·Basic Research·

Effect of heat shock protein-70 on lens epithelial cells in human diabetic cataract

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Abstract

• AIM: To study the effect of heat shock protein-70(HSP-70) on lens epithelial cells (LECs) in human diabetic cataract .

• METHODS: The expression of HSP-70 was assayed by using immunohistochemistry (streptavidin-alkaline phosph- atase, S-P) in human diabetic cataract LECs (23 cases) and human normal LECs (7 cases).

• RESULTS: The expression of HSP-70 (χ^2= 24.67, P< 0.01) was significant in diabetic human LECs (23 cases) but there was no expression of HSP-70 in normal human LECs (7 cases).

• CONCLUSION: HSP-70 may play a critical role in the development and formation of human diabetic cataract.

• KEYWORDS: heat shock protein/physiopathology; immunohistochemistry; human diabetic cataract/physiopathology

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INTRODUCTION

Up to now, the exact cyto-biological mechanism of human diabetic cataract remains dubious. To investigate the role of heat shock protein-70 (HSP-70) in the development and formation of human diabetic cataract, human diabetic cataract lens epithelial cells (LECs) were examined using immunohistochemical method and the results are reported as follows.

MATERIALS AND METHODS

Subjects Twenty-three patients with diabetic cataract hospitalized in the First Affiliated Hospital of Fujian Medical University, and operated on through small ultra-emulsive incision, were collected at random. These cases were averaged 38, ranging from 25 to 47 years in age. Among them, there were 12 males and 11 females. They were exclusively diabetic cataract patients. They were divided into several stages. Healon was injected into the anterior chamber during the operation, and the lens anterior capsular membrane was denudated continuously and circularly. The isolated membrane was placed in a 100mL/L neutral formalin solution right away, fixed for 48 hours and then embedded in paraffin. Seven accidentally injured cases served as the control group, whose lenses remained normal and transparent. The lens anterior capsular membrane was denudated and placed in a 100mL/L neutral formatin solution right away, fixed for 48 hours and then embedded in paraffin.

Methods Paraffin sections (3-5μm) were made out of the paraffin embedded tissues. Antihuman-monoclonal antibody HSP-70 and SP reagent were purchased from Fuzhou Maixin Company. HSP-70 expression in LECs was detected by SP 2 immunohistochemistry (following the operating instructions on the reagent box). PBS buffer solution took place of anti-HSP-70 as the negative control while the positive control was purchased from Fuzhou Maixin Company.

Statistical Analysis The substance that was tested positive by immunohistochemical staining was brownish-yellow particles. This HSP-70 positive substance was located inside the cell nucleus and cytoplasm. Fifty LECs were selected at random and examined with a slit-lamp microscope. A percentage was adopted to indicate the ratio of positive cells in the total lens cells and thus the degree of positive expression was calculated. The differences ranged from zero to three points, judged by the density of cell photographic reaction and the number of positive cells. The density was subdivided according to cell photographic reaction to the staining: unstained, 0 point; flavescent, 1 point; brownish, 2 points; dark- brown, 3 points, and according to the number of positive cells, less than 5%, 0 point; between 6%-25%, 1 point; between 26%-50%, 2 points; more than 50%, 3 points. The integrals of the two indexes were divided into 4 grades: negative (-), 0-1 point; weak positive (+), 2-3 points; positive

(++), 4-5 points; strong positive (+++), 6 points or more.

RESULTS

Even distribution of brownish-yellow particles were detected inside the diabetic cataract LECs nuclei and cytoplasm of the 23 cases. HSP-70 exhibited positive (Figure 1). No HSP-70 was expressed in the control LECs of the 7 cases. The two groups differed from each other significantly (χ^2=24.67, P< 0.01, Table 1).

DISCUSSION

The anterior-posterior subcapsular opacity marks an early pathological change in human diabetic cataract, and the LECs density seems significantly lower than that of other types of cataract[1]. It is known that cell apoptosis is a cellular basis for the development of cataract, and the abnormal reproduction, migration and trans-differentiation of the LECs are the pathological basis for sub-capsular cataract [2]. Some recent studies have indicated that the lens can exhibit a series of heat shock proteins (HSP). HSP plays a critical role in maintaining lens stability and opacity [3]. Its structure and abnormal expression are closely correlated with the diabetic cataract development and formation.

HSP was discovered by Ritossa, a geneticist, in 1962 when he was studying Drosophila Melannogaster (fruit fly) salivary gland. Under various stress reactions (physical, chemical as well as microorganisms), organisms are able to activate a special series of genes-heat shock genes-to produce a special type of proteins with conservative structure-Heat Shock Protein [4]. This special protein can strengthen the protection of its own cells from being injured by the stress factors [5]. According to the molecular weight, heat shock proteins are divided into two groups: the common lighter HSP sized about 15-30ku (including HSP-25 in lens and α-lens proteins) and the heavier HSP which is subdivided into 3 families (judged by the gene codes): HSP-110, HSP-90 and HSP-70. Chinese scholar Bu, was the first to find the mutant DNA-blinding domain of HSP-4 (a gene of coded HSP transcript factor) in congenital cataract. This gene regulates HSP of many types. Once mutation occurs to this gene, reproduction of HSP is reduced greatly or disappears totally, and thus triggers lens opacity and further the development of congenial cataract.

It is known that in most organisms, HSP-70 is one of the HSP families that exists in the maximum amount. HSP functions in many ways. It is involved in the control of cell reproduction and plays a critical role in growth, maturity and differentiation of the organism. Through immune mechanism, HSP-4 regulates enzymatic activity, influences

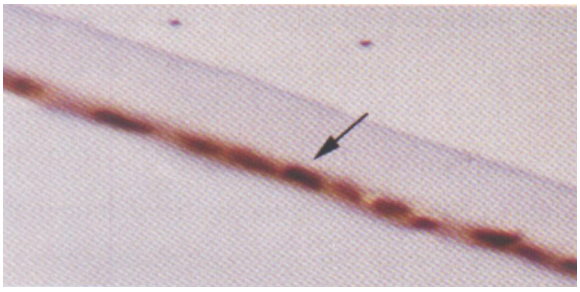

Figure 1 The positive expression of HSP -70 in human diabetic cataract LECs(SP×400)

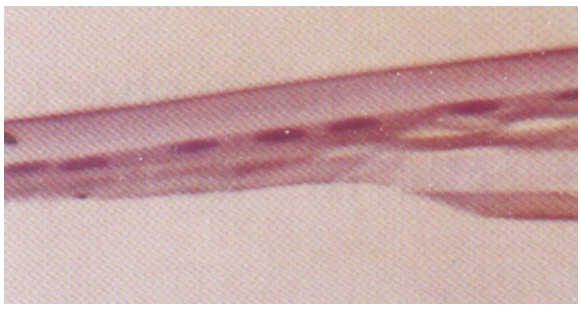

Figure 2 The negtive expression of HSP -70 in normal human LECs (SP×400)

Table 1 The expression of HSP-70 in different LECs groups

Groups	Expression of HSP-70				Total cases
	-	+	++	+++	
Normal LECs	7	0	0	0	7
Diabetic cataract LECs[b]	0	0	4	19	23

χ^2=24.67, [b]P<0.01 *vs* normal LECs

apoptosis-linked gene expression and thus takes part in cellular apoptosis at different levels and in different ways. It serves as molecular co-partner, helping the injured proteins (due to various stress reactions such as high heat, oxidation, high osmosis, etc.) to refold themselves, retain their configurations to function regularly and thus raising the survival rate of the cells. It was confirmed by Samali's experiments that with the same stimuli to induce cellular apoptosis, only a small number of HSP cultured cells died, but on the contrary, a large number of cultured cells in control died. All these suggest that HSP can inhibit cellular apoptosis.

In recent years, studies of lens HSP-70 began to attract researchers attention. Bagchi *et al* [6,7] discovered that HSP-70 expression increased in cultured mouse lens epithelial cells *in vitro* when the cells were stimulated by oxidation, high osmosis, etc. In our studies, diabetic cataract was induced with streptozotocin (STZ). No HSP-70 expression was detected in the control LECs while its expression was obvious in the diabetic cataract LECs. The expression increased with advancement of diabetic cataract. The

outcome is in agreement with what Bagchi found and is relevant to HSP-70 expression in human diabetic cataract LECs. Injury factors and stress reactions such as lens metabolic disorder, high osmotic pressure, can trigger the development of LEC abnormal apoptosis and reproduction, which further lead to cataract advancement. Meanwhile, stress reaction can increase HSP-70 expression in LECs. It was discovered in our study that no HSP-70 was expressed in normal human LECs whereas HSP-70 was expressed obviously in human diabetic cataract LECs, and that most HSP-70 gathered in the cellular nucleus and cytoplasm, and that HSP-70 expression increased with the lens opacity worsening.

HSP-70 expression increases in human diabetic cataract LECs under stress reaction such as high osmosis and high oxidation. Apart from accelerating LECs reproduction, it plays a role as molecular co-partner, helping to remove the denatured proteins, to restore cytoskeleton and to prevent oxygen free radical (produced due to stress reactions) from damaging the mitochondrial membrane. In doing so, HSP-70 in turn strengthens protection of itself. Meanwhile the highly exhibited HSP-70 acts in some way on the lens, interacting with chromatin in a special way to control cellular growth, reproduction, apoptosis, differentiation,etc. These stress reactions lead to the abnormal apoptosis of the LECs so that it can't maintain its rigidly-ordered three-dimensional image, causing great LECs reproduction, migration, differentiation and fibrosis. All these further lead to lens sub-capsular opacities and subsequent formation of diabetic cataract.

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