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Molecular mechanisms of skin ageing

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Abstract

Cutaneous ageing is a complex biological phenomenon consisting of two components; intrinsic ageing, which is largely genetically determined and extrinsic ageing caused by environmental exposure, primarily UV light. In sun-exposed areas, these two processes are superimposed. The process of intrinsic skin ageing resembles that seen in most internal organs and is thought to involve decreased proliferative capacity leading to cellular senescence, and altered biosynthetic activity of skin derived cells. Extrinsic ageing, more commonly termed photoageing, also involves changes in cellular biosynthetic activity but leads to gross disorganisation of the dermal matrix. The molecular mechanisms underlying some of these changes are now beginning to be unravelled and are discussed. As these mechanisms are identified, further insights into the underlying processes of skin ageing should emerge and better strategies to prevent the undesirable effects of age on skin appearance should follow. © 2002 Published by Elsevier Science Ireland Ltd.

Keywords: Skin; Photodamage; Sun-damage; Senescence; Ageing; AP-1; Extra-cellular matrix; Metalloproteinase

1. Introduction

Ageing of the skin is commonly associated with increased wrinkling, sagging and increased laxity, but when considering the underlying reasons for these changes, it is important to distinguish between the effects of true biological ageing and environmental factors, such as exposure to the sun.

Intrinsic skin ageing is largely genetically determined and clinically associated with increased fragility, loss of elasticity and has a transparent quality (Gilchrest, 1982). However, the vast majority of the skin's age related cosmetic problems are thought to be as a result of cumulative exposure to sunlight. Sun-exposed or photoaged skin is typically coarse and rough with deep lines and wrinkles and irregular pigmentation. Whilst their etiology is very different, some of the deleterious changes observed in sun-protected skin with age are similar to those that characterise photoaged skin. Increased matrix metalloproteinase (MMP) activity and reduced collagen I expression for example have been reported in both (Varani et al., 1998; Lavker, 1979; West, 1994). However, in sun-exposed areas these common processes are superimposed with specific changes in response to UV radiation, including massive elastosis and collagen degeneration.

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2. Intrinsic ageing

2.1. Introduction

The process of intrinsic skin ageing is similar to that occurring in most internal organs, involving slow deterioration in tissue function. The stratum corneum remains relatively unchanged, but the epidermis and dermis thins with a flattening of the dermo–epidermal junctions (Gilchrest, 1982). There is also a reduction in the number and biosynthetic capacity of fibroblasts (Bolognia, 1993) and progressive disappearance of elastic tissue in the papillary dermis (Francis and Robert, 1984). Skin collagen content decreases with age and the fine collagen fibres associated with infancy become increasingly dense and tightly packed and far more randomly orientated (Lavker et al., 1987).

2.2. Cellular senescence

The processes associated with intrinsic skin ageing are thought to result from a combination of events including (i) decreased proliferative capacity of skin derived cells; (ii) decreased matrix synthesis in the dermis; and (iii) increased expression of enzymes that degrade the collagenous matrix.

Decreased proliferative capacity of skin derived cells cultured from old donors versus those from younger individuals has been described in a number of studies. Keratinocytes (Gilchrest, 1983), fibroblasts (Schneider and Mitsui, 1976; Mets et al., 1983; Cristofalo and Pignolo, 1993) and melanocytes (Gilchrest et al., 1984; Medrano et al., 1994) all show an age-associated decrease in cumulative population doublings. These observations support the theory that cell division is a key driving force behind the eventual loss of replicative ability and ultimate change in cellular phenotype. This process has been termed cellular senescence and senescent cells have indeed been shown to accumulate in ageing human skin (Dimri et al., 1995).

Cellular senescence involves the arrest of cellular growth at G1 phase when cells reach the end of their replicative life span. The senescent cells cannot then be stimulated to enter S1 phase by physiological mitogens (Hayflick, 1965). This age-associated reduction in growth can in part be explained by the selective repression of several growth regulatory genes whose expression is important for G1 progression and DNA synthesis. In fibroblasts, these include c-fos proto-oncogene (Sesadri and Campisi, 1990), the helix-loop-helix Id-1 and Id-2 genes (Hara et al., 1994) and components of the E2F transcription factor (Dimri et al., 1994; Good et al., 1996), all of which are downregulated in senescent fibroblasts. Negative growth regulators are also overexpressed including the p21 and p16 inhibitors of cyclin dependent protein kinases (Noda et al., 1994; Hara et al., 1996). Other changes seen in senescent skin fibroblasts include increased expression of IL-1α (Maier et al., 1990) and of the EGF-like cytokine-heregulin that modulates the growth and differentiation of breast and other epithelial cells (Campisi, 1998). Thus senescent stromal cells are likely to influence the balance between growth and differentiation of the overlying epithelial cells.

Direct studies with keratinocytes have also demonstrated altered expression of growth-regulating molecules with age (Green et al., 1983). In particular, EGF binding and receptor phosphorylation is reduced and thought to be the result of age related changes in a critical downstream signalling element (Yaar, 1995). Other reported changes include enhanced expression of the IL-1 receptor antagonist and differentiation associated SPR2 (Gilchrest, 1994). Clearly there are many changes during cellular senescence, but whether these control senescence directly or whether they are a consequence of an as yet unidentified 'master switch' which also induces cellular senescence remains to be determined.

In addition to this irreversible growth arrest, senescent cells also require resistance to apoptotic death (Wang, 1995) and exhibit altered differentiation functions (Campisi, 1996). It is proposed that this leads to the accumulation of non-dividing senescent cells with altered gene expression and subsequent phenotype, eventually leading to a decline in tissue function and integrity that is characteristic of ageing (Campisi, 1997). It is this alteration in cellular differentiation that is

thought to account, in part, for the changes observed in matrix and metalloproteinase expression in aged skin.

In presenescent dermal fibroblasts, metalloproteinase activity is relatively low with collagenase (MMP1) and stromelysin (MMP3)—two key dermal extra-cellular matrix degrading enzymes, shown to be expressed at very low levels. In contrast, levels of matrix metalloproteinase inhibitors TIMP 1 and TIMP 3 are high, reducing degradative capacity further (Campisi, 1996). In senescent fibroblasts however, this is reversed with an increase in matrix metalloproteinase expression and a reduction in the expression of tissue inhibitors of metalloproteinase (West et al., 1989; Millis et al., 1992; Wick et al., 1994). Compounding these changes, the rate of collagen biosynthesis also falls with expression levels being markedly lower in the skin of elderly individuals than in foetal tissue or during the early postnatal years (Uitto et al., 1989). This change would tip the cell from a matrix-producing to a matrix-degrading phenotype and contribute to the reduction and disorganisation of collagen commonly associated with aged skin and the overall dermal atrophy.

Coupled with these changes, elastin gene expression is markedly reduced after the age of 40–50, as determined by mRNA steady state levels in cultured fibroblasts (Uitto, 1979), and there is progressive disappearance of elastic tissue in the dermis (Francis and Robert, 1984). The loss of oxytalan fibres in the papillary dermis seen as finger like projections towards the basement membrane is particularly noticeable. Again the reduced ability of aged cells to re-synthesise these fibres will present itself clinically as loss of recoil in the skin, another common feature of aged skin (Bravermann and Fonferko, 1982).

2.3. Oxidative damage

An alternative ageing hypothesis to that of cellular senescence is the oxidative stress theory of ageing (Sohal and Allan, 1990). This theory suggests that ageing is heavily influenced by external oxidative stresses which influence the genetic program through modulation of redox sensitive genes.

Cumulative oxidative damage as a causative factor in ageing is supported by a large body of experimental findings (for recent reviews, see Muscari et al., 1996; Sohal and Weindruch, 1996; Guyton et al., 1997). It is particularly relevant in skin given its high exposure to environmental agents such as ultraviolet radiation and ozone. Reactive oxygen species cause damage to lipids, proteins and DNA and also influence cellular senescence. Low doses of H_2O_2 have been shown to cause cells to enter a senescent-like state (Chen and Ames, 1994) presumably contributing to the proliferative and differentiation changes described above.

It has been proposed that this form of ageing may manifest itself as a process of terminal differentiation (Bayreuther et al., 1988), involving seven distinct morphotypes ranging from mitotic to post-mitotic and eventually degenerating cells. This morphological change is accelerated with advancing age and the application of oxidative stressors, with the degree of post-mitotic and degenerative cells increasing in both (Toussaint et al., 1992; Bayreuther et al., 1992; Alaluf et al., 2000). These findings suggest that ageing in skin may be associated with a shift in the ratio between post-mitotic and progenitor fibroblasts, resulting from an extended process of terminal cellular differentiation in vivo. As with the theory of cellular senescence, this switch is again likely to influence the overall phenotypic behaviour. The altered response of post-mitotic cells to chemoattractants, including matrix proteins, has already been demonstrated (Palka et al., 1996). In addition, free radicals also cause damage to connective tissue components of the dermis, particularly collagen (Dalle Cabonare and Pathak, 1994), which again is likely to influence cell behaviour via cell–matrix interactions.

The epidermis of skin possesses an extremely efficient antioxidant activity that is superior to most tissues (Kohen and Gati, 2000) and the reduction in efficiency of this system has been proposed as a factor in skin ageing. However, the role of reduced antioxidant capacity in ageing skin is still a matter of controversy. There are many reports describing the reduction of antioxidant enzymes in skin with age including Cu,Zn-

super oxide dismutase (SOD), catalase and glutathione peroxidase (Wei et al., 2001; Kohen and Gati, 2000), whilst others suggest that skin ageing is not due to a general decline in antioxidant capacity, demonstrating that in mouse skin at least, catalase, SOD and glutathione reductase activities remain constant with age (Lopez-Torres et al., 1994). However, all agree that the accumulation of free radicals throughout life most likely promotes cellular ageing as scavenging mechanisms are unlikely to be 100% efficient at any stage of life. Adding support to this, a recent study has demonstrated that fibroblasts from old donors are much more vulnerable to the accumulation of oxidised proteins following oxidative stress and are unable to remove them as efficiently as young fibroblasts (Merker et al., 2000).

3. Mechanisms of extrinsic ageing

3.1. Introduction

Extrinsic ageing primarily results from exposure to ultraviolet light. It has been suggested that as much as 80% of facial ageing is attributable to sun exposure (Gilchrest, 1989). Clinically, photodamaged skin is characterised by loss of elasticity, increased roughness and dryness, irregular pigmentation and deep wrinkling (Kligman and Kligman, 1986). Whilst changes do occur in the epidermis of photodamaged skin, including both an increase and decrease of epidermal thickness (corresponding to hyperplasia or severe atrophy respectively) and loss of epidermal polarity (Gilchrest and Yaar, 1992), changes in the proportion and/or functionality of the dermal extracellular components account for the major visible changes associated with UV-induced extrinsic damage.

There are three principle components involved namely, collagen fibres, the elastic fibre network and glycosaminoglycans. Collagen is the most abundant extracellular component accounting for 80% of the dry weight of skin and provides the strong tensile properties to the dermis (Oxlund and Andreassen, 1980). The elastic fibre network provide elasticity to the skin and accounts for 2–4% of the extra-cellular matrix in sun-protected skin (Uitto, 1979), whilst the glycosaminoglycan/proteoglycan macromolecules play a role in hydrating the skin and in biological signalling (Davidson, 1965). These comprise only 0.1–0.3% of the dry weight of skin. Changes in all three components have been described in photodamaged skin.

3.2. Connective tissue changes

The major histopathological sign of photoageing is the massive accumulation of so called 'elastotic' material in the upper and mid dermis. This material is comprised of extracellular matrix (ECM) components that make up the normal elastic fibre network. Elastin is considered the primary component as evidenced by positive staining of skin with the elastin-specific Verhoeff van Gieson stain (Chen et al., 1986; Bernstein et al., 1994). However, the microfibrillar scaffold protein fibrillin (Dahlback et al., 1990), the proteoglycan versican, and hyaluronic acid (Zimmermann et al., 1994; Bernstein et al., 1995; Kligman et al., 1985) are amongst the molecules also present. Thus, all the principle components of the elastic fibres are present, but despite this, their structural organisation and functionality are severely perturbed.

The process by which this elastotic material is first formed is still unclear, but is likely to involve degradation of existing elastic fibres and dysregulation of elastin and fibrillin production. Degradation of the existing fibre network is primarily attributable to the elevation in dermal elastase activity, arising from infiltration of inflammatory neutrophils and also from the dermal fibroblasts themselves in response to acute UV radiation (Labat-Robert et al., 2000). However, increases in metalloprotease activity, particularly MMP9, are also now known to play an important role (see below). Histochemically, the depletion of intact microfibrils and elastic fibres is clearly evident in photodamaged skin (Uitto et al., 1997; Watson et al., 1999).

Adding to this ongoing degradation, the deposition of newly synthesised elastotic material is also thought to occur. The upregulation of elastin

and fibrillin gene expression in photodamaged skin and fibroblasts derived from it has been reported (Bernstein et al., 1994), along with activation of the elastin promoter in a transgenic construct by UV irradiation (Uitto and Bernstein, 1998). Elastin gene expression is also upregulated in vitro in response to increased free radicals generated by a xanthine/xanthine oxidase system (Kawaguchi et al., 1997), conditions which no doubt occur in skin following acute UV radiation. Increases in other elastic fibre components have also been described. In particular, fibroblasts from photodamaged skin demonstrate increased versican synthesis which accumulates in abnormal elastic fibres in vivo (Bernstein et al., 1995).

More recently, studies analysing expression of elastin and fibrillin directly from photodamaged skin have contradicted the previously reported increases in RNA levels and indicate that chronic UV damage has no effect on the gene expression of either in vivo (Werth et al., 1997). The reasons for these conflicting data are unclear, but may represent differences in time between RNA analysis and previous sun exposure of the individual concerned. All considered, it is difficult to account for the extent of accumulation of amorphous elastotic material by the degradation of existing fibres alone. Overall these data suggest that an increase in elastic fibre synthesis does occur in photodamaged skin, but the material produced is clearly dysfunctional and contributes greatly to the formation of the amorphous mass so typical of sun-damaged skin.

In contrast with the elastic fibre network, components of the collagen fibre network, including collagen 1 and decorin are downregulated in photodamaged skin (Varani et al., 2001; Bernstein et al., 1995). This reduction in collagen fibre production is accompanied by the degeneration of the surrounding collagenous network (Bernstein and Uitto, 1996) and as with the breakdown of the elastic fibre network, MMPs have been implicated as the key mediators (Griffiths et al., 1993; Fisher et al., 1997). This accumulation of partially degraded collagen potentially has a negative impact on surrounding fibroblasts. Both fibroblast proliferative capacity and collagen synthesis are reduced when cells are exposed to degraded collagen in vitro (Varani et al., 2001).

3.3. Matrix metalloproteases and photoageing

The MMPs are a large family of degradative enzymes (Matrisan, 1992) and four in particular are thought to be important in matrix degradation in skin. The combined actions of collagenase (MMP1), 92 kDa gelatinase (MMP2), 72 kDa gelatinase (MMP9) and stromelysin 1 (MMP3) can fully degrade skin collagen and components of the elastic network (Birkadel and Hansen, 1987; Birkadel et al., 1993). More recently, elevated expression of MMP8 by infiltrating neutrophils following UV irradiation has been described, but is not thought to contribute substantially to UV-induced matrix degradation (Fisher et al., 2001). Collagenases are the only mammalian proteases capable of hydrolysing intact fibrillar collagen (Lui et al., 1995). Once cleaved in the triple helical domain, the denatured collagen can then be further broken down by both gelatinase and stromelysin. In addition, both MMP2 and MMP9 (Gelatinase A and B, respectively) have the potential to degrade the elastic fibre network. MMP-9 displays the greatest elastolytic and fibrillin-degrading activity, whilst MMP-2 shows greater specificity toward collagen III and is capable of degrading constituents of the dermo-epidermal junction (Berton et al., 2000). Neutrophils are also thought to be primarily responsible for production of the key elastolytic enzyme in skin, elastase, following the UV-induced inflammatory response.

As indicated earlier, the basal expression of these enzymes in 'normal' skin is relatively low, however, they can be markedly up-regulated by UV irradiation both in vivo and in cultured cells (Stein et al., 1989; Peterson et al., 1992; Fisher et al., 1997). Irradiation of human skin with just a single dose of UV light has been shown to increase the activities of MMPs, and this has been associated with significant degradation of collagen fibres. The levels of soluble type I collagen C-terminal cross linked telopeptides, quantitative measures of collagen degradation, were elevated 58% in UV irradiated skin after 24 h (Fisher et al., 1997). Irradiation induced expression of the three MMP genes occurred predominantly in the epidermis, but protein and enzymatic activity were

abundant in both the dermis and the epidermis indicating transport of protein through the basement membrane. This was particularly true for stromelysin as epidermal enzymatic activity was minimal. In conjunction with the upregulation of MMP activity, UV irradiation also induces a specific inhibitor of MMP activity, namely TIMP1, which helps to counter balance the degradative effects of the MMPs. Despite this, UV exposure clearly encourages a more degradative environment within the dermal tissue resulting in the destruction of both the collagen and elastic fibre network.

As referred to earlier, UVA and UVB irradiation also results in a significant increase in elastase activity, with chronic UVA radiation of mice producing an increase in skin elastase activity equivalent to that observed after 24 months of ageing in non-irradiated animals (Labat-Robert et al., 2000). The fragments arising from the degradation of the elastic fibres have also been shown to upregulate pro-collagenase-1 and stromelysin expression (Brassart et al., 2001) and may, therefore, play a role in the upregulation of these enzymes in vivo following chronic UV irradiation.

3.4. Signal transduction pathways

The molecular mechanisms underlying the induction of MMPs following UV irradiation are now beginning to be unravelled. The model proposed by Fisher et al. (1998) suggests that UV radiation activates growth factor receptors on the surface of fibroblasts and keratinocytes, resulting in signal transduction through a protein kinase cascade and subsequent activation of AP-1 in the nucleus. This then stimulates MMP production in both the dermis and epidermis and leads to the degradation of collagen and elastic fibres.

More specifically, it is proposed that UV activation of membrane receptors stimulates MAP kinase signal transduction pathways, via stimulation of GTP-binding proteins including ras, rac and cdc42. In support of this, activation of the three MAP kinases, ERK, jnk and p38 within 1 h of acute UV radiation has been demonstrated (Fisher et al., 1998). This activation is then succeeded by increased expression of the transcription factors c-jun and c-fos that, together with other protein factors form the transcription factor complex AP-1 (Karin and Hunter, 1995). Elevated levels of AP-1 are then responsible for inducing expression of key members of the MMP family responsible for the degradation of dermal matrix.

3.5. Dermal repair

The degradation of dermal matrix alone, however, is not considered enough to account entirely for the visible changes associated with photoageing. These are more likely to arise from defects in the subsequent repair processes, involving synthesis of new matrix proteins. Any defects in this process are likely to lead to permanent alterations in the structure and organisation of the collagen and elastin fibre network and impact substantially on the biomechanical properties of skin and its visual appearance. The processes of skin repair that follow the initial activation of degradative enzymes have been far less extensively studied and imperfect deposition of newly synthesised matrix proteins has yet to be proven. However, the induction of repair associated molecules in skin following chronic UV exposure and following treatment with retinoic acid has been demonstrated (Watson et al., 2001; Filsell et al., 1999; Kligman, 1996; Varani et al., 1998). The effects of retinoic acid treatment on skin are widely reported elsewhere (for reviews, see Fisher and Voorhees, 1996; Kligman and Leyden, 1993).

The influence of advancing age on the rates of wound repair in skin has been well documented (for review, see Gerstein et al., 1993), and many of the pathways described are likely to be involved in the repair of skin following UV insult. In particular, with increasing age, remodelling occurs more slowly with less collagen being produced in wounds and the collagen formed being qualitatively different, consistent with the reported reduction in the breaking strength of aged skin (Platt and Ruhl, 1972; Holm-Peterson and Zenderfeldt, 1971). If dermal repair processes, following UV induced damage, overlap with those described for wound healing, these age related changes will impact considerably on the ability of skin to repair itself following UV radiation. In

support of this, a delay in the wound healing response has been associated with an increase in fibroblast proteolytic activity resulting in greater contractility of collagen gels (Ballas and Davidson, 2001). A similar observation has been made in sun-damaged fibroblasts. Cultured fibroblasts in UV-exposed skin consistently show a greater contractility and subsequent force generation in collagen gels than those from a subject matched sun-protected site (Read et al., 1998). An association with elevated proteolytic activity in photo-damaged fibroblasts has, however, yet to be demonstrated.

4. Conclusions

It is clear that there are readily discernible differences between intrinsically aged skin and that aged by habitual exposure to sunlight, particularly at the macromolecular level. However, it is becoming increasingly evident that there are many consistent changes between the two at the molecular level. Changes seen with intrinsic ageing such as decreased cellular lifespan, reduced response to growth factors, disruption of matrix synthesis and elevation of proteolytic activity are all evident in photo-damaged skin. The changes are simply more pronounced.

Now that the molecular mechanisms underlying the skin's response to UV are beginning to be identified, they are also likely to provide further insights into those processes, which determine tissue ageing in general. Better strategies to protect skin against the undesirable effects of both sunlight and passage of time may soon follow.

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