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IMMUNOHISTOCHEMICAL STUDY OF SECONDARY CATARACTS USING ANTIBODIES TO CYTOSKELETAL ELEMENTS, THE HNK-1 EPITOPE AND MACROPHAGES

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Purpose To study the cytoskeletal elements and the presence of macrophages and the cell-adhesion related HNK-1 epitope in secondary cataracts after extracapsular cataract extraction and IOL implantation.
Methods Twenty-five formalin-fixed and paraffin-embedded pseudophakic human eyes obtained at autopsy were studied with 7 monoclonal antibodies (MAbs) to vimentin, cytokeratins 8 and 18, desmin, α-smooth muscle actin and to the HNK-1 epitope. Additionally, a MAb was used to detect macrophages. The specimens were immunostained using the avidin-biotinylated peroxidase complex (ABC) method and studied by light microscopy.
Results MAb Vim 3B4 to vimentin immunolabeled spindle-shaped cells in the secondary cataract in 17/18 eyes. Immunoreactive spindle-shaped cells were also found with MAb CAM5.2 to cytokeratin 8 in 14/19 secondary cataracts, but with MAB CY-90 to cytokeratin 18 only in 1/19 secondary cataracts. No immunoreaction was seen with MAb D33 to desmin, whereas MAb 1A4 to α-smooth muscle actin immunolabelled spindle-shaped cells in 16/19 secondary cataracts. A granular immunoreaction with MAbs HNK-1 and NC-1 to the HNK-1 epitope was present within the secondary cataract adherent to the lens capsule in all cases. This immunoreaction was attributed to the extracellular matrix. MAb PG-M1 revealed few round cells representing macrophages in 8/19 secondary cataracts, and granular immunoreaction representing disrupted macrophages was seen in additional 5 eyes. The lens capsule was not labeled with any of the MAbs used.
Conclusions Immunoreaction for vimentin, cytokeratin 8 and α-smooth muscle actin revealed spindle-shaped cells in the secondary cataract of the human eye. Based on their antigenic profile, these cells are myoepithelial in nature and probably represent metaplastic lens epithelial cells.

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ANTIPROLIFERATIVE EFFECT OF ACLACINOMYCIN A ON PORCINE LENS EPITHELIAL CELLS IN VITRO

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Purpose: Capsular opacification is the most common late complication of uncomplicated extracapsular cataract extraction. Proliferation of lens epithelium on the posterior capsule occurs in about 50% of adults after 3-5 years and extremely often in children. Aclacinomycin A (ACA) is an antiproliferative agent that has been used in the treatment of acute myeloic leucemia for many years. We wanted to investigate whether a single exposure of ACA to porcine lens epithelial cells could be sufficient to inhibit longterm cell proliferation.
Methods: Primary cultures were started with cells separated from the anterior lens capsule of pig eyes. In 2 experimental set-ups (E1 and E2) cells of second passage were exposed to different concentrations of ACA for 5 min. (E1: 2, 4, 8, and 12 µg/ml; E2: 0.5, 1, 1.5, and 2µg/ml). In E1 cells were counted 19 days after treatment, in E2 1, 2, 3, and 4 weeks after exposure (5 flasks for each concentration and duration of culture). Data were analyzed using the Tukey's Studentized Range (HSD) Test.
Results: E1: logarithms of cell numbers were inversely correlated to drug concentration. No cells treated with 12µg/ml survived 19 days. Already 2µg/ml reduced the cell number significantly to about 10% of those of control cultures. E2: After 1 week, cell numbers were already significantly reduced ($p=0.05$) compared to control cultures for all concentrations tested. Within the next two weeks there was a further albeit insignificant decrease in cell number in all cultures treated with ACA. Control cultures reached a 1.5fold increase within this time. After 4 weeks, however, cell counts were significantly higher ($p=0.05$) than after 3 weeks in cultures treated with 0.5,1, and 2µg/ml, those incubated with 0.5µg/ml even exceeding the number of originally seeded cells.
Conclusion: A single exposure to ACA for 5 min. at 1µg/ml appears sufficient to markedly reduce the number of lens epithelial cells in an in vitro-system and to inhibit cell proliferation for at least 3 weeks. Thereafter, however, re-proliferation possibly occurs. A treatment with 12µg/ml for 5 min. may be lethal for all cells in culture.

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PHOSPHORYLATION OF HSP 25 DURING LENS CELL DIFFERENTIATION.

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Purpose: The patterns of phosphorylation and dephosphorylation of α-crystallins A and B change during lens cell differentiation. Phosphorylated forms of both polypeptides are significantly more abundant in differentiated fiber cells than in their parent epithelial cells. α-Crystallins share several biological properties with the small heat shock proteins (Hsps). They have homologus amino acid sequences, similar stress induced expression pattern and similar chaperone-like properties. Furthermore, α-crystallins and Hsps are phosphorylated *in vivo* at sites with homologous amino acid sequences. To ascertain whether small Hsps undergo phosphorylation in the lens cells during differentiation, a comparative analysis of the Hsp 25 phosphorylation pattern in epithelial and fiber cells was undertaken.
Methods: Analysis of phosphorylated and non-phosphorylated forms of Hsp 25 was carried out in cell extracts from rat lens epithelium and cortex by isoelectric focusing and Western blot using an antibody specific for the recombinant rat protein. The phosphorylated forms were identified by their isoelectric points and the characteristic shift upon *in vitro* dephosphorylation with phosphoprotein phosphatase 2B (PP2B).
Results: Non-phosphorylated Hsp 25 and two phosphorylated forms were detected in epithelial and fiber cells extracts. The phosphorylated forms were present at significanty higher concentration in the fiber cell extracts. Phosphorylated Hsp25 was sensitive to dephosphorylation by PP2B in both cell extracts but the dephosphorylation rate was remarkably slower in the fiber cell extracts.
Conclusions: The results demonstrate that Hsp 25 is phosphorylated in the lens in vivo, where it occurs at least in two phosphorylated forms. The phosphorylation state of Hsp 25 changes with lens cell differentiation, resulting in a relative increase in phosphorylated forms in the fiber cells. This suggests that Hsp 25 phosphorylation may be important in lens cell differentiation.

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RABBIT AND MONKEY LENS EPITHELIAL CELL (LEC) PROLIFERATION *IN SITU* AFTER LENS EXTRACTION

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Purpose: LEC proliferation is the main cause of posterior capsule opacification (PCO) after cataract surgery. The thymidine analogue bromodeoxyuridine (BrdU) was used to determine at which time after surgery rabbit and monkey LEC were most active and preparing for cell division by entering the S-phase.
Methods: Extracapsular lens extraction was performed in NZW rabbits and *Macaca fascicularis* monkeys. At various times from 8 hours to 2 months after surgery, the rabbits received an i.p. injection of BrdU. After additionally 2 hours the animals were killed and the eyes were enucleated. The number of monkey LEC in S-phase were evaluated in the same manner at 1, 3, 5, 8 and 13 days after surgery and these animals were sacrificed three hours after BrdU injection. All eyes were prepared for immunohistochemical analysis of S-phase incorporated BrdU.
Results: The relative number of BrdU positive rabbit LEC nuclei peaked at day 1-2 with 30 and 20 % labelled cells respectively. The percentage of labelled cells declined to 1 % at day 7, and stayed at this low level throughout the two-month study. Monkey LEC had a lower rate of proliferating cells with a maximal BrdU incorporation of 3 % at day 5 with a less defined peak of cells in S-phase.
Conclusions: Although PCO in the rabbit appears months after surgery, rabbit LEC proliferation is a surprisingly early event with most of the cells in S-phase the first days after surgery. However, in the monkey eyes, the longer lag-phase and the fewer cells preparing for cell division agree with the slower development of PCO in a primate eye.