DonnishJournals 2041-1180

Donnish Journal of Medicine and Medical Sciences
Vol 2(3) pp. 011-018 April, 2015.
http://www.donnishjournals.org/djmms

Original Research Article

Studies on Gene Expressions at the RNA Level Associated with the Senile Lens Changes in Human Lens Cataract

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Accepted, 18th March, 2015.

Senile lens changes in response to various stimuli are initiated by eye lens proteins coded by genes. In this study, we investigated twelve genes expression at the level of RNA, including those associated with age-dependent changes of lens (Sirt1 and Sirt3 genes), heat shock genes family (HSP70, GRP78, Acry AA genes), inflammatory reaction (TNFα and NF-kβ genes), heat production in mitochondria (UCP1 gene) and oxidative reaction (GSR gene). Those associated with apoptotic and cell senescent stimuli (p53, Caspase3, and p27 genes) were also identified. The genes expression was examined using quantitative Real Time PCR (qRT-PCR) and results were confirmed by conventional RT-PCR. Our results showed that the relative changes in gene expression of Sirt1, p53, HSP70 as well as Caspase 3 were low, Sirt3 and Acry AA were very high, and GSR and GRP78 genes were high, while the other five genes were moderate. All genes were normalized with GAPDH expression. From the twelve gene expression profiles, we concluded the three types of stimuli involve in human lens cataract, oxidation, heat, and inflammation, may contribute to the clouding of the senile lens cataract in Indonesia. We finally hypothesized that not only did the proteins were maintained in the cells of senile lens cataract, but also the mRNAs, especially Sirt3, Acry AA, GSR, and GRP78 mRNAs.

Keywords: senile, lens, senescent, cataract.

INTRODUCTION

One form of the degenerative diseases in the eye is cataract, commonly called senile cataract. Cataract is a clouding of the lens due to the inability of the eye lens protein to maintain its function in facing various stimuli, such as physico-chemical and biological stressors, causing the lens to change from its transparent appearance to a cloudy one. However, the mechanisms involved in the regulation of protein production in the eye lens and its maintenance in whole human life remain poorly understood.

The aging process depends on the age of cells and the ability of cell's renewal. These capabilities are in varying degree according to the types of cells or tissues. Lens of the eye is a unique organ which consists of one type of cells, the epithelial cells, which differentiate into lens fiber cells (Lovicu and McAvoy, 2005).

The lens fiber cells contain proteins that are continuously present in our whole life. Cells that are long lifespan require a well-regulated biological processes in order to function well and to maintain their homeostasis including maintaining mRNA

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level. Until now, there has been a lack of knowledge on the comprehensive mechanisms regulating the gene expression in the human eye lens cataract in Indonesia. There have been many publications on pathogenesis-related cataract lens proteins such as crystalline, glutathione reductase and others, but none related to cataracts in Indonesia was reported.

The proteins encoded by genes can response to several stimuli. Gene expression is an initial response of a cell to stimulation, either from the outside (extracellular) or within the cell (intracellular). External stimulation on the eye lens can come from oxidation, radiation, and heat (Urbak and Vorum, 2010). Infectious diseases that are still quite high in Indonesia are also one of the external factors that can stimulate an inflammatory reaction and induce the related genes expression. Unfortunately, until now there have been no data from Indonesia reported. Besides genetic disorders, another internal stimulation can come from degenerative diseases, including age-related cataract. In this disease, age still remains as the dominant risk factor for the changes which take place in the lens with aging (Truscott, 2000). As has been reported by previous researchers, the human lens is the ideal tool for studying the aging process, because the cells in the central area of the lens still contain fetal cells that remain present since birth (Korlimbinisetal., 2009; Brennan *et al*., 2012). This characteristic allows the lenses to be used as adaptive models to study the function of RNA in cataract (Wu *et al*., 2012).

There is a specific family of gene that plays an important role in the aging process, the Sirtuin genes. In mammals, there are seven types of sirtuin, SIRT1-SIRT7 (Kelly, 2010) located in the various compartments of cells (Finkel *et al*., 2009). Although the sirtuins influence in lengthening the life span, but in some organisms there are still controversies about this function. This is due to the incomplete understanding on the biochemical pathways and regulatory mechanisms of the aging process, in particular about the defense against cellular stress in the lens of the human eyes.

To understand the related pathway, it has also been reported that there is a link between aging and the accumulation of reactive oxygen species (ROS) in mitochondria (Ito *et al*., 2006; Balaban *et al*., 2005; Wallace, 2005). Almost 90% of ROS in the cells derived from mitochondria (Balabanetal, 2005), and this is due to mitochondria being a main target of oxidative processes. In this respect, SIRT3, a protein acetylation, localized to mitochondria regulates the level of ATP, thus influencing the enzymes, which have functions in metabolic activities mediated by calorie restriction (Someya *et al*., 2010; Ahn *et al*., 2008). The biochemical pathway related to this function leads to increased NADPH levels and an increased ratio of reduced glutathione to oxidized glutathione in mitochondria (Someya *et al*., 2010). The expression of Sirt3 can be reduced excessively by the oxidative stress (Brown *et al*., 2013), leading to increase aging process.

If we refer to cell stress, which occurs before the lenses become cataracts, the first defense mechanism initiated by the lens is through heat shock proteins (HSP). The oxidative process in mitochondria produces heat, facilitated by a specific protein located in the inner membrane of mitochondria called uncoupling protein 1 (UCP1). The UCP1 may be associated with heat shock gene family that was triggered by the increase of cellular temperature.

To date, no systematic molecular analysis of essential gene expression associated with several stimuli and no systematic explanation of those stimuli inducing the senile lens changes. The aim of this study is to clarify the profile of twelve genes expression in human lens cataract. Here we report several gene expressions at mRNA level isolated from lens epithelial cells of the eye cataracts obtained from Indonesian patients. We examined the gene expressions of SIRT1, SIRT3, and GSR that in most organs of the human body were decreasing with age (Someya *et al*., 2010; Brown *et al*., 2013; Finkel *et al*., 2009) and p27 whose role in cellular senescence (Garcia-Fernandez *et al*., 2014; Majumder *et al*., 2008), of the lens cataract have not been reported yet. We describe a novel report on the UCP1 gene expression associated with senile lens changes and other genes induced by heat. In addition, we also determined the expression of two genes involved in the apoptotic pathway (p53 and Caspase 3), and two genes involved in inflammatory reaction (TNFα and NF-kβ). All these gene expressions were compared to the housekeeping gene, GAPDH.

MATERIALS AND METHODS

Clinical Samples

Patients were age 40-71 years old (n=9) diagnosed with cataract NC4 senilis up to NC6 (NC=nuclear cataract). All samples were collected after obtaining informed consent from the patients. The patients had received pre-operative treatment relevant to the Ophthalmology procedures. The extracted lenses were collected from the human lens cataracts by means of the small incision cataract surgery (SICS) in RS Cicendo Bandung, Indonesia. The extracted lenses were transferred into sterile tubes containing RNA later solution (Ambion, USA). Total number of samples were 9 lens senile cataracts. This study was approved by the Research Ethics Board of the Faculty of Medicine, Padjadjaran University, Bandung, Indonesia.

RNA Isolation

RNA was extracted from the lens material by the method of Trizol reagent (Invitrogen, USA). Lens material was homogenized in 4M guanidine thiocyanate containing 0.1% Tris-HCl and 0.97% β-mercapto ethanol. The homogenized solution was centrifuged at 14,000 *g* using column tube according to the manufacturer's instructions. RNA pellets were recovered and purified by phenol-chloroform extraction and ethanol precipitation and underwent conventional RT-PCR (Promega, USA).

Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)

qRT-PCR was performed using the 1-step qRT-PCR kit according to the manufacturer's instructions (Kapa Biosystems, USA), using forward and reverse primer for Sirt1, Sirt3, GSR, p27, TNFα, HSP70, GRP78, and AcryAA, p53, caspase 3, UCP1, and NF-kβ (table 1). The qRT-PCR reaction was subjected to reverse transcription (RT) for 5 minutes at 42°C, followed by inactivation of the RT enzyme at 95°C for 3 minutes and the cycle of PCR for 40 cycles. The result can be seen in the form of threshold cycle (Ct curve), by applying the relative changes in targeting genes expression (Livak and Schmittgen, 2001). Ct (threshold cycle) is reported as the PCR cycle number that crosses an arbitrarily placed signal threshold (Schmittgen and Zakrajsek, 2000). The average Ct was calculated for both target gene and internal control (GAPDH) and the ∆Ct was determined. We then categorized the Ct values varying from 15 to 40 (score 1 to 5); 15-20 was very high expression (score 5); **>**20-25 (score 4) was high

Table 1. Primers used for Real Time and RT-PCR

Gene	forward primer (5'-3')	reverse primer (5'-3')
GAPDH	TGTTCCAATATGATTCCACCCAT	AGCCACACCATCCTAGTTGC
HSP70	TCTTGTGTGGGTGTTTTCCA	CACCACCATAAAGGGCCAAT
TNFα	GAGCTGAGAGATAACCAGCT	CGTTTGGGAAGGTTGGATGT
GRP78	GAGGACAAGAAGGAGGACGT	GAACGGCAAGAACTTGATGTC
SIRT1	ATGAGGAGGATAGAGCCTCA	CTTGACCTGAAGTCAGGTATT
SIRT3	GATGTAGCTGAGCTGATTCG	CACTCTCTCAAGCCCATCGA
CryAA	TCGTCATCTTCCTCGATGTG	GAAGCAAAGGAAGACAGACAC
p27	CTGCGCTATTTCCAGGCTGC	AGCTCCTGACTCACTTCAGG
UCP1	GAAGGGCGGATGAAACTCTA	GTGTAGCGAGGTTTGATTCC
NF-kβ	GTTATGTATGTGAAGGCCCAT	TGTCACATGAAGTATACCCAG
p53	TCTGAGTCAGGAAACATTTTC	CTGGGAAGGGACAGAAGATGA
Caspase 3	ATTGTGAGGCGGTTGTAGAA	CATGTATGATCTTTGGTTCCAA
GSR	TGGCTTTCCAAGTTGAGG	CTGCATGGCCACGGATGATT

expression; **>**25-30 was moderate expression (score 3); **>**30-35 was low expression (score 2), and **>**35-40 was very low expression (score 1). All these categories were confirmed with gel electrophoretic data.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

RT-PCR was performed with a one-step system (Promega BioSci., San Luis, CA, USA) using primers described previously (Table 1). RT-PCR mix were subjected to RT for 45 min at 45°C, followed by inactivation of RT-enzyme at 94°C (2 min) and PCR (40 cycles) consisted of 94°C (30 sec), 60°C (1 min), and 68°C (2 min) with a final extension at 68°C (10 min). The products were then run on a 3% agarose gel. Gel doc equipment was used to determine the intensity of bands on agar electrophoresis by using mass ruler as a marker (BioRad).

RESULTS

mRNA expression profiles in the lens cataract

Of the 12 genes identified in lens fiber cells, 4 genes were involved in age senile cataract (Sirt1, Sirt3, p27, GSR), 4 genes were involved in expansion of heat activated gene expression (HSP70, GRP78, CryAA, UCP1), two genes were involved in apoptotic pathway (p53 and Caspase 3), and two genes were involved in inflammatory reaction (TNFα and NF-kβ). In addition, the GAPDH was selected as an internal control gene, the gene was relatively stable to microenvironment changes.

The mRNA expression in the cataract lens was determined by Real-time PCR, which identified 12 genes expressions (table 2) and data evaluation results in cycle of threshold (Ct) values. In figure 1, the variation of gene expressions were devided into 5 scores of Ct value related to the electrophoretic data. In table 2, of the twelve gene expressions, five genes were +∆Ct, varying from 1.10 to 6.10. Those genes were Sirt1, HSP70, TNFα, p53, and caspase3. Seven genes were -∆Ct, varying from -0.19 to -11.03, those genes were Sirt3, GSR, p27, GRP78, CryAA, UCP1, and NF-kβ. These threshold cycle (Ct) values were then further categorized (table 3 and table 4). The higher score value, the higher gene expression level (table 2). Our results showed that the expression level of Sirt1, p53, and Caspase 3 were low, but the expression levels of Sirt3 and CryAA were very high. In particular, the expression level of UCP1 and HSP70 were moderate and low, respectively (Figure 1A).

Heat-associated transcript levels were determined through analysis of UCP1 mRNA using semi-quantitative RT-PCR from 9 human lens fiber cell samples. This analysis identified the measurable expression in lens fiber of heat shock gene family including HSP70, GRP78, and AcryAA (table 3).

The level of TNFα and NF-kβ expressions were moderate and exhibited an elevated level of expression relative to the level of apoptotic pathway including p53 and Caspase 3 ($P<0.001$).

In table 3, of the 4 genes identified in lens cataract, Sirt 1 and Sirt 3were involved in age-dependent cataract and p27 was associated with cellular senescence (Majumder *et al*., 2008; Garcia-Fernandez *et al*., 2014). One additional gene, glutathione synthase reductase (GSR) was also involved. The expression of Sirt1 was lower than that of Sirt3 expression (Figure 1B). In figure 1C, p53 and Caspase3 were in the same level (low expressions or score 2), but qualitatively the Caspase3 was ~30-fold higher than p53 (table3).

HSP70 and the others heat shock genes (GRP78 and Cry AA) were listed in table2, that their expressions were low, high, and very high, respectively. The Cry AA was the same level as the Sirt3 expression (score 5) was shown in figure 1A, whereas in figure 1D the Cry AA and GSR were compared to GAPDH expression.

DISCUSSION

The process of clouding of lens is largely dependent on the integrity of the lens epithelial cells. Human lens epithelial cells

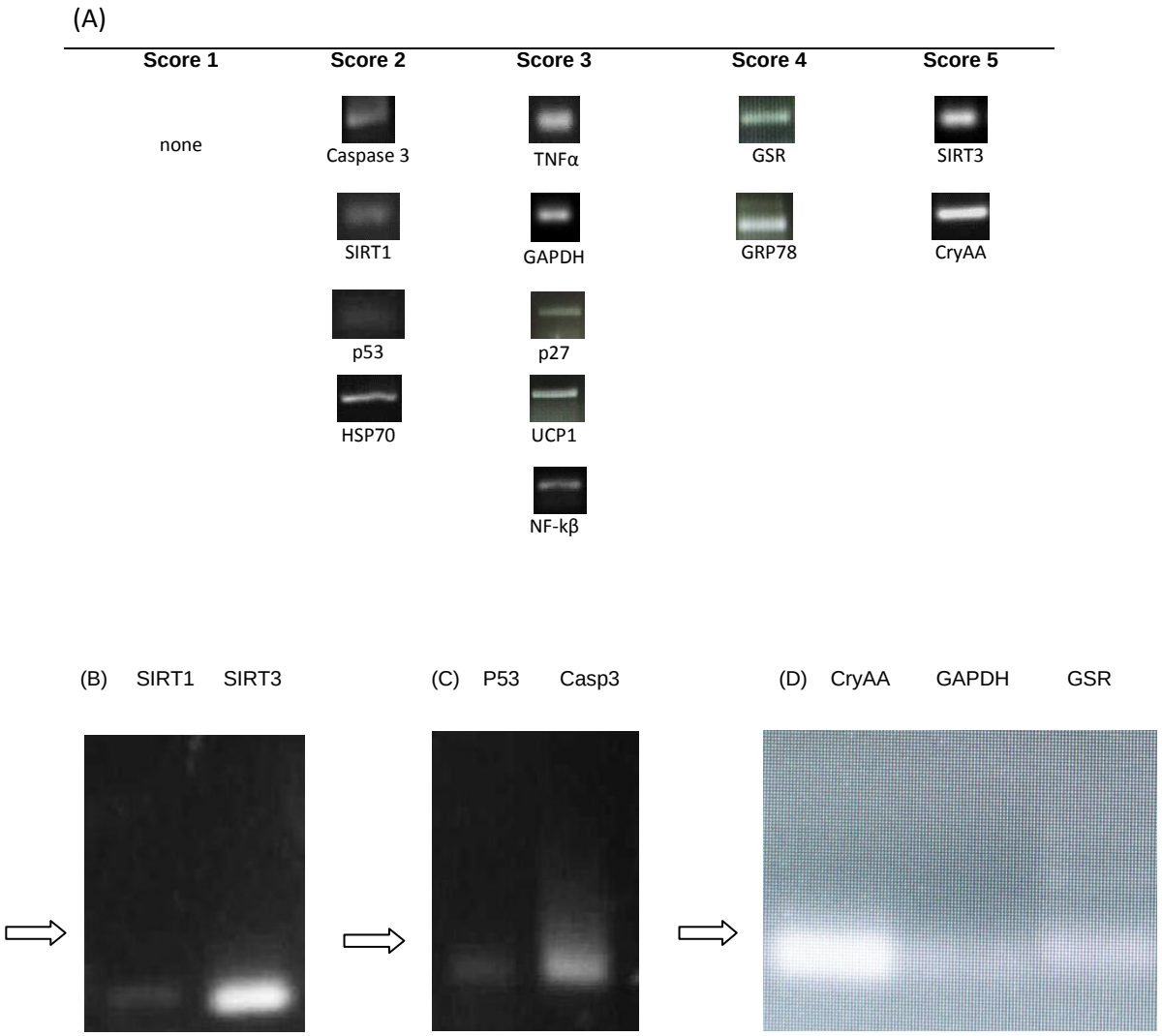

Figure 1. Electrophoretic data of the gene expressions in the cataract lens. (A) no one gene expression was found in score 1. Score 2 showed the three gene expressions with their Ct values varied from >30 to 35. Score 3 showed the six gene expressions with their Ct values from >25 to 30. Score 4 showed the two gene expressions with their Ct values from >20 to 25 and the score 5 was two gene expressions of >15 to 20. (B) Sirt1 was compared to Sirt3 gene expressions. (C) p53was compared to Caspase3 gene expressions. (D) CryAAand GSR were compared to GAPDH gene expressions.

are exposed to many types of stressors or stimuli such as temperature variation, radiation and photo-oxidative stress. In normal condition the temperature of cell is maintained by producing heat in mitochondria facilitated by UCP (Nelson and Michael, 2008). Within the human lens, the variation of genes expression also varies with the value of cycle threshold (Ct) and categories of genes expression (table2). The score of expression was recorded from 1 to 5, from low to very high expression. Relative quantification describes the changes in expression of target genes (twelve genes) relative to GAPDH expression that is as a reference gene (the category was moderate and the score was 3).

Four genes, TNFα, p27, UCP1, and NF-kβ were moderate expressions, categorized in the same group with GAPDH (score 3) (figure 1A). Caspase3, p53, and Sirt1 were expressed lower than GAPDH and the score were 2 (low expression), suggested apoptosis stimuli might be low or weak. Sirt1, a gene coding for Sirt1 protein that play a role in aging was also low expression, suggested the stimuli-inducing Sirt1 gene was not able to increase its expression. According to previous publication by Yang, Sirt1 is sensitive to the states of redox and cellular metabolism (Yang *et al*., 2007). The expression of Sirt1 is known to be controlled at transcriptional and post-transcriptional levels.

Based on our results, the expression of Sirt1 was low and its low expression might be related to the expression of NF-kβ (moderate expression) (Salminen *et al.,* 2013). In addition, we reported Sirt3 was very high expression (score 5), the same trend as AcryAA expression, indicating that these two genes were stimulated strongly at the transcriptional level in senile

cataract. In figure 1B, the comparison of RT-PCR data between Sirt1 and Sirt3.In several tissue Sirt3 is suppressed with aging (Brown *et al*., 2013), but in our result showed the expression of Sirt3 was very high in cataractous lens (~ thousands-fold increase compared to GAPDH), the same trend as CryAA expression. In lens cataract suggested both Sirt3 and AcryAA might be stimulated as a compensated mechanism to improve their regenerative capacity in senile cataract.

GSR is a gene coding for GSR enzyme.Catalyzing GSR to GSH whose expression in this study was high, indicating the oxidative process in lens cataract was also high.

Another response was shown by family of heat shock genes, including HSP70, GRP78 and AcryAA. mRNA level of these three genes were different categories from one to another. We can see that HSP70 with low category of expression while GRP78 was high and the AcryAA was very high. With high GRP78 expression, we speculated in lens senile cataract there was increasing endoplasmic reticulum stress.

Expression of TNFα and NF-kβ, were moderate expressions (score 3), with a similar level as GAPDH. In Indonesia at which the incidence of infectious disease are still high, the moderate expression of these two genes might indicate a cellular response of those genes to a moderately prolonged inflammatory reaction.

In table 3, we analyzed the relative gene expressions using the $2^{-\Delta\Delta Ct}$ method (Livak and Shcimttgen, 2001). Of the five genes (TNFα, HSP70, Caspase3, Sirt1 and p53), the status of two genes (TNFα and HSP70) were ~2-2.5 fold lower than the GAPDH and the status of three genes expression, caspase3 and sirt1 were relatively low compared to GAPDH expression, and the p53 was the lowest expression (~100 times lower than GAPDH) suggesting the three genes were suppressed stronger than those of TNFα and HSP70 genes. In figure 1B, Sirt1 was lower than Sirt3 and in figure 1C the caspase3 and p53 were low expressions. The consequences of this suppression, could show that the apoptotic pathway might be also weak.

Further analysis, we calculated the $2^{-\Delta\Delta Ct}$ of seven gene expressions, the state of p27 that was no relative change of gene expression (by one fold), suggested p27 expression that maintain cell senescent was relevant with GAPDH activity. It was a surprise that the relative changes of six gene expressions (UCP1, NF-kβ, GSR, GRP78, Sirt3, AcryAA), UCP1 showed relative high expression by more than 10 fold (table3). We suggested that in lens cataract, the cells were stimulated by stressors or stimulators inducing UCP1 gene expression producing heat. With the same perspective, NF-kβ that was in the moderate category showedthe relative high expression by more than 10 fold GAPDH, suggesting inflammatory stimuli occurred in lens cataract inducing NF-kβ gene expression. If we looked at another gene that play a role in the oxidative process (GSR gene) of the eye lens, we can see that this gene was high expression by more than 16 fold GAPDH, suggested that the eye lens was in oxidative stress. Another interesting finding of Sirt3 and AcryAA showed very high gene expressions by more than 1000 fold, or even by more than 2000 fold GAPDH.

Figure 1B showed expression of Sirt3 compared to Sirt1 and 1D showed expression of AcryAA compared to GAPDH and GSR, respectively. How this mRNA are present at a much higher level than those of other genes still remain for further observation. From all these findings, we speculated two major stressors that were accumulated in lens cataract. The first, was a very high stimulation of oxidative stress that resulted in the increase of Sirt3 and AcryAA gene expressions could prolong our life. The second might be the gradated process of mRNA were very slow in lens cell. All these processes can be stressors for mitochondria and endoplasmic reticulum, leading to induce the very high Sirt3 and high GRP78 expressions, respectively.

In this study, the senile lens cataract showed different expression of sirtuin genes, Sirt1 was low expression, but Sirt3 was very high expression (figure 1B). Thus, the profile of the two gene expressions in eye lens were different from those genes in another organs, at whichSirt3 is a primary mitochondrial fidelity gene that lost its activity with aging (Schwer *et al*., 2009; Park *et al*., 2011). In response to aging, p27 gene expression also acts as a maintenance of cell senescent, and the cell avoid from apoptosis by decreasing the level of caspase 3 expression (Ct value is low) (table2).

Sirt1 can stimulate the expression of antioxidants via the Fox O pathways which is also involved in combating oxidative stress (Salminen *et al*., 2013). But in our data, its expression was low, indicating ROS may affect mRNA production of Sirt1. Sirt1 and Sirt3 are enzymes that have deacetylation activity, but with different localization in the cell (Ahn *et al*., 2008). Sirt1 is abundant in the cell nucleus, while Sirt3 is abundant in mitochondria. Sirt3 is active in mitochondria, suggesting that the activity of Sirt3 have a relationship with the free radical theory of aging events (Park *et al*., 2011). It is well known that mitochondria are organelles where ATPs are produced in cells and this energy formation is also formed as a byproduct of ROS which is produced from an incomplete reduction of dioxygen. Heat that results from ROS can cause "oxidative stress". In our data Sirt3 was very high expression, while caspase3 was low expression, suggested in eye lens cataract the apoptosis pathway may be very weak or not active.

This can be understood, as the release of mitochondrial Sirt3 protects BAX into mitochondria thus preventing apoptosis (Kincaid and Bossy-Wetzel, 2013), leading to cellular senescence that is supported by p27 expression (moderately expression). In this report, the Sirt3 gene expression is induced strongly that may protect the lens from damage. The Sirt3 expression is in relevance with the expression of GSR, GRP78, and AcryAA gene expression which support the lens protection.

In table 2, the UCP1 gene was moderately expressed (Ct value >25–30) suggested that heat was actively produced in mitochondria. As we know UCP1 protein requirement associated with heat production in mitochondria. This protein is one of the molecular targets of lens Sirt3 (Kelly, 2010), whose category was a moderate expression (Ct>25-30). Although this mechanism is still not well understood, it might be related to H^+ transport through UCP1 in the inner mitochondria membrane that produces heat. In normal condition, lens temperature is lower than whole body (Truscott, 2001).

We speculated the warmer temperature in the lens that accumulate throughout life, contributes to the development of senile lens cataract. Here, HSP70 was low expression in cataractous lens, its protective ability to maintain protein folding was also decreased, impacting to lens dysfunction. This mRNA expression level might be induced by daily temperature variation. We suggested that prolong repeat increase of intraocular temperature more than normal, contributes to the stress-related genes inducing transcription of heat shock genes family, which also leads to eye lens clouding. Strong sun shines in the tropical country like Indonesia, is photo-oxidative, one of the external factors that can trigger thermal-induced heat shock gene expression.

Table 2. Categorization of genes expression at the mRNA level based on the threshold cycle (Ct) by using qRT-PCR.

Targeting genes	genes Ct	categories of expression	score of expression
TNFα	29.69 ± 1.34	Moderate	3
HSP70	30.06 ± 4.45	Low	2
caspase3	30.32 ± 2.37	Low	2
SIRT1	31.07 ± 3.55	Low	2
p53	34.69 ± 2.30	Low	2
GAPDH	28.59 ± 1.90	Moderate	3
p27	28.39 ± 1.68	Moderate	3
UCP1	25.81 ± 2.35	Moderate	3
NF-kβ	25.19 ± 1.88	Moderate	3
GSR	24.52 ± 1.06	High	4
GRP78	21.93 ± 1.40	High	4
SIRT3	18.54 ± 0.68	very high	5
CryAA	17.55 ± 0.66	very high	5

Table 3. The order of twelve targeting genes was placed in the order from lowest to highest based on their Ct values compared to GAPDH. The ΔCt value that was higher than GAPDH were placed in a group of positive ΔCt (+ΔCt) and that which is lower than GAPDH were placed in a group of negative ΔCt (-ΔCt). -ΔCt suggested a higher expression than GAPDH and +ΔCt suggested a lower expression than GAPDH. Calculation of data was adapted from Livak and Schimttgen (2001).ΔΔCt = ΔCt (target gene) - ΔCt (GAPDH).

Targeting genes	GAPDH Ct	genes Ct	ΔCt		ΔΔCt	Normalized target gene relative to GAPDH
			(avg. target gene Ct - avg. GAPDH Ct)			$2^{-\Delta\Delta Ct}$
(n=9)	(n=9)	(n=9)	+ΔCt	-ΔCt		
TNFα		29.69 ± 1.34	1.10 ± 0.56		1.10	0.47
HSP70		30.00 ± 4.45	1.41 ± 2.55		1.41	0.38
caspase3		30.32 ± 2.37	1.74 ± 0.47		1.74	0.30
SIRT1		31.07 ± 3.55	2.48 ± 1.65		2.48	0.18
p53		34.69 ± 2.30	6.10 ± 0.4		6.10	0.01
GAPDH	28.59 ± 1.90	28.59 ± 1.90	0.00		0.00	1.00
p27		28.39 ± 1.68		-0.19 ± 0.22	-0.19	1.14
UCP1		25.81 ± 2.35		-2.78 ± 0.45	-2.78	6.87
NF-kβ		25.19 ± 1.88		-3.40 ± 0.02	-3.40	10.56
GSR		24.52 ± 1.06		-4.06 ± 0.84	-4.06	16.68
GRP78		21.93 ± 1.40		-6.66 ± 0.5	-6.66	101.13
SIRT3		18.54 ± 0.68		-10.05 ± 1.25	-10.05	1136.20
CryAA		17.55 ± 0.66		-11.03 ± 1.24	-11.03	2091.03

Nevertheless, the increasing of lens temperature is not a single factor that causes cataract, since there are multiple pathological conditions likely to be responsible for the clouding of the lens. Human lens opacity results from a combination of factors, including cellular damage (Brennan *et al*., 2012).

The cryAA that belongs to the heat shock gene family is characterized by a strong induction at high temperature (Horwitz, 1992; Jakob *et al*., 1993; van Monfort *et al*., 2002; Kim *et al*.,1998). There are also considerable differences in the tissue localization and expression levels of crystallin AA (Basha *et al*., 2011). The temperature-dependent chaperone activity, including αA-Crystalline markedly improves with an increase in temperature (Reddy *et al*., 2000). We speculated that this action through inducing of CryAA gene expression.

The specific function of crystalline AA may play role in maintaining lens transparency. Although we do not know the exact mechanisms, we suggested that lens cell has the ability to maintain the mRNA in the quiescent state. These RNAs were maintained for supporting the protein synthesis or for gene expression mechanism regulated by micro RNA (Wu *et al*., 2012).

It was previously reported that HSP70 can inhibit apoptosis by binding to Apaf1 forming an apoptosometospur Caspase3 (Beere *et al*., 2000). In this study the gene expression of Caspase3 was not strong that means there is not enough activation of apoptotic pathways. This finding supported the data showed by Weber and Menko (2005). In this perspective, we hypothesized that in maintaining lens cell survival is required the cellular network analysis, including cell differentiation (the changing of cuboidal lens cells into the fiber lens cells); cellular senescence and cell death signals that all of them are involved in cellular networks of lens homeostasis.

Other evidence showed that the oxidative gene expression GSR is strong enough to reduce oxidative substances. All of these events will have an impact on CryAA protein, a protein encoded by the gene CryAA. This means the strong expression of CryAA may be due to oxidative processes that can undergo denaturation of this protein solubility. The CryAA gene expression that is very strong might be as a cellular compensation to maintain protein synthesis of the lens.

It appears that strong Sirt3 expression is a protective mechanism against damage to the lens. Some reports have suggested when a cell undergoes mild stress, the synthesis of protein in this cell will be inhibited, but later end up with recovery (Knowlton, 2007). Instead, consistent severe stress will lead towards apoptosis. Cataracts a real occur preceded by endoplasmic reticulum (ER) stress (Mulhern *et al*., 2006; Teodore-Morrison *et al*., 2013) in the lens, which was indicated by increasing of GRP78 gene (Bip) expression. With high expression of GRP78, there would be a small possibility for cells to undergo apoptosis because the protein folding can be maintained in physiological state. In this study, we showed that the eye lens functions is maintained by GRP78, CryAA, the enzyme GSR and Sirt3.

Figure 1D showed the expression of CryAA and GSR compared to GAPDH genes. In addition, the decrease of Sirt1 activity enhances NF-kβ expression (Salminen *et al*., 2013), as shown in table 2 in which the expression of NF-kβ was moderately high. We suggested a slow and moderate intensity of inflammatory reaction may be one of the stressors that accumulate for years induce the formation of lens opacity in Indonesia.

Further investigation of biochemical pathway and cell signaling of the eye lens is required to understand the mechanism of cataract.

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