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Dexamethasone impact on vimentin in human lens epithelial cells

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ABSTRACT

Purpose. To study the expression of vimentin on the impact of dexamethasone (Dex) in human lens epithelial cells (HLECs), and the role of vimentin expression in steroid-induced cataract (GIC) formation.

Material and methods. HLECs were exposed to DMEM and DMEM + 1umol/L Dex for 7 days. Real-time PCR and Western-blot were applied to identify the expression of vimentin at protein and transcription levels. The distribution and expression of vimentin in HLECs were measured by immumoflorescence staining.

Results. Real-time PCR results showed that Dex group than the control group vimentin mRNA expression is decreased significantly different between groups ($p<0.05$), the difference was statistically significant. Western-blot results show that Dex group than the control group vimentin protein expression is decreased significantly different between groups ($p<0.05$), the difference was statistically significant, suggesting Dex can reduce the expression of vimentin. Immunofluorescence staining showed that Dex group than the control group vimentin expression is decreased significantly, which consistent with the Western-blot results.

Conclusion. Dex can induce expression of vimentin in HLECs, which may cause abnormal lens epithelial cell proliferation and differentiation, eventually leading to the formation of GIC.

Key words: *Dexamethasone, vimentin, GIC, HLECs.* ■

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РЕФЕРАТ

Влияние дексаметазона на виментин в клетках эпителия хрусталика человека

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Цель. Исследовать экспрессию виментина (vimentin) при воздействии дексаметазона (Декс) в эпителиальных клетках хрусталика человека (ЭКХЧ), и роль экспрессии виментина в образовании катаракты, вызванной стероидами (КВС).

Материал и методы. Эпителиальные клетки хрусталика человека (ЭКХЧ) помещались в среду DMEM и среду DMEM + 1umol/L Dex (Декс) на 7 дней. Для выявления экспрессии виментина на уровни белка и транскрипции применялась методика ПЦР в реальном времени (Real-time PCR) и Вестерн-блот-анализ (Western-blot). Распределение и экспрессия виментина в ЭКХЧ измерялись с помощью иммунофлуоресцентного окрашивания.

Результаты. Результаты ПЦР в реальном времени показали, что в группе Декс по сравнению с контрольной группой экспрессия виментина мРНК снижается статистически достоверно с разницей между группами ($p<0,05$). Результаты Вестерн-блот-анализа показывают, что в группе Декс, по сравнению с контрольной группой, экспрессия белка виментина уменьшается, существенно отличаясь между группами ($p<0,05$), различие было статистически достоверно, предполагаем, что Декс может снизить экспрессию виментина. Иммунофлуоресцентное окрашивание показало, что в группе Декс экспрессия виментина значительно снижалась по сравнению с контрольной группой, что согласуется с результатами при использовании Вестерн-блот-анализа.

Заключение. Дексаметазон может вызывать экспрессию виментина в эпителиальных клетках хрусталика человека, что может являться причиной аномальной пролиферации и дифференциации эпителиальных клеток хрусталика, в конечном счете приводящим к образованию стероидной катаракты.

Ключевые слова: *дексаметазон, виментин (vimentin), КВС, ЭКХЧ.* ■

Авторы не имеют финансовых или имущественных интересов в упомянутых материале и методах.

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Paper highlights: As a result of the GIC single factor influence, making its drug treatment with clear goals, if we can further understand the pathogenesis of GIC, research and development a target design drugs can prevent the happening of the GIC.

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Long-term systemic or local application of corticosteroids can cause the posterior subcapsular opacities (PSO), known as glucocorticoid induced cataract (GIC). Related to the pathogenesis of GIC, there are many hypotheses, but none of them can explain the formation of PSO, so there is no effective means in the prevention and treatment. New view thought that hormones may through its receptor mediated vimentin changes to affect lens epithelial cell proliferation and differentiation, eventually leading to the formation of GIC. Therefore, to study the influence of vimentin impact on lens epithelial cell proliferation and differentiation may be a new direction for research in the pathogenesis of GIC. We proposed by dexamethasone (Dex) impact on the human lens epithelial cells (HLECs), to investigate the influence of the expression of vimentin protein changes and discussed which in the mechanism of GIC, especially the formation of the PSO.

MATERIAL AND METHODS

1.1. Material

HLECs purchased from the American Cell Bank (ATCC), fetal bovine serum FBS (U.S. Gibco), Dex (U.S. Sigma), reverse transcription kit (United States Life), real-time PCR kit (United States Life), QPCR instrument (American Life). Mouse anti-human vimentin IgG (USA Santa Cruz), rabbit anti-mouse IgG (USA Santa Cruz), SDS-PAGE gel kit (Pik days Reagent Company), microplate reader (US Bio Rad), EVOS inverted fluorescence microscope (USA AMG), gel imager act.

1.2. Methods

1.2.1. HLECs vitro

HLECs cultured in DMEM medium containing 20% fetal bovine serum, 100 U ml / 1 penicillin, 0.1 mg ml / 1 streptomycin and 2μM L- glutamine. They were divided into two groups: the hormone group (Dex Group): DMEM + 1umol / L, Dex, the control group (Control Group): DMEM + 0umol / LDex.

1.2.2. Analysis of vimentin mRNA by Real-time PCR

Total cellular RNA extraction and real-time PCR reactions carried out in accordance with classical methods previously [1]. The upstream primer of vimentin was 5'-ACCGCTTTGCCAACTACAT-3', the Under primeris was 5'-GTCCCGCTCCACCTC-3'.

1.2.3. Analysis of vimentin by western blotting

(1) Extraction of total cellular proteins: Digestion of each group cells to extract total proteins of cells.

(2) Westren-blot: Taking protein samples to take SDA-PAGE gel electrophoresis, transferred to a membrane to wet nitrocellulose membrane (NC film), the NC membrane placed in blocking buffer overnight at 4° C. They were then incubated overnight at 4° C with the primary vimentin antibody(mouse anti-human vimentin antibody and mouse anti-human GAPDH antibody, 1:1000). After washing five times with TBST, membranes were incubated for 2 h with Rabbit anti-mouse secondary antibodies(Rabbit anti-mouse IgG, 1:1000). Images were detected with the Alkaline phosphatase color kit. The scanned images were quantified using Gel documentation system.

1.2.4. Immunofluorescence staining analysis for vimentin

The cells were passaged to confocal dish, cultured for 12h after washing twice with PBS, each time 10min. They were joined 4% paraformaldehyde solution to fixed 15-20min at room temperature, then washing 3 times with PBS; joined 0.2% Troton X-100 to permeabilize cell 7min, then joined 4% BSA to block 1h they were then incubated overnight with the primary vimentin antibody (mouse anti-human vimentin antibody) 1:400. After washing three times with PBS, they were incubated for 30 min with fluorescent secondary antibodies 1:400 at room temperature from light. Washing three times with PBS, then were observed under fluorescent microscope.

1.2.5. Image Acquisition and Analysis

The results of Real-time PCR detected by Quantity One program statistics, obtaining CT value of each set of PCR, using the calculation method of gene expression △△ Ct strength. The images of Western-blot were measured by the Image J program to strip the central density.

1.3. Statistical methods

Using SPSS19.0 statistical software for statistical analysis. All the experiments were repeated three times, all data as x±s, groups were compared using ANOVA, with $p<0.05$ was considered statistically significant.

RESULTS

2.2.1. The results of Real-time PCR and Western-blot

HLECs after Dex induced 7d, vimentin mRNA expression is reduced, the significant difference between groups ($p<0.05$) (figure 1a). HLECs after Dex induced 7d, vimentin protein expression was significantly decreased,

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the significant difference between groups ($p<0.05$). GAPDH express was no statistically significant difference between groups ($p>0.05$) *(figure 1b)*.

2.2.2 The results of Immunofluorescence staining

Vimentin expression in the cytoplasm in the HLECs, nuclear weeks radiating interwoven into a network structure to the peripheral connected to the cell membrane, distribution range and cell shape contour *(figure 2)*. The control group vimentin fiber bundle of thick, dense, dense mesh structure, suggests the expression of vimentin is high *(frgure 2a)*. Dex group of vimentin fiber bundle sparse, slender, sparse mesh structure, suggests that the expression of vimentin is low *(figure 2b)*, consistent with the results of western-blot.

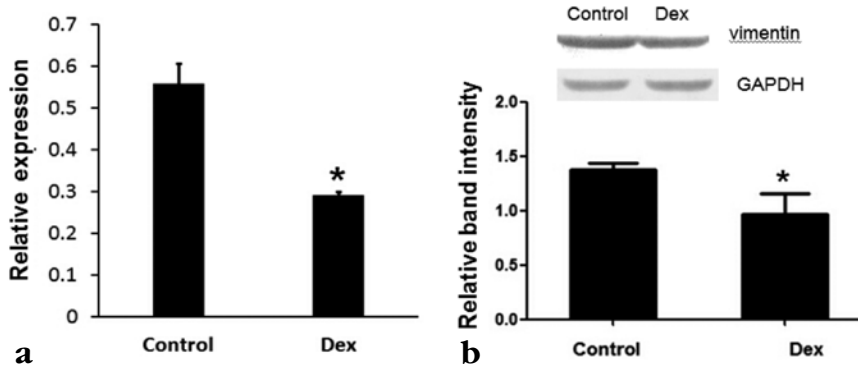

Figure 1. Real-time PCR and Western -blot were applied to identify the expression of vimentin at protein and transcription levels in HLECs exposed to Dex. A: HLECs after Dex induced 7d, vimentin mRNA expression is reduced, the significant difference between groups ($p<0.05$). B: HLECs after Dex induced 7d, vimentin protein expression was significantly decreased, the significant difference between groups ($p<0.05$). GAPDH express was no statistically significant difference between groups ($p>0.05$)

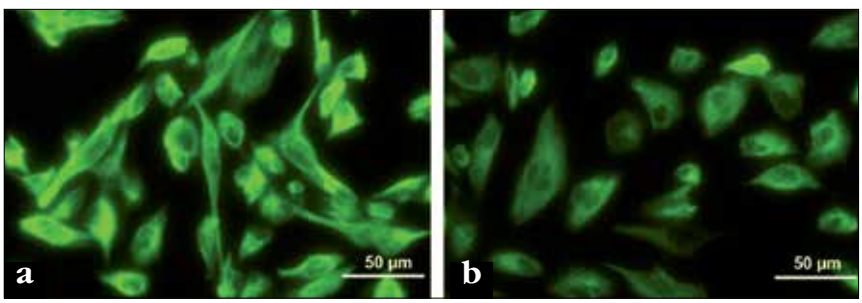

Figure 2. Observe the expression and distribution of vimentin in HLECs in the Dex group and the control group with inverted fluorescence microscope cell immunofluorescence x200. Vimentin expression in the cytoplasm in the HLECs, nuclear weeks radiating interwoven into a network structure to the peripheral connected to the cell membrane, distribution range and cell shape contour. A: the control group vimentin fiber bundle of thick, dense, dense mesh structure, suggests the expression of vimentin is high. B: Dex group of vimentin fiber bundle sparse, slender, sparse mesh structure, suggests the expression of vimentin is low

DISCUSSION

Glucocorticoid induced cataract (GIC) is the main complications in the eye of the application of hormone [10]. Epidemiological and clinical observations have been confirmed, systemic, topical corticosteroids and inhaled corticosteroid therapy are likely to cause cataracts [2]. There were many hypotheses about GIC formation mechanism [11]. Studies have shown that changes in GR-mediated vimentin could direct impact on the shape and function of lens cells, which in turn leads to the formation of GIC.

Typical performance of GIC was PSO. The main morphological changes were [6]: posterior subcapsular opacities cortical, lesions tend to be clearer boundary, that the whole appearance was granular gravel. Histological changes characteristic were [5]: anterior lens capsule, the front cortex and nucleus – relatively normal, the posterior pole of lens fiber cell swelling, disorganized nuclear elongation, posterior subcapsular cortex lesions visible in varying degrees round.

Vimentin as an important cytoskeletal protein, which is expressed in an appropriate amount of lens cells, is important to maintain normal morphology and function [12]. Recent studies have shown that the expression of vimentin was related to glucocorticoid receptors [1]. But, the exact role of vimentin in biology within the lens is unknown.

Hong Yan first reports to demonstrate that dexamethasone can reduce the expression of vimentin in rats lens epithelial cells [9]. Our study shows that dexamethasone can significantly reduce the expression of vimentin in HLECs, which indicates GR involved the change of human lens vimentin expression levels. This is the first report showed that, Dex inducing of cataract was related to the decreased expression of vimentin. Previous studies have also confirmed that vimentin was involved of lens epithelial cell signal transduction, structural changes in cells, and cell differentiation and apoptosis [4]. It can be presumed that the reduction of expression of vimentin in HLECS caused by Dex could affect the structure and function of lens epithelial cells, thereby leading to changes in lens transparency [13].

Glucocorticoid treatment of human lens epithelial cells results in activation of the GR and subsequent modulation of target gene expression via the mitogen activated protein kinases (MAPKs) and phosphatidylinositol 3 kinase/protein kinase B (PI3K)/AKT) pathways [3, 14]. And then likely to affect many cellular functions, such as proliferation, differentiation, apoptosis, survival, or migration. All of these are possible related to the formation of GIC. It is also interesting to note that involvement of both the PI3K/AKT and MAPK pathways has been reported for vimentin expression and activity [6]. Our studies have shown that Dex can induce HLECs in decreased expression of vimentin.

Our studies already proved that Dex can induce expression of vimentin in HLECs, which may cause abnormal lens epithelial cell proliferation and differentiation, eventually leading to the formation of GIC.

In summary, this study has proved in the gene level, protein level and in terms of morphology and distribution of proof that Dex can induce human lens epithelial cells vimentin content decreased. Which could speculate that the possible mechanism of Dex inducing GIC maybe by inducing reduce GR-mediated vimentin content, resulting in abnormal lens epithelial cell differentiation, signal transduction, survival and apoptosis, ultimately inducing the formation of GIC. Future experiments will a further study the relationship between the expression of vimentin protein with the changes of hormone concentrations, and further study the specific pathogenesis of GIC, in order to research the GIC prevention and drug treatment targets.

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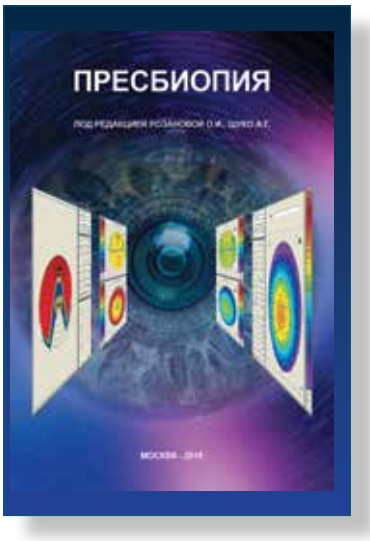
КНИГИ



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Монография посвящена актуальной проблеме офтальмологии – пресбиопии. В книге последовательно отражены современные представления о механизмах старения организма, описаны структурно-функциональные изменения в зрительной системе при развитии пресбиопии, с позиций общей патофизиологии представлена концепция формирования пресбиопии. Особое внимание в монографии уделено закономерностям изменения зрительного восприятия при утрате аккомодации и современным методам лечения пресбиопии. Книга предназначена для послевузовского образования, рассчитана на врачей-офтальмологов, врачей-патофизиологов, интернов и клинических ординаторов.

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