The Ovine Lens Cytoskeleton

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Joshua D. McDermott

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Joshua D. McDermott

The lens of the eye is a vital tissue in the visual system, responsible for the collection and focusing of light on to the retina. Comprised of epithelial cells at differing stages of differentiation, the transparency of the lens is dependent on the highly ordered crystalline structure of lens proteins. The lens consists of several proteins including crystallins (α, β, γ) that make up 90% of the soluble protein, and the lens cytoskeletal proteins. Cytoskeletal proteins contribute only a fraction of the total lens protein, but are thought to play an important role in the establishment and maintenance of transparency. Calpain-induced degradation of these proteins may be involved in the development of cataracts. This has been an area of research at Lincoln University where a flock of sheep genetically predisposed to cataract maintained as a cataract development model.

The aim of this research was to investigate the distribution of cytoskeletal proteins in the lens, and to examine the effects of calpain proteolysis on these proteins, with the goal of establishing the role of the lens cytoskeletal proteins in the ovine cataract model. A combination of techniques was used including immunohistochemistry, which required the development of a specific protocol for ovine lenses. Cytoskeletal proteins were identified using immunohistochemistry in lens tissue sections and exhibited characteristic distributions. Actin displayed preferential distribution in the short sides of the fibre cells in the cortex of the lens but was absent in the lens nucleus, while spectrin in the cortex and nucleus was associated with the fibre cell membrane. Filensin was observed in the outer cortex of lens sections associated with the fibre cell membrane and cytoplasm, although the pattern of localisation was indistinct due to the abundance of filensin breakdown products. Vimentin displayed membrane and cytoplasmic association in the outer cortex that diminished toward the lens nucleus, with membrane associated vimentin only persisting in the deeper regions of the cortex and nucleus.

Additionally, the effect of novel calpain inhibitors (*Cat0059* and *Cat811*) in preventing proteolysis of lens cytoskeletal protein was investigated and compared with calpain inhibitors developed elsewhere (*SJA6017*). The inhibitors were tested at between 10 and 0.1 μM (100 nM). All inhibitors were effective at 10 μM. *SJA6017* provided significant protection to vimentin at 1 μM. *Cat0059* was found to protect spectrin and filensin at 1 μM, but not vimentin, while inhibitor *Cat811* was found to protect spectrin only. *SJA6017* added to assays at 100 nM offered significant protection to spectrin, and *Cat0059* was found to protect filensin and spectrin to a significant degree at 100 nM, indicating the novel inhibitors were comparable to those developed elsewhere in terms of their effectiveness.

Taken together, the evidence presented in this thesis shows the cytoskeletal proteins as crucial elements in the lens. Their pervasive presence coupled with evidence that lens cytoskeletal proteins are sensitive to calpain-induced proteolysis that is inhibited with novel calpain inhibitors suggests that the lens cytoskeletal proteins may be useful targets in cataract prevention for future research.

Keywords: Lens, immunohistochemistry, cytoskeleton, actin, spectrin, vimentin, filensin, fluorescence, microscopy, calpain, proteolysis, inhibitor, cataract.

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ABBREVIATIONS

ATA	aurintricarboxylic acid
AQP-	aquaporin
BCA	bicinchoninic acid
Bfsp2	human CP49 gene
CP49	phakinin
CP115	filensin
Cx-	connexion
DTT	dithiothreitol
E64	trans-epoxysuccinyl-L-leucylamido-4-guanidino-butane
F-actin	filamentous actin
FITC-lectin	fluorescein isothiocyanate conjugated lectin
g	gravity
G-actin	globular actin
IgG	immunoglobulin G
IHC	immunohistochemistry
LBCF	lens based cell free

Lectin	*Triticum vulgaris* lectin
LDS	lithium dodecyl sulphate
Lp-	lens specific calpain isoforms
MDa	mega Dalton
MeOH	methanol
MIP	major intrinsic protein
NFDM	non-fat dairy milk
O.C.T.	optimal cutting temperature
O.D.	optical density
PBS	phosphate-buffered saline
PI	propidium iodide
PFA	paraformaldehyde
R^2	coefficient of determination
SJA6017	*N*-[4-fluorophenylsulfonyl]-L-valyl-L-leucinal
TBS	tris-buffered saline
TTBS	tween-20/tris-buffered saline
Tris-HCl	tris-hydrochloride

TRITC	tetramethylrhodamine isothiocyanate
TRITC-lectin	tetramethylrhodamine isothiocyanate conjugated lectin
Tween-20	polyoxyethylene (20)sorbitan monolaurate
USF	urea soluble fraction
v/v	volume per volume
w/v	weight per volume