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Immunohistochemical Localization of MUC 1 Glycoprotein in the Retina

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Glycoconjugates on the photoreceptor cell surfaces and in the interphotoreceptor matrix (IPM) are involved in a variety of cell functions. The O-linked glycoconjugates, seen in the filaments of IPM by electron microscopy,[1] may connect the neural retina with the retinal pigment epithelium (RPE) in the subretinal space.[2] These O-linked glycoconjugates are found on the surfaces of both cone and rod photoreceptors except for the basal one-fourth of the inner segments, about the height of Müller cell fiber baskets.[3] These observations led us to speculate that O-linked glycoconjugates in the IPM may link the RPE-processes to the Müller cell fiber baskets, on which some acceptors for glycoconjugates may be present.

MUC 1 glycoprotein, the first mucin to be cloned, is localized on the apical surfaces of epithelial cells as a transmembrane, polymorphic glycoprotein.[4] We recently examined the immunohistochemical distribution of MUC 1 in various tumors of the eyelid, orbit, and eyeball.[5] During examination of the eyeball tumors, we noted a preferential distribution of MUC 1 in the retinal region that was free of tumor cells. We report this finding because MUC 1 appears to be one of the acceptors on Müller cell fibers for the O-linked glycoconjugates in the IPM.

One eyeball with retinoblastoma and two eyeballs with choroidal melanoma were enucleated and histopathologically examined; immunohistochemical examination of MUC 1 was done on the tumor-free retinal areas. The eyeballs were immersion fixed with buffer formalin at 4°C overnight, rinsed in Dulbecco's phosphate buffered saline (PBS) at 4°C overnight, dehydrated in an ethanol series, and embedded in paraffin. Five-micrometer sections of the specimens were cut and mounted on silane-coated glass slides. The sections were deparaffinized in xylene and hydrated in a graded series of ethanol. The sections were then immersed in 0.01 mol sodium citrate buffer (pH 6.0) and boiled for 4 minutes in a pressure cooker to unmask antigens, following the protocol of Novocastra Laboratories (Newcastle, UK). Endogenous peroxidase activity was blocked by treatment with 0.2% H_2O_2 in methanol for 30 minutes. Nonspecific binding was blocked by incubating the sections with 3% horse serum (diluted with PBS; Sigma) for 30 minutes at room temperature. The tissue sections were then overlaid with a monoclonal antibody, NCL-MUC1 (mouse IgG; Novocastra Laboratories; 1/100 diluted with PBS) in a humidified chamber for 1 hour at room temperature. A control experiment was done by incubating serial sections with a nonimmune mouse serum. Slides were washed in PBS for 30 minutes, incubated with biotinylated antimouse IgG (1/100 diluted with PBS; Vector Laboratories, Burlingame, CA, USA) for 1 hour at room temperature, washed in PBS for 30 minutes, and developed for binding with the Vectastain Elite ABC kit and diaminobenzidine as the peroxidase substrate following the standard protocol of Vectastain kits (Vector Laboratories). The slides were dehydrated, coverslipped, and examined for the location of brown reaction product.

In the tumor-free regions of the retina, the antibody against MUC 1 glycoprotein bound to the basal portion of the photoreceptor inner segments (Figures 1A, 1C, 1D). Because there was little reaction product observed in the retina incubated with the

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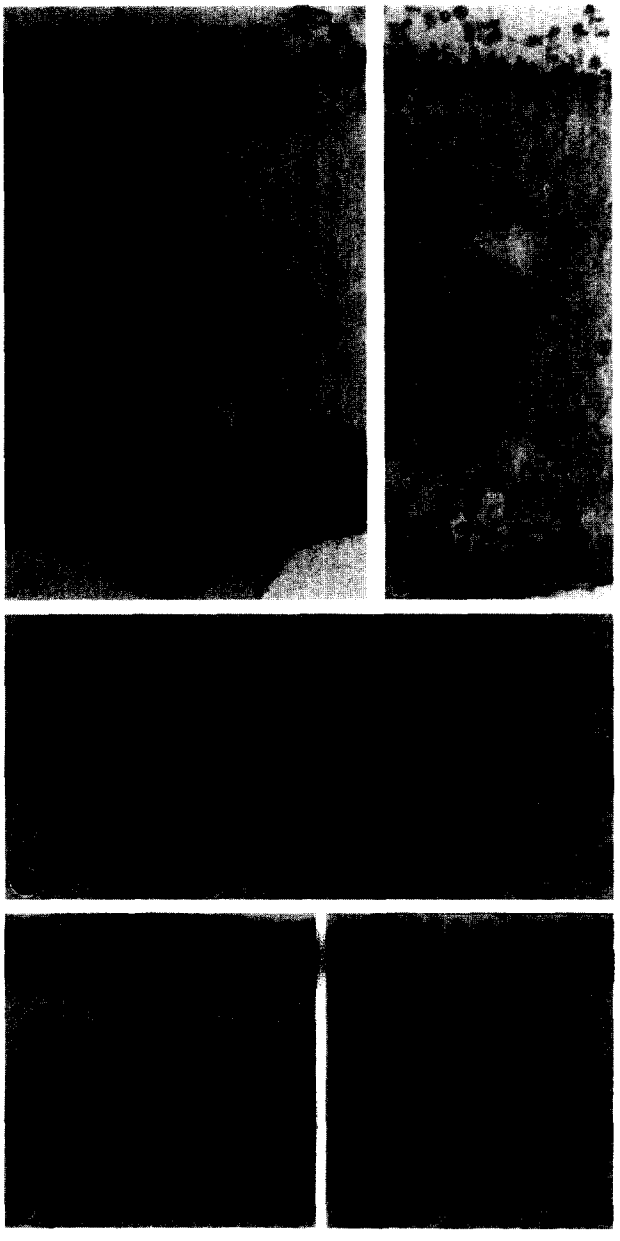

Figure 1. Immunohistochemical localization of MUC 1 glycoprotein in the human retina. (**A**, **B**, **C**) Tumor-free retinal areas of the eyeball enucleated because of retinoblastoma. (**D**, **E**) Morphologically preserved areas of retina in an eyeball enucleated because of choroidal melanoma. (**A**, **C**, **D**) Reaction product is localized in the basal portion of photoreceptor inner segments (arrow). (**B**, **E**) Control sections incubated with nonimmune mouse serum have little reaction product. PE: retinal pigment epithelium. Bar = 10 μm.

nonimmune serum (Figures 1B, 1E), the staining in the basal inner segments was considered to be specifically induced by the antibody-binding.

MUC 1 glycoprotein has been shown to be localized at the apical surfaces of epithelial cells as a transmembrane glycoprotein.[4] Retinal cells whose apical surfaces are present in the region of the basal inner segments are considered to be Müller cells. Therefore, it is possible that the area that is positive for MUC 1 may be the apical surfaces of the Müller cell fiber baskets. MUC 1, anchored as a transmembrane glycoprotein in the Müller cell fibers, may be connected with the O-linked glycoconjugates detected in the IPM (except the basal inner segments) by the previous lectin histochemistry.[3] Further investigation by electron microscopy and binding assay is required to confirm this speculation.

MUC 1 in the basal inner segments may also function as a barrier in the outer limiting membrane because mucins are implicated in cellular protection.[4] The restricted distribution of MUC 1 in the retina may be an important clue in understanding the physiological roles of MUC 1.

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References

1. Sameshima M, Uehara F, Ohba N. Specialization of the interphotoreceptor matrices around cone and rod photoreceptor cells in the monkey retina, as revealed by lectin cytochemistry. Exp Eye Res 1987;45:845–63.
2. Uehara F. Molecular cell glycobiology of the retina. Nippon Ganka Gakkai Zasshi (J Jpn Ophthalmol Soc) 1993;97:1370–93.
3. Uehara F, Ohba N, Sameshima M, Unoki K, Okubo A, Yanagita T, et al. Binding of amaranthin in photoreceptors of monkey retina. Jpn J Ophthalmol 1994;38:360–63.
4. Carraway KL, Fregien N. Mucin structure and function: Insights from molecular biology. Trends in Glycoscience and Glycotechnology 1995;7:31–44.
5. Uehara F, Ohba N, Yonezawa S. Glycopathological study of eyelid tumors. Nippon Ganka Gakkai Zasshi (J Jpn Ophthalmol Soc) 1996;Suppl 100:191.