SCIENTIFIC REPORT

Neoplastic transformation of ciliary body epithelium is associated with loss of opticin expression

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Background: Opticin is a recently discovered glycoprotein present predominantly in the vitreous humour. It is synthesised and secreted by the ciliary body epithelium (CBE) from the initiation of CBE development in the embryo, and production continues throughout life.

Aim: To determine whether a variety of ciliary body tumours synthesise opticin to characterise further its role in ciliary body health and disease.

Methods: Immunohistochemistry was used to determine the distribution of opticin in normal human CBE, and in hyperplastic and neoplastic CBE lesions.

Results: Opticin was immunolocalised to the basal cell surface and basement membrane material of the non-pigmented CBE in nine donor eyes as well as four hyperplastic lesions of the CBE (Fuchs's adenoma). By contrast, none of eight neoplastic lesions (two adenoma and six adenocarcinoma) of CBE stained for opticin.

Conclusion: The present series supports the theory that opticin is produced by the non-pigmented CBE throughout adult life. Loss of opticin expression by this tissue is associated with and could contribute towards neoplastic transformation.

Opticin is a member of the small leucine-rich repeat proteoglycan/protein (SLRP) family of extracellular matrix molecules.¹ Various functions have been ascribed to the SLRP family, including regulation of matrix assembly, binding to growth factors and modulation of cellular activities including suppression of growth of neoplastic cells.^{2–3} Opticin was first identified in the vitreous humour of the eye¹ and seems to be expressed predominantly in the eye.^{4–6} Opticin associates with vitreous collagen fibrils and may, by binding heparan sulphate proteoglycans of the inner limiting lamina, be involved in vitreoretinal adhesion.⁷ Opticin may also bind growth hormone and thereby regulate its activities in the eye.⁸

Immunolocalisation of opticin in the adult human eye,⁹ as well as in situ hybridisation of mRNA in both developing and adult mouse eyes and adult human eye,^{5–10} have shown that the non-pigmented ciliary body epithelium (CBE) produces opticin. As opticin expression is first detected at the time of initiation of ciliary body development and this expression then localises to the CBE where it continues at a high level throughout adult life, opticin may represent a marker for ciliary body and CBE differentiation.^{5–10}

To investigate the possible association between opticin and CBE differentiation, and to further characterise the place of the protein in ciliary body health and disease, we used immunohistochemistry to assess the presence of opticin associated with a variety of ciliary body tumours, ranging from hyperplasias to malignant neoplasms.

MATERIALS AND METHODS

Specimens

Specimens of 12 ciliary body tumours, diagnosed after surgical excision (n = 8) or enucleation (n = 4) were identified during a

retrospective audit of the ocular pathology archive at the Royal Liverpool University Hospital, Liverpool, UK. The patients were aged 7–81 years. Seven were female and five were male. Anonymised, coded sections were prepared for immunohistochemical investigation in accordance with, and with the approval of, the Liverpool Research Ethics Committee. Histopathological examination of the 12 tumours showed that 4 were non-neoplastic pseudoadenomatous hyperplasia of the CBE (Fuchs's adenomas). The other eight were neoplastic. Two of these were adenomas of the CBE, four were adenocarcinomas of non-pigmented or mixed CBE and two were adenocarcinomas of pigmented epithelium. In addition, nine normal human donor eyes (age range 17–95 years), obtained from the local eye bank, were assessed to confirm that opticin associated with the normal ciliary body.

Antiserum

OPT-A antiserum, as used and characterised by Ramesh *et al.*,⁹ was used for immunohistochemistry. This is a polyclonal rabbit antiserum raised against a synthetic peptide (VLNPDNYGEVIDLSNYYEELTDYGDQLPEVK) corresponding to an opticin-specific sequence in the amino terminal region of human opticin.

Immunohistochemistry

Paraffin-wax-embedded sections, 5-µm thick, were used for analysis. They were first dewaxed and hydrated through xylene and graded alcohols to water. Sections were incubated for 10 min in serum-free protein block (Dakocytomation, Glostrup, Denmark), to reduce non-specific binding. They were incubated for 1 h at room temperature with either OPT-A antiserum, diluted 1:10 000 in TRIS-buffered saline, or diluted immunoglobulin fraction of normal rabbit serum (Dakocytomation), as a negative control. Sections were thoroughly washed in TRIS-buffered saline before incubation for 30 min with polymer-alkaline phosphatase, the secondary antibody in the EnvisionAP kit (Dakocytomation). After a further wash, specimens were stained with fast red chromogen from the same kit, to which levamisole was added, to a final concentration of approximately 0.2 mmol/l to counteract endogenous alkaline phosphatase activity. Sections were counterstained with haematoxylin and then mounted with an aqueous mount. In grading the opticin immunoreactivity in each tumour, the section stained for opticin was compared both with its negative control and with positive staining in adjacent normal tissue or in the eyes stained for comparison. Each was recorded as ++ staining for maximum immunoreactivity (at the level of intensity of staining in positive control tissue), as – staining for the same as background and + staining for immunoreactivity between control and background. These assessments were made by two observers (DCA and PSH) independently and subsequently averaged by adding the scores and dividing by two, as described previously.¹¹

Abbreviations: CBE, ciliary body epithelium; SLRP, small leucine-rich repeat proteoglycan/protein

Table 1 Number of specimens staining for opticin, according to nature of lesion

Strength of stain	Non-pigmented CBE (n = 9)	Hyperplasia (Fuchs's adenoma) (n = 4)	Adenoma (n = 2)	Non-pigmented/mixed adenocarcinoma (n = 4)	Pigmented adenocarcinoma (n = 2)
++	9	4			
+			2	4	2
-					

++, staining for maximum immunoreactivity; +, staining for immunoreactivity between control and background; -, staining for the same as background CBE, ciliary body epithelium.

RESULTS

Consistent with the findings of Ramesh *et al.*,⁹ immunoreactivity to the opticin antiserum was observed most intensely in the vitreous base and along the retinal internal limiting membrane. It was consistently found in a dense layer covering the basal surface of the non-pigmented CBE of the ciliary body. It was not similarly observed at the basal surface of the pigmented iris

epithelium or pigmented ciliary epithelium. In addition, it was found in the walls of some retinal blood vessels, along the posterior surface of the lens, and there was less intense staining of ciliary body and iris stroma, similar to that reported previously.¹² No staining was seen in other ocular tissues. Replacement of the primary antibody with non-immune rabbit serum abolished the staining.

Figure 1 (A) Immunolocalisation of opticin in pseudoadenomatous hyperplasia of the ciliary body epithelium (CBE). Note areas of red staining between the blue counterstained epithelial cells. (B) Negative control for (A), using non-immune serum. (C) Haematoxylin and eosin-stained section showing the morphology of the lesion stained in (A). (D) Lack of immunoreactivity for opticin in neoplastic adenoma of ciliary epithelium. Note localisation for opticin on the basal surface of adjacent normal non-pigmented CBE. (E) Negative control for (D). (F) Haematoxylin and eosin-stained section showing the morphology of the lesion stained in (D).

Table 1 shows the intensity of opticin staining for the specimens included in the series. They have been grouped according to the nature of each lesion. As can be seen, pseudoadenomatous hyperplasia of the CBE (Fuchs's adenomas) consistently showed moderate immunoreactivity for opticin. The staining was localised to the basal cell surface and within the adjacent basement membrane material produced by these cells (fig 1A–C). Conversely, all the neoplastic lesions in the series failed to show staining. Figure 1D–F shows an example of a neoplastic ciliary adenoma, where, in contrast with the labelled adjacent normal epithelium, the tumour shows no immunoreactivity.

DISCUSSION

In this study, we confirm the localisation of opticin, as reported previously,⁹ on the basal surface of non-pigmented CBE. We have also shown the presence of opticin in basement membrane material between proliferating epithelial cells in pseudoadenomatous hyperplasia of the CBE (Fuchs's adenomas). Conversely, within the limits of immunohistochemical localisation, we found no opticin in any of the eight neoplastic lesions of CBE. These included adenomas and adenocarcinomas, as well as both non-pigmented and pigmented lesions. This is, however, a small series, and further studies may show other neoplasms that are positive for opticin.

Takanosu *et al*⁸ have shown the expression of opticin mRNA in the mouse embryo in the region of the presumptive ciliary body, at a stage earlier than ciliary body differentiation can be distinguished morphologically. They suggest that opticin mRNA may represent a marker for ciliary body differentiation. In the adult mouse, they found such expression confined to non-pigmented CBE. In our study in human eyes, the localisation of opticin on the basal surface of the non-pigmented CBE and in the vitreous from eyes of a wide age range shows production throughout life. It is not therefore surprising that the hyperplastic CBE lesions in adults, which retain a differentiated state, continue to produce opticin.

Our results suggest that opticin expression may be lost in association with neoplastic transformation of the CBE. It is interesting to note that other members of the SLRP family have been shown to retard the growth of a wide variety of tumour cells. Much work, particularly on animal models and in vitro studies, has shown the potential for decorin to inhibit tumour growth. Reed *et al*¹³ have recently shown a marked reduction in breast cancer growth and metastasis after injection of decorin protein core into rats with a breast carcinoma xenograft. They had similar results using an adenoviral vector containing the decorin transgene. Previous studies have shown the ability of decorin to inhibit other tumours, including colon and squamous carcinoma,¹⁴ lung carcinoma, ovarian cancer and glioma.¹⁵ Investigations into the mechanism for this anti-oncogenic effect have shown that decorin can bind to and inhibit transforming growth factor- β , while up regulating p21 expression (through activation of the epidermal growth factor receptor) and leading to cell cycle arrest.¹⁶ It has also been shown to interfere with ErbB2 activity.¹⁷ Clinically, Troup *et al*¹⁸ showed an association between reduced expression of decorin and poor outcome in invasive breast cancer. Interestingly, they showed the same association for another of the SLRPs, lumican. Vuilleumoz *et al*¹⁹ have shown inhibition of melanoma progression by lumican. Other members of the SLRP family may therefore share the anti-oncogenic role of decorin. We hypothesise that opticin could act to inhibit tumour growth and that loss of opticin expression by non-pigmented CBE may itself be permissive to neoplasia.

Decorin has been shown to inhibit angiogenesis.²⁰ As opticin is concentrated in the vitreous, which is an avascular tissue, it is tempting to speculate that opticin may also play an anti-angiogenic part and contribute to keeping the vitreous free from blood vessels.

In conclusion, our series lends support to the theory that opticin is produced by the non-pigmented CBE throughout adult life. Loss of opticin expression by this tissue may be associated with neoplastic transformation, or vice versa. It is emerging that other members of the SLRP family may be important in regulation of cellular proliferation and cancer progression.²⁰ Whether opticin also has an effect on this process warrants further investigations which may lead to a better understanding of the pathobiology and management of eye diseases that depend on uncontrolled cell proliferation.

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Competing interests: None.

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