Development of the rat iris

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Development of the rat iris was studied electron microscopically. Development of the rat iris began on the sixteenth prenatal day and differentiated actively during the three days before birth. The iris showed mature structure by the end of the postnatal second week. The sequence of the smooth muscle formation from the neuroepithelial cell was specifically described. The differentiation began with formation of the basal infoldings. Myofilaments were found in the cells which had formed a basement membrane around the infolded basal and lateral cell membranes.

Key words: rat iris, smooth muscle formation, basal infoldings, myofilaments, lateral cell membranes.

Although the fine structure of the iris has been reported by several authors, development of the mammalian iris has scarcely been described in detail. Since the iris has several unique structures which are determined by its embryonal origin, elucidation of the developmental process seems to be important for the further understanding of this tissue. The developmental sequence of the smooth muscles from the neuroepithelial cells was specifically emphasized in this investigation.

Materials and method

Black hooded rats of Long-Evans and albino rats of Charles River C. D. strain were studied.

Fetuses of thirteenth, fifteenth, and sixteenth through twentieth days and postnatally developing rats of newborn, second day, one week, and two weeks were killed by decapitation. The frontal portion of the eye was excised and fixed in four per cent glutaraldehyde solution in 0.15M pH 7.2 phosphate buffer at room temperature. During the 20 minute initial fixation in the glutaraldehyde, the tissue was dissected carefully under an operating microscope. Small pieces of the anterior margin of the optic cup measuring not more than 1 mm. in any direction were excised and transferred to one per cent osmium tetroxide in the same buffer solution at a cold temperature. After 90 minutes of fixation in osmium, the tissue was dehydrated in ethyl alcohol, treated with propylene oxide, and embedded in an epoxy resin. Tissues were carefully oriented so as to show the anteroposterior cross-sections. Thin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope.

Sections, 0.5μ thick, were also obtained from the same block and stained with toluidine blue for light microscopic study.

Observation

The optic cup is made of two layers of neuroepithelium: single cell thickness outer layer and the inner, a few cells thick. The two layers are connected in a hairpin turn fashion at the tip and form a round margin.
Fig. 1. Anterior margin of the optic cup on the fifteenth day of gestation. The tip is extending into the anterior chamber. (Toluidine blue. x250.)

Fig. 2. A, Sixteenth prenatal day. B, Eighteenth prenatal day. C, Fourteenth postnatal day. Schematic drawings show the sequence of muscle differentiation. A, The first sign of differentiation is formation of basal infoldings in the anterior layer neuroepithelium. B, Basal lamina substance and fine filaments are formed in the intercellular spaces as the second step. C, Sphincter muscle is completely formed by the postnatal second week.
The major part of the inner layer of the neuroepithelium differentiates rapidly to form the retina immediately following the formation of the cup.

For two to three days, the anterior portion of the newly formed cup is filled with abundant mesenchymal cells including blood vessels. On the thirteenth prenatal day, this area begins to lose cells to form the anterior chamber. Simultaneously, thin vascular mesenchymal tissue begins to extend from the connective tissue outside the outer margin of the optic cup. This is the first sign of development of the iris. The neuroepithelial cells begin to extend about two days later.

**Development of the sphincter muscle.**

On the fifteenth day of embryonal life, the round margin of the optic cup becomes slightly pointed and begins to extend in an anterior direction, following the mesenchymal tissue (Fig. 1). On the sixteenth day, the marginal zone of the optic cup is a two-cell layer measuring about 50μ in length, forming a loop configuration at the tip. Cells at the anterior tip margin of the loop begin to increase in density in nuclei and cytoplasm. On the seventeenth day, the two-cell layer extension of the neuroepithelium is about 100μ long, and the cells at the loop margin begin to proliferate. At the time of birth, the proliferating cells form a cluster which protrudes anteriorly from the neuroepithelial layer. The cluster of cells increases in size continuously until the second postnatal week and becomes the sphincter muscle (Fig. 2).

The earliest sign of differentiation of the muscle cell is demonstrated on the sixteenth day, when a few cells at the anterior margin of the loop begin to increase the basal infoldings. The infolded basal cell membrane begins to show patchy condensation in several places at the same time (Fig. 2, A). These cells also have increased numbers of mitochondria, rough-surfaced endoplasmic reticulum, and glycogen particles.

On the seventeenth to eighteenth day of gestation, the muscular differentiation becomes more obvious (Fig. 3). Mitotic figures are found frequently in this cluster. Basal infolding of the cell becomes more

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Fig. 3. Prenatal seventeenth day. Basal portion of the anterior neuroepithelium at the margin of the cup. Cell membranes are markedly infolded (arrow). Mitosis is active in this area. (Original magnification ×16,800.)
Fig. 4. Prenatal eighteenth day. Spaces formed between the infolded lateral cell membranes accumulate basement membrane and fine filaments. (Original magnification x34,400.)

abundant and spaces between the infoldings become larger. The lateral cell membranes also separate in several places, and intercellular spaces of various sizes are formed. Basal lamina substance is found not only at the original basal surface of the cell but also in the deep infoldings and lateral intercellular spaces (Figs. 2, B, and 4). Fine filamentous substance is also formed abundantly in the lateral intercellular spaces (Fig. 4). Although the cytoplasm of these cells shows no myofilaments yet, the general appearance of the cell shape is unquestionably that of the muscle cell: Nuclei are dense in chromatin and indented, the cell has many surfaces. During
the last two or three prenatal days, formation of the basement membrane continues until the whole cell surface, except small portions of the apical membrane, is covered with it. Fine collagen fibers begin to show in large intercellular spaces. No firm attachment between the stroma connective tissue and the epithelial layer is formed before the day of birth.

At the time of birth, the cluster of cells at the loop margin is divided into a few smaller groups by fine connective tissue. Numerous large mitochondria and rich rough-surfaced endoplasmic reticulum are found in the general area of the cytoplasm. These microorganelles begin to decrease in the basal area and are replaced by fine myofilaments (Fig. 5). Also, the amount of glycogen particles increases in this area. Some proliferated cells have lost the apical junctions with the posterior epithelium. Melanin pigment and melanosomes are still present in the differentiating muscle cells.

On the second postnatal day, the cluster of cells has mostly finished its muscle differentiation (Figs. 6 and 7). The cells are uniformly filled with thin myofilaments and have less microorganelles. Patchy aggregations of myofilaments (dense bodies) and thick myofilaments begin to show in the cytoplasm. Muscle cells are separated by basement membranes but attached to each other by small tight junctions at several places. Many muscle cells in the anterior portion of the cluster have lost their attachment to the neuroepithelium by this period. However, some anterior epithelial cells are intermingled with the differentiating muscle cells. Fibroblasts and blood vessels are frequently found between the groups of muscle cells. The basement membranes of the muscle cells attach firmly to the stromal connective tissue of this stage.

The appearance of the sphincter muscle
Fig. 6. Postnatal second day. Muscle cells contain massive myofilaments. Thin and thick filaments are distinguishable. Lateral cell spaces contain filamentous connective fibers and the basement membrane. (Original magnification x39,000.)

Development of the dilator muscle. The anterior layer neuroepithelial cell forms the dilator muscle in its basal portion at about the same time that the sphincter muscle differentiates. However, the process of development of the dilator muscle is slower than that of the sphincter.

On about the sixteenth embryonal day, the basal cell membrane of the anterior layer epithelium begins to make infoldings in a fashion similar to that of the sphincter muscle. The tips of the infoldings begin to thicken and form patches on the seventeenth or eighteenth day (Fig. 6). The cells do not proliferate and the lateral and apical junctions remain unchanged.

On about the day of birth, the basal infoldings of the anterior cell begin to spread out and fine basal lamina material is formed around them. Myofilaments are seen in the basal cytoplasm on the second postnatal day (Fig. 9). The rest of the cell contains many melanin granules and mitochondria. The dilator muscle of the rat iris is not as conspicuous as is that of other animals. There is no further development of the dilator muscle after the age of one week.

Development of the epithelium. The
sphincter muscle cells differentiate from a cluster of cells at the marginal zone of the neuroepithelial loop and the dilator muscle develops within the basal processes of the anterior layer cells. The neuroepithelium ceases active mitosis on about the eighteenth prenatal day and the cell layer begins to stretch. The cytoplasm of both layers contains rich mitochondria, numerous melanin granules, and rough-surfaced endoplasmic reticulum (Fig. 10). The apical membranes of both cells are tightly joined in several locations. There are moderate interdigitations between two cells. The lateral junctions of the epithelial cells become less conspicuous as development progresses.

Small intercellular spaces containing electron-lucent fluid are found between the apical cell membranes from the earliest stage of the development (Fig. 10). On about the seventeenth prenatal day, marked infoldings are frequently found in apical cytoplasms of both epithelial cells. However, these infolds do not form myofilaments nor basement membranes. The num-

Fig. 7. Postnatal second day. Cells of the anterior layer of the neuroepithelium have developed into muscle cells and form a large mass. Muscle cells are divided by fine connective tissue. The posterior epithelium is still attached to the muscle cells. (Original magnification x6,000.)
Fig. 8. Prenatal seventeenth day. Central portion of the iris. The cells of the anterior neuroepithelium showed marked basal and lateral infoldings of their cell membrane (black arrows). Apices of these cells are joined with the posterior cells by numerous tight junctions (white arrows). The epithelium and the stroma are loosely attached. (Original magnification ×7,300.)
Fig. 9. Postnatal first day. The basal infoldings of the anterior neuroepithelial cells are covered with the basement membrane. The cytoplasm has not developed myofilaments. Microtubules (mt) are seen in the infolded cytoplasm. (Original magnification ×38,500.)

...ber of microorganelles in the cytoplasm of the posterior cells increases gradually until two weeks after birth. Melanosomes of albino rats decrease in number considerably around the second postnatal week. Melanin pigment granules of pigmented rats remain permanently.

**Development of the stroma.** The initial mesenchymal tissue which has extended from the connective tissue at the outer marginal portion of the optic cup on the thirteenth or fourteenth day of gestation is mainly vascular. Around the blood capillary, fibroblast-like cells begin to appear on the fifteenth day. On the eighteenth day, the tissue, about 35 μ thick, contains several blood vessels and many fibroblasts and is loosely attached to the neuroepithelium (Fig. 8). Some mesenchymal cells arrange themselves in a layer at the anterior margin. But these cells develop neither junctional apparatus nor basal lamina, and there are large spaces between the cells (Fig. 11). The developing tip of the connective tissue also has no limiting structure. Growing blood vessels and loose connective tissue end abruptly at the pupillary end. Cells in the developing stroma contain rich rough-surfaced endoplasmic reticulum and mitochondria and extend numerous fine processes in all directions. Also, there are several cells which are heavily pigmented. The blood vessel wall is formed by thick endothelial cell cytoplasm. Pericytes have electron-dense nuclei and cytoplasm (Fig. 11). The basement membrane around the blood vessels and beneath the anterior epithelium increases in thickness and begins to form a firm attachment of the epithelium about the eighteenth day. Loose collagen fibers are seen in the stroma on the sixteenth day, and they gradually increase in number. At the time of birth, collagen fibers are found abundantly in the vicinity of the developing muscles and around blood vessels. Nerve fibers appear in the stroma by this stage and extend along the blood...
vessels. Fine nerve fibers do not extend into the space between muscle cells until the second postnatal day.

Comment

Although the iris sphincter muscle is structurally pure smooth muscle, it differentiates from the neuroepithelial cell rather than from the mesenchymal cell. The dilator muscle also develops within the neuroepithelium and forms a typical myoepithelium. Similar to glandular myoepithelium which is known to develop from an ectodermal origin, iris smooth muscle does not differentiate from a mesenchymal but from a neuroepithelial origin.

Differentiation of the sphincter muscle begins at the basal portion of the cell. As the first sign of the differentiation, the basal infolding is formed in the anterior epitelium. Basal lamina is then formed between basal infoldings and in the space between the lateral cell membranes. Thus the neuroepithelial cell loses its polar orientation before the cellular differentiation starts. Following the formation of basement membranes on the surfaces, the cells lose microorganelles and differentiate into smooth muscle.

The striated muscle cell differentiates first by formation of myofilaments in the cytoplasm. Ciliary muscle, which is the smooth muscle of mesodermal origin, also forms myofilaments as the first step of differentiation (Fig. 12). The iris neuroepithelium develops myofilaments only after the cell has formed the basal structure at the lateral cell membrane. Once the cell begins to form filaments, the manner of differentiation is found to be identical to

Fig. 10. Prenatal nineteenth day. Apical junctional zone of the neuroepithelium. Both anterior and posterior epithelial cells contain numerous melanosomes. Cell membranes form tight junctions in several locations. Minute spaces into which fine processes protrude are seen (*) between the two cells. m, Mitochondria. (Original magnification x21,300.)
Fig. 11. Postnatal first day. Stroma consists of blood capillaries, fibroblasts, nerve fibers, and loose collagen fibers. Capillaries are surrounded by darkly staining cells. AC, anterior chamber. (Original magnification x7,100.)

Fig. 12. Ciliary muscle cell on the prenatal nineteenth day. Fine myofilaments are formed in the cytoplasm without formation of the basement membrane on the outside of the cell (arrows). (Original magnification x19,200.)
other muscle cells. Thin filaments are formed first and followed by thick filaments.

The dilator muscle develops in a similar fashion. However, its process is slower than that of the sphincter muscle. The dilator muscle is formed only in the basal portion of the anterior epithelium. Similar to other myoepithelium, the muscle components develop in the basal portion of the cell. Because of its unique manner of development, iris epithelium has two cell layers which are attached by their apical ends. There are minute spaces between the apical membranes of the epithelium. Since these spaces correspond to the original neurovesicle lumen and are present throughout developmental and adult life, they may have an important function in the iris epithelium.

The posterior free surface of the iris epithelium is covered with a basement membrane. This unique structure appears to be determined by the developmental characteristics and has been pointed out earlier.

Stromal connective tissue begins with an extension of a vascular tissue two to three days earlier than the epithelial growth. The anterior limit of the iris is made of mesenchymal cells. No endothelial cell has developed.

Although the developmental process of the iris muscle takes place simultaneously with differentiation of the retina, lens, and cornea, substantial development occurs in a later stage (seventeenth to nineteenth prenatal day). Also, this is the period during which the whole eyeball becomes substantially larger and the cellular differentiation of other parts becomes apparent. Sequential processes of the development of the ocular tissue have already been demonstrated histologically.

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REFERENCES