related diseases, and provide new ideas in order to solve problems in the field of apoptosis clinical medicine. Endoplasmic reticulum stress is closely related to the occurrence and development of tumors. Further study on the mechanism of endoplasmic reticulum stress can deepen our understanding of these diseases at the molecular level, and contribute to further breakthroughs in physiology and pathology. RNF13 is involved in many cell activities. Recently, studies have found that the expression of RNF13 is up-regulated during the development of many types of cancers, such as liver cancer, colon cancer, esophageal cancer, ovarian cancer, renal cell carcinoma and melanoma [51-53]. The function of RNF13 in apoptosis may be closely related to tumorigenesis. In this study, we found that the expression of Inc-RNF-13-2 was decreased in HLECs (α A-crystallin

overexpressed) at some concentrations and at some time periods which may indicate that α A-crystallin can depress the expression of lnc-RNF-13-2.

In addition to, we established alphaA-crystallin overexpression primary cell and perform western blot and real-time quantitative PCR to verify specific anti apoptotic proteins and related lncRNAs, and the results were consistent with HLECs groups.

Overall, the results of our study suggest that the overexpression α A-crystallin can increase the expression of lnc-TMPRSS15, TMPRSS15 and related CDH12, and decrease the expression of HSD11B1, lnc-RNF13-2 and lnc-cdh2-1, which may result in the significant high expression of anti-apoptotic proteins such as HSP60, survivin and P53. Our results showed that α A-crystallin can promote the expression of some anti-apoptotic proteins and lnc-TMPRSS15 plays an important role in this process. The function of lnc-TMPRSS15 will be investigated in future studies.

Declarations

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Ethics approval and consent to participate

Not available.

Consent for publication

Not available.

Availability of data and materials

The data accession numbers/web links is GSE193629 study at:
<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE193629>

Competing interests

The authors declare that they have no conflicts of interest.

Funding

This work was supported by grants from the National Natural Science Foundation of China (Grant No. 81700818) to Lin Wang, Heilongjiang Postdoctoral Research Initiation Fund (Grant No. LBH-Q15105) to Lin Wang, and the Research and Innovation Fund of the First Hospital of Harbin Medical University (Grant No. 2017B016) to Lin Wang.

Authors' contributions

Lin Wang, Wencheng Zhao and Ping Liu planned the study. Lin Wang, YuQing Wang ,Wencheng Zhao, and Ping Liu conducted the experiment. Lin Wang, YuQing Wang ,Wencheng Zhao, Ping Liu wrote and submitted the article.

Acknowledgements

Not applicable

Confirmed statement

Not applicable

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Tables

Table 1. The primers used for qPCR detection of selected lncRNAs and mRNAs

Gene name	Forward	Reverse
lnc-TMPRSS15-1:1	AGAAACCAGTGCCATGAGTAAA	GGCTTGAACAGAAAAGGAGTAGA
lnc-CDH2-1:2	ACACCTATGACCTTGGCTTCG	TGTTGACCCTGGCACTCTTCT
lnc-RNF13-2	TTGAGTTGGAATATGTTCTGGC	TGCTGATAGGAGGAAAGGCT
tmprss15	ATGGGGTCGAAAAGAGGCATA	CTGCACCTCGTTGGGATTCC
cdh12	TTTGATGGAGGTCTCCTAACACC	ACGTTTAACACGTTGGAAATGTG
HSD11B1	AGCAGGAAAGCTCATGGGAG	CCACGTAAGTGAAGGAAAGTTGAC
beta-actin	CTACCTCATGAAGATCCTCACCGA	TTCTCCTTAATGTCACGCACGATT

Figures

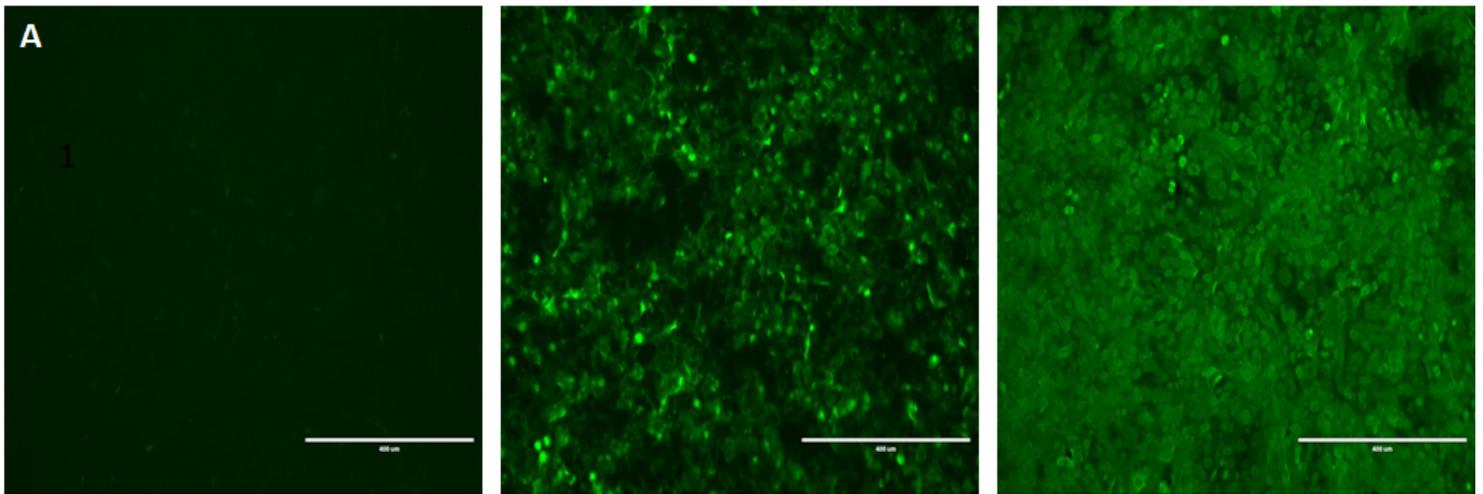


Figure 1

A. The fluorescence photo of cultured HLECs; B. The fluorescence photo of stable overexpression of α A crystallin; C. The fluorescence photo of stable overexpression of α B crystallin.

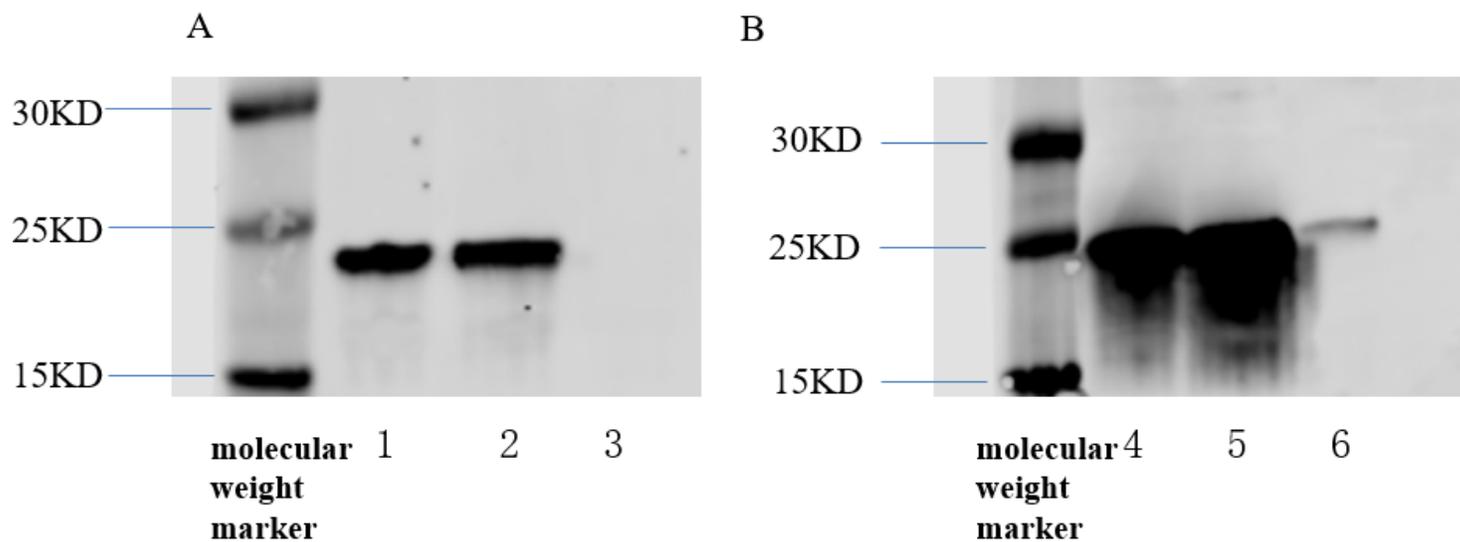


Figure 2

Western blot verification of over expression of α A and α B crystallins: 1, 2: over expression of α A crystallin [20KD]. 3: Negative control. 4,5: over expression of α B crystallin [22KD]. 6: Negative control.

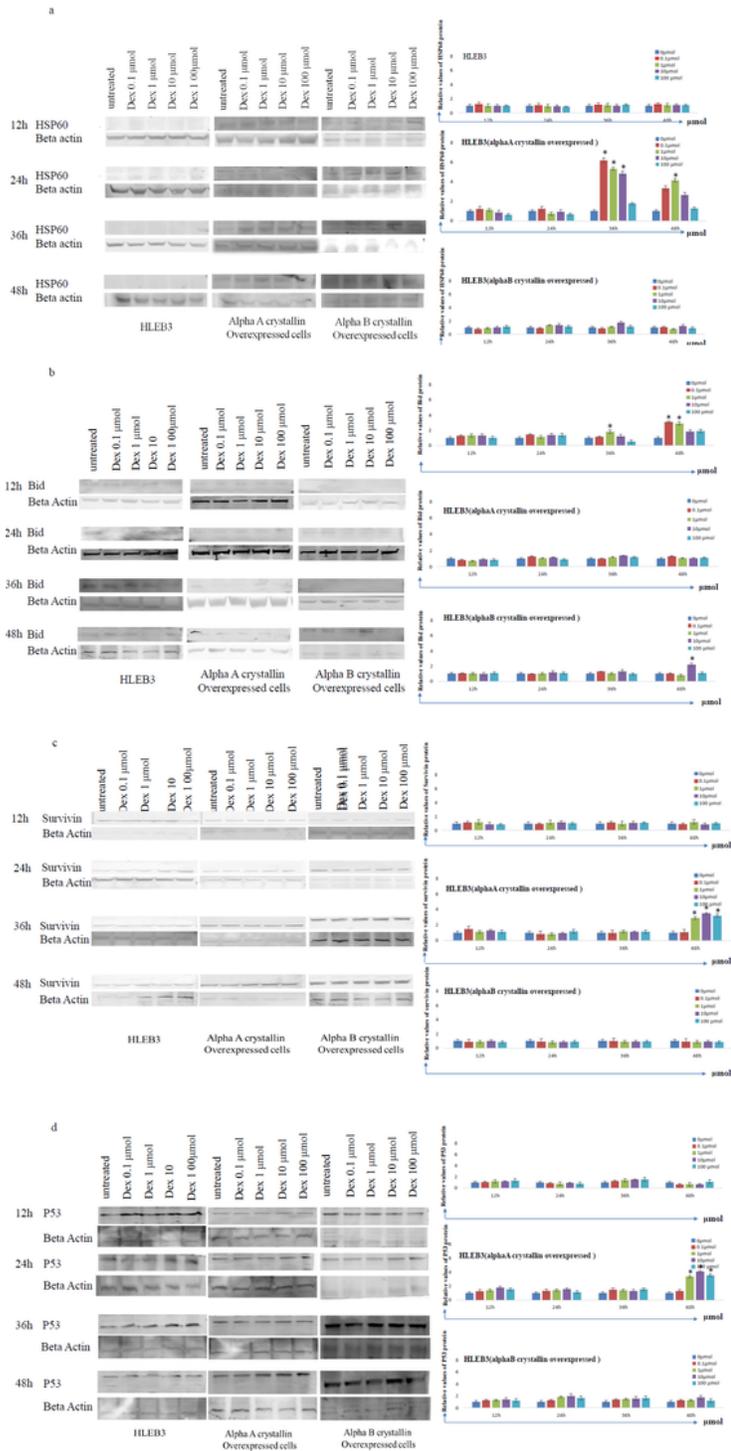


Figure 3

a. The anti- HSP60 antibody, showed positive reactions to HSP60 in Western-blot of HLECs at the expected molecular size for HSP60 of approximately 58 KD. When HLECs were exposed to the drugs, the expression of HSP60 protein was variable and results from NIH image (ver.163) are shown in Fig. 3a. These data indicate that the expression of HSP60-protein significantly increased when HLECs (alphaA crystallin overexpressed)were exposed to 0.1 \times 10 μ mol Dex for 36 h; 1 μ mol Dex for 48 h.

b. The anti- Bid antibody, showed positive reactions to Bid in Western-blot of HLECs at the expected molecular size for Bid of approximately 21 KD. When HLECs were exposed to the drugs, the expression of Bid protein was variable and results from NIH image (ver.163) are shown in Fig. 3b. These data indicate that the expression of Bid -protein significantly increased when HLECs were exposed to 1µmol Dex for 36h and 0.1 µmol Dex for 48 h; the expression of Bid -protein significantly increased when HLECs(alphaB crystallin overexpressed) were exposed to 10µmol Dex for 48h. However, after exposed to Dex, the expression of Bid in HLECs (alphaA crystallin overexpressed) have not significantly expression.

c. The anti- survivin antibody, showed positive reactions to Survivin in western-blot of HLECs at the expected molecular size for Survivin of approximately 16.5KD. When HLECs were exposed to the drugs, the expression of Survivin protein was variable and results from NIH image (ver.163) are shown in Fig. 3c. These data indicate that the expression of Survivin -protein significantly increased when HLECs (alphaA crystallin overexpressed)were exposed to 1µmol,10µmol,100µmol Dex for 48 h. However, after exposed to Dex, the expression of Survivin in HLECs and HLECs (alphaB crystallin overexpressed) have not significantly expression.

d. The anti- P53 antibody, showed positive reactions to P53 in Western-blot of HLECs at the expected molecular size for P53 of approximately 43 KD. When HLECs were exposed to the drugs, the expression of P53 protein was variable and results from NIH image (ver.163) are shown in Fig. 3d. These data indicate that the expression of P53 -protein significantly increased when HLECs (alphaA crystallin overexpressed)were exposed to 1µmol and 100µmol Dex for 48 h; However, after exposed to Dex, the expression of Survivin in HLECs and HLECs (alphaB crystallin overexpressed) have not significantly expression.

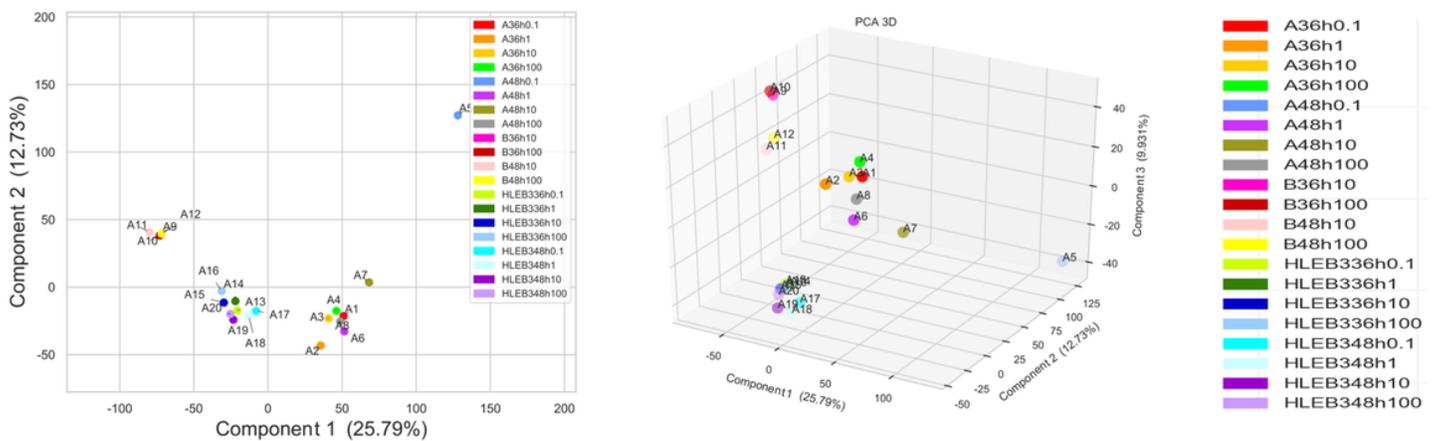
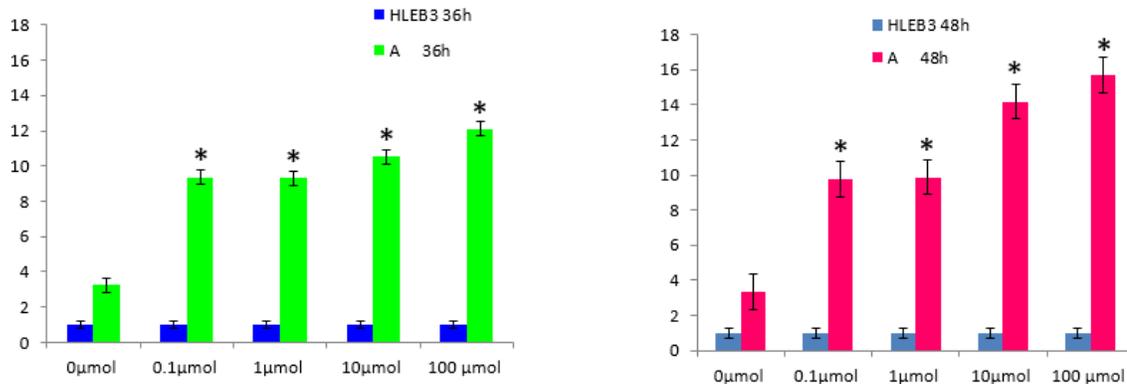


Figure 4

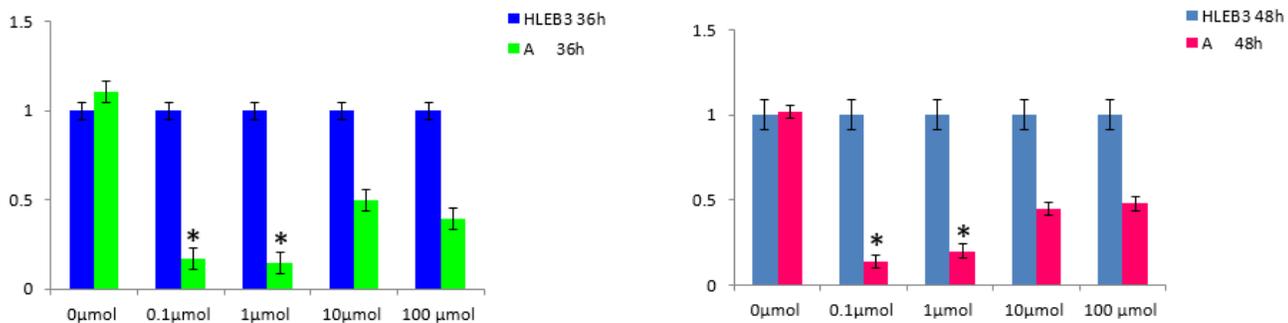
Based on the results of western blot, the RNA samples of HLECs (alphaA crystallin overexpressed) exposed to 0.1 ,1,10,100µmol DEX for 36h and 48h ; HLECs (alphaB crystallin overexpressed) exposed to 10,100µmol DEX for 36h and 48h ; HLECs exposed to 0.1 ,1,10,100µmol DEX for 36h and 48h were performed for lncRNA and mRNA chip analysis. We use the expression of genes to conduct principal

component analysis (PCA), observe the distribution of samples, explore the relationship between samples or verify the experimental design. PCA can show the relationship between samples from different dimensions. The 2D figure could be seen in Figure4a and 3D PCA figure could be seen in Figure4b. The PCA figures showed the samples in the same group are relatively close.

lnc-TMPRSS15-1



lnc-CDH2-1



lnc-RNF-13-2

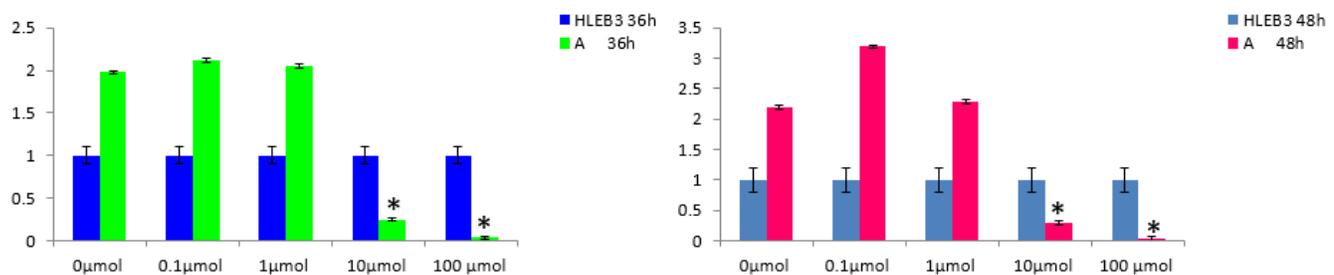
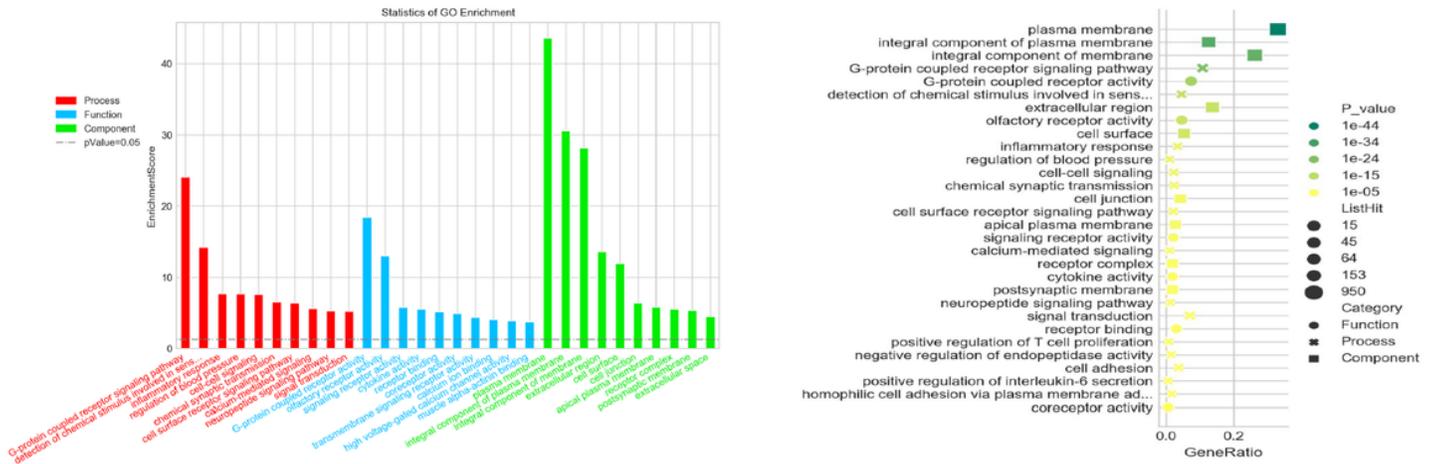


Figure 5

After the deep analysis of the lncRNA and mRNA chip results. A, we found when HLECs (alphaA crystallin overexpressed) exposed to 0.1 ,1,10,100μmol DEX for 36h and 48h, the expression of lnc-TMPRSS15 were significantly increased . B, We found when HLECs (alphaA crystallin overexpressed) exposed to 0.1 and 1μmol DEX for 36h and 48h, the expression of lnc-CDH2-1 were significantly decreased .C,We found when HLECs (alphaA crystallin overexpressed) exposed to 10 and 100μmol DEX for 36h and 48h, the expression of lnc-RNF-13-2 were significantly decreased .

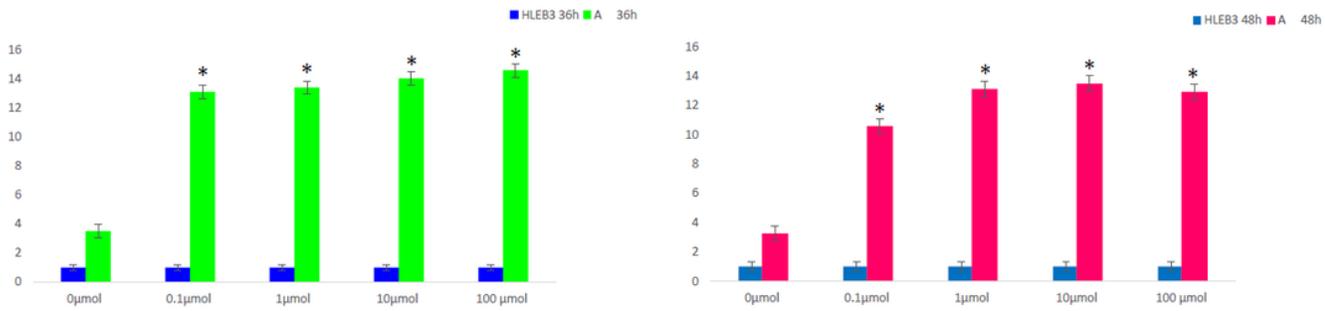


Term_ID	Term_description	ListHit	ListTotal	PopHit	PopTotal	GeneRatio	BgRatio	FoldEnrichment	GeneSymbols	P_value	FDR_bh	Category
GO:0016021	integral component of membrane	371	1627	3655	19805	0.228027044	0.184549356	1.235588399	TMPRSS15	2.38E-06	0.000214	Component

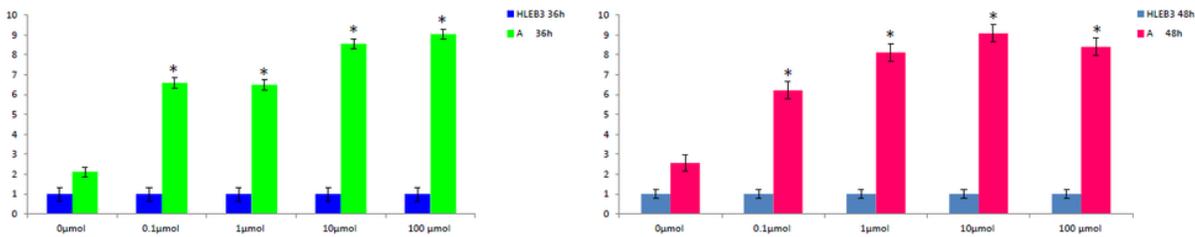
Figure 6

For lncRNA-TMPRSS15 has the most expressed significance in the 8 groups. Then, we analyzed the difference genes by go and KEGG enrichment to determine the biological functions or pathways that the difference genes mainly affect. After analysis, the function of tmprss15 is integral component of membrane (CC) which located in is the most significant top 10 GO entry. However, based on the KEGG analysis, we did not find significance entry for TMPRSS15. For RNF-13-2 and CDH2-1, we did not find significantly GO and KEGG entry for them and the results could be seen Figure 6.

mRNA-TMPRSS15



mRNA-CDH12



mRNA-HSD 11B1

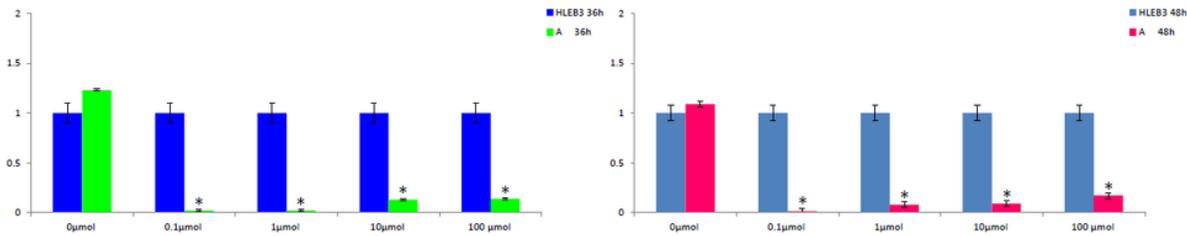


Figure 7

The expressed changes of TMPRSS15 were shown in Figure 7a. When HLECs (alphaA crystallin overexpressed) exposed to 0.1, 1, 10, 100 μmol DEX for 36h and 48h, the expression of TMPRSS15 were significantly increased. When HLECs (alphaA crystallin overexpressed) exposed to 0.1, 1, 10, 100 μmol DEX for 36h and 48h, the expression of CDH12 were significantly increased. When HLECs (alphaA crystallin overexpressed) exposed to 0.1, 1, 10, 100 μmol DEX for 36h and 48h, the expression of HSD11B1 were significantly decreased. To verify the results of mRNA microarray, we detected the expression of TMPRSS15, CDH12 and HSD11B1 using qPCR. The results showed that the results were consistent with the lncRNA array analysis and the results could be seen in Figure 7a, b and c.

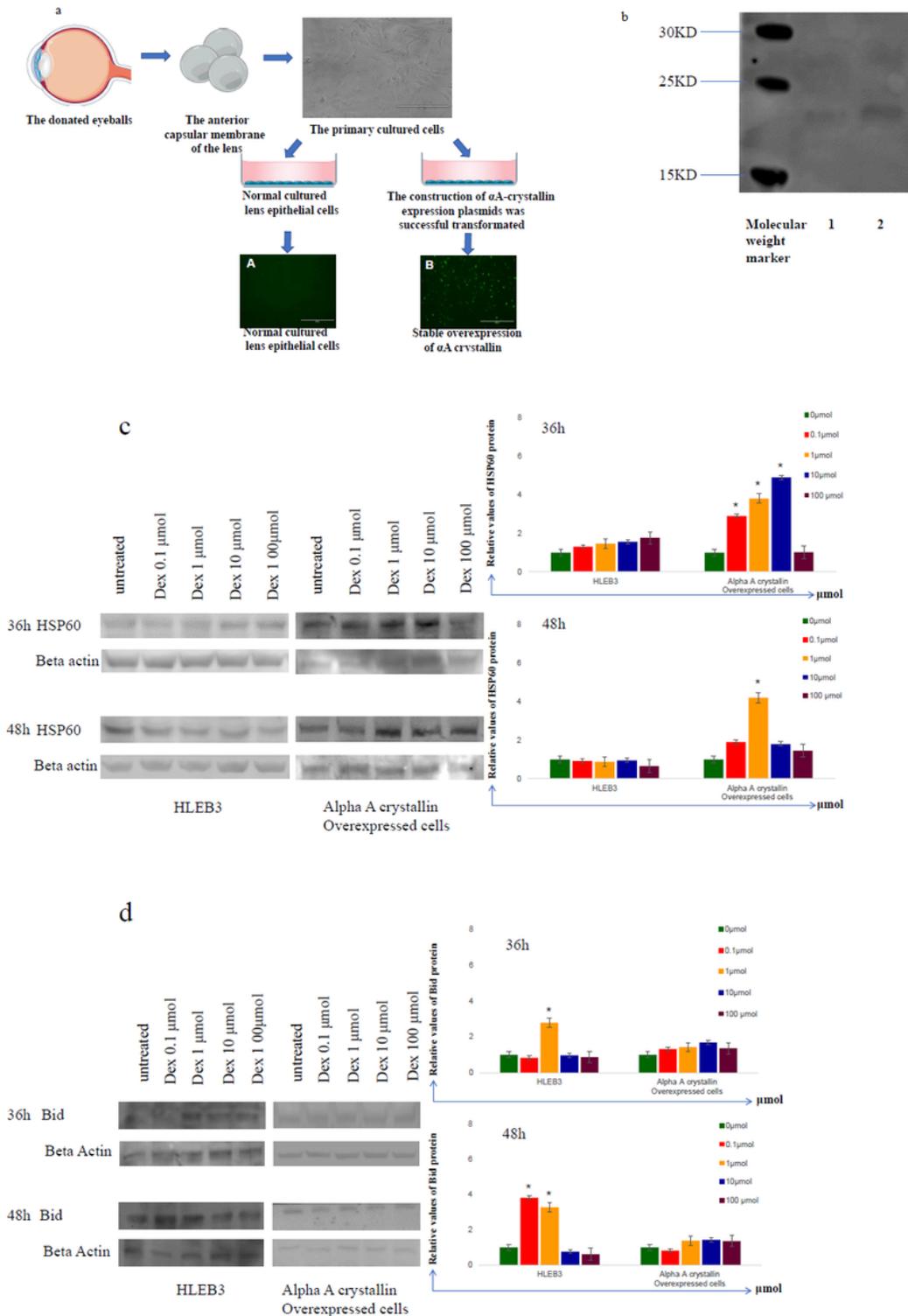


Figure 8

a . The donated eyeballs were removed from the eye bank of the eye hospital of the First Affiliated Hospital of Harbin Medical University, and the lens was separated. The anterior capsular membrane of the lens was removed with capsulorhexis forceps and immediately placed in RPMI-1640 medium. The primary cultured cells can be subcultured when they are 80% - 90% full of the bottom of the bottle. The

cells with good growth in the second to fourth generations are used in the experiment. Transfection was detected by fluorescence microscopy and the photo results are shown in Figure 8a

b. Western blot verification of over expression of α A :1 , Negative control 2: over expression of α A crystallin (20KD)

c. The anti- HSP60 antibody, showed positive reactions to HSP60 in Western-blot of HLECs at the expected molecular size for HSP60 of approximately 58 KD. When HLECs were exposed to the drugs, the expression of HSP60 protein was variable and results from NIH image (ver.163) are shown in Fig. 8c. These data indicate that the expression of HSP60-protein significantly increased when HLECs (α A crystallin overexpressed) were exposed to 0.1 μ M Dex for 36 h; 1 μ M Dex for 48 h.

d. The anti- Bid antibody, showed positive reactions to Bid in Western-blot of HLECs at the expected molecular size for Bid of approximately 21 KD. When HLECs were exposed to the drugs, the expression of Bid protein was variable and results from NIH image (ver.163) are shown in Fig. 8d. These data indicate that the expression of Bid -protein significantly increased when HLECs were exposed to 1 μ M Dex for 36h and 0.1 μ M Dex for 48 h. However, after exposed to Dex, the expression of Bid in HLECs (α A crystallin overexpressed) have not significantly expression.