Expression of heat shock protein 27 in *in vitro* cultured human lens epithelial cells***☆

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Abstract

BACKGROUND: Special anatomical location makes eye lens expose to stressful situation in a long term. Whether the environmental stress can up-regulate the expression of heat shock proteins in human lens epithelial cells? Whether the synthesis increase occurs in the level of transcription or translation, remains unclear.

OBJECTIVE: To observe the expression and location of heat shock protein 27 (HSP27) in human lens epithelial cells under the conditions of high temperature and oxidative stress, and to investigate the pathogenesis of the cataract.

METHODS: Human lens epithelial cells cultured *in vitro* were exposed to heat (45 ℃) and oxidative stress (50 mmol/L H_2O_2) for 30 minutes, respectively, then allowed to recover normal conditions. At different intervals (0, 2, 4, 6, 16, 24 hours), immunocytochemistry and reverse transcription polymerase chain reaction were used to determine the expression and localization of HSP27.

RESULTS AND CONCLUSION: HSP27 was shown to express in both physiological and stressful conditions. The expressions of HSP27 mRNA and protein were remarkably increased at 2 hours following heat and oxidative stress, and reached the peak at 6 hours. HSP27 could maintain a high level for 16 hours. The stress-induced HSP27 protein positive particles transferred from the cytoplasm to the nucleus, and gradually shift back to the cytoplasm along time. It is proved that HSP27 exists in lens epithelial cells and can be increased after stress. The data suggested it may play an important protective role in lens epithelial cells in respond to cellular stress.

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INTRODUCTION

The optimal vision is highly dependent on the clarity of the ocular lens. It has been generally accepted that the lens transparency is maintained by the supramolecular structure of its constituent proteins. Preservation of this structure is maintained by the fidelity of native proteins. Quarternary protein structure depends on proper folding of newly synthesize polypeptides, and adequate protection from environmental or pathophysiological stress[1-2]. Destabilization of native protein organization induces opacity of the lens. Thus a detailed understanding of how lens proteins retain their native structure for sustained period of time is essential.

The majority of animal and plant cells respond to stress by overexpressing heat shock proteins[3-6]. Therefore, in this study, we examined the experssions of heat shock protein 27 (HSP27) at the transcription or translation levels, in the human lens epithelial cells cultured *in vitro* with response to exogenous stress applied to the lens. The experiments showed that environmental stress could induce overexpression of HSP27 in the human lens epithelial cells, which probably plays a role on protection to the lens.

MATERIALS AND METHODS

Design: An observation of single sample.

Time and setting: The experiment was carried out in the Key Laboratory of Congenital Malformation, Ministry of Pubic Health, China Medical University between September 2003 and September 2004.

Materials

Human lens epithelial cells B3 were obtained from the Department of Ophthalmology in the First Hospital Affiliated to China Medical University. Confluent human lens epithelial cells were grown in DMEM, supplemented with 15% fetal bovine serum, in a humidified incubator at 37 ℃.

Methods

Experimental grouping

Heat group: The human lens epithelial cells were heated in thermostatic waterbath box for 30 minutes at 45 ℃.

Oxidative stress group: The human lens epithelial cells were exposed for 30 minutes in DMEM containing 50 mmol/L H_2O_2, then recovered to normal conditions for further culture. Some stress cells were allowed to recuperate after exposure to stress in normal culture medium at 37 ℃ for 0, 2, 4, 6, 16 and 24 hours. Immunocytochemistry and reverse transcription polymerase chain reaction (RT-PCR) were used to determine the level and localization of HSP27.

Immunocytochemistry

The cells on the glass coverslips were rinsed with 0.02 mol/L phosphate buffered saline 3 times, then air dried at room temperature, fixed with propylaldehyde for 30 minutes, rinsed once with phosphate buffered saline, and placed in a −20 ℃freezer for the preservation. The cells were dyed by the immunohistochemistry reagent box (Boster company). The slide was flushed with the distilled water, and added 3% hydrogen peroxide for 10 minutes to eliminate the endogenous peroxide enzyme. The antigen repairing fluid Ⅰ was added for 5−7 minutes. The normal goat blood serum was used

to seal up for 20 minutes. Then incubated with concentrated rabbit anti-human HSP27 antibody for 1 hour at 37 ℃, then colored for 20 minutes with SABC, re-dying with the haematoxylin, dehydration and mounting, then observed under the microscope. The yellowish brown particles in the cells were positive. The image gray values were scanned and analyzed by the MetaMorph/DP10/BX50 micro image analyzer.

RT-PCR

Total RNA was isolated from freshly prepared cultured human lens epithelial cells by the guanidinium thiocyanate-phenol-chloroform extraction method. The A_{260}/A_{280} was measured using ultraviolet spectrophotometer. The RNA concentration was calculated according to A_{260}, and then diluted to 1 g/L. After confirming the structural integrity of the total RNA samples by electrophoresis on 1% agarose gels, 1 μg of RNA was reverse transcribed using oligo(dT) as a primer in the presence or absence of reverse transcriptase according to the manufacture's instruction. PCR was then performed in the total volume of 50 μL using cDNA as a template in the presence of primer pairs as described.
The primer sequence of HSP27 (216 bp) are as follows:
F 5'-AAG GGT CGC AGC AGT TGT-3',
R 5'-CGT CTT GTG CAG GTG GTT A-3'.
The primer sequence of β-actin (690 bp) are as follows:
F 5'-GAT TGC CTC AGG ACA TTT CTG-3',
R 5'-GAT TGC TCA GGA CAT TTC TG-3'.
10×reaction buffer and Taq polymerase were obtained from Eppendorf.
PCR conditions were used: cDNA was melted at 94 ℃ for 10 minutes to denature the DNA, and the reaction was then run through 30 cycles at 94 ℃ for 45 seconds, 52.5 ℃ for 1 minute, and 72 ℃ for 1 mibnute as a thermocucler for the progradation. After the last cycle, the polymerization was extended at 72 ℃ for 7 minutes to complete all stands. PCR performed on each sample of RNA that had not been reverse transcribed to cDNA was used as a negative control and showed no amplified product. PCR amplification products 6 uL were separated by 2% agarose gel electrophoresis, and stained with ethidium bromide for visualization with an imager workstation. Kodak 1D digital science gel electrophoresis gel image analysis software was applied to analyze the results of gel electrophoresis, and to calculate the relative content of target gene mRNA, repeated four times.

Main outcome measures

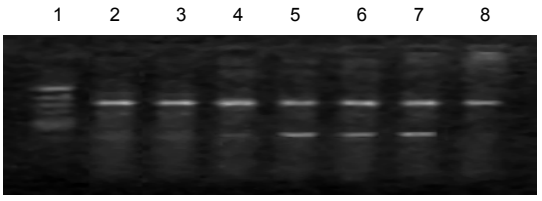
The mean gray value of the HSP27 expression in human lens epithelial cells.

Statistical analysis

The data were calculated to Mean±SD and analyzed by one-way ANOVA using SPSS 10.0 software by the second author.

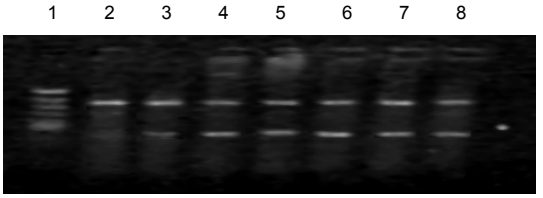
RESULTS

HSP27 were shown to express in the human lens epithelial cells cultured *in vitro* at the transcription and translation levels. The levels of HSP27 mRNA were increased 2 hours after heat shock and oxidative stress, reached the peak at 6 hours. Subsequently they were decreased gradually in each group, but they all could maintain a high level at 16 hours (Figures 1, 2).



1: standard molecular weight; 2: pro-treatment group; 3–8: after exposure to stress in normal culture medium at 37 ℃ for 0, 2, 4, 6, 16, 24 hours (β-action: 612 bp; heat shock protein 27: 216 bp)

Figure 1 The expression of heat shock protein 27 mRNA at each time in heat shock group



1: standard molecular weight; 2: pro-treatment group; 3–8: after exposure to stress in normal culture medium at 37 ℃ for 0, 2, 4, 6, 16, 24 hours (β-action: 612 bp; heat shock protein 27: 216 bp)

Figure 2 The expression of heat shock protein 27 mRNA at each time in oxidative stress group

The expressions of HSP27 protein were increased after both heat shock and oxidative stresses. And the variations of the protein at each interval were basically as same as that of transcriptional level (the mean gray values of each group are seen in Table 1). The stress-induced HSP27 protein positive particles transferred from the cytoplasm to the nucleus, and coming back to the cytoplasm gradually along with the time.

Table 1 The mean gray value of heat shock protein 27 by the immunocytochemistry in each group ($\bar{x}$±s)

Time	Heat shock group	Oxidative group
Pre-stress	146.70±1.84	146.83±1.39
0 h	146.43±2.39	146.86±2.06
2 h	148.39±0.98[a]	148.65±1.41[a]
4 h	149.79±0.66[a]	150.00±0.60[a]
6 h	150.79±1.01[a]	150.29±0.67[a]
16 h	150.17±1.05[a]	149.21±1.03[a]
24 h	150.15±1.02[a]	147.47±1.87

[a]P < 0.05, *vs*. pre-stress

DISCUSSION

The complex pathogenesis of the cataract is poorly understood, and there is no efficient therapy or prevention to date without operation. As evidenced in human eyes, lens epithelial cells in cataract may be lost by apoptosis[4]. The ability of various cellular stress factors that may be implicated in pathogenesis of

cataract, including hydrogen peroxide, blue light, and so on to induce apoptosis has been demonstrated *in vitro*. Many stress stimuli that are capable of triggering apoptosis such as heat shock or oxidative stress, induce the synthesis of different heat shock proteins that confer a protective effect against a wide rang of cellular stress[7-9]. Recent results indicate that HSP also influences the cascade of apoptosis[10]. HSP is originally identified on the basis of their increased synthesis after cellular exposure to elevated temperature and are ubiquitously expressed in various tissues[11-12]. They are roughly grouped into several classes according to their size and sequence homologies. Most of them exert strong cytoprotective effects and behave as molecular chaperones for other proteins[13-14]. But there was no evidence that environmental stress induced them increasing in human lens epithelial cells. And whether the increased synthesis is regulated at the transcriptional level or at the translational level is not known.

A rapid increase in the synthesis of HSP, HSP27 is a common cellular response to environmental stress[15]. To find out whether HSP functions as a stress-inducible protein or marker for cellular stress in human lens epithelial cells, our experiments were performed *in vitro* on human lens epithelial cells. The cells were subjected to elevated temperature, the classic stimulus for induction of HSP. Next, we sought to determine whether a stimulus implicated in the pathogenesis of cataract leads to an increased expression of HSP. In attempt to reproduce such pathologic conditions, we chose to study the expression of HSP after human lens epithelial cells injury with a oxidizing stimulus. The results showed that such a response was also found in human lens epithelial cells exposed to the both stresses. The response was time dependent, since no elevation of HSP mRNA was found in cells at the end of 30 minutes at 45 ℃. It was, however, readily apparent at 2 hours after the stress. But the expressions of HSP27 got to the summit at different intervals from 2 hours to 6 hours in each group. Subsequently they were decreased gradually in each group, but they all can maintain a high level at 16 hours.

A detailed knowledge of how proteins retain their native structure is essential for lens transparency. Protein denaturation induced the synthesis of HSP. HSP are considered to be molecular chaperones that bind to unfolded and denatured proteins, promoting their re-folding and correct assembly[16-18]. We studied the effect of environmental stress on mRNA levels and protein levels of HSP27. HSP27 encodes the 27 KDa HSP family or the small HSP, which function as molecular chaperones in thermotolerance. Furthermore, HSP27 expression is related to cell growth and differentiation[15, 19-21]. The exogenous stress-induced expression of HSP27 indicated that HSP27 involved in the anti-stress injury of the human lens epithelial cells. We believed that the over-expressed HSP may delay the development of cataract and provide a new idea to clinical therapy.

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体外培养人晶状体上皮细胞 HSP27 的表达***☆

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摘要

背景：晶状体独特的解剖位置使其长期暴露于应激环境中。但环境应激能否引起人晶状体上皮细胞中热休克蛋白表达增加？并且这种合成的增加是发生在转录水平，还是在翻译水平，至今尚不知。

目的：观察在高温、氧化应激条件下，HSP27 在人晶状体上皮细胞中的表达和定位情况。探讨白内障的发病机制。

方法：体外培养人晶状体上皮细胞，分别在高温(45 ℃)、氧化(50 mmol/L H_2O_2)条件下培养 30 min 后，恢复至正常条件。于 0，2，4，6，16，24 h 不同时间段，采用免疫细胞化学、RT-PCR 法检测 HSP27 的表达情况。

结果与结论：晶状体上皮细胞在生理和应激情况下均有 HSP27 的表达。热休克和氧化应激后 2 h 导致 HSP27mRNA 和蛋白表达明显增加，6 h 达最高峰，16 h 仍维持在较高水平。应激导致的 HSP27 蛋白阳性颗粒由胞浆转移至胞核，并随着时间逐渐转移回胞浆。证实晶状体上皮细胞中存在 HSP27。应激情况下，诱导 HSP27 合成增加，其作为一种对抗应激的蛋白质可能对晶状体上皮细胞起着重要的保护作用。

关键词：应激；热休克蛋白 27；晶状体上皮细胞(LEC-B3)；眼组织工程；体外培养

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