

## CLINICAL CASE

# Histopathology of Subcapsular Cataract in a Patient with Atopic Dermatitis

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### ABSTRACT

**Purpose.** To report the histopathological features of anterior subcapsular cataract associated with atopic dermatitis.

**Case Report.** A 29-year-old man with atopic dermatitis presented with bilateral anterior subcapsular cataract. After routine cataract surgery, the anterior subcapsular cataractous tissue was obtained as an anterior capsulorhexis flap and prepared as a wholemount for histological analysis. The wholemount consisted of a well-demarcated central grayish-white plaque surrounded by transparent capsule, corroborating the slit-lamp biomicroscopic appearance. Higher magnification of the plaque revealed a fibrous and amorphous mass, most likely extracellular matrix owing to the presence of irregularly arranged bundled strands of fibrils, typical of collagen. Lens epithelial cells at the plaque were densely packed and myofibroblast-like and immunoreactive for alpha-smooth muscle actin. In contrast, lens epithelial cells more distant from the plaque retained their regular cuboidal arrangement and regular spacing, and were not labeled for alpha-smooth muscle actin, similar to lens epithelial cells obtained from a non-cataractous case.

**Conclusions.** The presence of alpha-smooth muscle actin-reactive elongated cells at the plaque suggests that the cuboidal lens epithelial cells making up the anterior subcapsular cataract have transdifferentiated into spindle-shaped myofibroblastic cells that produce and deposit aberrant extracellular matrix. This transdifferentiation process, more commonly known as an epithelial-mesenchymal transition, contributes to a fibrotic response leading to the development of human anterior subcapsular cataract. (Optom Vis Sci 2017;94:00-00)

**Key Words:** anterior subcapsular cataract (ASC), atopic dermatitis, myofibroblast, transforming growth factor-beta (TGFβ), epithelial-mesenchymal transition (EMT)

Anterior subcapsular cataract, a characteristically dense, white opacity directly beneath the anterior lens capsule, is often associated with atopic dermatitis, uveitis, and mechanical or chemical ocular trauma.<sup>1</sup> The prevalence of anterior subcapsular cataract is relatively uncommon in Europe and North America at 2 to 3% of cataract<sup>1</sup> compared to 4.8% in Asian countries.<sup>2,3</sup> There is little in the literature regarding the age-specific incidence of anterior subcapsular cataract; however, unlike more common forms of cataract such as nuclear or cortical, it seems to be more prevalent in younger populations; 74% of patients with anterior subcapsular cataract are younger than 60 years of age with an average age at diagnosis of 51.9 years.<sup>2</sup>

There is a compelling body of evidence implicating epithelial-mesenchymal transition in the pathogenesis of anterior subcapsular cataract, characterized by transdifferentiated myofibroblastic cells surrounded by an accumulation of extracellular matrix, immediately below the anterior lens capsule.<sup>3</sup> Numerous animal models such as cultured embryonic chick lens cells, rat lens epithelial explants, whole rat lens culture, and transgenic mice have been utilized to model the pathogenesis of anterior subcapsular cataract; however, there has been a paucity of research using human tissue.<sup>4</sup> Hence, histopathological examinations of excised human lens tissue are fundamental in translating these animal studies to the human condition. In this case report, we describe the histopathological features of a human lens capsulorhexis with anterior subcapsular cataract.

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### CASE REPORT

A 29-year-old man of Asian descent presented with bilateral dense anterior subcapsular cataract (Fig. 1A, B) with best-corrected visual acuity of 20/60 in the right eye and 20/120 in the left. He was first diagnosed with cataract at 21 years of age after reports of symptoms

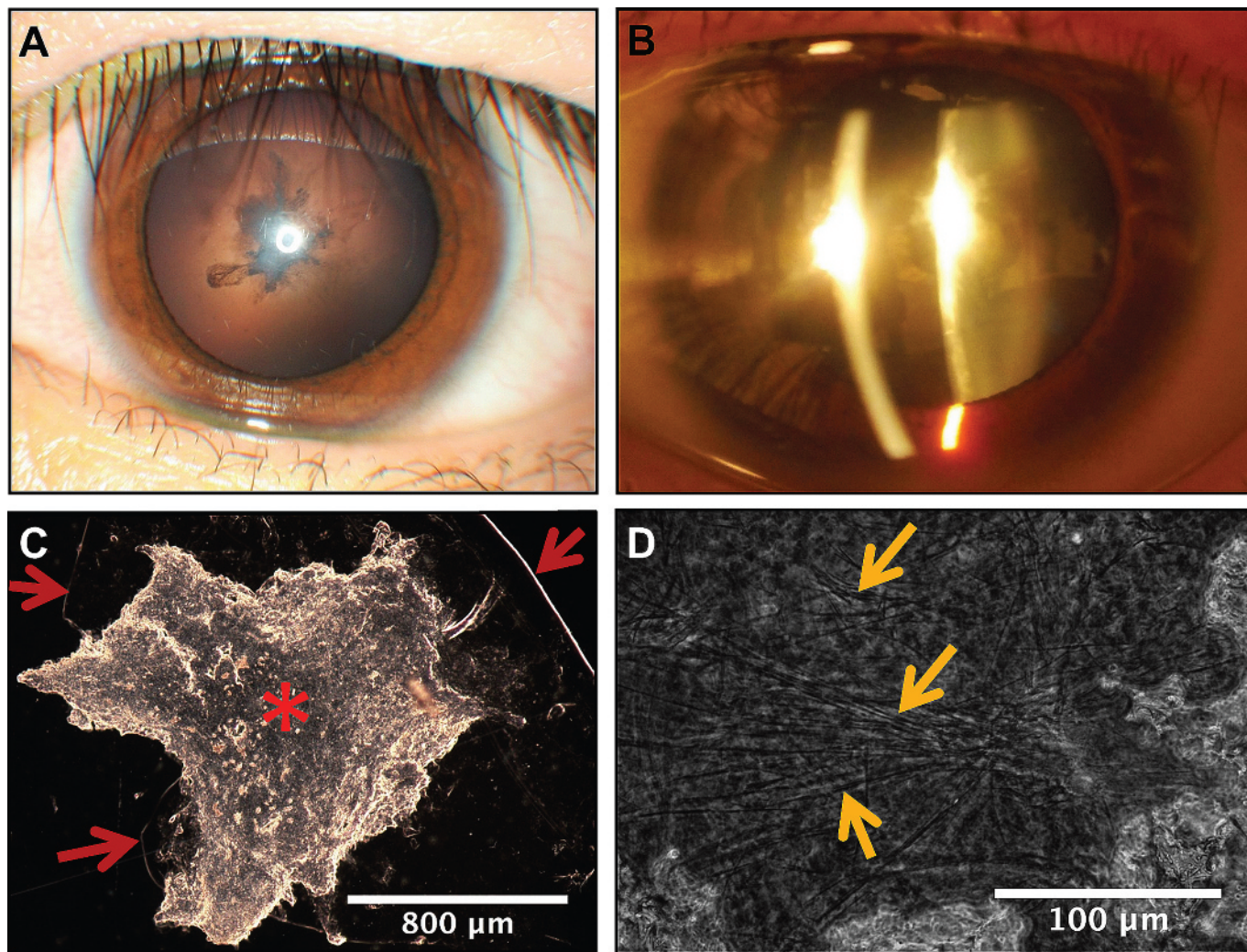


**FIGURE 1.**

Slit-lamp biomicroscopic photographs of the anterior subcapsular cataract in the left eye with diffuse illumination (A) and optic section (B). Phase-contrast microscopy of the anterior subcapsular plaque shows a white, amorphous mass evident centrally (\*) within the capsulorhexis specimen (edges denoted by red arrows) in (C). A magnified view of the surface of this plaque (D) under phase contrast shows regular strands (yellow arrows) representing fibrils that are typical of collagen that comprises the abundant extracellular matrix of the plaque.

of glare and decreased vision. His medical history was significant for atopic dermatitis, especially on his face and neck, for which he was prescribed topical betamethasone dipropionate cream for occasional use. On clinical examination, the patient had no other identifiable eye pathology.

The anterior subcapsular cataractous tissue was obtained as an anterior capsulorhexis flap during routine cataract surgery involving phacoemulsification and intraocular lens implantation in the left eye. There were no postoperative complications and the best-corrected visual acuity was 20/20 in the left eye after surgery. A non-cataractous control tissue was obtained from a 68-year-old man of Asian descent who underwent cataract surgery of the right eye for a dense nuclear and cortical cataract.

The anterior capsules with adherent cells, including lens epithelial cells, were fixed immediately in neutral buffered formalin (10%) and prepared as a wholemount. Phase-contrast microscopy was used to examine morphological features. Detailed cellular spatial characteristics were analyzed by staining cell nuclei with Hoechst 33342 (3  $\mu$ g/mL; Calbiochem, USA). To determine

whether these lens epithelial cells had undergone an epithelial-mesenchymal transition, the myofibroblastic marker, alpha-smooth muscle actin (1:100 dilution; A2547, Sigma-Aldrich, MO, USA), tagged using an Alexa Fluor 594-conjugated goat anti-mouse secondary antibody (1:1000 dilution; Molecular Probes, Invitrogen, CA, USA), was visualized using epifluorescence microscopy (Leica DMLB, Wetzlar, Germany). Given the capsulorhexis contained normal (circular nuclei) and pathological (elongated nuclei) lens epithelia, we had an internal negative control for the alpha-smooth muscle actin labeling. The specificity of the alpha-smooth muscle actin antibody used is validated by many of our past studies using western blotting and immunofluorescent labeling where it has been featured.<sup>5,6</sup>

The wholemount consisted of a well-demarcated central grayish-white plaque (Fig. 1C) surrounded by transparent capsule (margins outlined by arrows), corroborating the slit-lamp biomicroscopic appearance (Fig. 1A). Higher magnification of the plaque revealed a fibrous and amorphous mass of extracellular matrix consistent with the presence of irregularly arranged bundled strands, which appeared to be typical of collagen fibrils (Fig. 1D). Without further

studies specifically labeling for collagen, we cannot further characterize or confirm the exact nature of these fibrils.

The anterior lens epithelium showed a gradual transition in cell morphology extending from the plaque to more peripheral

non-plaque regions. Using a Hoechst stain, lens epithelial cells at the plaque (Fig. 2A) show more elongate cell nuclei that are unevenly distributed, with many nuclei apposed and overlapping each other, indicative of cell multilayering (Fig. 2B). These cells

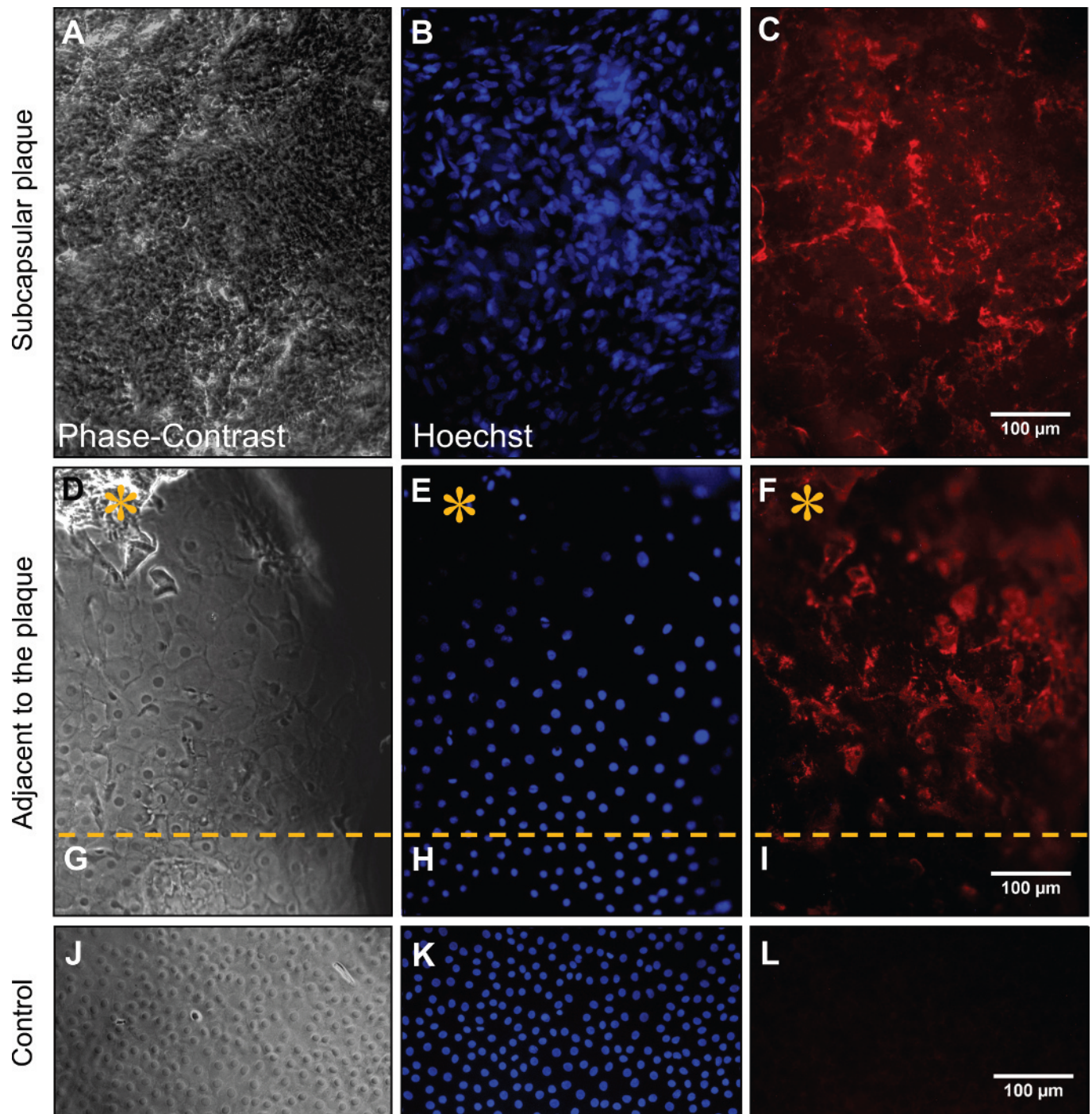


**FIGURE 2.**

Comparison of regional differences between lens epithelial cells at the plaque and adjacent to the plaque. The dashed line demarcates the transition of normal lens epithelial cells (below line) into myofibroblastic cells (above line) as they approach the plaque (\* denotes the edge of the plaque). Cells at the plaque (A) with Hoechst staining (B) appear denser, display more closely associated elongate cell nuclei and multilayering and are immunoreactive for alpha-smooth muscle actin (C). Directly adjacent to the plaque (D\*), lens epithelial cells appear more flattened and regularly spaced, maintaining the rounded appearance of their nuclei (E), but still express alpha-smooth muscle actin (F). Directly beyond this region, a population of well-defined, more regularly spaced cobblestone epithelial cells is evident (G) exhibiting uniform round nuclei (H) and notably little to no expression of alpha-smooth muscle actin (I). Lens epithelial cells of a non-cataractous patient appear more compact and uniform (J) compared to the anterior subcapsular cataractous tissue with regular and circular nuclei with no evidence of multilayering (K) and no immunoreactivity for alpha-smooth muscle actin (L).

were immunoreactive for alpha-smooth muscle actin (Fig. 2C). Lens epithelial cells in the transition zone between the plaque and non-plaque regions (Fig. 2D) displayed a more regular organization (Fig. 2E) and a sparsity of alpha-smooth muscle actin labeling compared to the plaque region (Fig. 2F). The images in this transition zone are focused on the plane of the lens epithelial cells adjacent to the plaque, and hence, the upper region of the images where the edge of the plaque is evident (\*) is out of focus because of the greater thickness of the plaque. Lens epithelial cells at the non-plaque regions (Fig. 2G) were evenly distributed with more uniformly rounded nuclei, also more compact spacing (Fig. 2H), and little if any expression of alpha-smooth muscle actin (Fig. 2I). The control tissue of the non-cataractous patient displayed this same cell uniformity (Fig. 2J) to the non-plaque region of the anterior subcapsular cataractous tissue. Although the cell nuclei are a similar size and shape (Fig. 2K) compared to the non-plaque region (Fig. 2H), the control lens epithelial cells are more tightly spaced with higher cell density and exhibit no immunoreactivity for the myofibroblastic marker, alpha-smooth muscle actin (Fig. 2L).

## DISCUSSION

Atopic dermatitis is a chronic inflammatory skin disease characterized by erythematous plaques, intense pruritus, and cutaneous hypersensitivity to environmental triggers.<sup>7</sup> The prevalence of atopic dermatitis has been steadily increasing, with 10 to 20% of children and 1 and 3% of adults affected globally.<sup>7</sup> The incidence of cataract development in patients with atopic dermatitis has been reported from 5 to 38% with the age of onset ranging from adolescence into adulthood.<sup>7</sup>

The precise pathogenic mechanisms underlying cataract formation in atopic dermatitis remain elusive. It has been suggested that patients with atopic dermatitis have an intrinsic defect in their epithelial cells, resulting in defective barrier function and greater susceptibility to oxidative stress and inflammatory mediators such as interleukin-6, tumor necrosis factor, and transforming growth factor-beta.<sup>7</sup> Corticosteroid therapy has also been associated with cataract formation; however, this usually results in the posterior subcapsular type.<sup>7</sup> Interestingly, the incidence of cataract in corticosteroid-naïve atopic dermatitis patients was no different compared to patients with a history of both topical and systemic corticosteroid use, suggesting that the incidence of posterior subcapsular cataract formation in patients with atopic dermatitis cannot be explained by the use of corticosteroids alone.<sup>8</sup>

Evidence from animal research supports the role of epithelial-mesenchymal transition in the pathogenesis of anterior subcapsular cataract.<sup>9</sup> Epithelial-mesenchymal transition is a phenomenon whereby polarized epithelial cells undergo morphologic and molecular changes to give rise to elongate, motile, matrix-producing mesenchymal cells (Fig. 3A).<sup>10</sup> In the ocular lens, epithelial-mesenchymal transition drives lens epithelial cells to relinquish their cobblestone morphology and transdifferentiate into spindle-shaped myofibroblastic cells that elongate and migrate across the lens capsule leading to anterior subcapsular cataract (Fig. 3B).<sup>9</sup> During this process, lens epithelial cells lose their characteristic epithelial membrane markers, such as E-cadherin, and acquire mesenchymal markers such as alpha-smooth muscle actin and express aberrant extracellular matrix proteins.<sup>9</sup> Transforming growth factor-beta has been identified as a potent inducer of this epithelial-mesenchymal transition.<sup>9</sup>

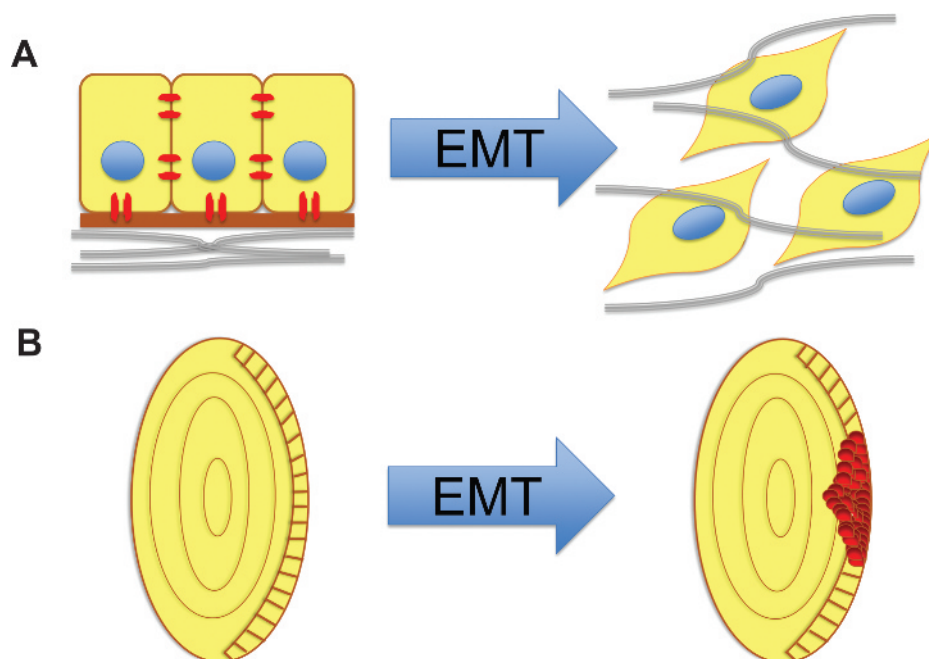


**FIGURE 3.**

Schematic diagram of epithelial-mesenchymal transition and anterior subcapsular cataract. During epithelial-mesenchymal transition (A), lens epithelial cells lose their polarity and normal cellular adhesions and transdifferentiate into spindle-shaped mesenchymal cells that secrete extracellular matrix. The normal human lens (B, left) consists of a monolayer of lens epithelial cells overlying a mass of fiber cells, all encased within a thick basement membrane, the lens capsule. Epithelial-mesenchymal transition also leads to anterior subcapsular cataract (B, right) with aberrant accumulation of mesenchymal cells and extracellular matrix (in red).

The present case reveals variations in cell density between plaque and non-plaque regions of the anterior subcapsular cataractous tissue, which supports the role of the epithelial-mesenchymal transition process in the pathogenesis of anterior subcapsular cataract. As lens epithelial cells undergo epithelial-mesenchymal transition, they appear to migrate towards the region of the plaque and, in doing so, lose their uniform arrangement and begin to flatten across a wider area before ultimately becoming incorporated into the fibrotic mass of multilayered cells and extracellular matrix at the plaque. It is not clear whether the expression of alpha-smooth muscle actin precedes, proceeds, or is concomitant with the cell shape changes observed (reflected in the change in shape of cell nuclei). Future studies will be aimed at elucidating the temporal changes in protein expression and correlating this to cell morphology by analyzing anterior subcapsular cataract specimens presenting at different stages of severity. This is one advantage of *in vivo* transgenic mouse models that exhibit anterior subcapsular cataract, as they enable the study of not only the induction but also the spatial and temporal progression of anterior subcapsular cataract in a reproducible manner. With this, we can more effectively translate to human anterior subcapsular cataract pathology.

This anterior subcapsular cataract case demonstrates many molecular and morphologic similarities to previous animal and human research involving transforming growth factor-beta-induced epithelial-mesenchymal transition in the lens. Treatment with transforming growth factor-beta resulted in myofibroblastic transdifferentiation and production of aberrant extracellular matrix in both rat<sup>9</sup> and human lens epithelial explant systems.<sup>11</sup> *In vivo* models including intravitreal injection of active transforming growth factor-beta into rodent eyes<sup>12</sup>, ectopic overexpression of mature transforming growth factor-beta-1 in transgenic mouse lenses,<sup>13</sup> and adenoviral gene delivery of transforming growth factor-beta into the mouse anterior chamber<sup>14</sup> have all induced anterior subcapsular cataract with characteristic epithelial-mesenchymal transition features. Transforming growth factor-beta has also been implicated in the pathogenesis of another fibrotic lens event, posterior capsular opacification that arises from the transdifferentiation of residual lens epithelial cells across the capsular bag after cataract surgery.<sup>11</sup> Accompanying this are elevated levels of active transforming growth factor-beta-2 and its receptors that have been detected in donor human postmortem capsular bag tissue.<sup>11</sup>

It has been postulated that anterior subcapsular cataract development in patients with atopic dermatitis may be directly related to constant trauma secondary to eye rubbing.<sup>7</sup> Both transforming growth factor-beta-1 and transforming growth factor-beta-2 isoforms are constitutively expressed by lens epithelial cells and exist in the ocular media in their latent, inactive forms.<sup>15</sup> After trauma, active levels of transforming growth factor-beta isoforms become dramatically elevated.<sup>16</sup> In a mouse lens injury model of anterior subcapsular cataract, capsular puncture has been found to upregulate the transforming growth factor-beta/Smad signaling pathway and induce myofibroblastic transdifferentiation of lens epithelial cells.<sup>16</sup> Smad proteins are intracellular molecules involved in transforming growth factor-beta signal transduction.<sup>9</sup> Once activated, transforming growth factor-beta receptors phosphorylate receptor-regulated Smad2 and Smad3 that associate with the common Smad (Smad4) to form a heteromeric complex.<sup>9</sup> This complex translocates into the nucleus to regulate transcription of transforming growth factor-beta-responsive genes.<sup>9</sup> Pretreatment with transforming growth factor-beta-neutralizing

antibodies before puncture wound injury inhibited the nuclear translocation of Smad3 and Smad4 and subsequent lens fibrosis.<sup>16</sup> Moreover, injury-induced epithelial-mesenchymal transition was inhibited in Smad3 knockout transgenic mice, supporting the role of Smad signaling in lens fibrosis.<sup>16</sup> However, some alpha-smooth muscle actin expression was still detected in lenses of Smad3 null mice overexpressing transforming growth factor-beta-1, suggesting that alternative transforming growth factor-beta-signaling pathways may be involved in this lens fibrosis.<sup>17</sup>

Although the Smad signaling pathway represents the canonical signaling pathway for transforming growth factor-beta, several non-Smad intracellular signaling cascades have been implicated in regulating epithelial-mesenchymal transition in the lens.<sup>9</sup> The mitogen-activated protein kinase pathways including extracellular-signal-regulated kinase, stress-activated p38, and Jun N-terminal Kinase are involved in transforming growth factor-beta-induced epithelial-mesenchymal transition in the lens.<sup>9</sup> Recently, Sprouty, an antagonist of mitogen-activated protein kinase, has been identified as a negative regulator of transforming growth factor-beta signaling in the lens.<sup>18</sup> Conditional knockout of Sprouty from the lens of mice resulted in anterior subcapsular cataract with subpopulations expressing alpha-smooth muscle actin and a concomitant loss of E-cadherin indicating loss of the epithelial phenotype.<sup>5</sup> Interestingly, overexpression of Sprouty in lens epithelial cells rendered the cells less responsive to transforming growth factor-beta signaling and maintained lens transparency.<sup>6</sup> Moreover, the Rho/Rho kinase pathway has been shown to relay transforming growth factor-beta signals leading to cytoskeletal reorganization, cell motility, and invasion in the lens.<sup>19</sup> Transforming growth factor-beta also signals through the mTOR<sup>20</sup> and the phosphoinositide 3-kinase/Akt pathway<sup>21</sup> to regulate cell growth inhibition and induction of epithelial-mesenchymal transition. Wnt signaling has also been found to promote transdifferentiation in the lens of transgenic mice overexpressing transforming growth factor-beta-1 with increased nuclear  $\beta$ -catenin detected in anterior subcapsular cataract plaques.<sup>22</sup> More research is required to characterize the signaling pathways involved in transforming growth factor-beta-induced epithelial-mesenchymal transition to develop potential therapeutic agents for the prevention of anterior subcapsular cataract and posterior capsular opacification.

It is interesting to note that elevated levels of transforming growth factor-beta have been implicated in other ocular pathologies, including corneal opacification, proliferative vitreoretinopathy, and primary open angle glaucoma.<sup>23</sup> However, these pathologies are not typically associated with a corresponding anterior subcapsular cataract. This may be because the lens normally has protective mechanisms such as Sprouty that may prevent the initiation of the epithelial-mesenchymal transition process, even if transforming growth factor-beta signals are transduced in surrounding ocular tissues.<sup>5</sup> It is likely that a dysregulation of such protective mechanisms within the lens may render it susceptible to undergoing epithelial-mesenchymal transition with resultant anterior subcapsular cataract.

In the present case, cells of the human anterior subcapsular cataract plaque were strongly immunoreactive for alpha-smooth muscle actin. Although alpha-smooth muscle actin is an established myofibroblastic marker,<sup>10</sup> its role in lens fibrosis seems to conflict in animal and human studies. In bovine and rat lens epithelial-mesenchymal transition models, the expression of alpha-smooth

muscle actin has been found to correlate with increased matrix contraction and consequent capsular wrinkling.<sup>9</sup> In contrast, Dawes et al. found that alpha-smooth muscle actin expression did not correlate with matrix contractility and, surprisingly, alpha-smooth muscle actin knockdown using small interfering RNA technology enhanced matrix contraction.<sup>24</sup> This species difference should be an important consideration when modeling human disease and evaluating putative therapeutic agents. Future studies should be aimed at further elucidating the functional differences in alpha-smooth muscle actin between species and characterizing the proteins involved in matrix contractility in various experimental systems.

With all case reports, we are hindered by the limited tissue and hence restricted in making major conclusions relating to the general molecular and morphologic phenotype of human anterior subcapsular cataract. The use of immunolabeling of a wholemount anterior subcapsular cataract proved beneficial in examining a large surface area to obtain valuable information regarding regional diversity between the plaque and surrounding intact lens epithelia; however, this manipulation was detrimental to the structural integrity of the anterior subcapsular cataractous tissue, and we were reluctant to further characterize the anterior subcapsular cataract histologically by subsequently embedding and sectioning tissue that may not be reflective of its native state. This will be taken into account for future studies with the objective of correlating both wholemount and corresponding sectioned material for further analysis. Although the use of a non-cataractous tissue was important in establishing the validity of the alpha-smooth muscle actin antibody, it should be noted that although the lens epithelial cells appear similar in appearance, the control specimen is from a much older patient because of the limited availability of “normal” lens tissue from younger age-matched patients.

## CONCLUSIONS

The process of epithelial-mesenchymal transition is complex involving not only morphological and cytoskeletal reorganization but also extensive biochemical and genetic changes. Here, we highlight some of these morphologic and molecular traits accompanying epithelial-mesenchymal transition in human anterior subcapsular cataract, traits that have also been well characterized in animal models of epithelial-mesenchymal transition-driven cataract. Although we have shown some of this cellular heterogeneity, further characterization of human anterior subcapsular cataract cases is required in patients with atopic dermatitis. By translating the morphologic and molecular events mediating epithelial-mesenchymal transition from animal research to the human lens, novel pharmaceutical alternatives can be developed for the treatment of anterior subcapsular cataract and potentially posterior capsular opacification.

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