

## Lens Epithelial Cell Differentiation in the Anterior Capsular Plaque Obtained from a Patient with Radiation-Induced Cataract

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### Abstract

We report a case of a 63-year-old male who was presented with hypermature cataract in the left eye. History revealed that he was suffering from left-sided oral cancer for which he had received radiation therapy before six months. While performing cataract surgery, the presence of anterior capsular plaque (ACP) was noted. The ACP was processed for the localization of markers for cell proliferation and differentiation. The ACP consisted of extracellular matrix positive to collagen type I, fibronectin and  $\beta$ -crystallin indicative of abnormal differentiation of lens epithelial cells. Many cells of plaque were positive to PCNA suggesting high proliferation activity.

**Keywords:** Radiation Cataract; Anterior Capsular Plaque; Oral Cancer; Radiation Therapy; Crystalline Lens; Lens Epithelial Cells

### Introduction

The eye is the second most susceptible organ to the radiation after the skin [1]. During the radiation therapy of tumours located in the head region, eyes are exposed and cornea and the crystallin lens gets most affected [2]. There is no report of lens capsular plaque associated with the radiation-induced cataract. In the present case report, we have evaluated proliferation and differentiation status of the cells of anterior capsular plaque (ACP) obtained from radiation induced cataract.

### Case History

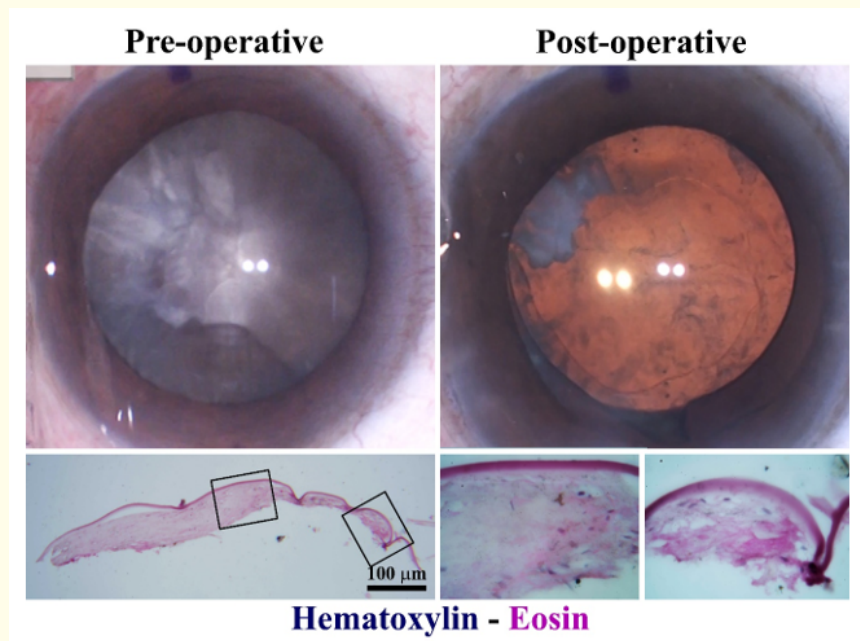
A 63-year-old diabetic male patient was presented with complaints of blurring of vision in both eyes. His best-corrected visual acuity was 20/200 in the right eye and hand movement in the left eye. Anterior segment examination of the right eye showed an immature senile cataract and the left eye showed the presence of hypermature cataract along with anterior capsular plaque (ACP). Intraocular pressure (IOP) was 12 mmHg in both eyes. The posterior segment examination of RE was unremarkable. B-scan of the left eye revealed a well-attached retina with clear vitreous.

The detailed report of his cancer and radiation therapy was obtained from the Institute where he underwent treatment. He was suffering from carcinoma of left tongue and buccal mucosa, Stage 1. He received a single field external radiation therapy as a curative treatment in a form of 30 cycles of 200Sv radiation for 1.88 seconds for 30 days. Hence the total radiation given was 6000Sv. Soon after radiotherapy, he noticed that his left eye vision is deteriorating which has rapidly reduced over the last four months.

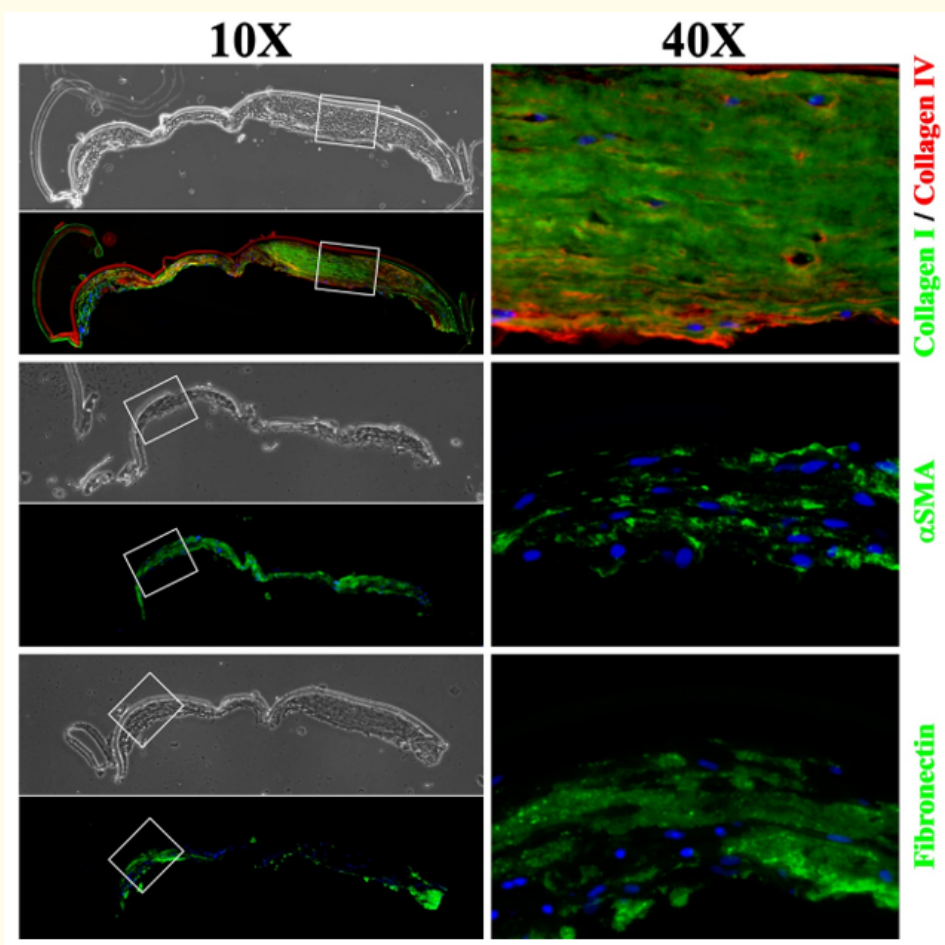
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He was advised to undergo cataract surgery using standard phacoemulsification with implantation of Toric IOL in the left eye under guarded visual prognosis. Cataract extraction was performed with a standard phacoemulsification technique using a 2.2 mm incision. The ACP was differentiated using trypan blue staining. The ACP was cut with the help of microscissors and rhexis was completed from the opposite side with the help of forceps. The anterior capsule along with harboring ACP was collected in phosphate buffer saline (PBS). The ACP was immediately fixed in 2% paraformaldehyde in PBS for 2 hours and then processed to obtain 5 µm thick paraffin sections. Sections were mounted on silane-coated slides and deparaffinized, hydrated and washed with PBS. Some sections were processed for standard hematoxylin-eosin staining. For immunofluorescence studies, sections were stained for collagen type I, collagen type IV, αSMA, β-crystallin, fibronectin and PCNA using appropriate dilution of antibodies [3]. Sections were then incubated in the second antibody tagged with AlexaFluor 488 and AlexaFluor 546 containing 0.1 µm DAPI. Sections were then mounted in a polyvinyl alcohol-glycerol mounting medium containing 2.5% diazobicyclo-octane (DABCO). The sections were observed under an epifluorescence microscope (Axioskope II; Carl Zeiss, Germany) and images were taken with a cooled CCD camera (Cohu, San Diego, CA, USA). The specimen was examined according to the suggestions of the institutional ethics committee and was according to the tenets of the Declaration of Helsinki. Informed consent was obtained from the subjects.

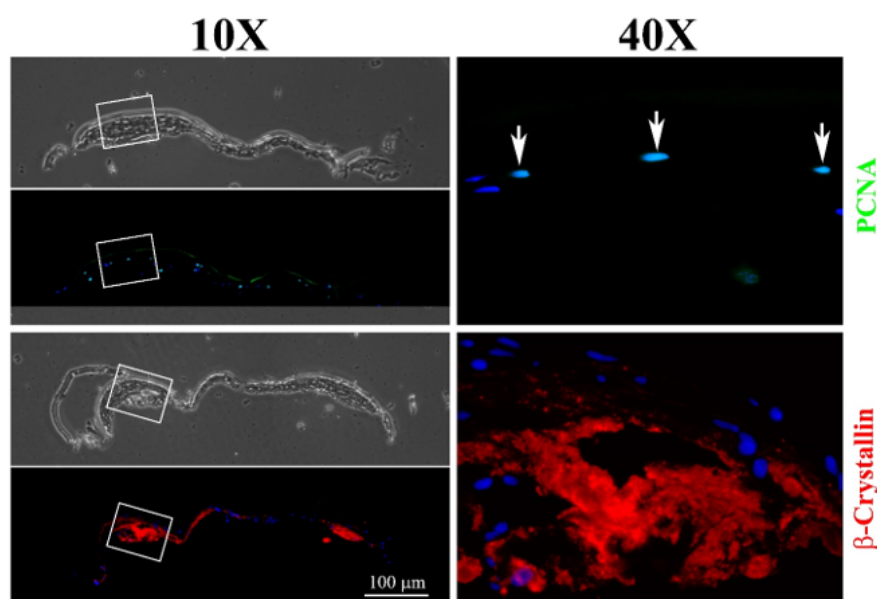
The ACP was well demarcated, multifocal, white, opaque and consisted of multiple lobes located underneath the lens capsule. The ACP mainly consisted of ECM with few scattered cells. The nuclei of the cells were spindle-shaped and elongated. The ECM was both fibrous and amorphous. The fibrous ECM consisted of bundles arranged like a ribbon (Figure 1). Under the fluorescence microscope, these fibers appeared to be made of collagen type I (Figure 2). The ECM stained almost entirely for collagen type I (Figure 2). The collagen type IV was present in the lens capsule but was also found in the ECM (Figure 2). Besides collagen, ECM also contained fibronectin (Figure 2). The cells of plaque were also positive to αSMA (Figure 2). The ECM located towards the lens fibers side and the periphery of plaque stained strongly for β-crystallin (Figure 3). Almost half of the cells of plaque were positive to PCNA (Figure 3).



**Figure 1:** Morphology of radiation-induced adult anterior capsular plaque. Hematoxylin-eosin (HE) and Periodic acid Schiff-hematoxylin (Pas-H) stained sections of plaque. The plaque consisted of a large amount of extracellular matrix (ECM) and few cells. ECM was in a form of bundles arranged parallel to lens capsule. These bundles were less eosinophilic but stained heavily with PAS. Cells suspended in a bundle for ECM were spindle-shaped. Bar = 100 µm.



**Figure 2:** Expression of trans-differentiation markers in the radiation-induced adult anterior capsular plaque. The plaques consisted of an extracellular matrix chiefly composed of collagen I. Collagen IV was present around the cells and in the lens capsule. Most of the cells of plaque were positive to αSMA. Fibronectin was mainly present in the extracellular matrix of plaque. Nuclei were counterstained with DAPI. Bar = 100 μm.



**Figure 3:** Expression of proliferation and fiber cell differentiation markers in the radiation-induced adult anterior capsular plaque. PCNA was selected as a proliferation marker and plaque contained many PCNA positive cells (arrow). β-crystallin is a marker of fiber cell differentiation. Many areas of plaque particularly the peripheral regions were strongly positive to β-crystallin. Nuclei were counterstained with DAPI. Bar = 100 μm.

## Discussion and Conclusion

The presence of collagen I and fibronectin in the ECM of plaque and  $\alpha$ SMA in the cells is indicative of the epithelial-mesenchyme transition (EMT) of the LECs [4-7]. Many cells in the ACP were also positive to the PCNA, which is a well-established marker of cell proliferation [8]. We have also noted the presence of  $\beta$ -crystallin, which is a marker of fiber cell differentiation [9]. Taken together, the results suggest that radiation-induced ACP forms due to high proliferation and abnormal differentiation of the LECs [3].

Radio-sensitivity is highest in cells which are highly mitotic or undifferentiated such as the lens epithelial cells [10]. Development of cataract can occur with doses less than 10 Gy [11]. ICRP recommendation regarding threshold dose values of ionizing radiation for damage to eyes is 0.5 - 2 Sv SI Unit or 50 - 200 REM Conventional Unit (CU) for detectable opacities and is 5 Sv SI Unit or 500 REM CU for visual impairment (cataract). Whereas for highly fractionated or protracted radiation exposure, the threshold value is > 8 Sv SI Unit or 800 REM CU for cataract formation [12]. Our patient received up to 200 Gy SI Units of radiation therapy which is 25 times more than the recommended threshold dose as per ICRP guidelines. The radiation-exposed patients of head and neck cancers need to be counseled for regular periodic ophthalmological examination and surgeons should watch for the presence of ACP during surgery.

## Key Messages

- Radiation exposure may leads to the development of anterior capsular plaque associated with cataract.
- Anterior capsular plaque results due to high proliferation and abnormal differentiation of lens epithelial cells.

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