Expression of CD18, CD49b, CD49c and CD49e on lens anterior capsules in human cataracts

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Abstract

Purpose To examine the lens epithelial cells obtained from the anterior lens capsules removed during cataract surgery and detect various subclasses of the cell surface adhesion molecules known as integrins.

Methods The circular sections of anterior capsules with attached lens epithelial cells (LECs) were obtained during cataract surgery from 28 patients. The lens capsules were immunohistochemically stained.

Results CD49b, CD49c, CD49e, and CD18 were detected in varying degrees in specimens obtained from human cataractous lenses. The positive percentages were 33, 75, 33, and 20%, respectively. The most striking feature was the increasing staining profiles towards the edges of the capsules (away from the central part of the lens capsules) for CD49c, suggesting that the LECs showed higher immunoreactivity for this antibody. The immunoreactivity for CD49b and CD49e was weaker. This was absent for CD18 in the central part of the lens capsules.

Conclusion The positive expression of antibodies suggests that specific integrin subunits were expressed in LECs of human cataracts. These results suggest that lens epithelial cells expressing CD49b, CD49c, CD49e, and CD18 might be precursors in the process of anterior lens epithelial cell (A cell) adhesion, and hence play a role in anterior capsule opacification or in subsequent migration and a possible role in posterior capsule opacification.

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Introduction

Following cataract surgery posterior capsular opacification (PCO) is the most common complication, affecting up to 50% of all adult patients after 2 years and virtually all paediatric cases.^{1,2} It is secondary to the migration of lens epithelial cells (LECs) from the lens equator onto the posterior lens capsule or lens epithelial metaplasia into myofibroblasts (fibrosis).³ The blocking of $\alpha_v \beta_3$ integrin expression was noted to inhibit specifically LEC migration and proliferation in an animal model.⁴ Various authors have also suggested the role of TGF- β in accelerating transdifferentiation and contraction of the capsular bag.^{5,6} These results suggest that a common feature of anterior subcapsular cataract (ASC) and PCO may be induction of an epithelial–mesenchymal transition by TGF- β . Epithelial cell proliferation, collagen deposition, basement membrane duplication, and lens fibre regeneration subsequently result in capsular opacification and visual compromise.^{7,8} The anterior lens epithelial cells (A cells) are the source of any form of anterior subcapsular cataract. The primary type of response of the A cells to any stimulus is to proliferate and form fibrous tissue by undergoing fibrous metaplasia, sometimes termed ‘pseudofibrous metaplasia’.³

Several studies suggest that the residual LEC proliferation, differentiation, and migration onto the posterior capsule lead to PCO.^{3,7,8} Anterior capsular shrinkage and ultimate constriction (capsulorhexis contraction syndrome or capsular phimosis) of the anterior capsulectomy opening, excessive zonular traction and its sequelae, IOL decentration, and retinal detachment can also occur.^{9–11}

Anterior lens capsules discarded during cataract surgery contain a monolayer of epithelial cells. It has been reported that the migration of LECs in the human eye is dependent on the extracellular matrix (ECM), which could promote the adhesion of LECs.^{3–5}

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Integrins are cell adhesion molecules that have been implicated in the migration of cells to the posterior capsule of the lens. They are a family of heterodimeric membrane glycoproteins expressed on a diverse range of cell types. The glycoproteins function as the major receptors for ECM proteins and as cell–cell adhesion molecules. All integrins consist of noncovalently associated α and β subunits. They bind to a variety of ECM proteins such as collagen, fibronectin, von Willebrand factor and to members of the immunoglobulin superfamily.

However, the various subtypes of lens adhesion molecules involved in anterior and posterior capsular opacification have not been identified. As the knowledge of these processes increases, the possibility of developing a number of therapeutic agents will also increase.

In this article, we report the expression of integrins in anterior capsules removed during cataract surgery. An immunohistochemical technique was used to detect the presence of integrin receptors for lens capsule components.

Patients and methods

Prior Ethics Committee approval from the Argyll and Clyde health board was obtained. The circular sections of 28 circular capsules with attached LECs were obtained during surgery from 28 patients with age-related cataract. The average age of the patients was 75 years $\pm$ 2.49 (SD), and of the 28 patients 15 were women and 13 men. The type of cataract (six anterior subcapsular cataracts, five nuclear sclerosis, three posterior subcapsular cataracts, five cortical cataracts, and nine anterior and posterior subcapsular changes with nuclear sclerosis) prior to surgery was also noted. In the present study, no information about the intraoperative CCC size or postoperative results were available. Each capsule was immediately placed on a silane-coated glass slide.

Antibodies

All anti-integrin antibodies used were monoclonal (mAb) (Novocastra lab, Newcastle upon Tyne, UK) and are listed in Table 1. The site of primary antibody binding was detected using a peroxidase-based avidin–biotin

complex (ABC) system (Vector labs, Peterborough, UK)—in which biotinylated-immunoglobulin and peroxidase were utilized.

Immunostaining

The specimens on the glass slide were fixed in acetone for 5 min, washed in phosphate-buffered saline (PBS), and blocked with 3% aqueous hydrogen peroxide for 10 min at room temperature. After rinsing, the specimens were stained with primary antibodies α_2 , α_3 , α_5 , β_1 , and β_2 for 60 min at 37°C.

Primary antibody was removed (with Gielson) and diluted biotinylated antibody (Vectastain ABC kit, Vector labs, Burlingame, CA, USA) was added to each slide. The specimen was incubated with Vectastain elite ABC reagent at room temperature for 30 min. Peroxidase activity was visualized by incubating with diaminobenzidine (DAB) for 10 min. The specimens were counterstained with haematoxylin, mounted with DPX, and observed under light microscopy. Negative control staining was performed by omission of the primary antibodies.

Results

A total of 28 human capsules obtained from cataractous lenses were analysed for immunoreactivity for CD49b, CD49c, CD49e, and CD18 antibodies. The CD49b, CD49c, CD49e, and CD18 antibodies specifically test for the integrin subunits α_2 , α_3 , α_5 , and β_2 , respectively. The integrins were observed in some specimens and are portrayed in Figure 1. The individual subtypes of integrin antigens α_2 , α_3 , α_5 , and β_2 and their CD49b, CD49c, CD49e, and CD18 antibodies were analysed. The positive percentages were 2/6(33%), 6/8(75%), 3/9(33%) and 1/5(20%), respectively. There was no staining in the negative control slides. Table 2 summarizes the immunostaining results obtained from the various subtypes of integrin dimers used. CD49c ($\alpha_3\beta_1$ dimer) showed a significantly higher proportion of positive results ($\chi^2 = 4.72$, $df = 1$, $P = 0.03$) in comparison to CD49b, CD49e, and CD18.

The most striking staining profiles were obtained at the edges of the capsules for CD49c. The edge staining was not an artefact, as indicated by the absence of staining

Table 1 Human integrin antibodies and possible dimers

Antigen	Possible dimer(s)	Possible ligand(s)	Antibody (Source)	Dilution
β_2	$\alpha_1\beta_2$	ICAM-1,2; C ₃ b ₁	CD18 (Novocastra)	1:25
α_2	$\alpha_2\beta_1$	Collagen I, IV; laminin	CD49b (Novocastra)	1:25
α_3	$\alpha_3\beta_1$	Laminin, fibronectin, collagen I	CD49c (Novocastra)	1:25
α_5	$\alpha_5\beta_1$	Fibronectin	CD49e (Novocastra)	1:25

Figure 1 Expression of (a) CD49b, (b) CD49c, and (c) CD18 antibodies on the LECs of human cataract anterior capsules: (d) Negative control slides with no staining. The cell nucleus stained blue and a positive reaction produced a brown reaction (original magnification $\times 40$). Note the intense brown reaction with CD49c compared to CD49b and virtually no reaction with CD18.

Table 2 Individual expression of integrins in cataract LECs

Antigen	Antibody (source)	Cases	Positive	Negative	Positive percentage (%)
$\beta_1\alpha_2$	CD49b (Novocastra)	6	2	4	33
$\beta_1\alpha_3$	CD49c (Novocastra)	8	6	2	75
$\beta_1\alpha_5$	CD49e (DAKO)	9	3	6	33
$\alpha_2\beta_2$	CD18 (Novocastra)	5	1	4	20

when control immunoglobulin was substituted for anti-integrin antibodies and by the specific staining pattern (brown colour) obtained with the anti-integrin antibodies.

Discussion

Our results suggest that integrins of two different subfamilies are expressed in LECs. Integrins are transmembrane heterodimers consisting of noncovalently associated α and β subunits. Colocalization of α and β subunits implies the presence of the corresponding heterodimer.¹²

We report here that human cataract LECs express α_2 , α_3 , α_5 , β_1 and β_2 integrins in specimens removed during cataract surgery. There was a higher positive expression of CD49c ($\alpha_3\beta_1$ dimer) (75%) in comparison to $\alpha_2\beta_1$, $\alpha_5\beta_1$, and $\alpha_1\beta_2$ subunits (33, 33, and 20%, respectively). In a previous report by Zhang *et al*¹³ the positive percentages of β_1 , β_2 , α_2 , α_3 , and α_5 integrins were 70, 65, 75, 70, and

80%, respectively. In the present study, the higher positive expression of α_3 and β_1 and a relatively poorer yield of α_2 , α_5 , and β_2 subunits may be secondary to the characteristics of the subtypes of integrins. The α_3 and β_1 subunits are considered to be responsible for the adherence of cells to ECM components such as fibronectin, vitronectin, collagen type IV, and laminin. The α_2 , and β_2 subunits are considered to be responsible for cell-to-cell adherence. During cataract surgery, there is a breakdown of lens epithelial barrier representing altered cell surface receptor and cytoskeleton rearrangement similar to wound healing.

In 1998, Saika *et al*¹⁴ reported finding prolyl 4-hydroxylase subunits, which are needed for collagen production, in human capsules with IOLs showing that LECs are able to produce collagen. Recent studies indicate type IV collagen, laminin, and fibronectin and vitronectin as important ECM proteins promoting lens epithelial cell adhesion.^{15,16} Integrins and other cell adhesion molecules may activate intracellular signal

pathways, altering the response of lens epithelial cell–cell adherence and cell–ECM interaction. Residual LECs come in contact with the lens capsule or with the IOL after surgery. These cells undergo fibrous metaplasia and produce prostaglandin E₂, resulting in capsular fibrosis and blood–aqueous barrier disruption.^{17,18}

Continuous curvilinear capsulorrhexis has been postulated to affect the degree of anterior capsular opacification leading to capsule contraction syndrome.¹⁹ After CCC and IOL implantation, the area of the anterior capsule opening seems to decrease gradually for up to 3 months postoperatively.^{20–24} One possible explanation is the more important sphincter effect with a small capsulorrhexis. In the present study, no information about the intraoperative CCC size or postoperative results was available. Therefore, we could not correlate with the immunohistochemical staining profile at the edge of the capsulorrhexis and a subsequent role in capsule shrinkage leading to anterior capsular opacification.

The sandwich theory of PCO¹⁵ states that an IOL made of a bioactive material allows a single LEC to bond to the IOL and the posterior capsule at the same time, producing a sandwich pattern. This includes the IOL, cell monolayer, and posterior capsule. The PCO rates would hence depend on the bioactivity of IOL, ECM properties, and LEC proliferation, differentiation and migration. A sealed sandwich may prevent the epithelial in growth through this space.

In the present study, the most striking feature was an increasing staining profile obtained towards the capsulorrhexis edges of the anterior capsule. Our results suggest numerous integrin subunits being expressed prominently by proliferating cells, this being maximum in LECs towards the pre-equatorial zone. We suppose that the formation of PCO has a relationship with differentiation that includes more than the proliferation of LECs.

Cell adhesion molecules, especially integrins, play an important role in gene expression, signal transducers within the cell, as well as receptors for ECM. In our study the positive percentages of integrin expression tested were not 100%, which may be the result of individual characteristics of each patient. More studies are necessary to determine the presence of unique cellular receptors on human LECs for the ECM proteins, namely fibronectin, type IV collagen, and laminin. This would therefore help us to understand the mechanisms by which these membrane proteins promote adhesion and migration of these cells. This would have clinical implications for the development of new therapeutic approaches to inhibit PCO and postoperative inflammation caused specifically by residual LECs.

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