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Ageing: collagenase-mediated collagen fragmentation as a rejuvenation target

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We are honoured to provide this celebratory editorial marking the 125th anniversary of the *British Journal of Dermatology*.

Human skin, like all organs, undergoes cumulative deleterious alterations that result in a decline of function due to the passage of time. However, unlike other organs, with the exception of the eye, skin experiences cumulative damage due to ultraviolet (UV) irradiation from the sun. Deleterious changes due to sun exposure are superimposed on natural ageing, giving rise to photoageing on sun-exposed skin. Photoageing and natural ageing can be viewed as variations on a theme, making them distinguishable by clinical, histological and molecular features. However, the underlying common theme, namely fragmentation of the dermal collagenous extracellular matrix and its impact on fibroblast function, justifies photoageing being considered a form of accelerated or premature ageing.

During the last 30 years, largely owing to the ability to study the effects of acute exposure of human skin to solar-like UV, much progress has been made regarding the molecular basis of photoageing. Knowledge gained from the study of photoageing has greatly informed our understanding of natural ageing. While photoageing and natural ageing affect more than the dermis, alterations to collagen are among the most prominent and are likely the primary causes of the common appearance-related and clinical manifestations of aged skin. This editorial will therefore focus on the collagenous microenvironment as a target for damage during the ageing process and as a driving force for perpetuating both photoageing and natural ageing.

Photoageing begins with the absorption of energy and the generation of reactive oxygen species

The ability of solar UV radiation, a form of electromagnetic energy, to affect the biology of the skin is dependent on the absorption of the energy by molecules in the skin. Macromolecules, including nucleic acids, proteins and lipids, are able to absorb solar UV radiation. The absorbed energy can directly alter the chemical nature of the absorbing molecule or it can be transferred to other molecules. For example, DNA can be mutated by direct absorption of solar UV radiation, and energy can be transferred to molecular oxygen to create reactive oxygen species (ROS).

The mammalian ultraviolet response and activation of tyrosine kinase receptors

Scientists investigating basic mechanisms showed that UV exposure stimulates intracellular signal transduction pathways identical to those that are activated by the family of cell surface receptors that possess tyrosine kinase activity (i.e. the ability to add phosphates to tyrosines in proteins). The term 'mammalian UV response' was coined to refer to the activation of cellular signaling pathways and downstream changes in gene expression.[1] Discovery of the mammalian UV response was followed closely by direct evidence that UV exposure rapidly activates tyrosine kinase receptors and that this activation is the driving force for the mammalian UV response.[2] The UV response in human skin activates tyrosine kinase receptors for growth factors and cytokines.[3] This activation is among the earliest biochemical events following exposure to UV radiation.[4]

Ultraviolet exposure activates tyrosine kinase receptors by inhibiting protein tyrosine phosphatases

Understanding how UV exposure activated tyrosine kinase receptors emerged from basic studies focusing on the balance between protein tyrosine kinases (attaching phosphates to tyrosines) and protein tyrosine phosphatases (removing phosphates from tyrosines).[5] Sequencing and biochemical studies revealed that all protein tyrosine phosphatases employ the same chemistry to catalyze phosphate removal.[6] Importantly, this chemistry is highly susceptible to inhibition by ROS.[7] Exposure of tyrosine phosphatases to ROS completely inhibits activity. These findings, combined with knowledge about photochemical generation of ROS, the mammalian UV response and activation of tyrosine kinase receptors, provided the impetus to investigate protein tyrosine phosphatases as mediators of the actions of UV in skin.

Regulation of the tyrosine kinase epidermal growth factor receptor (EGFR) was chosen for study because of its prominence in skin physiology and rapid, robust induction of epidermal growth factor tyrosine phosphorylation by UV exposure. Furthermore, pharmacological inhibition of EGFR activation substantially reduces UV-induced signal transduction in human skin.[8] Systematic identification and testing of members of the receptor-type protein tyrosine phosphatases family that are expressed in human keratinocytes resulted in the discovery that protein tyrosine phosphatase kappa (RPTP-κ) controls tyrosine phosphorylation of EGFR.[9]

RPTP-κ was shown to counterbalance EGFR tyrosine kinase activity, maintaining EGFR in a nonphosphorylated and therefore inactive basal state. ROS generated by UV irradiation directly inhibited RPTP-κ activity, resulting in rapid EGFR tyrosine phosphorylation. In essence, RPTP-κ acts as a sensor for ROS that confers UV activation of EGFR.[10] In addition to receptors for EGFR, receptors for cytokines such as interleukin-1β and tumour necrosis factor-α are also rapidly activated by UV exposure. Activation of these receptors initiates inflammatory cascades, which are critical features of skin's response to UV. Taken together, we now have a basic understanding of signal transduction that is elicited by UV exposure.

Induction of matrix metalloproteinases couples the mammalian ultraviolet response to photoageing

Downstream effectors of UV-induced signalling pathways include transcription factors that act to alter skin cell gene expression. A critical downstream transcription factor is activator protein-1 (AP-1). Exposure of human skin to suberythemal doses of UV rapidly induces AP-1.[4,11] Among the genes directly regulated by AP-1 are certain members of the matrix metalloproteinase (MMP) family of enzymes, including MMP-1 (collagenase), MMP-3 (stromelysin) and MMP-9 (gelatinase).[12] Induction of AP-1 and MMPs occurs primarily in the epidermis; however, these MMP proteins are secreted and pass through the basement membrane to accumulate in the dermis.[13]

MMP-1 specifically initiates fragmentation of collagen fibrils, cleaving at a single location. MMP-3 and MMP-9 have broader substrate specificities, further fragmenting MMP-1-cleaved collagen fibrils.[14] Importantly, cross-linking of collagen fibrils, which normally occurs during formation and maturation of collagen fibrils, prevents their complete degradation and removal.[15] Cross-linked regions of fibrils are nearly impervious to MMP actions, and therefore remain irreversibly present within the dermal collagenous matrix.

In addition, UV exposure represses collagen synthesis, thereby preventing new collagen production that could otherwise counteract ongoing collagen fragmentation.[11] Thus, the actions of UV-induced MMPs results in both a net reduction of collagen due to loss through degradation of non-cross-linked regions of collagen fibrils, and concomitant generation of residual cross-linked collagen fragments. With chronic UV exposures, the chain of events described above is repeated, resulting in accumulation of collagen damage. This long-lived damage is critical because it impairs the structural and mechanical integrity of the collagen and the function of cells that reside within it.

Accumulation of collagen fibril fragmentation creates a dermal microenvironment that perpetuates ageing/photoageing

The dermal collagenous matrix is simultaneously a source of mechanical strength and resiliency, and a dynamic scaffold to which dermal cells attach. These two properties are inseparable, and the impact of collagen fibril fragmentation on fibroblasts, whose function is dependent on interactions with the collagen, provides a basis for understanding the pathophysiology of both photoageing and natural ageing. Fibroblasts have the capacity to both produce and degrade collagen. The balance between these two opposing processes is regulated by the dermal microenvironment.

Fibroblasts need to attach to specific sites on collagen fibrils in order to stretch and achieve optimal mechanical force (Fig. 1).[16,17] Collagen fibril fragmentation causes loss of the binding sites on collagen fibrils, thereby preventing fibroblast attachment, with concomitant reduction of stretch and mechanical force (Fig. 1).[18] In this less-stretched state, fibroblasts upregulate MMPs and downregulate the production of collagen. With repeated UV exposure, or the passage of time, accumulation of collagen fibril fragmentation shifts the balance in favour of MMP expression and collagen fibril fragmentation. A self-perpetuating cycle is created that permanently resets collagen homeostasis. In essence, accumulation of cross-linked collagen fragments becomes a driving force for further collagen fibril degradation by increased MMP levels, and further inhibition of new collagen production.[19]

We have recently shown that this cycle of decline can be reversed by enhancing structural support within the dermis of aged or photoaged human skin.[20,21] Injected cross-linked hyaluronic acid (CL-HA), which is used as a dermal filler for aesthetic purposes, creates pockets within the dermis that exert pressure on surrounding loosely-packed, fragmented collagen fibrils. This internal mechanical force stretches fibroblasts that are adjacent to CL-HA pockets and stimulates the production and deposition of new collagen fibrils. Stretch-induced collagen production is mediated by stimulation of a major regulatory pathway (the transforming growth factor-β pathway), which is downregulated in aged/photoaged human skin.

The scenario of self-perpetuating collagen fragmentation also applies to natural dermal ageing. With the passage of time, MMP-mediated collagen fibril cleavage results in accumulation of cross-linked fragments. This accumulation initially occurs slowly owing to very low levels of MMP activity in young, sun-protected dermis. The average half-life of collagen in sun-protected skin is 15 years.[22] However, as mentioned above, cross-linked collagen fragments remain in the skin for considerably longer. As cross-linked collagen fragments accumulate with ageing, they deleteriously affect fibroblast function and collagen homeostasis, as observed in photoaged skin. In naturally aged skin, like photoaged skin, collagen fragmentation causes loss of fibroblast binding-sites, bringing about reduced stretch and mechanical force (Fig. 1). MMP expression by fibroblasts rises, collagen production falls, and pro-inflammatory cytokine synthesis increases. Accumulation of cross-linked collagen fragments is a critical driving force for the pathophysiology of both natural ageing and photoageing.

Recently, we demonstrated the validity of this concept in newly developed transgenic mouse models. In mice, specific inducible expression of human MMP-1, or an upstream

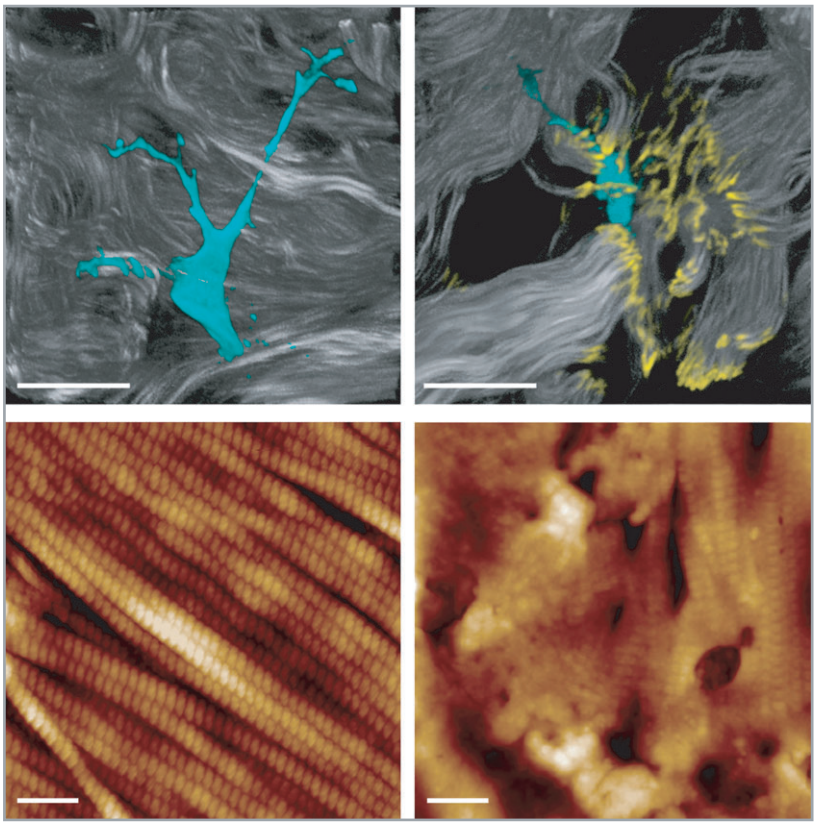

Fig 1. (Upper left) In young human skin, a fibroblast (blue) is spread by attachment to densely packed bundles of collagen fibrils (grey). Spreading of fibroblasts, with attendant mechanical force, promotes collagen production and suppresses matrix metalloproteinase (MMP)-mediated collagen fibril fragmentation. (Lower left) Nanoscale image of intact, tightly packed collagen fibrils in young skin. (Upper right) In aged human skin, fibroblast (blue) spreading is reduced by loss of attachment sites due to fragmentation of collagen fibrils (grey with fragmented ends highlighted in yellow). Reduced fibroblast spreading, with attendant decreased mechanical force, suppresses collagen production and promotes MMP-mediated collagen fibril fragmentation. (Lower right) Nanoscale image of fragmented, disorganized collagen fibrils in aged skin. Photoaged skin displays similar features as shown for representative images of sun-protected aged skin. Images in upper panels are adapted from second harmonic generation multiphoton fluorescence microscopy; scale bar = 20μm. Images in lower panels are from atomic force microscopy; scale bar = 200nm.

regulator that stimulates expression of MMPs in fibroblasts, yields a phenotype that closely resembles photodamaged/aged human skin. The dermal collagenous extracellular matrix is fragmented and disorganized, MMP expression is enhanced, collagen production is downregulated and the skin is thin, fragile, has reduced elasticity and is wrinkled (unpublished data).

It is important to appreciate that the dermal filler studies and mouse models address the quality of the collagenous extracellular matrix, irrespective of age or the state of the cells within the dermis. In human studies, enhancement of internal mechanical force by injection of dermal filler elicits 'youthful' collagen production by fibroblasts in photodamaged and aged sun-protected skin. In mouse models, fragmentation of the collagen yields an aged phenotype, despite the skin cells being young. Two obvious lessons emerge: (i) the dermal microenvironment is a critical effector of the ageing process in skin; and (ii) dermal ageing is an active process rather than simple global decline. Both of these features provide opportunity for therapeutic intervention.

Treating ageing/photoageing: now and in the future

Clearly, the quest for a youthful appearance marches on. It is estimated that as a result of rising consumer incomes and changing lifestyles, the global skincare products industry is forecasted to reach around $265 billion by 2017, with skincare as the largest segment.[23] How will our understanding of the molecular basis of skin ageing enable development of new, effective therapies to improve the appearance and function of aged skin?

Given the pathophysiology of skin ageing, it is not surprising that all existing effective treatments stimulate the production of skin collagen. Examples are lasers, coarse grit microdermabrasion, topical 5-fluorouracil and injection of CL-HA dermal filler.[24–26] Among topical treatments, vitamin A (retinol) and its active metabolite all-*trans* retinoic acid have been the most widely studied.[27] Following up on incidental findings of improved skin appearance in persons using all-*trans* retinoic acid for acne,[28] a placebo-controlled, double blind

clinical study revealed significant wrinkle reduction, and overall improvement of both texture and pigmentation.[29] Furthermore, it was shown that improved skin appearance was accompanied by new collagen production.[30]

The striking clinical and biochemical results from this seminal study set the stage for acceptance and development of the science of human appearance within dermatology and skin research.[29,30] In the more than 25 years since this first controlled clinical study, no other topical treatment has been rigorously studied or shown to approach the effectiveness of all-*trans* retinoic acid. This situation may reflect the complexities and costs of drug development, the nature of the skin care industry and the intransigence of the biology of skin ageing. Clearly, our understanding of the molecular basis of skin ageing and now the availability of new inducible mouse models of dermal ageing, provide fertile soil for the development of new ways to treat and retard the inevitable decline of skin function and appearance.

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Department of Dermatology, University of Michigan, Ann Arbor, Michigan 48109, U.S.A.
E-mail: gjfisher@umich.edu

G.J. Fisher
D.L. Sachs
J.J. Voorhees

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