The role of the retinal pigment epithelium: topographical variation and ageing changes

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Abstract

The retinal pigment epithelium (RPE) is a single layer of post-mitotic cells, which functions both as a selective barrier to and a vegetative regulator of the overlying photoreceptor layer, thereby playing a key role in its maintenance. Through the expression and activity of specific proteins, it regulates the transport of nutrients and waste products to and from the retina, it contributes to outer segment renewal by ingesting and degrading the spent tips of photoreceptor outer segments, it protects the outer retina from excessive high-energy light and light-generated oxygen reactive species and maintains retinal homeostasis through the release of diffusible factors. The ageing characteristics of the RPE suggest that in addition to cell loss, pleomorphic changes and loss of intact melanin granules, significant metabolic changes occur resulting, at least in part, from the intracellular accumulation of lipofuscin. This pigment has been shown to be highly phototoxic and has been linked to several oxidative changes, some leading to cell death. While the aetiology of age-related macular degeneration is complex and is as yet unresolved, it is likely that accelerated ageing-like changes in the RPE play a fundamental role in the development of this condition.

The retinal pigment epithelium (RPE) is a hexanocuboidal layer of cells which is essential for the maintenance and survival of the overlying photoreceptor cells and is thought, in addition, to regulate the integrity of the choroidal capillaries. In its quest for optimal retinal function the RPE performs a number of critical functions, i.e. formation of the outer blood-retinal barrier, transepithelial transport of nutrients and waste products, transport and storage of retinoids, phagocytosis and degradation of spent outer segments, protection against light and free radicals and the production of growth factors. The structure and function of the RPE varies dependent on both location in the retina (macula vs periphery) and age-related changes.

Structure of the RPE

The RPE is a continuous monolayer of cuboidal post-mitotic epithelial cells located between the photoreceptors and Bruch's membrane.^{1,2} The apical surface of these cells contains two types of microvilli: long thin microvilli 5–7 µm in length which are believed to maximise the area of apical plasma membrane available for transepithelial transport and specialised microvilli termed photoreceptor sheaths. Rod sheaths have a cylindrical conformation and cover only the tip of the outer segment while cone sheaths are more complex and can extend further into the interphotoreceptor matrix in order to reach the cone outer segments. The basal surface of the RPE contains numerous invaginations which, like their apical counterparts, increase the overall surface area for the absorption of nutrients. The lateral membranes of these cells are joined by a continuous belt of tight junctions which form the outer blood-retinal barrier.^{3,4} In addition, RPE cells are metabolically coupled via intercellular gap junctions.⁵ Cells contain a basal nucleus and a plethora of organelles which reflect the high metabolic rate of the RPE. The cells are particularly rich in smooth endoplasmic reticulum and lysosomes, which are distributed throughout the cell, while abundant mitochondria are located towards the base of the cell. In addition, cigar-shaped melanin granules 2–3 µm in length are, for the most part, vertically orientated, lying parallel to one another at the base of, or within, the apical processes.

The size, shape and appearance of the cells vary with retinal location.^{1,6,7} The cells in the macula measure about 14 µm in diameter by 12 µm tall. The cells become flatter and wider away from the macula, with cells at the ora serrata measuring up to 60 µm in diameter with cell size and shape becoming particularly irregular.¹ The darker pigmentation of the macula has been attributed to the greater distribution of melanin granules in this region.⁸

Topographically, melanin density shows a slight decrease from the peripheral retina to the posterior pole but with an increase in the

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macular region. However, the peak in melanin density may be attributed in part to the denser packing of melanin per unit area (cells in the macula are taller and narrower compared with extra-macular cells) rather than an increased number of melanin granules per cell.

Transepithelial transport

The RPE in its role as the outer blood–retinal barrier regulates the transepithelial transport of ions, fluid and metabolites via lateral plasma membrane barrier junctions and specialised transport proteins within the apical and/or basal plasma membranes.^{9,10} The asymmetric distribution and regulation of these proteins ensures that the RPE can determine the microenvironment bathing the photoreceptors. In particular, the RPE regulates the passage of a variety of ions (e.g. Cl^- , K^+ , Na^+ , HCO_3^-) to and from the retina. This is important in the polarisation/hyperpolarisation of cell membranes, in fluid transport and the regulation of pH. A number of amino acids including taurine, alanine, glutamine and leucine are actively absorbed across the RPE.^{11,12} Glucose is passively transported via the GLUT1 glucose transporter which is located in both the apical and basolateral RPE membranes.¹³ Trans-RPE transport is regulated by a variety of external signals of both paracrine and endocrine origin.⁹ Gases diffuse freely across the cell layer with high levels of oxygen moving from the choroid to the photoreceptors.

Retinoid transport and storage

The RPE plays a fundamental role in both the transport and storage of retinoids essential for maintenance of the visual cycle.¹⁴ In brief, retinol is derived from the blood where it is bound to serum retinol binding protein (RBP). Retinol is transferred into the cell via specific RBP binding sites localised to the basal and basolateral surfaces of the RPE. Once within the cell all-*trans*-retinol is immediately bound to cellular RBP and becomes esterified. The resultant all-*trans*-retinyl ester provides a non-toxic substrate for isomerisation to the 11-*cis* conformation, which becomes bound to cellular retinaldehyde binding protein. 11-*cis* retinol is converted to 11-*cis* retinal and transported via interphotoreceptor matrix binding protein (IRBP) to the photoreceptors where, in rods, it combines with opsin to produce rhodopsin. A complete loop is formed by the transfer of retinol back to the RPE either conjugated to IRBP or as a function of the phagocytosis of rod outer segments. Esterification of retinol also provides an intracellular store of retinoids.

Phagocytosis and degradation of outer segments

Throughout life the spent tips of photoreceptor outer segments are ingested by the underlying RPE. It is estimated that in man each RPE cell is exposed to 45 photoreceptor outer segments and that the turnover rate for rod outer segments is in the region of 10 days.¹⁵ The

recognition signals for outer segment binding and ingestion are poorly understood although a number of putative receptor molecules have been identified. These include the mannose receptor,¹⁶ $\alpha_v\beta_5$ integrin,¹⁷ CD38¹⁸ and Mertk.¹⁹ Once ingested the phagosome fuses with a lysosome to form a secondary phagosome or phagolysosome. The ingested outer segments are digested by a combination of over 40 lysosomal enzymes which are capable of degrading proteins, polysaccharides, lipids and nucleic acids. The major lysosomal proteases are members of the cathepsin family, of which cathepsin D is prominent in the RPE.²⁰ The end-products of degradation are either recycled or voided via the choroidal capillaries. In contrast, protease inhibitors cause a build-up of lipofuscin-like material within RPE cells and provide support for the view that lipofuscin, at least in part, results from the incomplete degradation of rod outer segments.^{21–23}

Both the phagocytic load and lysosomal enzyme activity vary with retinal location. The phagocytic load is dependent on both the type and density of the overlying photoreceptors.²⁴ The RPE cells in the fovea will phagocytose cone outer segment tips almost exclusively, while those in the remaining areas will phagocytose primarily rod outer segments. It has been reported that the mean ratio of photoreceptor cells per RPE cell is higher in the macula compared with perimacular or equatorial regions;²⁴ however, this was not substantiated by a subsequent study.²⁵ Lysosomal enzyme activity appears to be highest in the central retina and lowest in the periphery.²⁶ Whether this is a reflection of phagocytic load has yet to be proven.

Protection against light and free radicals

The constant exposure of the RPE to visible light and high local oxygen tensions together with the high metabolic activity of the RPE provides an ideal environment for the formation of reactive oxygen species with the potential to damage proteins, DNA and lipids. Furthermore, phagocytosis of photoreceptor outer segments involves the generation of reactive oxygen species.²⁷ To limit oxidative damage the retina/RPE has evolved three lines of defence. The wavelength and intensity of light reaching the RPE can be attenuated by macular pigment which filters out reactive blue light²⁸ and by melanin which will act as a neutral density filter and reduce light levels entering the RPE.⁸ The RPE contains both enzymic (e.g. superoxide dismutase and catalase) and non-enzymic (e.g. glutathione, ascorbate, α -tocopherol, carotenoids, melanin) antioxidants which neutralise reactive oxygen species before they can cause damage to cellular macromolecules.^{29,30} Despite these controls some oxidative damage is inevitable. However, the cell is able to recognise damaged lipids, protein and nucleic acids, which it rapidly repairs or replaces.³¹

Growth factors

The RPE has been shown to produce a wide variety of growth factors and cytokines both *in vitro* and *in vivo*.³² These include FGF, TGF- β , IGF-I, PDGF, VEGF, TNF- α and members of the interleukin family. Many of these peptides are constitutively expressed although their function in the normal retina remains unclear. FGF and IGF-1 may act as neuroprotective agents,^{33,34} TGF- β may regulate synthesis and turnover of matrix,³⁵ while VEGF may control metabolic activity via the flt-1 receptor.³⁶ In addition to synthesising growth factors the RPE also expresses receptors for most, suggesting an autocrine loop. Since growth factors play a major role in a variety of retinal pathologies, it seems logical to implicate the RPE in some of these events. For instance (a) RPE atrophy can cause choroidal capillary dysfunction suggesting a role for diffusible factors,³⁷ (b) RPE cells synthesise VEGF³⁸ which may be implicated in sub-retinal neovascularisation, (c) downregulation of neurotrophic agents such as FGF may make the retina cells susceptible to apoptotic cell death³³ and (d) a variety of growth factors and cytokines are upregulated in RPE cells localised to epiretinal membranes.³⁹

Age-related changes to the RPE

The human RPE is a non-dividing system which, with increasing age, is seen to undergo structural changes, lose melanin, accumulate the age pigment lipofuscin, have reduced antioxidant capacity and progressively accumulate deposits on, or within, the underlying Bruch's membrane. These age-related changes appear to be strongly associated with the pathogenesis of age-related macular degeneration (AMD).

Morphology

There have been numerous studies to determine changes in RPE cell density with age. However, reports remain contradictory and the extent of cell loss with age is unclear.^{6,7,24,25,40} The most comprehensive study has been undertaken by Panda-Jonas and colleagues⁴¹ who reported that RPE cell density decreases by about 0.3% per year with increasing age. It is reasonable to hypothesise that RPE cell loss will be greater in the macula compared with the periphery due to higher metabolic activity in the central retina. In support of this some workers have reported that cell density decreases with age, with one of the studies showing that cell loss was slower in whites than in blacks.²⁴ By contrast, one study found no age-related change in cell density.²⁵ If RPE cell loss does occur, and is at a different rate to photoreceptor loss, an age-related change in phagocytic load would be anticipated. One study has reported that the ratio of RPE cells to photoreceptor cells decreases with age across the fundus²⁴ while a later study failed to reveal a significant age-related change.²⁵ There is considerable evidence of morphological change to the RPE in aged individuals; this includes loss of cell shape,

hyperplasia with regions of multilayered cells, atrophy (particularly overlying drusen) and areas of both hyper- and hypopigmentation.^{42,43} Whether this represents normal age-related changes or age-related maculopathy is unclear.

Pigmentation

There is considerable change in the pigmentation of the RPE with age.^{2,8} This is evident from fundus examination, in which the RPE appears darker in the young. This is due to a combination of loss of electron-dense melanin granules and the accumulation of less dense lipofuscin granules.

A decline in the total numbers of pure melanin granules is observed in all regions of the retina after the age of 40 years.^{44–46} The decline in melanin granules in the macular RPE is about 35% between the early and late decades and will potentially result in decreased light absorption and a reduced antioxidant potential. The loss of true melanin granules correlates with the association of melanin granules with lysosomes and/or lipofuscin granules.⁴⁷ Melanolysosomes demonstrate loss of the typical cigar-shaped structure and probably represent melanin in the process of repair, modification or degradation. The origin of melanolipofuscin granules is unclear but has been suggested to result from the fusion of melanin and lipofuscin granules. However, the heterogeneity of melanolipofuscin granules suggests that this is unlikely and supports a more dynamic process whereby lipofuscin is actively deposited on melanin granules and vice versa.

Lipofuscin granules are lipid/protein aggregates which continuously increase in the RPE throughout life, eventually occupying up to 19% of cytoplasmic volume by 80 years of age.² Topographically, maximal accumulation of lipofuscin granules occurs in the posterior pole, albeit with a decrease at the fovea.⁴⁸ This correlates with the density distribution of rod photoreceptor cells and points to outer segments being a primary substrate for lipofuscin,^{7,48} although autophagy of spent organelles will also contribute. The molecular composition of lipofuscin has not been fully elucidated.⁴⁹ Evidence suggests that despite its name lipofuscin is predominantly protein, with as little as 25% being lipid. The only component of lipofuscin to be identified to date is the chloroform-soluble fluorophore, A2E.⁵⁰ A2E is a quaternary pyridinium salt that is the product of the reaction of all-*trans*-retinal and ethanolamine. The age-related accumulation of lipofuscin granules is considered by many to have an adverse effect on cell behaviour. To date two mechanisms have been proposed.² The first is that lipofuscin acts directly to cause RPE dysfunction by congesting the cytoplasm.⁷ The second advocates that lipofuscin is photoreactive and given the correct conditions can cause RPE cell death.^{51,52} We have previously demonstrated that lipofuscin is a photoinducible generator of a range of reactive oxygen species (i.e. superoxide anion, hydroxyl radical, singlet oxygen and lipid peroxides).^{52,53} The production of these

species is wavelength-dependent, being greatest in the blue light region of the visible spectrum. Analysis of blue light photoreactivity in isolated human RPE cells demonstrates a marked increase in oxidative activity as donor age increases, which is predominantly due to lipofuscin. Subsequent biochemical studies demonstrated that lipofuscin could photoinduce extragranular lipid peroxidation and inhibition of enzyme activity.⁵⁴ More recently we have confirmed the phototoxic potential of lipofuscin in a cellular system containing a full complement of antioxidants and implicated it in cell dysfunction such as occurs in ageing and retinal diseases.⁵⁵ In brief, exposure of lipofuscin-fed cells to short-wavelength visible light caused loss of lysosomal integrity, cytoplasmic vacuolation and membrane blebbing culminating in cell death. This correlated with lipid peroxidation and protein oxidation of cellular components.

Antioxidant status

There is considerable evidence that antioxidants are implicated in the ageing process. Transgenic experiments on *Drosophila* have shown that molecular genetic regulation of antioxidant systems can facilitate extended longevity, presumably by slowing down the ageing process.³¹ There is limited information of the changes in antioxidant levels within the RPE with age. Friedrichson *et al.*⁵⁶ reported an age-related decrease in vitamin E levels in the macular RPE after 70 years of age while levels in peripheral cells remained constant throughout life. Catalase activity has been shown to increase with age (perhaps indicative of excessive hydrogen peroxide generation) while SOD activity showed no correlation with donor age.⁵⁷ Castorina *et al.*⁵⁸ have demonstrated an age-related correlation between lipid peroxidation and antioxidant enzyme activity. Despite the potential association between oxidative stress and RPE dysfunction, the mechanism by which this may lead to cell damage is unclear. However, numerous studies have shown that oxidative stress can induce apoptosis in cultured RPE cells.⁵⁹

Molecular damage and senescence

Theories of ageing are manifold but tend to favour the accumulation of random error.³¹ This can occur through a variety of both enzymatic and non-enzymatic reactions which can cause molecular damage. Numerous studies have reported increased mitochondrial DNA, nuclear DNA, protein cross-linking and lipid hydroperoxides in aged cells compared with their younger counterparts. These changes which cause cell damage have yet to be confirmed for the RPE. However, exposure to oxidative stress can elicit such molecular damage in cultured RPE cells.^{55,60} Hjelmeland and colleagues^{61,62} have recently reported that the RPE in aged human eyes stains for senescence-related β -galactosidase. Using a cell culture model they have shown an association between

senescence and telomere loss. Whether telomere shortening plays a role in non-replicative RPE cells *in vivo* has yet to be ascertained.

Advanced glycosylation

There is gathering evidence that the age-related accumulation of advanced glycosylation end products (AGEs) in the retina may contribute to RPE dysfunction and AMD. AGEs accumulate within Bruch's membrane⁶³ and in addition may represent a component of lipofuscin which contains Schiff bases and fluorophores⁴⁹ – both features in the genesis of AGEs. Furthermore, AGEs may compromise lysosomal enzyme activity, since they have been shown to inhibit lysosomal enzyme activity in other cell types⁶⁴ Interestingly, overall lysosomal enzyme activity has been demonstrated to increase in the RPE with donor age, although this may simply reflect an increase in the number of lysosomes in response to lipofuscin accumulation.²⁶ The accumulation of AGEs may also affect growth factor production as they have been shown to elevate VEGF and PDGF production by RPE cells *in vitro*.^{65,66}

Relationship between ageing and disease

The association between natural ageing changes and age-related disease is complex.³¹ It is evident that many of these age-related changes are manifest preceding the onset of age-related maculopathy. Furthermore, it is becoming clear that ageing is a combination of both genetic and environmental factors which, in certain individuals, may predispose to disease.³⁰ Certainly, the intracellular accumulation of lipofuscin and changes in Bruch's membrane are prominent in eyes with AMD and represent excessive ageing changes.^{2,55}

References

1. Hogan M, Alvarado J, Weddell J. Histology of the human eye. Philadelphia: Saunders, 1971.
2. Boulton M. Ageing of the retinal pigment epithelium. In: Osborne N, Chader G, editors. Progress in retinal research. Oxford, New York: Pergamon Press, 1991:125–51.
3. Peyman G, Spitznas M, Straatsma B. Peroxidase diffusion in the normal photocoagulated retina. Invest Ophthalmol 1976;10:181–98.
4. Cunha-Vaz J. The blood–retinal barriers. Doc Ophthalmol 1976;41:287–327.
5. Stalmans P, Himpens B. Effect of increasing glucose concentrations and protein phosphorylation on intercellular communication in cultured rat retinal pigment epithelial cells. Invest Ophthalmol Vis Sci 1997;38:1598–609.
6. Streten B. Development of the human retinal pigment epithelium and the posterior segment. Arch Ophthalmol 1969;81:383–94.
7. Marshall J. The ageing retina: physiology or pathology? Eye 1987;1:282–95.
8. Boulton ME. The role of melanin in the RPE. In: Marmor M, Wolfensberger T, editors. The retinal pigment epithelium. Oxford: Oxford University Press, 1998:68–85.

9. Hughes B, Gallemore R, Miller S. Transport mechanisms in the retinal pigment epithelium. In: Marmor M, Wolfensberger T, editors. *The retinal pigment epithelium*. Oxford: Oxford University Press, 1998:103–34.
10. Rizzolo L. Polarity and the development of the outer blood–retinal barrier. *Histol Histopathol* 1997;12:1057–6.
11. Pautler E, Tengerdy C. Transport of acidic amino acids by the bovine pigment epithelium. *Exp Eye Res* 1986;43:207–14.
12. Miyamoto Y, Del Monte M. Na⁺-dependent glutamate transporter in human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 1994;35:3589–98.
13. Harik S, Kalaria R, Whitney P, Andersson L, Lundahl P, Ledbetter S, Perry G. Glucose transporters are abundant in cells with 'occluding' junctions at the blood–eye barriers. *Proc Natl Acad Sci USA* 1990;87:4261–4.
14. Chader G, Pepperberg D, Crouch R, Wiggert B. Retinoids and the retinal pigment epithelium. In: Marmor M, Wolfensberger T, editors. *The retinal pigment epithelium*. Oxford: Oxford University Press, 1998:135–51.
15. Young R. The renewal of rod and cone outer segments in the rhesus monkey. *J Cell Biol* 1971;49:303–18.
16. Boyle D, Tien L, Cooper N, Shepherd V, McLaughlin BJ. A mannose receptor is involved in retinal phagocytosis. *Invest Ophthalmol Vis Sci* 1991;32:1464–70.
17. Finnemann S, Bonilha V, Marmorstein A, Rodriguez-Boulan E. Phagocytosis of rod outer segments by retinal pigment epithelial cells requires alpha_v beta₅ integrin for binding but not for internalization. *Proc Natl Acad Sci USA* 1997;94:12932–7.
18. Ryeom S, Sparrow J, Silverstein R. CD36 participates in the phagocytosis of rod outer segments by retinal pigment epithelium. *J Cell Sci* 1996;109:387–95.
19. D'Cruz P, Yasumura D, Weir J, Matthes M, Abderrahim H, LaVail M, Vollrath D. Mutation of the receptor tyrosine kinase gene *Mertk* in the retinal dystrophic RCS rat. *Hum Mol Genet* 2000;9:645–51.
20. Rakoczy P, Sarks S, Daw N, Constable I. Distribution of cathepsin D in human eyes with or without age-related maculopathy. *Exp Eye Res* 1999;69:367–74.
21. Katz M, Shanker M. Development of lipofuscin-like fluorescence in the retinal pigment epithelium in response to protein inhibitor treatment. *Mech Ageing Dev* 1989;49:23–40.
22. Rakoczy P, Kennedy C, Thompson-Wallis D, Mann K, Constable I. Changes in retinal pigment epithelial cell autofluorescence and protein expression associated with phagocytosis of rod outer segments *in vitro*. *Biol Cell* 1992;76:49–54.
23. Wassell J, Ellis S, Burke J, Boulton M. Fluorescence properties of autofluorescent granules generated by cultured human RPE cells. *Invest Ophthalmol Vis Sci* 1998;39:1487–92.
24. Dorey C, Wu G, Ebenstein D, Garsd A, Weiter J. Cell loss in the ageing retina: relationship to lipofuscin accumulation and macular degeneration. *Invest Ophthalmol Vis Sci* 1989;30:1691–9.
25. Gao H, Hollyfield J. Aging of the human retina: differential loss of neurons and retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 1992;33:1–17.
26. Boulton M, Moriarty P, Jarvis-Evans J, Marcyniuk B. Regional variation and age-related changes of lysosomal enzymes in the human retinal pigment epithelium. *Br J Ophthalmol* 1994;78:125–9.
27. Miceli M, Liles M, Newsome D. Evaluation of oxidative processes in human pigment epithelial cells associated with retinal outer segment phagocytosis. *Exp Cell Res* 1994;214:242–9.
28. Beatty S, Boulton M, Henson D, Koh H-H, Murray I. Macular pigment and age-related macular degeneration. *Br J Ophthalmol* 1999;83:867–77.
29. Newsome D, Miceli M, Liles M, Tate D, Oliver P. Antioxidants in the retinal pigment epithelium. *Prog Retinal Res* 1994;13:101–23.
30. Beatty S, Koh H-H, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol* 2000;45:115–34.
31. Arking R. *Biology of ageing*. Sunderland, MA: Sinauer Associates, 1998.
32. Campochiaro P. Growth factors in the retinal pigment epithelium and retina. In: Marmor M, Wolfensberger T, editors. *The retinal pigment epithelium*. Oxford: Oxford University Press, 1998:459–77.
33. LaVail M, Unoki K, Yasumura D, Matthes M, Yancopoulos G, Steinberg R. Multiple growth factors, cytokines, and neurotrophins rescue photoreceptors from the damaging effects of constant light. *Proc Natl Acad Sci USA* 1992;89:11249–53.
34. Guan J, Gunn A, Sirimanne E, Tuffin J, Gunning M, Clark R, Gluckman P. The window of opportunity for neuronal rescue with insulin-like growth factor-1 after hypoxia-ischemia in rats is critically modulated by cerebral temperature during recovery. *J Cerebral Blood Flow Metab* 2000;20:513–9.
35. Roberts A, Sporn M. Transforming growth factor- β . In: Clark R, editor. *The molecular biology and cellular biology of wound repair*. New York, London: Plenum Press, 1998:275–310.
36. Guerrin M, Moukadir H, Chollet P, Moro F, Dutt K, Malecaze F, Plouet J. Vascutropin/vascular endothelial growth factor is an autocrine growth factor for human retinal pigment epithelial cells cultured *in vitro*. *J Cell Physiol* 1995;164:385–94.
37. Del Priore L, Hornbeck R, Kaplan H, Jones Z, Valentino T, Mosinger-Ogilvie J, *et al.* Debridement of the pig retinal pigment epithelium *in vivo*. *Arch Ophthalmol* 1995;113:939–44.
38. Shima D, Adamia A, Ferrara M, Yeo K-T, Yeo T-K, Allende R, *et al.* Hypoxic induction of endothelial growth factors in retinal cells: identification and characterisation of vascular endothelial growth factor (VEGF) as the mitogen. *Mol Med* 1995;1:182–93.
39. Charteris D. Proliferative vitreoretinopathy: pathobiology, surgical management, and adjunctive treatment. *Br J Ophthalmol* 1995;79:953–60.
40. Tso M, Friedman E. The retinal pigment epithelium. III. Growth and development. *Arch Ophthalmol* 1968;80:214–6.
41. Panda-Jonas S, Jonas J, Jakobczyk-Kmija M. Retinal pigment epithelial cell count distribution, and correlations in normal eyes. *Am J Ophthalmol* 1996;121:181–9.
42. Curcio C, Saunders P, Younger P, Malek G. Peripapillary chorioretinal atrophy: Bruch's membrane changes and photoreceptor loss. *Ophthalmology* 2000;107:334–43.
43. Garner A, Sarks S, Sarks J. Degenerative and related disorders of the retina and choroid. In: Garner A, Klintworth G, editors. *Pathobiology of ocular disease*. New York: Marcel Dekker, 1994:631–74.
44. Feeney-Burns L, Hilderbrand E, Eldridge S. Aging human RPE: morphometric analysis of macular, equatorial and peripheral cells. *Invest Ophthalmol Vis Sci* 1984;25:195–200.
45. Schmidt S, Peisch R. Melanin concentration in normal human retinal pigment epithelium: regional variation and age-related reduction. *Invest Ophthalmol Vis Sci* 1986;27:1063–7.
46. Weiter J, Delori F, Wing G, Fitch K. Retinal pigment epithelial lipofuscin and melanin and choroidal melanin in human eyes. *Invest Ophthalmol Vis Sci* 1986;27:145–52.
47. Feeney L. Lipofuscin and melanin of human retinal pigment epithelium: fluorescence, enzyme, cytochemical and ultrastructural studies. *Invest Ophthalmol Vis Sci* 1978;17:583–600.
48. Wing G, Blanchard G, Weiter J. The topography and age relationship of lipofuscin concentration in the retinal pigment epithelium. *Invest Ophthalmol Vis Sci* 1978;17:601–7.

49. Yin D. Biochemical basis of lipofuscin, ceroid, and age pigment-like fluorophores. *Free Radical Biol Med* 1996;21:871–88.
50. Eldred G, Lasky M. Retinal age pigments generated by self-assembling lysosomotropic detergents. *Nature* 1993;361:724–6.
51. Boulton M, Dontsov A, Jarvis-Evans J, Ostovsky M, Svistunenko D. Lipofuscin is a photoinducible free radical generator. *Photochem Photobiol B: Biol* 1993;19:201–4.
52. Rozanowska M, Boulton M, Burke J, Korytowski W, Jarvis-Evans J, Sarna T. Blue light-induced reactivity of retinal age pigment. *J Biol Chem* 1995;270:18825–30.
53. Rozanowska M, Wessels J, Boulton M, Burke J, Rodgers M, Truscott T, *et al.* Blue light-induced singlet oxygen generation by retinal lipofuscin in non-polar media. *Free Radical Biol Med* 1998;24:1107–12.
54. Wassell J, Davies S, Bardsley W, Boulton M. Photoreactivity of the retinal age pigment lipofuscin. *J Biol Chem* 1999;274:23828–32.
55. Winkler B, Boulton M, Gottsch J, Sternberg P. Oxidative damage and age-related macular degeneration. *Mol Vision* 1999;5:32.
56. Friedrichson T, Kalbach H, Buck P, van Kuijk F. Vitamin E in macular and peripheral tissues of the human eye. *Curr Eye Res* 1995;14:693–701.
57. Liles M, Newsome D, Oliver P. Antioxidant enzymes in the aging human retinal pigment epithelium. *Arch Ophthalmol* 1991;109:1285–8.
58. Castorina C, Campisi A, Di Giacomo C, Sorrenti V, Russo A, Vanella A. Lipid peroxidation and antioxidant enzymatic systems in rat retina as a function of age. *Neurochem Res* 1992;17:599–604.
59. Cai J, Nelson K, Wu M, Sternberg P, Jones D. Oxidative damage and protection of the RPE. *Prog Retinal Res* 2000;19:205–21.
60. Ballinger S, Van Houten B, Jin G, Conklin C, Godley B. Hydrogen peroxide causes significant mitochondrial DNA damage in human RPE cells. *Exp Eye Res* 1999;68:765–72.
61. Hjelmeland L. Senescence of the retinal pigmented epithelium. *Invest Ophthalmol Vis Sci* 1999;40:1–2.
62. Matsunaga H, Handa JT, Aotaki-Keen A, Sherwood SW, West MD, Hjelmeland LM. Beta-galactosidase histochemistry and telomere loss in senescent retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 1999;40:197–202.
63. Handa J, Verzijl N, Matsunaga H, Aotaki-Keen A, Luttj G, te Koppele J, *et al.* Increase in the advanced glycation end product pentosidine in Bruch's membrane with age. *Invest Ophthalmol Vis Sci* 1999;40:775–9.
64. Kasper M, Schinzel R, Niwa T, Munch G, Witt M, Fehrenbach H, *et al.* Experimental induction of AGEs in fetal L132 lung cells changes the level of intracellular cathepsin D. *Biochem Biophys Res Commun* 1999;261:175–82.
65. Handa J, Reiser K, Matsunaga H, Hjelmeland L. The advanced glycation endproduct pentosidine induces the expression of PDGF-B in human retinal pigment epithelial cells. *Exp Eye Res* 1998;66:411–9.
66. Lu M, Kuroki M, Amano S, Tolentino M, Keough K, Kim I, *et al.* Advanced glycation end products increase retinal vascular endothelial growth factor expression. *J Clin Invest* 1998;101:12219–24.