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Ultrastructural analysis of the human lens fiber cell remodeling zone and the initiation of cellular compaction

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ABSTRACT

The purpose is to determine the nature of the cellular rearrangements occurring through the remodeling zone (RZ) in human donor lenses, identified previously by confocal microscopy to be about 100 μm from the capsule. Human donor lenses were fixed with 10% formalin followed by 4% paraformaldehyde prior to processing for transmission electron microscopy. Of 27 fixed lenses, ages 22, 55 and 92 years were examined in detail. Overview electron micrographs confirmed the loss of cellular organization present in the outer cortex (80 μm thick) as the cells transitioned into the RZ. The transition occurred within a few cell layers and fiber cells in the RZ completely lost their classical hexagonal cross-sectional appearance. Cell interfaces became unusually interdigitated and irregular even though the radial cell columns were retained. Gap junctions appeared to be unaffected. After the RZ (40 μm thick), the cells were still irregular but more recognizable as fiber cells with typical interdigitations and the appearance of undulating membranes. Cell thickness was irregular after the RZ with some cells compacted, while others were not, up to the zone of full compaction in the adult nucleus. Similar dramatic cellular changes were observed within the RZ for each lens regardless of age. Because the cytoskeleton controls cell shape, dramatic cellular rearrangements that occur in the RZ most likely are due to alterations in the associations of crystallins to the lens-specific cytoskeletal beaded intermediate filaments. It is also likely that cytoskeletal attachments to membranes are altered to allow undulating membranes to develop.

1. Introduction

The differentiation of fiber cells in the cortex of human lenses is more complex than previously recognized. In addition to the degradation of membranous organelles to produce an organelle free zone that supports transparency of the lens core (Bassnett, 2009), the differentiating fiber cells undergo dramatic transformations about 100 μm from the surface within the remodeling zone (RZ) first described by Lim et al. (2009). This region, only 40 μm wide where nuclei are still found, shows extensive cellular disorganization by laser scanning confocal light microscopy. After immunohistochemical staining of membranes and nuclei, the observed complex cellular rearrangements and membrane undulations suggested the insertion of new membranes and the modification of intercellular junctions within the RZ. They noted that the radial cell columns, which were evident in the outer cortical layers where cells had the classical flattened hexagonal cross-section, were not visible in the RZ. The radial cell columns only appeared again in the deeper layer called the transitional zone (TZ), where cells still had complex irregular shapes without nuclei as they transitioned into the compacted cells of the adult nucleus more than 300 μm deeper. An important finding was that the RZ appeared at the same location regardless of the age of the lens over an age range of 16–76 years. This implies that all fiber cells in human lens nuclei must have undergone the cellular transformations in the RZ as part of a highly regulated differentiation process. Because the cells in the RZ appeared condensed and jumbled in confocal images, it was anticipated that this region might act as a barrier to diffusion; however, when an extracellular tracer (Texas red dextran) was applied, it readily diffused through the RZ and the TZ up to the adult nucleus, which appeared to be the physical barrier about 350 μm from the lens surface (Lim et al., 2009).

These remarkable observations about a narrow band within the cortex of adult human lenses invite numerous questions

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about dramatic changes in cell shape and interactions that can be addressed, in part, with high-resolution thin-section transmission electron microscopy (TEM). Unlike confocal imaging, which has a diffraction limited resolution of about 200 nm, thin sections can be prepared with about 2 nm resolution to reveal membranes and nuclei directly, as well as protein density and distribution indicated by cytoplasmic texture. Three factors were critical to obtain new structural insights using thin-section TEM. First, a new fixation procedure was employed that preserved whole lenses initially in formalin followed by paraformaldehyde that avoided the shrinkage reported for some formaldehyde fixations (Augusteyn et al., 2008) and that minimized any gradient of fixation. Second, the initial fixation was followed by Vibratome section processing, used extensively to analyze lens nuclear fiber cell membranes and cytoplasmic texture (Costello et al., 2008; Metlapally et al., 2008). Third, montages of thin sections allowed examination of fine details of cellular structure from the capsule, throughout the RZ and into the adult nucleus. This combination provided the excellent preservation and sufficient resolution to describe the initiating events in the formation of the RZ, formation of membrane undulations, changes in the membrane junctions and modifications in cytoplasm staining and texture that result in fiber cell compaction (Al-Ghoul and Costello, 1997; Al-Ghoul et al., 2001; Taylor et al., 1996). We confirmed the presence of the RZ at 100 μm from the lens surface over an age range of 22–92 years, similar to the original study, and found that the youngest donor lens at 22 years gave the clearest views of distinct cellular changes within the RZ, which supported the conclusion the cellular integrity was maintained within the RZ and there was no evidence for insertion of new membranes in the RZ.

Beneath the epithelium and elongating fiber cells, the fiber cells in humans have classical flattened hexagonal cross-sections with dimensions about 2 μm × 10 μm and close associations with adjacent fiber cells forming numerous specialized junctions and interdigitations (Kuszak and Costello, 2004). These include ball-and-socket interlocking devices (Dickson and Crock, 1972, 1975; Kuszak et al., 1988), edge processes (Taylor et al., 1996), tongue-and-groove interdigitations (Kuwabara, 1975; Taylor et al., 1996; Vrensen et al., 1992), square array junctions (Costello et al., 1985, 1989; Lo and Harding, 1984; Zampighi et al., 1982, 1989), and gap junctions (Costello et al., 1992; Goodenough, 1979; Kuszak and Brown, 1994; Lo and Harding, 1986). These specialized structures have important roles in determining cell shape, stabilizing intercellular contacts and minimizing extracellular space, dependent on lens species, lens age and age of the cell within the lens. It is reasonable to expect that gradual modifications of these specialized features transform the outer cortical fiber cells to the highly flattened fiber cells of the human adult nucleus where the interdigitations are more elaborate and the cytoplasm is more condensed, consistent with the higher refractive index of the lens core (Taylor et al., 1996; Jones et al., 2005). However, the presence of the RZ suggests critical stages of fiber cell differentiation might be more dramatic and more rapid than expected. Ball-and-socket interlocking devices are important in this process. Although young fiber cells of the outer cortex in humans and primates have relatively smooth cell surfaces with few ball-and-sockets (Kuszak et al., 1988; Taylor et al., 1996), a pronounced increase in these types of interactions is observed at the beginning of the RZ. Tongue-and-groove specializations are also critical and have been described as characteristic of older fiber cells, especially in the lens center where scanning electron microscopy has shown the undulating membrane topology to cover a major portion of the cell surface (Kuszak et al., 1988; Kuwabara, 1975; Taylor et al., 1996). We show here that formation of undulating membranes is initiated just after the RZ in the mid-cortex and are not present in any membranes within the RZ.

2. Materials and methods

Human donor lenses were obtained from the North Carolina Eye Bank, Winston–Salem, NC and the Ramayamma International Eye Bank, Hyderabad, India, within 24 h of death. Lenses were collected following the tenets of the Declaration of Helsinki. Whole lenses were immersion fixed in 10% formalin (neutral buffered containing 1–2% methanol) for 24 h followed by fixation in 4% paraformaldehyde in cacodylate buffer (pH 7.2) for 48 h following the protocol described earlier (Costello et al., 2012). Preserved lenses were shipped to University of NC for further processing by TEM using the Vibratome sectioning technique described previously (Costello et al., 2010). Briefly, pre-fixed lenses were sliced into 200 μm thick sections that were immersion fixed 12 h in 2.5% glutaraldehyde, 2% paraformaldehyde and 1% tannic acid in 0.1 M cacodylate buffer (pH 7.2) followed by en bloc staining in cold 0.5% osmium tetroxide for 60 min and 2% uranyl acetate (in 50% ethanol) in the dark for 30 min. Thick sections were dehydrated through a graded ethanol series, embedded in epoxy resin (Epon 812, EMS, Hatfield, PA) from which 70 nm thin sections were cut with a diamond knife (Diatome, EMS, Hatfield, PA). Thin sections were mounted on 300 mesh hexagonal grids and stained with uranyl acetate and lead citrate. Images were obtained using a FEI Tecnai G^2 (T12) TEM operated at 80 kV equipped with a Gatan slow scan CCD camera (1k × 1k, model 794, Gatan, Pleasanton, CA) and Digital Montage software (Gatan, Pleasanton, CA) for collecting up to 5 × 7 arrays of images used to construct extended montages.

3. Results

An advantage of the fixation protocol employed is that the short fixation in formalin appears to open access for subsequent fixatives to enter all regions of the lens. After paraformaldehyde fixation, whole lenses were uniformly hard and differences in mechanical properties at the capsule/epithelium and cortex/nuclear interfaces seemed to be minimized. The resulting whole fixed lenses were easily Vibratome sectioned and processed for TEM with no obvious distortion of cell shape due to osmotic or mechanical stress as illustrated in images of the equatorial plane showing the capsule, epithelium and elongating fibers from a transparent 22 y.o. donor lens (Fig. 1). Furthermore, the preservation of ultrastructure was excellent, revealed in part by the fine lamella of the capsule, the smooth interface between the capsule and epithelium, the good resolution of the epithelium-fiber cell-interface (Fig. 1, EFI) and the resolution of internal membranous structures. Clearly visible in this image are two nuclei (Fig. 1, N), having well-defined nuclear envelopes, and paired membranes of the irregular interface between adjacent epithelial cells (Fig. 1, arrowheads). In addition, internal organelles can be identified and numerous localized cellular defect vesicles (Fig. 1, black arrows) are visible that most likely represent secondary lysosomes or autophagic vesicles degrading and recycling cytoplasmic components (Costello et al., 2013). The mesa yielding these thin sections of epithelium was also used to prepare the subsequent montage of the cortex including the RZ and thus had the same resolution and preservation.

Images from thin sections of the cortex in the equatorial plane near the bow region contain the epithelium (EP) and classical fiber cells (FC) arranged in radial cell columns of flattened hexagonal cells that occasionally display a nucleus (Fig. 2A, cyan line, arrow; Fig. 2B). The thin section extends through the RZ where three regions show changes in cell shape, staining and formation of extensive finger-like interdigitations (Fig. 2A, magenta line; Fig. 2C,