ELSEVIER

LABORATORY INVESTIGATION

Cornea with Peters' Anomaly: Perturbed Differentiation of Corneal Cells and Abnormal Extracellular Matrix in the Corneal Stroma

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Purpose: We examined histopathologically the anterior ocular segment including the cornea and lens of an eye which had been enucleated in a patient with Peters' anomaly because of untreatable corneal perforation. Special effort was made to differentiate the corneal stromal and endothelial cells, and the stromal extracellular matrix.

Methods: Light microscopy, with hematoxylin and eosin staining, and transmission electron microscopy were employed.

Results: Corneal endothelial cells and Descemet's membrane were not detected in the central cornea, where there were immature cells with a fibroblastic configuration. The inner surface of the peripheral cornea was covered with cells containing pigment granules in the cytoplasm. Cell density in the central corneal stroma was relatively high. The diameter of the stromal collagen fibrils was not uniform. A mature collagen fibril-free area was also seen in the central corneal stroma.

Conclusions: Differentiation of neural crest-derived cells in corneal stroma and endothelium might have been perturbed in the cornea of this patient with Peters' anomaly, inducing the defect in the corneal endothelium and the qualitative and quantitative abnormalities of the extracellular matrix. **Jpn J Ophthalmol 2003;47:327–331** © 2003 Japanese Ophthalmological Society

Key Words: Extracellular matrix, neural crest-derived cell, Peters' anomaly, ultrastructure.

Introduction

Peters' anomaly is characterized by defects in the corneal endothelium and Descemet's membrane associated with central corneal opacity, and adhesion between the cornea and the anterior surface of the crystalline lens or iris.[1–4] Kupfer et al described the ultrastructural abnormalities in the corneal endothelium and Descemet's membrane in the cornea with Peters' anomaly.[3] About 50% of the cases with this anomaly are reportedly associated with goniodysgenesis with or without development of congenital glaucoma.[3,4] Most of the cases develop bilaterally.

During normal embryonic development, periocular neural crest cells migrate into the area between the surface ectoderm and lens vesicule, and then differentiate into corneal endothelial cells and keratocytes. They also construct the extracellular matrix (ECM) of the corneal stroma and Descemet's membrane. Perturbed migration and differentiation of embryonic periocular neural crest cells are reportedly the most likely of the possible causes of Peters' anomaly.[5–7] This ocular anomaly may be associated with systemic anomalies presumably caused by dysfunction of neural crest cells during embryonic development, supporting the hypothesis that neural crest cell dysfunction may cause Peters' anomaly. The systemic anomalies include congenital cardiovascular disease, cleft palate and craniofacial anomalies.[6,7]

In the present study, we examined the anterior ocular segment including cornea and lens of a globe, using light and transmission electron microscopy, which had been enucleated in a patient with Peters' anomaly because of untreatable corneal perforation. A special focus was put on the differentiation of corneal stromal and endothelial cells and the architecture of the stromal ECM.

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Case report

The patient was born on July 1st, 1996 as the youngest triplet after a 32-week pregnancy. Her body weight at birth was 1143 grams. Immediately after birth, opacity was seen in the center of both corneas. Her parents came with the patient 2 days after birth to consult us. At the first consultation, we found stromal opacity with neovascularization in the center of both corneas (Figure 1A). The diameters of both corneas were 7 mm × 7 mm. There was no abnormal finding in the conjunctiva and eyelids. Although the corneal opacity made it impossible to observe the intraocular structures directly, a B mode echographic examination showed a structural abnormality in the anterior chamber which was connected to both cornea and lens, and the absence of any structural abnormality in the vitreous cavity, retina and optic nerve. We diagnosed the patient's condition as Peters' anomaly. Intraocular pressure measured by Tonopen XL (Mentor, Norwell, MA, USA) was 16 mm Hg in the right eye and 18 mm Hg in the left eye. The patient had congenital heart disease involving defects in the septa of the atrium and the ventricle and also hydrocephalus associated with a defect in the corpus callosum. The other two triplets had no ocular or systemic disorders. Two months later, we found that the center of the right cornea had become thin with an enlargement of the corneal diameter without an increase in the intraocular pressure. The globe was enucleated on October 1, 1996, 12 hours after central corneal perforation had occurred.

Histological examination of the enucleated globe

The enucleated globe was fixed with 2.0% glutaraldehyde in 0.1 M phosphate buffer. After hemisectioning, one half was processed for paraffin embedding and the other was postfixed with 1.0% osmium tetroxide for 90 minutes for Epon embedding. Paraffin sections were stained with hematoxylin and eosin (HE) and observed under light microscopy. After dehydration through a graded ethanol series, the specimen was embedded in Quetol 812 (Nisshin EM, Tokyo). Sections were stained with toluidine blue and observed under light microscopy. Ultrathin sections were observed under transmission electron microscopy after electron staining with uranyl acetate and lead citrate.

Results

Binocular microscopy of the enucleated globe showed thinner central corneal stroma with a spontaneous perforation (Figure 1A). Hemi-sectioning of the globe visualized the presence of the adhesion between the corneal endothelial face and the protruding anterior pole of the crystalline lens, and the thin iris tissue adherence to the corneal endothelial surface, all characteristic of Peters' anomaly (Figure 1B). No abnormal structure was seen in the vitreous cavity (not illustrated).

Light microscopy of the HE-stained section revealed defects in the corneal endothelium and Descemet's membrane at the center of the cornea (Figure 2A). The inner surface of the central and mid-peripheral cornea was covered with cells of fibroblastic configuration or cells with the cytoplasmic pigment granules of iris tissue, respectively (Figures 2B and 2C). The distance between the neighboring corneal stromal cells seemed shorter with a presumably decreased amount of the ECM (Figure 2B). Neovascularization and infiltration of the inflammatory cells were also occasionally seen in the affected corneal stroma, presumably induced by epithelial defect-related

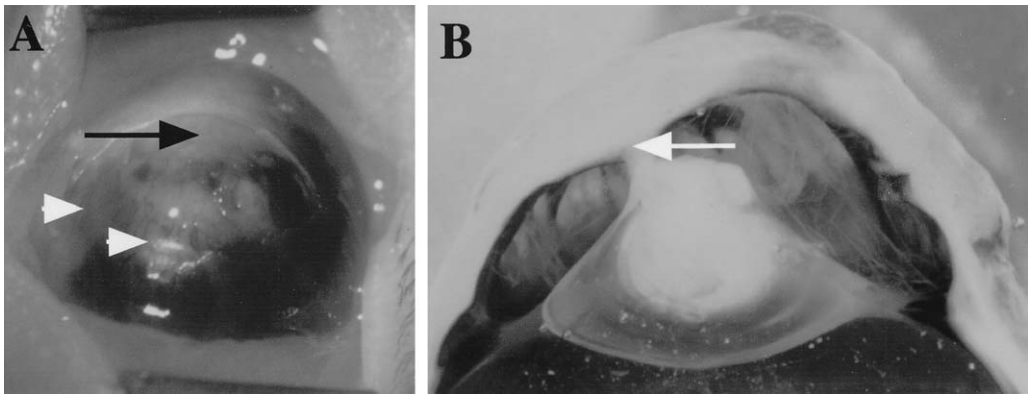

Figure 1. **(A)** Photograph of the right cornea 2 months after the first consultation shows corneal neovascularization (arrowheads) with a central opacity (black arrow). **(B)** The right globe has abnormal iridolenticular adhesion (white arrow) with nuclear cataract.

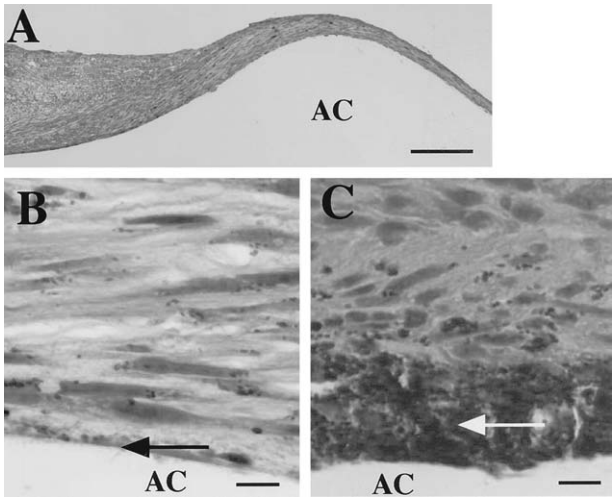

Figure 2. **(A)** Light microscopy of the HE-stained section reveals the thin central cornea. **(B)** The inner surface of the central cornea lacks the endothelium and Descemet's membrane and is covered with the cells of fibroblastic configuration (black arrow). **(C)** The inner surface of the peripheral cornea is covered with the cells with cytoplasmic pigment granules (white arrow). These pigmented cells are thought to be iris tissue which has adhered to the inner corneal face. The distance between the neighboring corneal stromal cells seems shorter with a presumably decreased amount of the ECM. AC: anterior chamber. Bar = 500 μm (A); 5 μm (B, C).

inflammation (not illustrated). On the other hand, the inner surface of the most peripheral cornea was found to be covered with a line of endothelium and Descemet's membrane (Figure 3). In this area a thin anterior chamber was seen without the adhesion between the inner corneal face and iris tissue (Figure 3).

Ultrastructural observations revealed defects of the corneal endothelium and Descemet's membrane in the central region of the cornea, and confirmed that the inner surface of the central cornea was covered with fibroblastic cells similar to corneal stromal cells (Figures 4A and 4B). Similar to the findings by light microscopy, the density of the stromal cells seemed greater with a presumably decreased amount of ECM. There was also a collagen fibril-free area in the stroma (Figures 4A and 4B). The diameters of the stromal collagen fibrils were not uniform (Figure 4C). The anterior lens surface was not covered with lens capsules. Lens epithelial cells were found to form a multilayer, being mixed with lenticular fibers (Figure 5).

Discussion

In the present study, light and electron microscopy showed an increased density of corneal stromal cells and

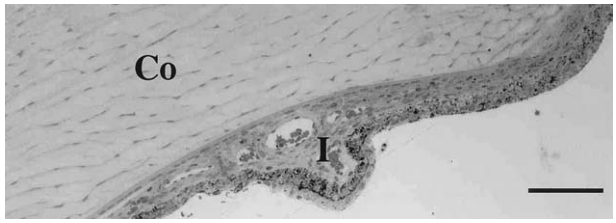

Figure 3. The anterior chamber angle of the globe with Peters' anomaly. Descemet's membrane and a line of the corneal endothelium are observed in the peripheral region of the cornea, where the anterior chamber is retained. A few inflammatory cells, presumed leukocytes, are seen behind the iris (I). Co: corneal stroma. Bar = 100 μm.

defects in the corneal endothelium and Descemet's membrane in the central area of the cornea, typical of Peters' anomaly. Increased density might indicate the decreased accumulation of ECM. Moreover, the diameters of the stromal collagen fibrils were not uniform. Matrix degrading enzyme secreted by corneal cells and/or inflammatory cells which might remodel the stromal ECM in the infiltration of inflammatory cells, presumably induced by corneal perforation, was detected in the corneal stroma of this case. However, there is also a possibility that the abnormal collagen fibrils might be primarily present in the corneal stroma of this case of Peters' anomaly. Although an abnormal ECM composition, such an increment of the fibronectin in the corneas of cases of Peters' anomaly, has been reported by Lee et al, they did not indicate whether the ultrastructural collagen fibrinogenesis in the corneal stroma was disturbed or not.[8] Abnormal keratan sulfate proteoglycan (KSPG) protein expression is a possible cause of the lack of uniformity in the collagen fibrils. Chakravarti et al and we have independently ablated the gene encoding lumican, one of the corneal keratan sulfate proteoglycan proteins, and revealed that the loss of lumican results in the presence of abnormal collagen fibrils with a corneal opacity.[9,10] On the other hand, the abnormality of the heparan sulfate/dermatan sulfate PG in the cornea may not lead to the abnormality of the collagen fibril because the loss of decorin does not induce the lack of uniformity of corneal stromal collagen fibrils in mice.[11] The expression pattern of KSPG proteins in the cornea with Peters' anomaly should be investigated. There is also a possibility that abnormal cytokine regulation of the neural crest-derived cells is present, inducing the lack of uniformity of stromal collagen fibrils. We have reported that the expression of KSPG proteins is abnormal in the cornea of the embryos of transforming growth factor â2 (TGFâ2)-null mice which have globe anomalies similar to those seen in human cases of Peters' anomaly, while the

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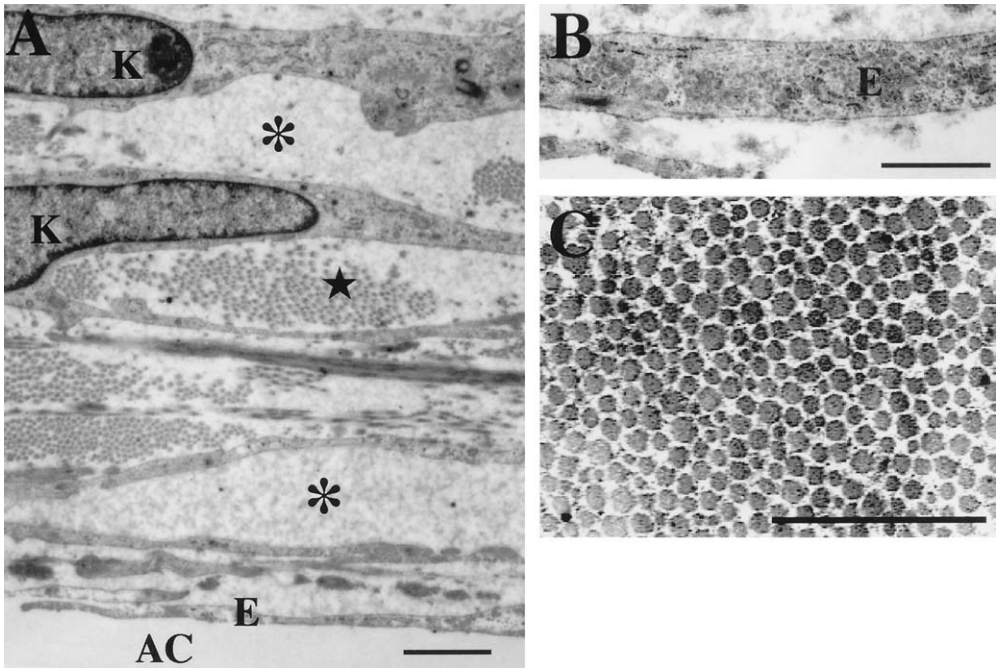

Figure 4. (A) Ultrastructural findings of the posterior part of the central cornea. Fibroblast-like cells (K) are surrounded by extracellular matrix with (star) or without (asterisks) collagen fibrils. The inner surface of the cornea is covered with cells (E) with a fibroblastic configuration. **(B)** A high magnification picture shows that the cells lining the inner surface of the central cornea exhibit fibroblastic configuration (E). **(C)** The diameters of collagen fibrils in the stroma are not uniform. AC: anterior chamber. Bar = 2 μm (A); 1 μm (B, C).

globes of TGFâ1- or TGFâ3-deficient mouse embryos are normal.[12] It remains to be investigated whether periocular neural crest cells in Peters' anomaly may be responsible for TGFâ2 signaling. We have confirmed that the expression of keratocan, one of the corneal KSPG proteins,[13] is decreased as compared to that of lumican in keratocytes of a TGFâ2-null mouse embryo.[12] Keratocan is believed to be an important protein for corneal integrity because mutation of the gene encoding this protein, *KERA*, leads to an abnormally shaped cornea.[13]

Histological observations by both light and electron microscopy showed the presence of fibroblastic cells lining the inner surface of the cornea where there was no Descemet's membrane, whereas the peripheral cornea had an obvious Descemet's membrane and endothelium. Loss of differentiated endothelium might induce adhesion between the inner corneal face and iris tissue, considering that the inner surface of the peripheral cornea with Descemet's membrane and endothelium does not adhere to iris tissue, retaining the anterior chamber space. This indicates the perturbed differentiation of neural crest-derived cells into the corneal endothelium in the central region of the cornea. Endothelial differentiation in human eyes may also be regulated by TGFâ2 because delayed differentiation of the corneal endothelial cells is also

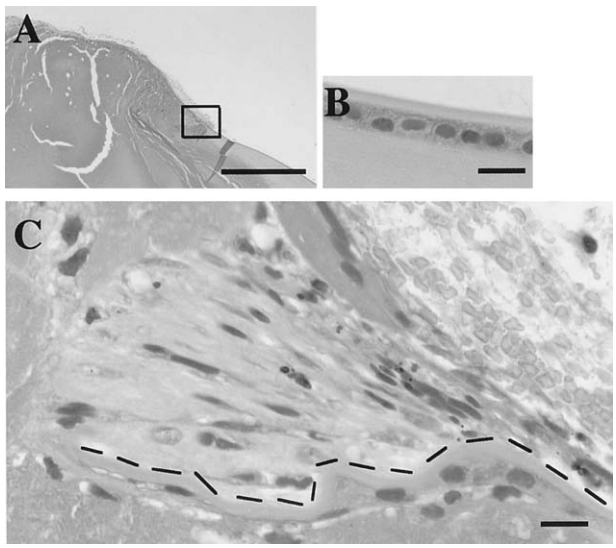

Figure 5. The crystalline lens of the globe of Peters' anomaly. **(A)** A low magnification photograph of the anterior surface of the lens. The central anterior part of the crystalline lens lacks the lens capsule. The rectangular area indicates the end of the anterior capsule. **(B)** Lens epithelial cells beneath the normally formed peripheral lens capsule. The cell lies in a monolayer showing an epithelial configuration. **(C)** Higher magnification of the boxed area in frame A shows multilayered elongated lens cells. Hematoxylin and eosin staining, Bar = 1 mm (A); 10 μm (B, C).

seen in TGFâ2-deficient mouse embryonic eyes. On the other hand, genes responsible for the anomalies in the anterior chamber of the human eye have been reported. Although the exact mechanism of the abnormal ocular development in the absence of TGFâ2 remains unknown, further study is needed to clarify the possible signaling crosstalks between TGFβ2-derived signals and genes related to anterior segment anomalies of the eye.

In the present study, the central cornea became perforated during the follow-up intervals although perforation of the cornea is relatively rare in Peters' anomaly. Goniodysgenesis might reduce the drainage of the aqueous humor, and induce the extension of the globe to buffer the increased intraocular pressure. Extension of the central thin corneal stroma and the corneal epithelial damage secondary to the loss of function of the corneal endothelium might have caused the perforation of the central cornea in this case.

References

1. Peters A. Ueber angeborene Defektbildung der Descemetschen Membran. Klin Monatsbl Augenheilkd 1906;44:27–40.
2. Traboulsi EI, Maumenee IH. Peters' anomaly and associated congenital malformations. Arch Ophthalmol 1992;110:1739–1742.
3. Kupfer C, Kuwabara T, Stark WJ. The histopathology of Peters' anomaly. Am J Ophthalmol 1975;80:653–660.
4. Heath DH, Shields MB. Glaucoma and Peters' anomaly. Graefes Archive for Clinical & Experimental Ophthalmology 1991; 229:277–280.
5. Beauchamp GR, Knepper PA. Role of the neural crest in anterior segment development and disease. J Pediatr Ophthalmol Strabismus 1984;21:209–214.
6. Bolande RP. The neurocristopathies. A unifying concept of disease. Hum Pathol 1974;5:409–429.
7. Shirai S. Developmental mechanisms of congenital eye abnormalities. Nippon Ganka Gakkai Zasshi (Acta Soc Ophthalmol Jpn) 1991 Dec;95(12):1206–1237.
8. Lee C-F, Yue BYJT, Robin J, Sawaguchi S, Sugar J. Immunohistochemical studies of Peters' anomaly. Ophthalmology 1989;96: 958–964.
9. Chakravarti S, Magnuson T, Lass JH, Jepsen KJ, LaMantia C, Carroll H. Lumican regulates collagen fibril assembly: skin fragility and corneal opacity in the absence of lumican. J Cell Biol 1998;141:1277–1286.
10. Saika S, Shiraishi A, Saika S, et al. Role of lumican in the corneal epithelium during wound healing. J Biol Chem 2000;275:2607–2612.
11. Danielson KG, Baribault H, Holmes DF, Graham H, Kadler KE, Iozzo RV. Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. J Cell Biol 1997;136:729–743.
12. Saika S, Saika S, Liu C-Y, et al. TGFβ2 in corneal morphogenesis during mouse embryonic development. Dev Biol 2001 Dec 15; 240(2):419–432.
13. Liu C-Y, Shiraishi A, Kao CW-C, et al. The cloning of mouse keratocan cDNA and genomic DNA and the characterization of its expression during eye development. J Biol Chem 1998;273: 22584–22588.
14. Pellegata NS, Dieguez-Lucena JL, Joensuu T, et al. Mutation in KERA, encoding keratocan, causes cornea plana. Nature Genetics 2000;25:91–95.