BRIEF REVIEWS ON ASPECTS OF AGING AND THE EYE

Aging and the cornea

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Aging, the persistent decline in age specific fitness of an organism as a result of internal physiological deterioration, is a common process among multicellular organisms.[1] In humans, aging is usually monitored in relation to time, which renders it difficult to differentiate between time dependent biological changes and damage from environmental insults. There are essentially three types of aging at work in any adult tissue; the aging of long lived proteins, the aging of dividing cells, and the aging of non-dividing cells.[2] Dividing cells may be derived from renewing populations in which the rate of cell loss and division is great. An example is the corneal epithelium in which complete turnover occurs within 5–7 days after terminal differentiation.[3 4] Conditional renewal populations, which normally have an extremely low proliferation rate, can also produce dividing cells in response to extrinsic stimuli. Stromal keratocytes are a prime example of a conditional renewing population.[5] Corneal endothelial cells retain the capacity to undergo mitosis and conditional renewal in humans although they very seldom do so.[6–9] Non-dividing cells are those from static cell populations (exemplified by cerebral neurons) which never divide during adult life.[3]

Corneal aging produces both structural and functional changes. These changes in turn can affect the ability of the organ to refract light, to repair itself, and to protect itself and the internal structures of the eye.[10] A variety of corneal aging changes have been reported. However, as it is difficult to distinguish age specific deterioration from degenerations modified by environmental and genetic factors, we think it is helpful to consider these alterations within the broader framework of the aging process. The study over the past 30 years of isolated cells in culture as a model system for aging changes has greatly advanced our understanding of these concepts.

Cell aging

Normal adult cell populations do not divide indefinitely either in the culture dish or in the body.[3 11] Cellular senescence or replicative failure is the process that imposes a limit on their replicative lifespan, and it is thought that cell senescence acts as a powerful tumour suppression mechanism which thus lengthens the healthy reproductive lifespan of the organism.[12] However, the emergence of senescent cells also contributes to the aging process in mitotically competent tissues. This theory, the cell hypothesis of aging, proposes that the gradual accumulation of senescent cells is the primary event that leads to the development of age linked degenerative changes in tissue.[13 14] A key feature of this hypothesis is the presence of senescent cells, but it says nothing about the mechanism that causes the cells to become senescent in the first place. These mechanisms are considered below.

What are senescent cells?

A concept of cell senescence can perhaps best be appreciated after a consideration of what it is not. Senescence is distinct from quiescence, a transient growth arrest state, also known as contact inhibition. Rather confusingly, both senescence and quiescence are referred to as the G0 phase of the cell cycle (sometimes more helpfully distinguished as G0Q and G0S).[15] Senescence is also distinct from cell death, occurring either by apoptosis or necrosis, and it is not a form of terminal differentiation.[16 17] The phenotypes of growth and senescence are totally distinct cell cycle compartments; there is no such thing as a half senescent cell. Cells that enter replicative senescence acquire two phenotypes: they leave the cell cycle with a G1 DNA content,[18] and they undergo a characteristic series of changes in biology and gene expression that alters the function of the cell.[13 19] In this latter situation some genes are transcriptionally repressed, some gene expression is upregulated, and some totally senescent specific genes are turned on.[20] These changes cover practically every aspect of cell physiology and occur in a highly selective manner.[2] As many of the changes occur in genes coding for secreted products the senescent cell can potentially affect the surrounding microenvironment. This altered function of senescent cells may thus be the critical phenotype that compromises tissue function and integrity. As these changes have been largely studied in vitro it is important to examine the means by which cells become senescent and to question their significance within aged tissue.

Where could senescent cells come from within the cornea?

There are two main routes by which a cell may become senescent; they are explained below.

CONSTITUTIVE CELL SENESCENCE

Replicative failure is often visualised as the cell counting a fixed number of divisions and then entering senescence but, while conceptually straightforward, this is a misleading oversimplification. Rather than simply counting its way to senescence, each time a cell divides it has a given chance of never dividing again, a chance that increases each time the cell replicates until senescence becomes a certainty.[21 22] However, since the process is controlled by chance, a cell that has divided only once can still be unlucky and enter senescence. The constitutive process is thus rather like playing Russian roulette, the chance of the fatal bullet is fixed, but the outcome is uncertain. Unlike Russian roulette, each time the cell divides extra bullets are loaded into the revolver. In tissue culture the end result of tens of thousands of chance decisions are cell populations which have a mixture of growing and senescent cells, the proportions of which shift in the direction of total senescence as the culture is passaged and the cells divide.[23] In tissue, even very limited division can thus begin to produce senescent cells. The kinetics of constitutive senescence can be explained in terms of the inheritance of chromosomes with progressively shortened telomeric DNA sequences.[24]

REACTIVE CELL SENESCENCE

This pathway to the senescent state was demonstrated recently and provides a fascinating alternative to the

constitutive route. Current data show that this stage is essentially identical to constitutive senescence but happens in a matter of hours. It has been shown that the induction of the activated H-ras oncogene into growing fibroblasts can trigger senescence.[25] This suggests that, rather like apoptosis, senescence can be induced by mutation or mitogenic overload; this may implicate topical treatment with anticancer drugs in the induction of senescence. In contrast with the constitutive route, this pathway appears to require little if any cell division. Thus, senescent cells may appear much more frequently in quiescent tissue than previously thought, particularly if that tissue is in a mutagenic environment. This may be clinically relevant with the increasing use of potentially mutagenic agents such as 5-fluorouracil and mitomycin C to prevent scarring after pterygium excision or glaucoma filtration surgery; particularly since mitomycin C has been shown to rapidly induce senescent-like changes in cultured fibroblasts.[26] These drugs soak into sclera, conjunctiva, and cornea, particularly after subconjunctival injection, but probably also after sponge application.[27] We have seen prolonged effects on the tissue fibroblasts in the drug treated area that seem unable to divide further despite maximal serum stimulation.[28] It is possible that some of this growth arrest is reactive cell senescence although this remains to be proved. This is an important distinction as senescence is irreversible, unlike prolonged growth arrest seen in vitro that may recover.[29 30] The clinical importance of these observation is that accelerated senescence may thus cause disease within the affected tissue that may not become apparent for many years.

Particularly interesting from the perspective of senescence is the recent observation that removal of the corneal epithelium can trigger apoptosis in the underlying anterior keratocytes.[31] These cells are then replaced after re-epithelialisation by division and migration from the posterior stroma. This apoptosis repopulation process is believed to form a line of defence against invading viruses,[32] it also provides a mechanism by which cell division, and hence the constitutive senescence pathway, may be activated. These processes may be manifest as a decline in the density of keratocytes with advancing age.

The cornea is saturated in light that is potentially mutagenic and reactive senescence can potentially occur in any of the cell layers. Cell turnover within the epithelium is continuous, and thus constitutive senescence has the potential to occur in both the transient amplifying population or, more seriously, in the dividing stem cells. Corneal epithelium has not been examined for the presence of senescent cells but studies of skin strongly suggest they will be present.[33 34] The behaviour of cultured keratocytes from old donors is also consistent with an elevated fraction of senescent cells in the biopsy rather than a reduced number of starting cells.[35] An age related increase in the number of senescent cells in human endothelium has been observed.[36 37]

How does cell senescence affect the cornea?

Structural and functional alterations documented in the aging cornea are listed in Table 1. Although insufficient information exists on the senescence of epithelial cells to draw more than speculative observations, senescence is associated with a decreased ability to resist a wide range of physiological stresses. Changes in the ocular surface render the aging cornea more susceptible to infection for various reasons. There is an increase in epithelial permeability with age that may either represent a breakdown of epithelial barrier function[47] or an increased tear contact time.[46] Changes in the distribution of integrin subunits in the epithelium could also reduce the epithelial barrier function. The α6 subunit and the β4 subunit, components of hemidesmosomes, become discontinuous with age. However, the number and distribution of hemidesmosomes along the basal lamina do not appear to change with age.[48] A reduced ability of corneal cells to upregulate adhesion molecules and a reduced phagocytic ability of reactive polymorphonucleocytes in response to infection also occur with aging,[43 44] and this could impair the ability to eliminate a bacterial infection. Epithelial disease, in turn, has the potential to contribute to cell loss within the endothelial layer.[49]

The major cellular component of the corneal stroma is the keratocyte. Few studies have been conducted on these cells, but in vitro studies of senescent dermal and lung fibroblast-like cells have demonstrated constitutive overexpression of collagenase, stromolysin, and elastase.[50 51] Simultaneously, the expression of tissue inhibitors of metalloproteinases (TIMP 1 and TIMP 2)[2 52] are greatly reduced, as is collagen mRNA.[53] Fibronectin is produced in an altered form which is a less efficient substrate for cell adhesion,[54] proteoglycan synthesis falls,[49] and the migration rate[55] and the ability of fibroblasts to contract a collagen lattice in vitro also decline.[56] Lipofuscin and endogenous ceramide levels increase.[57 58] Gap junction assembly times increase by an order of magnitude and membrane permeability increases sharply.[59] Specific inhibitors of calcium dependent membrane currents are induced.[60] In addition, the glycation of corneal collagen produces an increase in intramolecular spacing.[42] The overall result of these changes is a radical shift of the senescent cell into a highly catabolic phenotype[2] and, in aging skin, senescent cells have been demonstrated in close proximity to degen-

Table 1 Alterations seen in the aging cornea

Characteristic	*Result of change*
(1) Changes in shape and optical properties	(i) Steepening of keratometry and a shift from with the rule to against the rule astigmatism[38 39] (ii) Transparency is unaffected in central cornea in absence of scar or degeneration[40] (iii) Collagen intramolecular and interfibrillar spacing increases—possibly via increased protein glycation[41 42] (iv) Increased thickness of Descemet's membrane
(2) Corneal degenerations (influenced by environmental and genetic factors)	(i) Cornea farinata (ii) White limbus girdle (iii) Mosaic degeneration (iv) Deep crocodile shagreen (v) Hassall–Henle bodies (vi) Arcus senilis
(3) Physical properties	(i) Resistance to infection reduced (ii) Failure to upregulate ICAM-1 and reduced inflammatory cell infiltration[43] (iii) Reduced phagocytically active cells after infection[44] (iv) Decline in high energy metabolism[45] (v) Increased tear contact time[46] (vi) Increased epithelial permeability to flourescein[47]

erative and disorganised collagen fibrils.[33] The reduced keratocyte density within the aging cornea, the breakdown of collagen fibres, and the appearance of collagen-free spaces may reflect similar changes within this tissue.[61 62] The increase in lipofuscin granules seen in the aged stroma (corneal farinata) may represent deposition of products from senescent cells.

Some of these factors could adversely affect wound repair. A reduced number of fibroblasts, an inability of many of those cells to divide, the decreased ability for migration, a reduced ability to contract a wound lattice, and the depressed synthesis of collagen are believed to contribute strongly to impaired wound healing in the aging dermis. While no direct evidence for this currently exists in the normal cornea, patients with Werner's syndrome—a hereditary disease characterised by premature fibroblast senescence[63]—show severely impaired corneal wound healing following cataract surgery.[64] The implication that senescence may have a detrimental effect on the outcome of corneal surgery is thus strong. Decreased healing of wounds in the aged may be advantageous in some circumstances such as glaucoma filtration surgery, but age has also been identified as the most important individual variable affecting the outcome after refractive surgery; the amount of effective aggression decreasing proportional to increasing age.[65–67]

Age related changes in the corneal endothelium have been examined clinically and experimentally. It has been estimated that between the ages of 20 and 80 years the annual reduction in cell density averages approximately 0.6%, with concomitant increases in polymegethism and pleomorphism.[68–71] However, as the mean age of the population sample increases, there is an increased spread in the range of the endothelial cell density counts.[72] This means that the measurement of endothelial cell density is not a reliable index of the chronological age of the cornea, and suggests an environmental influence. Changes in cell density and shape with age have been observed to occur in the human,[73] monkey,[74] rat,[75] cat,[74 76] dog,[77] and rabbit,[78] but in each of these species the adult mean cell density (about 2500 cells/mm^2) is remarkably constant. Interestingly, it has been noted in the rat, which has the ability for endothelial cell division, that the total reduction in endothelial cell numbers is of the same order of magnitude as occurs in humans, but the cell loss is compressed into the shorter life span of this species.[75] The biological mechanisms behind the gradual endothelial cell loss during aging remain to be elucidated, but might involve hormonal changes or environmental influences such as ultraviolet irradiation and chemical toxicity. In particular, the degradation of enzymes in the anterior segment that normally metabolise and detoxify hydrogen peroxide and other free radicals may lead to progressive damage to the endothelial layer.[79] Reduction in endothelial cell numbers and the increased variability in cell size and shape that accompany normal aging may adversely affect endothelial function,[80] although this reduced function may also be the result of a decline in high energy metabolism with age.[45] The aged cornea is slower to recover from hypoxic stress,[81] and grafts from older donors usually require a longer postoperative period to attain their final thickness.[82 83] Although advanced donor age does not preclude the use of a cornea for grafting, the life span of a transplanted endothelial cell is, as yet, unknown. In rabbits, where the endothelial cell layer is able to regenerate, the pattern of corneal endothelial wound healing after transcorneal freezing is slower and less extensive in corneas from adult animals than from young animals.[84]

Conclusions

A wide range of changes occur in the aging cornea, some of which can be linked to the changes seen in aging cells in culture. The lack of uniform culture systems for corneal epithelium and endothelium has limited the study of senescence phenotype in these cell types. Although the growth arrest phenotype of senescence is universal among different cell types the changes in function that accompany it are not, with many growth arrest genes showing high tissue specificity. Only studies of these cell types will allow firm conclusions to be drawn. Simple inactivation of the mechanism of replicative failure is intrinsically undesirable since it apparently functions as an antitumour mechanism. A more sensible strategy appears to be to define in greater detail the functional phenotype of senescence and then to attempt to modify this through therapy, an intriguing clinical possibility for the future.

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