The Cellular and Molecular Biology of Skin Aging

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Background: The dramatic alterations in the appearance of the integument with increasing age are due in part to a progressive destruction of the delicate architecture of the connective tissue components of the dermis. Both collagenous and elastic components display a degeneration consistent with the overexpression of proteolytic activity. Recent advances in the field of molecular gerontology, using in vitro models of cellular aging, are yielding clues as to the fundamental causes of dermal aging.

Observations: Dermal fibroblasts possess a finite replicative capacity of 50 to 100 doublings, then cease replicating in response to growth factors. Cells cultivated to the end of their replicative lifespan in vitro display alterations consistent with their playing a role in aging in vivo. In particular, senescent dermal fibroblasts overexpress metalloproteinase activities that may explain the age-related atrophy of extracellular matrix architecture.

Conclusions: The recent discovery of a structural change in the telomeric region of the genome with cellular aging and new insights into DNA damage checkpoint mechanisms offer new opportunities to uncover both the molecular mechanisms regulating cellular aging and possibly to devise new strategies to manipulate these molecular events for therapeutic effect.

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THE RAPID growth in the aged sector of the population is changing the practice of dermatology. Life expectancy has increased from a mere 47 years in the United States in 1900 to approximately 75 years in 1990. While there were only three million people in the United States over 65 years of age in 1900, by 1990 the number had grown to 32 million and the aged continue to be the most rapidly growing segment of the population.[1] This demographic shift will result in a marked increase in age-related dermatologic disease in the coming decades and highlights the need for an improved understanding of the fundamental molecular mechanisms of skin aging.

Cutaneous aging presents as an insidious and progressive degenerative process, inevitable in course, and predictable in outcome. On a macroscopic level, the changes include the following: dermal atrophy, wrinkling, and a loss of elasticity and subcutaneous fat. The gross appearance of aging skin, in turn, heralds dramatic underlying histologic alterations. There is a profound atrophy of the architecture of the dermis with destruction of both collagenous and elastic components. There is also a disregulation of the proliferative homeostasis of numerous cell types resulting in benign and malignant lesions.

The histopathologic findings of aging skin have been carefully described in previous articles; however, there have been no attempts to review the data in the light of cellular aging models. In this review, we will discuss the salient features of aging skin and describe recent advances in the molecular genetics of cellular aging that are supplying important new insights into its etiology.

Numerous reviews have adequately described the difference between normal cutaneous aging and damage from solar exposure. The prior is referred to as intrinsic aging, and the latter as actinic aging. In this article, we will, therefore, emphasize only the intrinsic process and the data discussed will be limited to protected regions of skin from identical sites and different ages.

From the Geron Corp, Menlo Park, Calif.

HISTOLOGIC ALTERATIONS ASSOCIATED WITH CUTANEOUS AGING

The Epidermis

The epidermis is classified as stratified squamous epithelium. Keratinocytes originate from a stem cell layer (stratum basale) in contact with the basal lamina. The basal cells replicate with one remaining in contact with the basal lamina and maintaining the stem cell phenotype, and the other dividing a few times and then differentiating into a terminally nondividing cell. During this differentiation process, the cells migrate into the stratum spinosum (characterized by pronounced desmosomes), then into the stratum granulosum (named for the visible accumulation of keratohyalin that forms a watertight barrier), and finally into the stratum corneum (containing flat keratinized cells).

The epidermis is a highly mitotic tissue, and it is commonly assumed that the basal keratinocytes are dividing thousands of times during the lifespan of the individual. However, recent studies performed in pig and rodent skin suggest that epidermal stem cells may divide far fewer times.[2] The number of doublings required is dependent on the nature of the replicative cascade. A tangential cascade proceeding from a single stem cell doubling (**Figure 1**, left) would require thousands of stem cell doublings to produce the number of keratinocytes observed in normal aging skin. In contrast, an exponential cascade, shown in Figure 1, right, would allow a single stem cell doubling to produce hundreds of differentiated keratinocytes. Since the flattened keratinocyte has many times the surface area of the small basal keratinocyte, it is reasonable to assume that, under the exponential scheme, a single basal keratinocyte division followed by a chain of four to six mitotic events could generate enough differentiated keratinocytes for 1 to 3 years. In support of the exponential model, suprabasilar mitotic cells are well documented in pig and human skin.[3] Also, the basal cells appear to be organized into epidermal proliferative units,[4] with about 11 basal cells underlying each flattened differentiated cell, and a single central basal cell (presumably the stem cell) proliferating only rarely. In further support of the exponential model, basal stem cells are observed at the base of rete ridges, where they rarely divide, giving rise to a burst of transit proliferative cells carrying out further exponential amplification.[3,5] Many of these transit laterally along the basal lamina, as shown in Figure 1, right. These observations suggest that epidermal stem cells may divide less than one hundred doublings during the course of human aging. This, in turn, has important implications for the role of cellular aging in the aging of the epidermis.

It is frequently reported that there is a thinning of the epidermis during aging.[6,7] However, these observations are disputed by other investigators.[8,9] No perceptible changes have been reported in the ultrastructure of the stratum spinosum or stratum granulosum, and the thickness of the stratum corneum appears to remain unchanged.[6] On an ultrastructural level, only the cells of the stratum basale display alterations in morphologic appearance. Aging skin stratum basale cells show an increased heterogeneity of size, morphologic appearance, and staining properties.[10] Similar changes are reported to occur in actinic aging and are occasionally referred to as diffuse epidermal dyscrasia.

The dermoepidermal junction shows well-documented alterations in aging skin.[11,12] There is a clear flattening of the junction resulting in a decrease in the junction surface area from 2.64 to 1.90 mm^2 in abdominal skin, with a corresponding decrease in interdigitating papillae.[13] This is likely due to a proportionate decrease in the number of rete ridges and dermal papillae per unit surface area of skin.[14] The young basal cells display villous cytoplasmic projections into the dermis that are strikingly reduced in aged skin.[6,15] This decreases the surface area of the junction of the dermis and epidermis decreasing the adhesion of the two layers. This effacement of the interdigitations predisposes the skin to shearing and bullae formation from stresses such as lichen planus or lichen sclerosus et atrophicus.

Aging epidermis shows a decrease in the number of melanocytes and Langerhans cells. The number of dopamine-reactive melanocytes has been reported to decrease from 8% to 20% per decade after 30 years of age.[16] The result is a weakened tanning response. The decreased proliferation of melanocytes, in turn, could result in increased UV exposure to the stratum basale, with a resulting increase in basal cell carcinoma. Paradoxically, there is a tendency for melanocytes to proliferate focally and to cluster and form lentigines. These hyperpigmented macules are designated senile lentigo, or "liver spots." While these lesions appear with increasing frequency with age and occasionally appear in unexposed areas, they are most frequently observed in regions chronically exposed to the sun.[17] The melanocytes are reported to be larger and morphologically more heterogeneous in aging skin.[18-20]

The Dermis

The dermis is mostly composed principally of extracellular connective tissue matrix of which collagen comprises some 70% of the dry weight.[21,22] The dermis can be divided into two layers: the papillary dermis, composed of relatively fine collagen fibrils roughly corresponding to the depth of the dermal papillae; and the reticular layer, which is deeper, and composed of more dense collagen fibrils. In contrast to the epidermis, the dermis shows striking alterations during aging. Studies of aging dermis have revealed that aging dermal matrix is more disorganized,[23,24] more avascular,[12] and more acellular. The disintegration of the matrix protein is characterized by a loss of the fascicular collagen fibrils and an increase in fibrils with a disorganized and granular appearance. Furthermore, collagen content, expressed as collagen per unit area of skin surface, has been shown to decrease during skin aging at a rate of approximately 1% per year throughout adult life.[25] There are also reports that collagen fibers become more compact perhaps due to a loss of proteoglycan ground substance.[15] There is reported to be a decrease in the amount of

"ground substance" or proteoglycans with age; specifically, decreased hyaluronic acid and dermatan sulfate.[26]

These results suggest that there may be a decrease in matrix collagen synthesis and/or an increase in proteolysis during aging. In support of the latter interpretation, while there is a marked decrease in procollagen synthesis from fetal to adult skin, from about 30 to 40 years of age onward there is no discernable decrease.[27] An overexpression of protease activity may explain not only the decreased collagen content, but also the widespread destruction of extracellular matrix architecture. Collagen imparts tensile strength to skin; therefore, the loss of integrity of collagen fibers may explain the proclivity of skin in elderly patients to tear under even moderate stress.

Elastin is a fibrous structural protein of the dermis. While comprising only about 2% of the total protein,[28] it supplies the skin with important elastic properties. There is a marked disintegration of elastin fibers in the papillary dermis during intrinsic aging.[29,30] This disintegration is increasingly apparent with age, such that by the age of 70 years the majority of the fibers are affected. The fibers are decreased in number and diameter and often appear fragmented, especially in the dermoepidermal region.[12] The fibers are frequently reported to have "fuzzy" margins consistent with damage by elastolytic enzymes.[15,30] In addition, focal differences (ie, patchy loss or proliferation of elastin fibers) become apparent. Actinic changes are similar, although more severe.[31] It is, therefore, widely acknowledged that intrinsic to the aging of skin is a pronounced autologous progressive destruction of the elastin fiber architecture that begins after the age of 30 years and becomes profound after the age of 70 years.[30,32] The role of elastolysis in the phenotype of skin aging is easily visualized in the case of inherited defects in elastin metabolism, such as cutis laxa (**Figure 2**). In this syndrome, there is a remarkable premature aging of skin and other connective tissues resulting in skin wrinkling, laxity, emphysema, osteoporosis, loss of subcutaneous fat, and cataracts.[33-36] In at least one case, the cause is attributable to elevated levels of proteolytic activity.[37] In summary, the age-related alteration in the mechanical properties of skin may be explained in part by the fragmentation of collagen and elastin fiber architecture and supramolecular organization that, in turn, may reflect increased extracellular proteolytic activity.

There is a decreased vascularity in the papillary dermis with age. The capillary loops that run perpendicular to the dermoepidermal junction are decreased in number.[12,38] In one study, the cross-sectional area of dermal venules decreased by 35%.[39] There is a reduction of mast cells in the dermis of about 50%. These cells provide angiogenic stimulus; therefore, their loss may play a role in the decreased vascularity. The vascular network of the dermis also shows evidence of less adventitial support. A decrease in the thickness of the wall of postcapillary venules and a corresponding decrease in the number of veil cells is reported in patients over 80 years of age.[40] Similarly, electron micrographic analysis of the elastic component of cutaneous arterioles revealed occasional granular degeneration in the aged samples. The loss of dermal vasculature leads to increased pallor and decreased temperature,[41] and the progressive loss of structural support for dermal vessels may lead to the increased bruising seen in the aged.

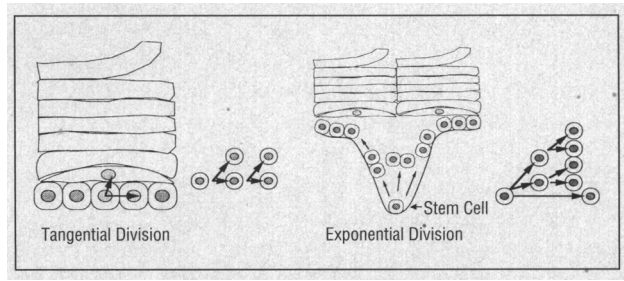

Figure 1. *Tangential and exponential epidermal cell kinetics. Left, A model of tangential basal epidermal stem cell replication. Right, A model of epidermal cells replicating and amplifying their number in an exponential manner thereby reducing the requisite number of doublings of the stem cell.*

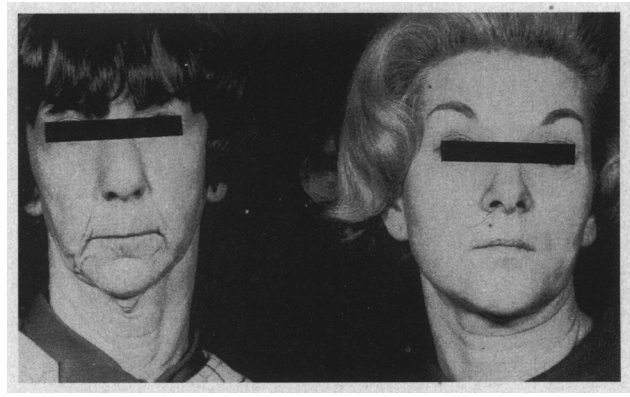

Figure 2. *Progeroid facies of cutis laxa. The patient at the left was diagnosed with cutis laxa at the age of 3.5 years. She presented with unusually lax skin on the face and neck that made her look approximately four times her actual age. In this photograph she was 16 years of age, while her mother on the right was 51 years old. (Courtesy of Victor A. McKusick, MD.)*

The eccrine glands decrease in number and show a decrease in organization[10] in aged skin. Concommitant with this degeneration is an increase in lipofuscin content in the epithelial cells.[42] Changes in the apocrine glands are not as well characterized. The secretion is reported to decrease,[43] and the glandular cells show increased lipofuscin deposition.[10,44] The sebaceous glands undergo unusual changes in aging. Sebum production parallels sebaceous gland size in the young. Both are high at birth when the fetal adrenal gland produces high levels of androgens; they subsequently decline with decreasing androgen levels until puberty when both increase again. The number of sebaceous glands changes little with age, however, they undergo a characteristic hyperplasia.[45] Sebum production in the elderly differs from the young in that it does not parallel gland size and decreases approximately 23% per decade in men and 32% per decade in women.[46] The changes in sebum production are usually attributed to decreased androgen production in the elderly,[47] although this is not consistent with the hyperplasia of the gland, an event usually also thought of as an androgen-mediated event. Sebaceous gland hyperplasia is a common

lesion in the elderly. The gland is profoundly enlarged with multiple hyperplastic lobules; the cause of these alterations in the sebaceous glands is poorly understood, although alterations in androgen metabolism have been implicated.[48]

Hair also undergoes numerous changes in aging. The number of terminal hairs and hair follicles decreases in both men and women.[49] It is estimated that 70% of all men over the age of 50 years have marked balding and the majority have some degree of graying.[50] Graying is a consequence of less melanin deposition. Gray hair is reported to have vacuolated, poorly pigmented melanocytes, and white hair has an absence of melanocytes in either the papillae or the shafts.[16]

In summary, skin exhibits numerous alterations with age that can be described as morpholytic. The most visible changes occurring in aging skin are seen in the dermis. Therefore, it seems logical to suggest that aging research should focus on the cells whose role is to synthesize and maintain this matrix. Fibroblasts are the most numerous and the most important cells regulating matrix metabolism in skin. It is no coincidence, therefore, that the cultured fibroblast has historically been the first choice as a cellular model for organismic aging.

THE BIOCHEMISTRY OF CONNECTIVE TISSUE MAINTENANCE

The fibroblast plays a pivotal role in the morphogenesis and dynamic remodeling of the dermis as well as other connective tissues. The remodeling process includes both a clastic or destructive aspect whereby the cells destroy the extracellular protein, and a blastic aspect whereby they synthesize new protein. Fibroblasts, like many other replicating cell populations, display at least two phenotypes depending on signals from the extracellular environment (**Figure 3**). In their normal maintenance mode, designated *quiescence,* they proliferate at a low rate and synthesize relatively low levels of proteolytic enzymes. In response to numerous stimuli, including inflammation and wounding, they dramatically alter their phenotype, re-enter the cell cycle, and upregulate the expression of proteolytic enzymes in a proenzyme form. This is frequently designated the *activated* phenotype. The proenzymes of the metalloproteins are then activated and begin to destroy extracellular matrix (Figure 3). The upregulation of collagenase synthesis is observed in both in vitro models and in inflammation and wound repair. For example, collagenase activity peaks on postoperative day 1 in sutured incisions and day 5 of large-defect full-thickness wounds and decreases progressively thereafter.[51]

Fibroblast activity is regulated by a host of cytokines and hormones. These regulate the synthesis of both structural proteins of the extracellular matrix and a family of proteases and protease inhibitors that sculpt the structural proteins during the construction of the extracellular matrix and catabolize it in remodeling. Connective tissue resorption is a normal part of cell migration, growth, angiogenesis, and wound healing and is normally a process tightly regulated by extracellular signals.[52]

The family of matrix metalloproteinases play a major role in the proteolysis of dermal extracellular matrix components. Collagenase is the rate-limiting enzyme in the degradative pathway of collagen.[53] It produces the initial cleavage of the molecule, thereby allowing it to "unwind" and leaving it to be further digested by gelatinases. Elevated collagenase activity is thought to play a role in numerous pathologic changes and examples of tissue remodeling, including arthritis, gingivitis, bone resorption, and postpartum involution of the uterus.[54] Stromelysins 1 and 2 possess a broader substrate specificity, with stromelysin 1 being the more potent.[55-57] They target such matrix components as fibronectin; proteoglycan core protein; the nonhelical regions of elastin; collagen types II, IV, and IX; laminin; procollagens I and III; and gelatin. Both collagenase and stromelysin must be converted to their active forms, probably by plasmin, the product of the plasminogen activator–mediated activation of plasminogen.[58-60]

There is evidence of continuing remodeling of elastic fibers as well.[30] Elastin fiber formation in a young adult has been documented.[61] The turnover time, however, is poorly measured and is reported to be several years for rodent aorta and lung.[62-64] Treatment of skin with pancreatic elastase resulted in protein that remained in the shape of short fibers, frequently in tangles with indistinct margins, with cysts and lacunae within the fibers virtually identical to those observed in normal aging. No such changes were observed using other agents such as collagenase, hyaluronidase, or dithiothreitol.[30]

THE MOLECULAR BIOLOGY OF CELLULAR AGING

In an essay written in 1881 called "The Duration of Life," the German naturalist August Weismann proposed a watershed concept in the history of biology. The theory, frequently referred to as the *immortality of the germ plasm,* stated that the cells of the germline were immortal having an infinite replicative capacity, whereas somatic cells from all of the bodily tissues except the germline were mortal and possessed a finite replicative capacity. "Death takes place because a worn-out tissue cannot forever renew itself, and because a capacity for increase by means of cell division is not everlasting but finite."[65]

The modern study of the cellular and molecular basis of age-related disease owes its origin to the pioneering work of Leonard Hayflick. In 1961, Hayflick and Moorhead reported that connective tissue fibroblasts possess a limited capacity for division, generally 50 to 100 population doublings depending on the age of the donor.[66-69] As shown in **Figure 4**, cells placed in tissue culture soon acclimate and enter a period of logarithmic growth. After a finite number of doublings, the cells slow their division rate and then become refractory to growth factor–induced proliferation. In **Figure 5**, it can be seen that young fibroblasts have a spindle-

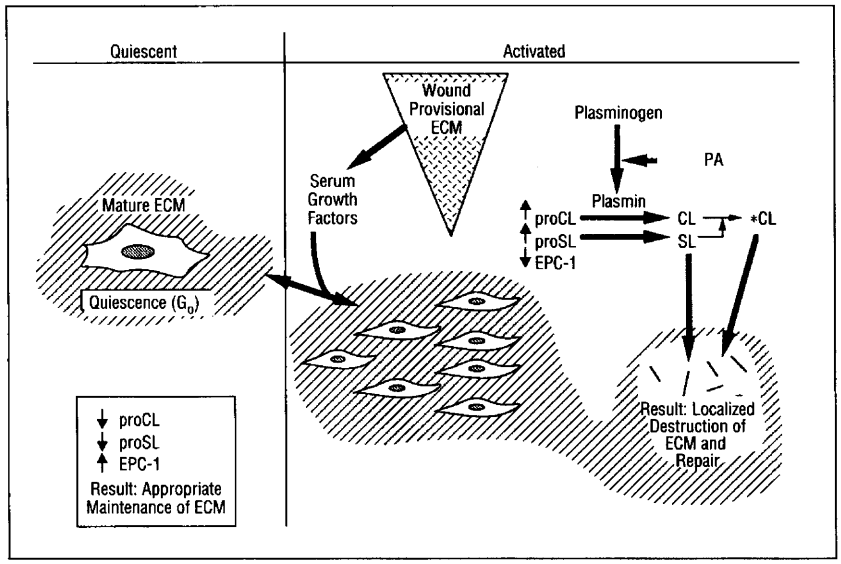

Figure 3. *Dynamics of young fibroblast function. Left, Young proliferation-competent cells such as dermal fibroblasts normally reside in quiescent nondividing states and secrete a pattern of proteins that maintains extracellular matrix (ECM). Right, In the case of activation from inflammation or wounding, the cells enter the cell cycle and upregulate the secretion of secreted protease activities that transiently destroy extracellular proteins as remodeling commences. Procollagenase (ProCL) and prostromelysin (proSL) are activated by plasminogen activator (PA). Activated stromelysin (SL) in turn cleaves activated collagenase (CL) into a super-activated form (*CL). Activated fibroblasts downregulate the expression of quiescent-specific proteins such as EPC-1.*

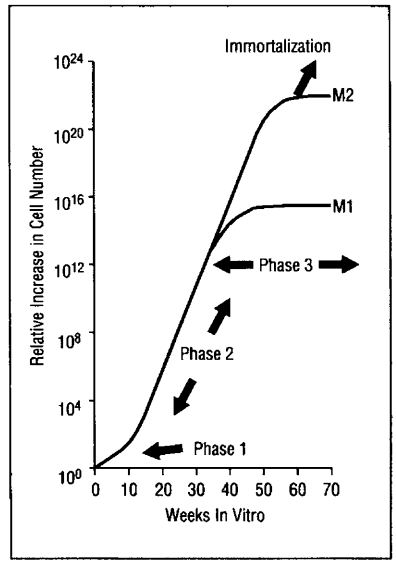

Figure 4. *Replicative senescence in vitro. The curve represents the replicative history of a typical culture of skin cells. After phase 1, which represents primary culture, a period of active growth commences—termed phase 2. After about 50 to 100 doublings, cell proliferation ceases in what has been termed phase 3 (now designated mortality 1 [M1]). In the presence of viral oncoproteins, M1 may be bypassed, which imparts to the cell an increased replicative capacity to a later horizon designated mortality 2 (M2). Rare clones of cells rising from populations of M2 cells may then become capable of indefinite growth designated "immortalization" (arrow).*

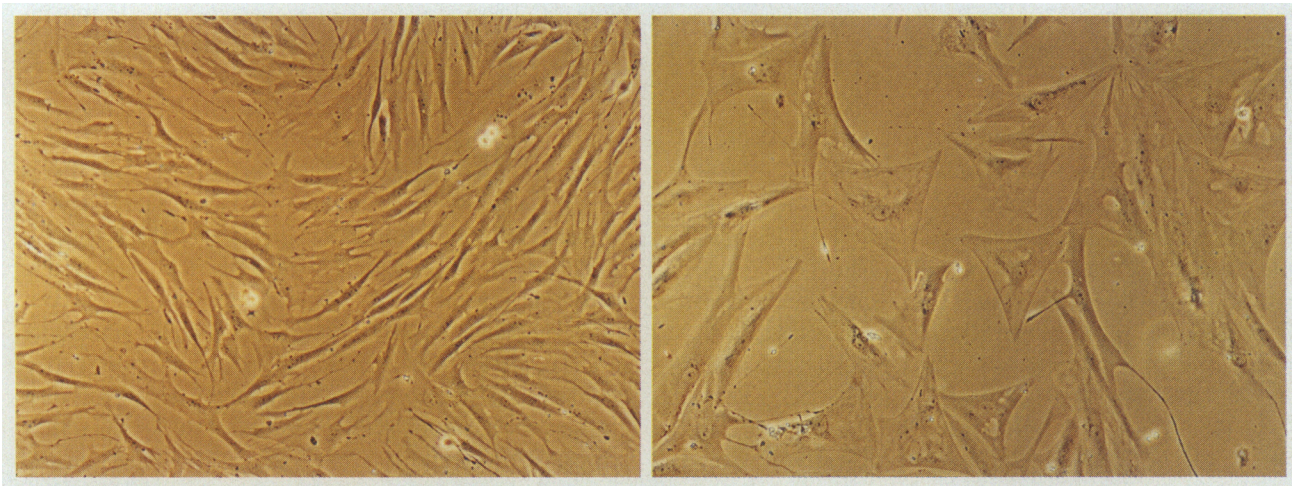

Figure 5. *Phase contrast photographs of young and senescent fibroblasts. Young human foreskin fibroblasts (left) were photographed after 28 population doublings in vitro. They are shown in a subconfluent monolayer grown in the presence of 10% fetal calf serum. After 64 doublings, the cells became senescent (right) and ceased replication despite adequate growth media.*

shaped morphologic appearance and actively replicate in the presence of 10% fetal calf serum. In contrast, senescent fibroblasts exit the cell cycle and display an altered morphologic appearance. For the last 30 years, the in vitro model of cellular senescence has been used to analyze the role of cellular aging in age-related disease separated from in vivo fluctuations in hormones and other parameters.

The most provocative discovery since the discovery that somatic cells possess a finite replicative capacity was that they progressively lose telomeric repeats from the end chromosomes. The telomere hypothesis[70,71] proposes that somatic cells lack sufficient amounts of activity of the enzyme telomerase to maintain the telomeric repeats in the face of the end replication problem. Therefore, with each round of cell division, mortal cells lose on average 50 to 200 base pairs (bp) of telomeric repeats with each cell division. Beginning with the fertilized zygote, the length of terminal restriction fragments is believed to be approximately 15 ki-

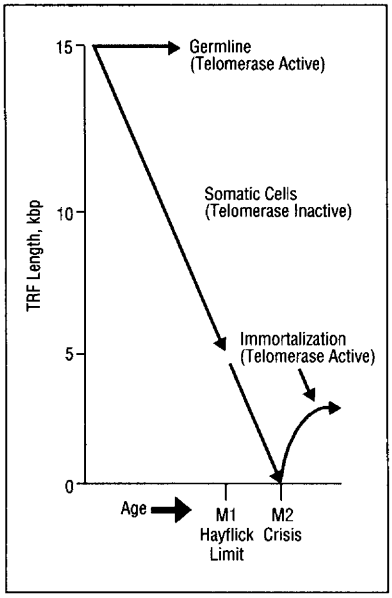

Figure 6. *The telomere hypothesis. Human germline cells display terminal restriction fragments averaging 15 kilobase pairs (kbp), which are maintained in the germline where telomerase activity is abundant. In contrast, somatic cells contain little or no telomerase activity and telomeric repeat sequences are progressively lost with cell division. The elimination of the gene products of the tumor supressor gene products p53 and pRb allows the extension of the replicative lifespan of cells, such that they now enter a second horizon of growth arrest designated mortality (M) 2, from which rare cells may immortalize by reactivating telomerase. The terminal restriction fragment length (TRF) represents the residual telomere fragments after digestion by restriction enzymes.*

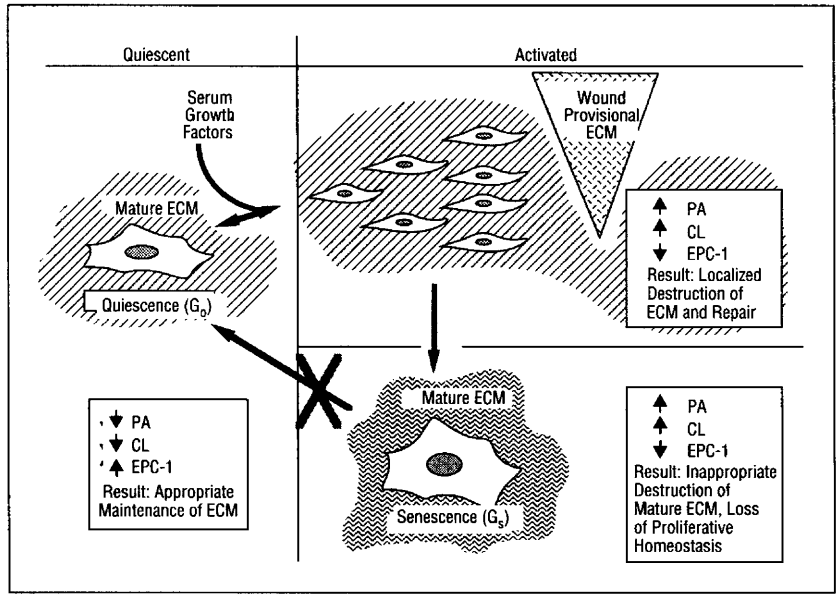

Figure 7. *Dynamics of young and senescent fibroblast function. Summary of the alterations observed in senescent fibroblast function in vitro and illustration of how such alterations may result in changes associated with the in vivo aging of skin. Left, A young fibroblast that has the capacity to enter quiescence (G_0) when in mature extracellular matrix (ECM) and low growth factors concentrations. Top right, Young fibroblasts can be induced by serum growth factors secreted from clotted blood, and the clot's provisional extracellular matrix, to become activated. Bottom right, Mortality 1 activation. Senescent fibroblasts lose responsiveness to growth factors in terms of both cell division and extracellular matrix metabolism. Senescent fibroblasts also lose the capacity to become quiescent and instead are blocked in a cell cycle phase distinguishable from G_0 designated G_s. The shift from G_0 to G_s results in an inappropriately fibroclastic activity toward mature extracellular matrix. PA indicates plasminogen activator; CL, collagenase.*

lobase pairs (kbp) (**Figure 6**). In the case of dermal fibroblasts, the length has decreased to about 10 kbp at birth, and subsequently shortens during aging, until it reaches senescence, now designated as mortality 1 (M1). The telomere hypothesis was an important advance in cellular gerontology as it allows researchers for the first time to carefully track the replicative life history of cells, and study the role of cellular senescence in age-related pathologic changes.

THE ROLE OF CELLULAR SENESCENCE IN SKIN AGING

The cells residing in the skin are believed to possess a finite replicative capacity that is gradually manifested during the course of aging. The dermal fibroblast is a discontinuously replicating cell. While normally quiescent and dividing only every 1 to 5 years, it retains the capacity to rapidly re-enter the cell cycle in response to a need signaled by wounding or inflammation. Subsequent to the repair response, the cells again quiesce. As shown in **Figure 7**, the fibroblast spends the majority of time in the quiescent state, which is characterized by a pattern of extracellular gene expression that results in the normal maintenance of the dermis. In response to activation, the fibroblast dramatically alters the pattern of extracellular gene expression consistent with the remodeling of the dermal matrix. Genes characteristically upregulated in the activated state are collagenase and stromelysin. An example of a gene downregulated is EPC-1.[72]

When senescent cells are compared with young cells, it can be seen that the senescent fibroblast has lost not only the ability of serum growth factors to induce cell proliferation, but also the ability of growth factors to modulate fibroblast extracellular function. Surprisingly, senescent cells do not appear to be blocked in a quiescent nonactivatable state, but instead are locked into what approximates a constitutively activated state, herein designated M1 activation.

The telomere hypothesis provides an intriguing explanation for M1 activation. The loss of telomeric repeats may trigger "checkpoint" mechanisms, mediated by the tumor suppressors p53 and pRb that both arrest cell division and upregulate gene expression normally stimulated by activation. In support of this mechanism of M1 activation, viral oncoproteins, such as SV40 T antigen that inactivate pS3 and pRb, allow cells to replicate past the M1 limit until they cease proliferation from presumably a loss of chromosomal integrity. This latter limit is designated mortality 2 (M2). Additional evidence that checkpoint mechanisms can lead not only to an arrest of proliferation but also the upregulation of secreted proteases is seen in studies where DNA damaging agents increase the expression of plasminogen activator and collagenase.[73-75]

Since telomere length predicts the replicative capacity of cells,[76] it may provide the best biomarker for cellular ag-

ing. Dermal fibroblasts obtained from neonates show a mean terminal restriction fragment length (TRF) of approximately 10 kbp and at replicative senescence in vitro a TRF length of about 5.0 kbp. When applied to total skin samples from donors aged 0 to 92 years of age, an inverse correlation between TRF size and donor age was observed with cells losing on average approximately 20 bp per year.[77] Similarly, dermal fibroblasts obtained from explanted skin of variously aged donors showed a progressive loss of TRF size with age, averaging about 15-bp loss per year.[76] It was also observed that dermal fibroblasts obtained from donors with the premature aging disorder Hutchinson-Gilford syndrome have TRF lengths of approximately 5.5 kbp compared with age-matched control subjects, whose TRF size was approximately 9.0 kbp.[76]

A model suggesting a role for replicative senescence in age-related changes in the skin implies that an increasing proportion of the dermal fibroblasts have reached M1 replicative senescence with advancing age. As a result of M1-activated gene expression, senescent fibroblasts display marked changes in the secretion of proteins relating to collagen metabolism. In support of this hypothesis, pig skin fibroblasts grown to replicative senescence in vitro showed decreased α1(I) messenger RNA synthesis.[78] Both a decrease in collagen synthesis and an increase in collagenolysis has been reported in rat skin fibroblasts cultured to senescence in vitro and aged in vivo.[79] Similarly, senescent lung fibroblasts show decreased synthesis of messenger RNA for α1(I) and α2(I) collagen[80] and decreased collagen production is reported in senescent gingival fibroblasts.[81]

In addition to decreased synthesis of collagen, senescent fibroblasts markedly overexpress collagenase activity.[82,83] Collagenase expression is linked with the growth state of the cell; therefore, it is important to compare the dynamics of expression in young and aged cells. Whereas early passage fibroblasts are capable of increased expression of collagenase in response to cytokines such as serum growth factors, senescent cells overexpress the gene more constitutively.[82] Therefore, the distinction between young and senescent collagenase expression is best described as aged cells expressing collagenase under inappropriate conditions, such as in an environment in which there is no extracellular signals for increased expression. In a third path leading to proteolysis, senescent cells express less TIMP-1, the naturally-occurring inhibitor of the metalloproteinases collagenase and stromelysin.[82,84] Therefore, in regard to collagen metabolism, skin fibroblasts grown to replicative senescence in vitro, underexpress procollagen, overexpress collagenase, and underexpress collagenase inhibitory activity. The result is that aged cells become increasingly proteolytic with age, a phenomenon consistent with the observed decrease in total collagen and structurally intact collagen fibers with aging in vivo.

Skin fibroblasts are reported to synthesize elastin and are believed to be the source of the protein in the dermis.[85,86] In part, this may be due to a decreased elastin production as observed in fibroblasts aged to senescence in vitro.[86] As in the case of the collagen fibers, the steady-state maintenance

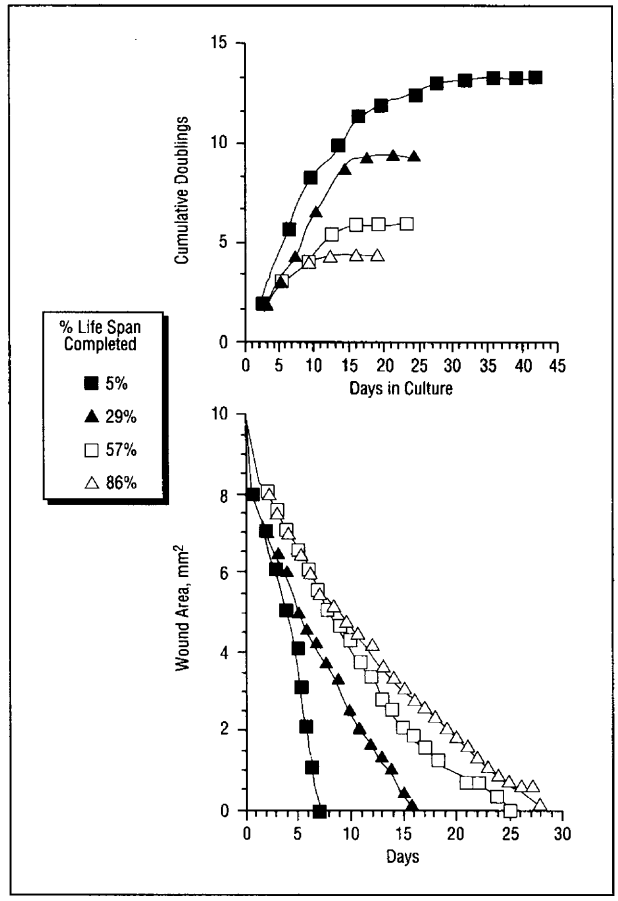

Figure 8. *Loss of proliferative capacity in dermal fibroblasts during aging in vivo. Dermal fibroblasts were cultured from Syrian hamsters to determine their maximum replicative capacity. Serial explants were obtained from a single animal as that animal aged. Top, A progressive loss of replicative capacity is observed during the aging of the animal. Bottom, Correlating with a progressive loss of replicative capacity is a progressive slowing of wound closure. (Courtesy of Sarah Bruce, MD, modified from Bruce and Deamond.[91])*

of elastic fibers is dependent on the balance of structural protein synthesis, and net proteolytic activity that is, in turn, dependent on the balance of protease secretion, activation, and inhibition by specific inhibitors. An imbalance in any of these pathways would lead to poor maintenance with resulting pathologic consequences. Numerous cells resident in the dermis secrete proteolytic enzymes capable of digesting elastin fibers; however, no increase in inflammatory cells has been observed.[30] A possible source is the activation of macrophage proelastase by senescent fibroblast-derived plasmin.

The cellularity of the dermis decreases during aging. The number of fibroblasts decreases at least 50% between birth and the age of 80 years.[14] The decreased numbers of dermal fibroblasts, mast cells, and Langerhans cells may simply reflect the increasing unresponsiveness of aging cells to normal growth signals. Even the changes observed in the basal layer of the epidermis may reflect cellular senescence. Given four to eight mitoses from the stem cell to the cornifying cell and the relatively larger surface area covered by the fully keratinized cell, 60 to 100 cell doublings by the stem cell may account for all of the keratinocytes produced in a lifetime, and the heterogeneity seen in the basal cells in advanced age may

reflect cells approaching senescence. Also, the appearance of the aging pigment lipofuscin in cells is observed during both in vivo and in vitro aging.

The progressive loss of replicative capacity of fibroblasts and keratinocytes may account for the impaired wound healing response of aged animals.[87-90] Longitudinal studies in aging hamsters show an age-dependent loss of replicative capacity in dermal fibroblasts coincident with an age-dependent slowing of wound closure (**Figure 8**).[91] Characteristics of senescent fibroblasts that may play a role in the slowed wound healing are as follows: impaired proliferative response to mitogens, diminished migration,[92-94] and alterations in secreted proteins are as described above.

The pattern of gene expression displayed by senescent skin fibroblasts is consistent with cellular aging playing a role in the degenerative alterations observed in aging skin. Therefore, agents that manipulate the pattern of gene expression to downregulate proteolytic activities and upregulate structural protein synthesis would be predicted to have therapeutic effect. Retinoic acid is now well known for its beneficial effects on aging skin. In this regard it is interesting that retinoids downregulate collagenase expression in human skin and synovial fibroblasts[95-98] and upregulate the expression of TIMP-1 messenger RNA.[98]

CONCLUSIONS

Normal skin ages in a characteristic fashion. While there are only subtle changes in the epidermis, there are profound alterations in the dermis. There is widespread destruction of extracellular matrix architecture characterized by decreased organization of collagen and proteoglycan ground substance and broken and frayed collagen and elastin fibers. Collagen imparts the tensile strength to the skin and elastin gives it resiliency and elasticity. The destruction of the architecture of these fibrillar proteins decreases the tensile strength of the skin, leaving it more vulnerable to sheer forces and tears, the loss of elasticity leads to sagging and wrinkling. The phenomenon of cellular senescence may provide new insights into the etiology of these age-related changes.

Progress in understanding the pathogenesis of age-related changes in skin has been unusually slow. This is due in part to the fact that age-related changes are manifold and complex. These new research tools may provide new insights into the pathogenesis of age-related changes in skin and related connective tissues. These insights may, in turn, lead to new strategies for biotechnological intervention.

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