Dysplastic Canine Retinal Morphogenesis

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Retinal dysplasia is a failure in normal retinal development. The morphologic sequence of the dysplastic processes was examined in fetuses and neonates from dogs affected with an inherited form of retinal dysplasia. The dysplastic change has its onset at 45–50 days' gestation and is most prominent in the dorsal peripapillary retina. Morphologic characteristics of involved sensory retina include the focal loss of cell junctions forming the external limiting membrane, folding of the sensory retina, disorganized proliferation of neuroblasts in the retina and subretinal space, and formation of rosettes composed of multiple layers of neuroblasts. These morphologic changes suggest that there is an intrinsic abnormality of neural retinal differentiation. Invest Ophthalmol Vis Sci 32:1492–1498, 1991

Retinal dysplasia is a failure in normal retinal development after a period of apparent normal development. It is characterized histologically by linear folding of the sensory retina and the formation of rosettes composed of variable numbers of neuronal retinal cells.1 This abnormality is associated with several spontaneously occurring and experimentally induced disorders of the developing eye in both humans and animals. Dysplastic retinal lesions in humans have been associated with x-linked hereditary-oculoacoustic cerebral degeneration,2 autosomal recessive vitreoretinal dysplasia,7 dominant exudative vitreoretinopathy,4 Meckel's syndrome,5 incontinentia pigmenti,6 trisomy,7,8 falciform retinal fold,9 and persistent hyperplastic vitreous.10 In utero exposure to irradiation11 and lysergic acid diethylamide12 have also been associated with congenital retinal dysplasia.

Heritable forms of retinal dysplasia have been reported in several breeds of dogs.1–18 Dysplastic lesions can also be induced in dogs and other species by infectious agents and x-irradiation.19–24 Retinal dysplasia in the English springer spaniel dog is characterized clinically by streaks and patches of hyperreflectivity in the superior fundus; additional changes can include retinal detachment and cataract.25 The condition is inherited as an autosomal recessive trait.26 The dysplastic lesion is present at birth and is characterized by folds and rosettes of neural retina.27 We determined the morphologic sequence of this dysplastic process.

Materials and Methods

A colony of healthy English springer spaniel dogs with ophthalmoscopically detectable fundic features of retinal dysplasia25 was used in this study (two males and ten females). A colony of ophthalmoscopically normal beagle dogs were used as controls. Female dogs were bred naturally or were inseminated artificially every other day during estrus. The first day that there were predominantly noncornified epithelial cells in the vaginal smear was considered the first day of gestation26; gestation length was 57–59 days. Fetuses were obtained by Cesarean section at embryonic (E) days 40, 45, 50, and 55. All fetuses were removed within 30 min of the attainment of a surgical anesthetic level in the dam and killed by intravenous injection of T-61 (Hoechst, Sommerville, NJ). At least ten eyes from animals in two litters were examined at each time. Puppies (two of each age) were obtained on the day of birth (P1) and at postnatal day 7 (P7). They were killed similarly. The use of animals in this study conformed to the ARVO Resolution on the Use of Animals in Research. The protocol was approved by the Animal Care and Use Committee, University of Illinois, Urbana.

Light Microscopy

The eyes were dissected free of extraocular tissue and fixed in Bouin's fixative. Before processing, all globes and retinas were examined with a stereomicroscope. The globes were hardened in 70% ethanol, hemisected in a dorsoventral plane, embedded in low-melt paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. A few globes were embedded
in methyl methacrylate, sectioned at 2 μm, and stained with hematoxylin and eosin.29

Electron Microscopy

The globes were fixed at 37°C for 15–30 min in 2% glutaraldehyde and 2% paraformaldehyde in phosphate or cacodylate buffer at pH 7.3. For transmission electron microscopy, strips (0.2 × 0.5 cm) of dorsal peripapillary retina were postfixed in buffered 1% osmium tetroxide and 1.5% potassium ferrocyanide. The tissue was dehydrated in graded alcohol, infiltrated with Epon 812 (Electron Microscopy Sciences, Fort Washington, PA), oriented in flat molds, and embedded in Epon 812. Thin sections (silver) were cut on an LKBIII ultramicrotome (LKB Inc., Rockville, MD) with a diamond knife, mounted on 100-mesh grids, and stained with methanolic uranyl acetate and lead citrate. They were examined in a Hitachi HU-12A electron microscope (Hitachi Inc., Gaithersburg, MD) at 75 kV. For scanning electron microscopy, the globes were hemisected in a dorsoventral plane. The specimens were postfixed in 2% buffered osmium tetroxide, dehydrated in ethanol and then acetone, and critical-point dried using liquid CO2 in a Polaron E3000 CPD device (Polaron Equipment Ltd., Watford, Hertfordshire, England). The retina was mounted on aluminum stubs, sputter-coated with gold-palladium, and examined in a Hitachi HHS-2R scanning electron microscope at 20 kV.

Results

Histologic evidence of early dysplastic change was identified in some fetal eyes at the E45 time point. All E40 globes were normal, and by E50–55, all globes had evidence of dysplastic change. The lesions were limited primarily to dorsal peripapillary sensory retina. The earliest abnormalities were characterized by an inward folding of the neuroblastic layer at the external limiting membrane (Fig. 1) or by loss of junctions forming the external limiting membrane (Figs. 2, 3) and disorganization of cells in the outer neuroblastic layer (Figs. 2–5). Loss of junctions and cell disorganization also occurred in folds of the sensory retina (Figs. 4, 5). The subjacent retinal pigment epithelium (RPE) was always present although in some areas was slightly hypertrophic directly under the folds.

By E50–55 and at P1 and P7, distinct retinal folds could be identified as numerous intraconnecting raised linear structures (Figs. 6, 7). These lesions were formed by large areas of disorganized neuroblasts in the retina and the subretinal space. Rosettes formed

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Fig. 1. (A) Early evidence of dysplastic change in a 45-day fetal English springer spaniel retina. There is an inflection of the outer neuroblastic layer in the dorsal peripapillary tapetal retina (arrowhead). The retinal pigment epithelium, choroid, and sclera are normal. H&E, bar = 100 μm. (B) Higher-power view of this early retinal fold. Note the loss of a distinct external limiting membrane (arrowhead). H&E, bar = 50 μm.
Fig. 2. Early dysplastic change in a 55-day fetal retina. There is loss of a distinct external limiting membrane (arrowhead) and disorganization of cells in the outer neuroblastic layer (double arrowhead). H&E, bar = 100 μm.

by neuroblasts around a central lumen were present in many folds (Fig. 8). These neuroblasts formed a distinct external limiting membrane and inner segments that extended into the rosette lumen (Fig. 9). Retinal vessels extended into these disorganized areas and occasionally were found in rosette lumens. The folds occasionally progressed to focal retinal detachment with slight hemorrhage in the subretinal space and associated RPE hypertrophy (Fig. 10).

Examination of the vitreal surface and the optic disc by scanning electron microscopy showed no evidence of fibrous attachments to the apex of retinal folds or aberrant hyaloid vessels.

Discussion

This study confirmed that retinal dysplasia in the English springer spaniel dog has its inception at days 45-50 of gestation. The dysplastic process is most prominent in the dorsal peripapillary tapetal region, involves only sensory retina, and is not associated with defects in the other coats of the eye. Reported postnatal lesions are similar, although focal retinal

Fig. 3. Disorganized and proliferating neuroblasts in the subretinal space of a P1 retina. Note the loss of a distinct external limiting membrane (arrowheads) and presence of the retinal pigment epithelium. H&E, bar = 50 μm.

Fig. 4. A developing fold in a E55 retina. Note the proliferative neuroblasts in the subretinal space (double arrowhead) and rosette formation in the neural retina (arrowhead). H&E, bar = 100 μm.

Fig. 5. Transmission electron micrograph from the apex of a retinal fold in a 1-day-old dysplastic retina. Neuroblasts are proliferating in a disorganized manner (arrowhead). Bar = 5 μm.
atrophy and diffuse retinal detachment are not found in the fetal lesion.

The earliest morphologic changes observed in fetal eyes are the loss of cell junctions forming a distinct external limiting membrane, inflection of the neuroblastic layer at the external limiting membrane, and disorganized proliferation of neuroblasts in the sensory retina and subretinal space. These lesions develop into folds and rosettes of the sensory retina.

Retinal dysplasia in humans and animals is often associated with abnormalities of the primary vitreous, retinal detachment, or coloboma and areas of RPE loss. These dysplastic processes are probably associated with the organizing role of the RPE. The choroid and sclera were morphologically normal; therefore, defects in these coats are probably not a cause of this dysplastic process. Retinal detachment was not a feature of the early lesion, and dysplastic regions were often in contact with the RPE. Although there was no evidence of morphologic abnormalities of the RPE, the spatial distribution of the lesion process to the dorsal peripapillary retina over nonpigmented epithelium may indicate a possible functional or metabolic role of the RPE in this region to the lesion process. A similar distribution has been identified in feline mucopolysaccharidosis VI where nonpigmented epithelium in the posterior pole is principally affected suggesting possible regional differences in RPE metabolism.

Abnormalities of the hyaloid vasculature or the retinal vasculature have been suggested as possible predisposing factors of retinal dysplasia. Vascular ab-
normalities were not identified in the incipiently dysplastic globes (E40 and E45) in the hyaloid region or along the vitreal surface of the retina. The vascular response seen in the retina in the dysplastic regions is probably in response to the proliferative retinal change.

Retinal dysplasia has been associated with necrosis or destruction of cellular elements of the retina, particularly with experimental viral diseases, x-irradiation, or toxins. The formation of rosettes in these cases may be an aspect of repair of injured neuroepithelium. There was no morphologic evidence in the eyes examined from these fetuses that necrosis was an initiating event or that there was a concurrent infectious agent.

The morphologic observations of loss of neuroblast polarity with disorganized proliferation and loss of junctions forming the external limiting membrane during the early lesion development without abnormalities of the RPE or other coats of the eye suggests that there is an intrinsic abnormality in retinal differentiation that may be associated with abnormalities in cell–cell adhesion in the outer neuroblastic region. O’Toole et al proposed that there was a defect in the development of Müller cells because they may act as a scaffold for neuroblast migration.

Cell surface adhesion molecules or glycoconjugates are thought to be important in mediating cell–cell interactions during organogenesis. Abnormalities in these factors may be a major contributor to dysplastic morphogenesis. Proliferative changes of the sensory retina similar to those seen here were produced in chick eyecup cultures by altering the sialic acid composition of neural cell adhesion molecule (N-CAM). Additionally, antibody to N-CAM will alter retinal development in chick retinal organ cultures. In a previous study of the external limiting membrane of incipiently dysplastic springer spaniel retina, a subjective decrease in the size and number of gap junction components was found in E46 retina. Correlating with this is evidence that N-CAM is important in the formation of gap junctional channels in neuroepithelium. The loss of junctions of the external limiting membrane may also be associated with changes in other adhesion molecules such as calcium-dependent cadherin which is associated with adherens-type junctions; antibodies to N-cadherin disrupted normal chick retinal histogenesis in vitro causing loss of the external limiting membrane and
rosette formation. Additionally, antibodies to R-cognin have caused interruption in the external limiting membrane and extensive cell disorganization when incubated with organ cultured chick retina. Inappropriate expression of cell adhesion molecules or glycoproteins on the neuroepithelium or Müller cells may lead to poor cell adhesion and the inability to form cell junctions or proper cell–cell communication, leading to subsequent disorganized proliferation of neuroblasts in the retina and subretinal space.

Retinal dysplasia in the English Springer spaniel dog is an autosomal recessive trait that causes proliferative retinal lesions and has its inception at a specific time during development. This appears to be a good model for studying factors affecting proper retinal morphogenesis.

**Key words:** retinal dysplasia, canine, development, eye, retina

**Acknowledgments**

The author thanks R. Bergstrom, M. Noonan, J. Scott, and L. Pitner for technical assistance; S. McLaughlin, P. Gerding, L. Helper, and B. Johnson for ophthalmic examination of the affected dogs; S. Young for advice; and the Word Processing Center for manuscript preparation. A portion of this work was done at Colorado State University.

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