·Basic Research·

The effects of laminin and fibronectin on human lens epithelial cells growth characters and vimentin expression

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Abstract

• AIM: To observe the morphologic characters and the expression of vimentin on human lens epithelial cells (hLECs) cultured on laminin and fibronectin.

• METHODS: The primary and secondary hLECs were cultured on plates treated with laminin or fibronectin. The feature of hLECS was observed and recorded using an inverse microscope with digital camera and statistical analysis was applied. MTT assay was used to show the growth curve of subcultured cells (the 3rd passage). Immunofluorescent staining was used to demonstrate the morphologic change in vimentin expression in the 3rd passaged hLECs.

• RESULTS: The hLECs in laminin or fibronectin group grew out significantly earlier than the untreated group. The cells could be subcultured fluendly in those two treated groups, while only a few survival cells were found in the untreated group. But the hLECs could undergo apoptosis in laminin group at the terminal phase of their life cycle, while lentoid bodies emerged in fibronectin treated group. There were more viable cells in the fibronectin group from the 4th to the 7th day by MTT reaction. Immunofluorescent staining showed a clear morphological difference between the two groups.

• CONCLUSION: Laminin provide a suitable growth microenvironment for the hLECs while fibronectin promotes the cells proliferation and differentiation.

• KEYWORDS: laminin; fibronectin; lens; crystalline; epithelial

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INTRODUCTION

Postoperative cataract is the most common late complication of uncomplicated cataract extraction, which seriously influences patients' vision recovery. Posterior capsular opacification (PCO) is its pathogenesis resulting from the lens epithelial cells adhesion, proliferation and migration on the inter surface of posterior capsule, fibrogenesis and extract of extracellular matrix (ECM). Human lens epithelial cells (hLECs) *in vitro* culture is a usual method to investigate the biologic and physiological characteristics of hLECs and the pathologic changes of PCO. However, the hLECs are terminally differentiated ones which is quite difficult to culture or subculture. Therefore the in vitro experiments are handled with an immortal cell line, such as HLE-B3 constructed by Andley[1] in 1994. The infinitely subcultured cells kept its epithelial characters steadily, which were quite different from the differentiated fibroblastic ones in PCO or some cataract[1-4].

Laminin and fibronectin are two components of the ECM which influence cellular growth and differentiation by conjugating integrin molecules [5]. Hence the elucidation of the molecular mechanisms contributing to cell adhesion is important in developing new drugs for treating the resulting diseases.

Vimentin is an intermediate filament protein (58 kiloDaltons) which is generally found in a variety of cells of mesenchymal origin and is developmentally regulated. Vimentin has been proved useful in the differential detection and is thought to be involved in the communication and transportation between the surface of a cell and its nucleus[6-8].

In this study, we cultured the human LECs on laminin or fibronectin coated dishes and observed the morphologic characters and the vimentin expression, in order to find a

more suitable culture model for PCO research.

MATERIALS AND METHODS

Materials Dulbecco's modified Eagle's medium/Ham's F-12 nutrient mixture (1:1, DMEM/F-12) and fetal bovine serum (FBS) were purchased from HyClone (Logan, UT). Laminin and fibronectin were purchased from GIBCO BRL (Invitrogen, Grand Island, NY). 3-[4, 5-dimethylthylthiazol-2-yl]-2, 5 diphenyltetrazolium broide (MTT) was purchased from Sigma (USA). The mouse monoclonal antibodies against vimentin and the FITC-conjugated secondary antibodies were purchased from Zymed (South San Francisco, CA). Olympus Bx51 immunofluorescent microscope, Olympus CK40 inverse microscope and LABsystems MK3 ELISA detector were applied in the study.

Laminin and Fibronectin Coated Plates Laminin and fibronectin of 30mg/L were used to coat the plastic plates at 5μg/cm^2. The plates were incubated at 4℃ overnight and blocked with 20mL/L bovine serum albumin (BSA) for 1 hour. Then rinsed the plates with DMEM/F-12 and dried them in the lamina hood. According to the coat components, the plates were divided into Ln and Fn group. The control group was treated with 20mL/L BSA only.

Cultivation of LECs Human tissue was handled complying with the Declaration of Helsinki of the World Medical Association. Twenty eight human eyes were obtained from China Medical University Eye Center (Shenyang, China). The lens anterior capsule were cut into small scraps after being rinsed with DMEM/F-12 plus 100mL/L FBS, they were seeded into the coated plastic plates that were coated or non-coated with the Laminin, Fibronectin. The epithelial scraps were incubated at 37℃ in 50mL/L CO_2 and the medium was changed every 3 days. At 70% -80% confluence, cells were digested with 2.5g/L trypsin plus 0.2g/L EDTA and then replanted into plastic plates at a density of 2×10^4 cells/cm^2. Cells were cultured for 8-10 passages to record the morphological characteristics and fixed for immunofluorescent staining.

MTT Assay The subcultured LECs (passage 3) were digested and counted. Then they were seeded in 96-well plates at the concentration of 2.5×10^7 cells/L, with 200μL in each well. The cells were incubated at 37℃ for 4 hours after 20μL MTT (5g/L) were added in the medium at 10 every morning. Then the supernatants were removed and 150μL DMSO was given. The optical density (A) of 570nm were detected for 7 days to draw the growth curve.

Immunofluorescent Staining The primarily and secondarily cultured LECs were collected and fixed in paraformaldehyde (40g/L). Slides were rinsed 3 times in 5g/L Triton-X100/PBS (v/v)for 15 minutes and then blocked in 100mL/L BSA at 37℃ for 50 minutes. The primary antibodies were applied and incubated in a moist chamber for 1 hour. Then the slides were rinsed in 5g/L Triton-X100/PBS (v/v) for 15 minutes and incubated with fluorescein-conjugated anti-mouse IgG in a moist chamber for 1 hour. The slides were preserved in glycerol-PBS (glycerol/PBS=9:1,v/v) for visualization. And the photos were taken with an Olympus microscope. Negative controls were incubated in mouse IgG instead of the primary antibody.

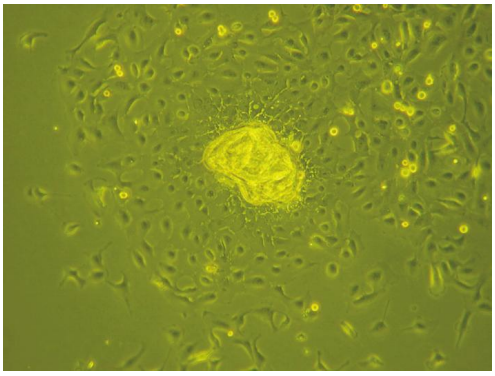

Figure 1 The primarily cultured hLECs on fibronectin. The primary hLECs on laminin were quite similar to this with fewer cells

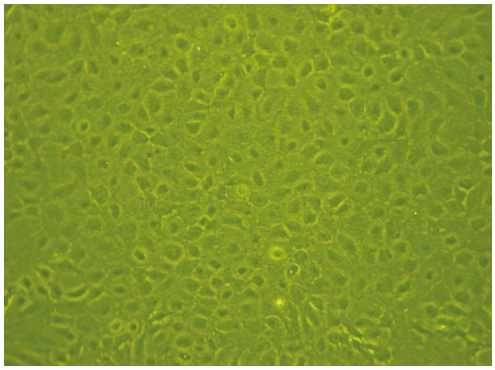

Figure 2 The subcultured LECs on laminin and almost all the cells were in epithelial form

Statistical Analysis The data were analyzed with Independent-Samples t test of Statistic Packages for Socies Science 10.0 (SPSS10.0).

RESULTS

Cell Culture The hLECs treated with laminin or fibronectin grew out significantly earlier than the control group (Figure 1). Almost all the subcultured cells took on an epithelial look in the laminin-treated group (Figure 2), while a fibroblastic phenotype in the control one. There were more cells in the fibronectin-treated group and fibroblastic cells

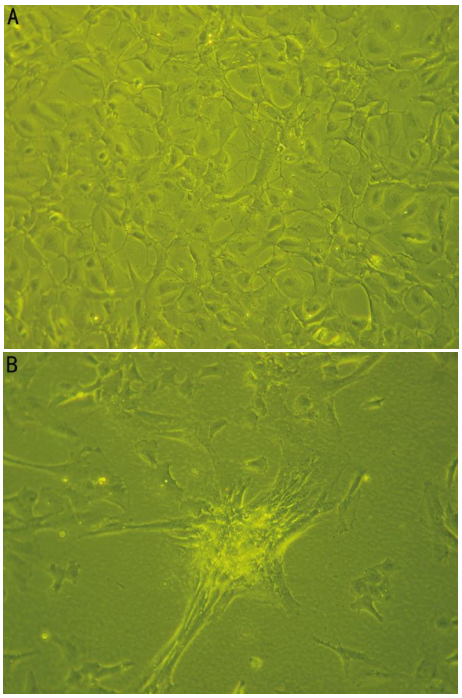

Figure 3 The 8th and 10th passage LECs A:The 8th passage LECs on laminin underwent apoptosis; B: The 10th passage LECs on fibronectin showed lentoid bodies

could be seen mixing with the epithelial ones. After subculturing 8-10 passages, cellular death and apoptosis were found in laminin-treated group, with the abnormal netting phenomenon (Figure 3A). The hLECs accumulated to form the characteristic lentoid bodies in the fibronectin-treated group while fibroblastic cells of the control group died out long before (Figure 3B).

MTT Assay There were similar absorptions in the laminin- and fibronectin-treated groups the first 3 days, but a significantly higher absorption was detected in the fibronectin-treated group from the 4th to 7th day (Figure 4).

Immunofluorescent Staining The positive expression of vimentin was seen in all samples. But the vimentin fibers were slim and tender, regular within the cell in the laminin group. And the filaments in fibronectin group were stick and rough, in irregular form (Figure 5A,B).

DISCUSSION

Human lens is an avascular tissue, devoid of innervations, and located posterior to the iris in the eye. This tissue is enclosed by a collagenous capsule so that the anterior capsule is approximately three times thicker anteriorly than the posterior capsule. The lens contains two major cellular types: the lens epithelium, limited to the anterior region of the lens, and the lens fiber cells which make up the rest of the lens [9]. A single layer of cuboidal epithelial cells which

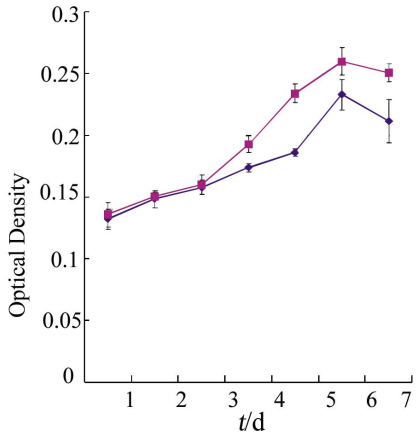

Figure 4 The growth curved by MTT assay (■–Fn Group, ◆–Ln group)

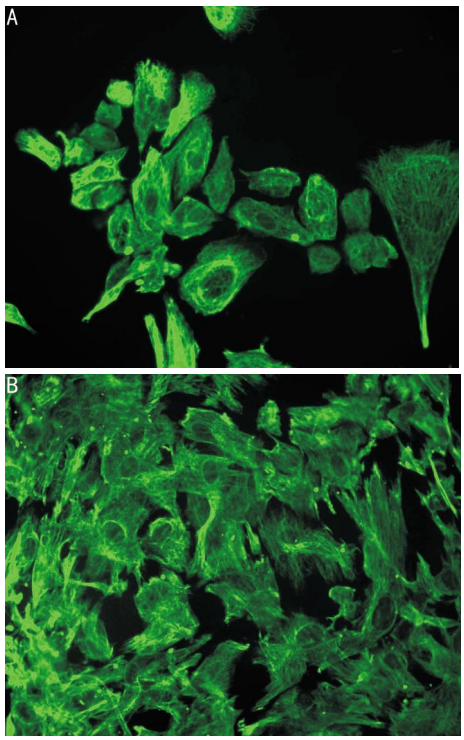

Figure 5 Expression of vimentin A:Expression of vimentin on laminin. The vimentin fibers were slim and tender, regular within the cells; B:Expression of vimentin on fibronectin. The vimentin fibers were rough and sticky, in irregular form

are nucleated and contain few organelles, line the anterior inner surface of the capsule. And the process of cell differentiation continues throughout the life time of the organism, resulting in fiber cells continually being formed at the elongation zone and displaced toward the center of the lens mass[9-11].

Previous reports revealed that it was quite difficult for the hLECs to survive in vitro culture medium with 100-200mL/L fetal bovine serum, even if many kinds of growth factors had been added. Few cells could be seen in the primary

cultured plates and they may entirely disappear when subcultured. So the immortalized cell lines, such as N/N 1003A LECs were used for *in vitro* researches on cataract or PCO [1, 10].

However, the most of the immortalized cells show more proliferative activity than the original ones, and the changed genotype or phenotype can be transmitted to the further generations. The features of infinite cell line could not resemble the development of cataract genosis or PCO which is a gradually transforming process from epithelial cells to fibroblast.

In our previous study, we first reported that laminin and fibronectin were used to coat the culture plates, which helped to make the microenvironment similar to the epithelial basal membrane, and better the culture surface character for cell adhesion and survival[9]. The hLECs spread smoothly and exuberantly along the coated surface and were sub-cultured stably till 8-10 passage.

There were some differences between the laminin-treated and the fibronectin-treated groups in morphology phenotype. Most of the hLECs in the laminin group maintained an epithelial state during the whole cycle and became apoptosis after 7 or 8 passages. The cells in the fibronectin group, however, proliferated much faster and changed into fibroblastic ones in the early stage. More viable cells could be seen with the irregular spindle shape, and formed the specific lentoid bodies after 8 or 10 passages. The MTT assay also demonstrated that the hLECs of fibronectin group had more proliferative activity than the laminin group. Laminin provides the suitable microenvironment for the hLECs to maintain the epithelial characters and fibronectin makes for survival or proliferation of the cells.

Fn showed promoting activity in cell proliferation and differentiation compared with Ln in the similar culture condition, and the subcultured cells in Fn group replicated quickly and transformed from epithelial cells to fibroblasic ones. After 8 or10 packages, the degeneration feature could be found in the Ln group with LECs replicating slowler with fewer cells surviving on the dishes, the apoptotic plasmid took on the netting phenomena. LECs in Fn group congested and accumulated with the organells of the inner layer cells disappearing to form the transparent lentoid bodies. It has been reported that lentoid is the finite LECs lines feature at terminal phase. After 20-90 days cultured *in vitro*, the LECs constructed the transparent organell-free bodies, made up of r-crystallin and MP26. It is regarded as one of the most important features of *in vitro* cultured LECs. But in our experiments, only the differentiated cells could accumulate to form lentoid bodies.

Ln is one of the most important extracellular matrix components to build up the lens anterior capsule. So it is understandable why Ln could help LECs remaintain in its cubical shape. It has been demonstrated that cultured LECs will develop into lentoid bodies under a number of stimuli including the presence of the lens capsule, growth factors, or retinal and vitreal extracts [10-13]. Wagner reported lentoid body formation as a result of the overexpression of PKC-α or PKC-γ in cultured N/N 1003A LECs [14,15]. Our results, as the LECs undergo apoptosis in Ln group and replicate to form lentoid bodies in Fn group, are quite in accordance with the reports. The lentoid bodies' formulation is composed of several steps. Firstly, Fn promotes LECs to proliferate and become slim and long, similar to fibroblastic cells. Secondly, these cells interact and adhere with each other. Thirdly, the inner part of the lentoid body degenerate and the organelles disappear. The specific phenomenon herein forms.

Vimentin is one component of the cytoskeleton intermediate filament. The normal vimentin expression is limited within the inner space of cytoplasmid, and there is a clear blank in the submembrane space, as shown in the light microscope as the separation of adjacent cells.

The fibers in Ln treated group were tender and in a regular form within the cell boundary, while those were rough in Fn group in our research. This difference helps to affirm that Ln may be better for epithelial cells growth and help to maintain the undifferentiated state of hLECs. It is suggested the different expression correlated with the cytoskeleton reconstruct during the epithelial cells transform to fibroblastic ones, and the signaling pathway of ECM-integrin molecules-cytoskeleton components mediated the signals.

This finding constructs a better *in vitro* model for the study of hLECs biologic characteristics. It may provide a novel way to research the relationship between the interaction of lens cellular-extracellular matrix and the genesis of primary and secondary cataract including PCO.

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