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Novel organelles in primate retinal epithelium

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ABSTRACT

We are investigating age-related changes in organelles in monkey retinal epithelium using transmission and analytic electron microscopy. We previously described a circular organelle in retinal epithelium with a diameter of about 0.5 μm. The organelle is unique in containing a single, round vacuole within an otherwise electron dense interior. We suggested that the organelle might be a melanosome with lysosomal properties. We now find that there are two similar organelles with such a single vacuole but which differ in their chemical composition, electron density, cell location and according to age.

Epon embedded sections from the macular epithelium of seven monkeys, ranging from 1 to 35 years of age, were examined by transmission electron microscopy. A seven year old monkey was processed for analytic electron microscopy to determine the chemical composition of the organelles. The number and location of the organelles in the retinal epithelium were determined.

The chemical composition of these two organelles was different. One of the organelles contained high mole fractions of oxygen and nitrogen and little phosphorous characteristic of melanin; the other had little oxygen and nitrogen and higher mole fractions of phosphorous uncharacteristic of melanin, but more common with lysosomal organelles. The latter had an electron dense rim around the vacuole, a less electron dense interior than the melanin containing organelle and also contained iron. The melanin containing organelle was more common in young monkeys and in the middle third of the cell. The organelle without melanin was more common in old monkeys and localized in the basal third of the cell.

Two similarly vacuolated organelles, not identified before in retinal epithelium, differ in their chemical composition. One contains melanin; the other does not. The former is more common in young and the latter more common in old monkeys. This suggests reorganization and or degradation of melanin-containing organelles with age. These changes show how analytic electron microscopy can distinguish major ultra-structural differences in organelles when mere observation fails to do so easily.

1. Introduction

The earliest signs of age-related macular degeneration are drusen, extracellular deposits in Bruch's membrane, commonly found in elderly humans and other primates with a macula. These deposits arise from protrusions of the basal membrane of the retinal epithelium. Drusen can enlarge and lead to degeneration of the neighboring epithelium and therefore should be considered as a possible factor in the pathogenesis of this disease. In addition to drusen, aging retinal epithelium accumulates large amounts of lipofuscin which may also play a role in the pathogenesis of this disease. The major factor leading to this retinal degeneration, however, is aging itself. For this reason we are examining how aging alters the ultra-structure of this non-replicating epithelial layer in rhesus monkeys, primates with a high prevalence of macular degeneration. We recently reported the presence of an organelle, about 0.5 μm in diameter, which was unique in containing a single, empty vacuole within an otherwise darker interior (Gouras et al., 2011). We suggested that this organelle contained melanin and was possibly lysosomal. To determine whether this organelle actually contained melanin we have now used analytic electron microscopy, which can detect melanin in ultra-structurally identified organelles (Biesemeier et al., 2011; Eibl et al., 2006).

2. Methods

All procedures were approved by the Institutional Animal Care and Use Committee of Columbia University and conformed to the Guide for the Care and Use of Laboratory Animals (8th edition,

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2011). After euthanasia, eyes from monkeys (Macaca *mulatta*), 1, 2, 7, 25, 29, 30 and 35 years of age, were removed within minutes and placed in a buffered solution of 4% paraformaldehyde. Diffusion of fixative was facilitated by piercing the eye at the limbus and injecting fixative into the vitreous. After a week or longer in fixative, the eyes were washed with a balanced salt solution and dissected with the aid of a surgical microscope. The anterior segment was removed and a square piece was cut out of the macula and prepared for electron microscopy. The segments from six of the monkeys were prepared for standard electron microscopy using heavy metal staining and examined with a JEOL 1200 electron microscope. The segment from the seven year old monkey was processed for analytic electron microscopy by not exposing it to heavy metal staining, osmium tetroxide, uranyl acetate and lead citrate. Ultra-thin (70 nm + 120 nm) sections of this unstained sample were mounted on uncoated aluminum grids and examined with a Zeiss 912 Omega transmission electron microscope equipped with an omega energy filter, a 2k × 2k CCD camera and an Oxford EDX detector with an ultrathin window and a digital pulse processor. Energy-filtered bright-field images were acquired and from them areas of interest were selected and investigated with analytical electron microscopy with a lateral resolution of 100 nm. The 100 nm spot was located to a central denser part of the organelles to avoid border-artefacts as described in (Biesemeier et al., 2011). The vacuoles were also measured individually in a number of samples, however as they were of more "epon-like" consistence probably due to wash out artefacts they were not further discussed.

Energy-filtered images, energy dispersive x-ray microanalysis and electron energy loss spectroscopy were applied as explained in (Biesemeier et al., 2011; Eibl et al., 2006). The Student's *T*-test was used to determine the statistical significance of measurements with an error probability of 5%.

3. Results

A typical area of the retinal epithelium in the macular area of a one year old monkey is shown in Fig. 1A. Besides melanosomes and mitochondria, typical organelles of this tissue, three dark circular structures with small empty vacuoles, can be seen at the upper right side of the photograph. Fig. 1B shows a magnified view of these three organelles. Additional magnified views of such organelles from other monkeys are shown in Fig. 1C. The chemical composition of the electron dense regions of these organelles showed high mole fractions of oxygen and nitrogen but phosphorous was at the detection limit, characteristic of melanin containing organelles (Table 1).

In Fig. 2, the same area of a 30-year old monkey is presented. At the upper right side of this nucleus are two circular organelles, each with an empty vacuole adjacent to two similar sized organelles without any vacuole. All of them are less electron-dense than the accompanying melanosomes in the apical part of the photograph. A magnified view of these structures is shown in Fig. 2B. Higher magnification of such organelles from other monkeys is shown in Fig. 2C. Chemical analysis of the interior of these organelles revealed lower oxygen and nitrogen mole fractions and relatively high phosphorous mole fractions (Table 1) uncharacteristic of melanin but consistent with the lighter electron density of the interior of these organelles and more common in lysosomal organelles. In addition, these organelles often had an electron dense ring around the rim of the vacuole which contained iron. (Table 1).

In both cases, the vacuolated internal structure lacked any specific elemental content and was usually of more "epon-like" composition. As this could be due to washout artefacts of water-soluble molecules within a watery compartment, they were not further discussed.

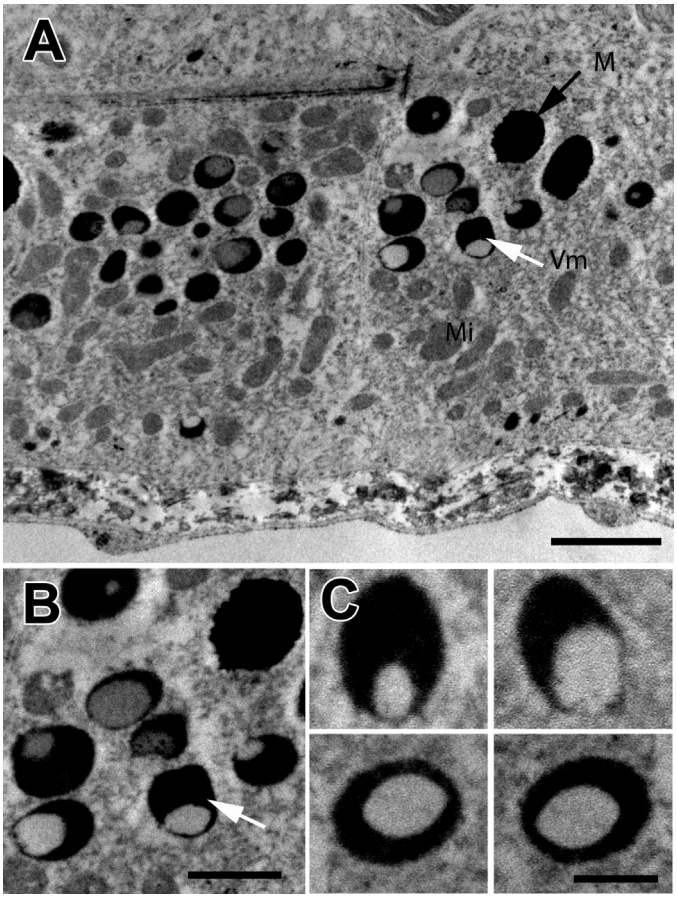

Fig. 1. Vacuolated vesicles with melanin: Fig. 1A shows the middle and basal side of a retinal epithelial cell from the macula of the one-year old monkey. The apical side of the cell is above and the basal side below. (The bar indicates 2 μm).Three dark circular structures with small empty vacuoles, supposed to be vacuolated vesicles with melanin as described in Table 1 (white arrow, Vm) can be seen at the upper right side of the photograph. They are surrounded by typical melanosomes (black arrow, M) of similar size and electron density but lacking any vacuole. Fig. 1 B shows a magnified view of these three "Vm" organelles (The bar indicates 1 μm). Additional magnified views of such organelles from other monkeys are shown in Fig. 1C. (The bar indicates 0.5 μm). M melanosome (black arrow), Mi mitochondrium.

The morphological differences, such as the lighter density of the interior and the presence of the electron dense ring around the vacuole of the organelle without melanin, allowed us to distinguish and track these two different organelles in the retinal epithelium. Fig. 3 shows these results for the two youngest and two of the older monkeys. The melanin containing organelles were more common in young monkeys and virtually absent in old monkeys ($p = 0.001$). On the other hand, the organelle without melanin was more common in the older monkeys and virtually absent in young monkeys. In addition to these age related differences, there was also a difference in the location of these organelles in the cell. The melanin containing organelles were more common in the middle third of the epithelial cell ($p = 0.02$) while the organelles without melanin tended to be more common in the mitochondria-rich basal third of the cell.

4. Discussion

This paper describes two novel organelles in the macular retinal epithelium of rhesus monkeys. They are always circular in all sections and therefore they must be spherical in three dimensions. They have a similar size and shape and always contain a single round vacuole that can sometimes occupy a large fraction of the