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Review

Skin aging and oxidative stress

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Abstract: Skin aging occurs through two main pathways, intrinsic and extrinsic. These pathways have significant interaction in contributing to the aging phenotype, which includes skin laxity, wrinkling, pigmentation irregularities, and the appearance of neoplastic skin lesions. Here, we review the critical role that oxidative stress plays in skin aging, including its effects on signaling pathways involved in skin matrix formation and degradation, proteasome activity, as well as DNA structure. Furthermore, we discuss the recent literature surrounding the prevention and treatment of skin aging. Although current research is suggestive of the role of antioxidants in anti-aging skin therapies, further research is much needed to explore its role in humans.

Keywords: Skin aging; photoaging; ROS; oxidative stress; antioxidants skin therapies

1. Introduction

Aging is a process of change every organ undergoes with the passage of time. It is characterized by decreased cellular proliferation and viability, increased cellular senescence and attrition, and is often accompanied by the accumulation of genetic mutations. In the skin, the aging process is divided into two main pathways, which are intrinsic (genetically determined) and extrinsic (environmentally mediated). Although extrinsic aging can be augmented or modulated to some degree, intrinsic aging cannot as it is determined by hereditary factors. However, both pathways have significant interaction and converge on common targets in the cellular machinery that contribute to the aging phenotype.

Clinical manifestations of skin aging include an increase in skin laxity, xerosis, wrinkles, pigmentation irregularities, presence of benign growths, such as seborrheic keratosis and/or malignant neoplasms such as basal cell or squamous cell carcinoma. On a cellular level, these clinical changes reflect a number of age-related functional deteriorations. These include a decrease in

keratinocyte proliferative capacity, decreased stratum corneum formation, decreased protective barrier regeneration attributable in part to reduced lipid synthesis, as well as defective thermoregulation due to changes in vascular responsiveness and the autonomic nervous system [1,2]. Although there are many factors at play that contribute to these processes, of the prime contributor to these changes within skin cells is the overproduction of reactive oxygen species (ROS), and resultant oxidative stress.

ROS are short lived molecules that can react readily with electron acceptors, such as oxygen, to convert them into free radicals in a chain reaction. They include singlet oxygen ($^1O_2$), superoxide anions (O_2^-), hydroxyl anions (OH•), and hydrogen peroxide (H_2O_2), amongst others. Their pathogenicity is largely attributable to their ability to react with cellular macromolecules in their vicinity such as lipids, proteins, and even DNA, significantly damaging their molecular structure and function [3].

Cellular homeostasis is maintained by intrinsic redox state modulating enzymatic pathways and antioxidants. These include, to name a few, vitamins C and E, glutathione (GSH), superoxide dismutase (SOD), catalase, uric acid, beta-carotene, the SPRR2 family of proteins, as well as CoQ10 and ferritin. Although these existing antioxidant systems counter the effects of ROS, when there is significant increase in ROS generation, these systems become overwhelmed, ROS begin to accumulate within cells resulting in what is termed oxidative stress.

2. Oxidative stress in the skin

As the major interface between the body and its surrounding environment, the skin is subject to an onslaught of environmental pollutants and toxins, as well as ingested xenobiotics and metabolites that drive the creation of ROS. Extrinsic sources of oxidative stress include ultraviolet irradiation (UVR), infrared irradiation (IRA), xenobiotics, and environmental pollutants [4]. Of these, UVR is perhaps the biggest offender. UVR is comprised of UVA (315–400 nm), UVB (280–315 nm), and UVC (100–280 nm); UVA accounts for roughly 95% of all UVR to reach earth. Both UVA and UVB penetrate the epidermis, however only UVA also penetrates the dermis. When UVR hits the skin, some of the photons are absorbed by natural chromophores, which include the porphyrins and flavins, vitamin K and B6 derivatives, bilirubin, urocanic acid (a component of the stratum corneum), advanced glycation end (AGE) products, and even DNA [5]. After excitation, these chromophores experience a transient change in molecular structure, causing them to become electron donors. As a consequence, they can react with other electron acceptors to form free radicals, ultimately resulting in oxidative stress of nearby targets.

Nonetheless, it is not only environmental agents such as UV that promote oxidative stress. In fact, many intrinsic processes also drive the creation of ROS. Cellular mitochondria steadily produce ROS as a byproduct of normal aerobic metabolism, wherein the electron transport chain (ETC) forms superoxide. Mitochondrial nitric oxide (NO) synthase produces NO, itself a free radical, which can combine with superoxide to form peroxynitrite ($ONOO^-$), a potent ROS. Although the mitochondria are oft-cited as the primary contributor of cellular ROS, many other cellular components also produce ROS. Other intrinsic sources of ROS include cytochrome p450, cyclooxygenases, NADPH oxidases, peroxisomal oxidases, and lipoxygenases (Figure 1) [6]. ROS production can be further amplified in response to infection, malignancy, and other inflammatory states. Mice which are deficient in Cu-Zn superoxide dismutase (SOD1), as well as SOD2, were found to exhibit multiple

aging-related phenotypes, including skin atrophy, related to excess superoxide levels [7-9]. As such, ROS are associated with a multitude of pathologies whether within the skin or in other organ systems. Many diseases, such as cancer and neurodegenerative disorders, have had oxidative stress implicated in their etiopathogenesis [10].

At low levels, it has been posited that ROS and free radicals are useful in cell signaling and may promote cell growth [10,11]. ROS, as well as reactive nitrogen species, are major components in pathogen defense systems in immune cells. A well-known example is NO, a prominent intracellular messenger, hormone, and cell cycle mediator well-known for its utility in vasodilation and blood pressure modulation. However, in the context of skin aging, the role of ROS in potentiating oxidative stress has tilted more toward its detrimental effects.

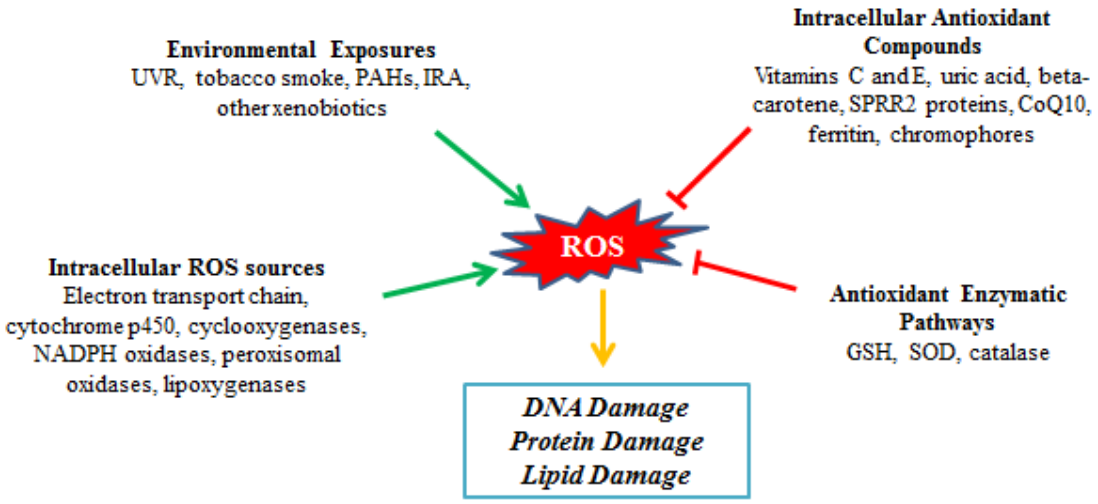

Figure 1. Summary of ROS and antioxidant sources within the cell. ROS: reactive oxygen species; UVR: ultraviolet irradiation; PAHs: polycyclic aryl hydrocarbons; IRA: infrared irradiation, GSH: glutathione; SOD: superoxide dismutase.

3. Oxidative stress and skin aging

Within keratinocytes and dermal fibroblasts, ROS are known to activate receptor-mediated signaling pathways of growth factors and cytokines. Downstream signaling induces c-Jun, which forms a component of activator protein 1 (AP-1), as well as NF-κB (Figure 2) [12,13]. The induction of these protein pathways leads to the overall effect of decreased collagen synthesis as well as increased collagen degradation through the activation of matrix metalloproteinases (MMPs), collagenases, and 92 kDa gelatinase, which work synergistically to degrade the skin matrix [14-16]. Collagen is an integral structural component of skin, thus its loss promotes sagging and the appearance of aged skin, as well as decreased skin hydration. Degraded collagen fragments, in turn, promote the further increase of ROS in human dermal fibroblasts, in a positive feedback method [17]. After episodes of oxidative stress, the dermis attempts to repair itself from the matrix damage it has experienced; however, such repair is often faulty. Imperfect dermal repair after an insult results in a "solar scar," appreciated histologically as solar elastosis and other dermal changes, and over time can lead to visible signs of skin photo-aging.

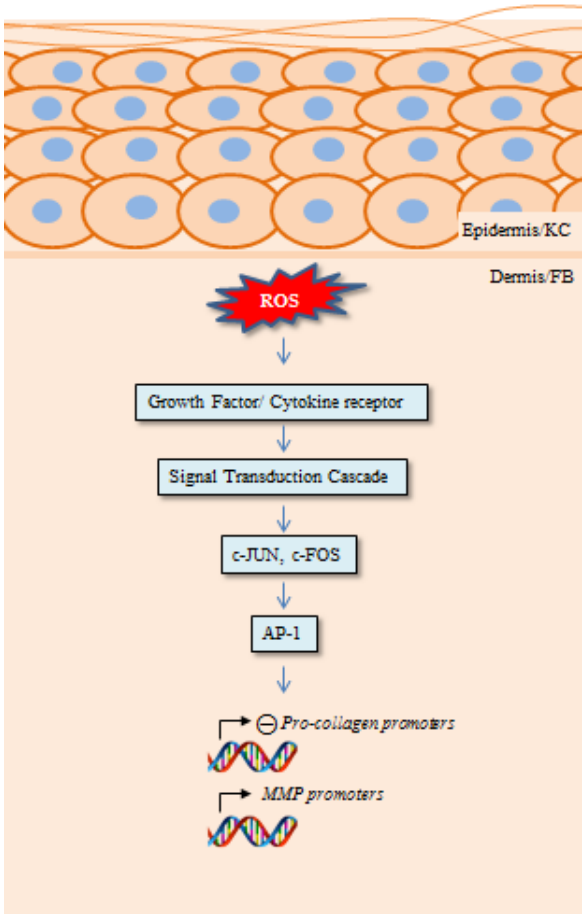

Figure 2. ROS signaling pathways contributory to skin aging. KC: keratinocyte; FB: fibroblast.

Considering UVR again, specifically, NF-κB activation by UV irradiation also results in the production of inflammatory cytokines that attract neutrophils, monocytes and macrophages. The activated cells can produce $^1O_2$ as well NO through the inducible nitric oxide pathway (iNOS). This contributes additional ROS production in skin tissue, increasing the oxidative stress in affected skin [18]. Neutrophils also contain collagenases which augment the effect of matrix degradation, and overall promote aging in the skin.

Another mechanism through which oxidative stress affects skin aging is by decreasing proteasome function. It is already known that in cultured keratinocytes, serial passages have an effect of decreasing proteasome activity [19]. Interestingly, the maintenance of normal proteasome function in human dermal fibroblasts has been associated with a delayed skin aging phenotype [20]. This suggests that aged skin, with its decreased proteasome activity, may have decreased protein degradation in comparison to younger skin. Similarly, UVR, a source of oxidative stress, has also been found to cause decreased proteasome peptidase activity [21]. Impeding the ability of the cell to degrade these products results in ROS accumulation and may contribute to the dysfunction of skin tissue during aging [22].

Oxidative stress may also potentiate skin aging through its effects on DNA. This involves formation of specific DNA lesions such as single-strand and double-strand DNA breaks, modification of DNA bases, loss of purines (leading to apurinic sites), and even damage to the DNA repair system [11]. ROS, particularly hydroxyl radicals, attack guanine at its C-8 position to yield 8-hydroxydeoxyguanosine (8-OHdG), a major DNA product of oxidative stress [11]. Other bases are

also affected by ROS, such as adenine, which is changed to 8- (or 4-, 5-) hydroxyadenine [11]. In many cell types, this type of DNA-damage is known to initiate cell cycle checkpoint arrest, thus limiting cellular proliferation [23].

Oxidative stress has been specifically implicated in replicative senescence in both human dermal fibroblasts (HDFs) as well as melanocytes [24]. In terms of cellular proliferation, it has been found to decrease cell doubling in cultured HDFs by 50% [25]. Furthermore, senescent HDFs were found to have 35% more steady state levels of 8-OHdG. As a treatment, alpha-phenyl-t-butyl nitrone (PBN), an antioxidant, was found to delay replicative senescence in a dose-dependent manner [25].

The effect of oxidative stress on DNA extends to the telomeres as well. Telomeres are terminal segments of the chromosome composed of repetitive DNA segments; they function to protect the chromosome from deterioration resulting from sequential replications. When telomeres are shortened to a critical length, the cell can no longer divide without compromising the DNA. Thus, the cell enters a state of senescence or apoptosis. In one study, H_2O_2 and Cu(II) ion, and SIN-1 (an effector of NO, O_2^- and $ONOO^-$ release), were used to introduce oxidative stress to DNA [26]. Through DNA sequencing, this addition was found to cause a 6-fold, and 4-fold (respectively) greater DNA damage in the telomere sequence of cells than in the non-telomeric regions assayed. Furthermore, the concentration of 8-OHdG was up to 4.5 times higher in the telomeric regions as opposed to the non-telomeric regions tested. This suggests that oxidative stress specifically damages telomeres, and thus may have a similar effect on skin aging as telomere shortening, with the overall effect of promoting cellular senescence.

Two other major extrinsic factors that affect skin aging include smoking, and environmental pollutants. Several epidemiological studies have evaluated the effect of smoking on skin wrinkling. They have found that smoking is an independent factor in skin aging [27-30]. Furthermore, studies have also revealed that the effect of smoking can be compounded with other determinants, such as UVR, to further augment the premature skin aging phenotype [28-30]. On a molecular level, the aryl hydrocarbon receptor (AhR) pathway, involved in the metabolism of xenobiotics, is critical in modulating the effect of tobacco on skin aging. This is mainly through its induction of MMP-1 in keratinocytes and dermal fibroblasts, which results in a breakdown of the skin matrix; however, ROS have also been demonstrated to be elevated in the skin of smokers [31]. Environmental pollutants, particularly those that contain polycyclic aromatic hydrocarbons (PAHs) also activate the AhR pathway and also increase the level of ROS within skin [32].

4. Prevention/treatment

Preventive approaches can be utilized in order to keep skin aging at bay, including modifying environmental exposures, dietary intake, as well as undertaking appropriate skin care. Oxidative stress is the target of many skin therapeutics that modulate extrinsic skin aging. As UVR is a major cause of skin aging, mediated in large part through its promotion of oxidative stress within the skin, decreased UVR insult is critical and can be achieved through established means of photoprotection: 1) sun avoidance during peak UV hours, 2) the use of broad-spectrum sun-protective agents; and 3) the use of photoprotective clothing/physical coverage and hats. Photoprotection alone can prevent a large source of oxidative stress within the skin.

Vitamins are also useful therapies in skin aging for their potent antioxidant effects. All trans retinoic acid (ATRA), a carboxylic acid formulation of vitamin A, is particularly beneficial in

reducing the skin effects of oxidative stress. It acts to inhibit the induction of c-Jun by UVR, thus preventing the assembly of AP-1 and the downstream effects of increased MMP and collagenase activity [12]. This ultimately results in a decreased appearance of aged skin over time. As lipid peroxidation is another source of ROS, its modulation is also useful for controlling oxidative stress within the skin. Alpha-tocopherol (vitamin E) prevents lipid peroxidation by scavenging tocopherols and tocotrienols, and is known to be photoprotective. Similarly, ascorbic acid (vitamin C), essential for normal collagen synthesis, also has a potent antioxidant effect. Topical long-term application of ascorbic acid has been found to decrease measures of photodamage in facial skin [33]. Another group found that the addition of vitamin C and collagen peptide was able to attenuate skin thinning in *Sod1*$^{-/-}$ mice, believed to be due to the antioxidant effects of both vitamin C and the collagen peptide [34]. Lastly, vitamin D, a nuclear receptor steroid hormone, is also known to modulate aging in part due to its anti-inflammatory actions as well as its role in oxidative stress reduction [35]. One recent study found that a large one-time dose of vitamin D was sufficiently anti-inflammatory to suppress macrophage-mediated iNOS production in nitrogen mustard treated mice skin, resulting in mitigation of local skin destruction [36]. This reinforces its potential in alleviating oxidative stress in the context of skin aging.

Other antioxidants useful in skin aging include polyphenols, present in green tea, which are known for their antioxidant and anti-inflammatory properties [37]. One derivative, known as epigallocatechin-3-gallate, is a potent inhibitor of oxidative stress and has been observed to confer photoprotection against UVB-mediated damage in normal HDFs, presumably through its effects on miRNA expression [38]. Another recent study found that a fruit blend made from blueberry extract, sea buckthorn (*Hippophae rhamnoides L.*), as well as collagen administered orally to UV-irradiated nude mice showed significant decreases in transepidermal water loss, decreased skin wrinkling, decreased MMP1 and MMP9 expression in the skin, as well as increased SOD activity [39]. Another group researching *Sod1*$^{-/-}$ mice found that oral supplementation of Melinjo (*Gnetum gnemon*) seed extract (MSE), which contains trans-resveratrol (RSV) and resveratrol derivatives, was able to reverse skin thinning and increase mRNA expression of *Col1a1* whilst decreasing *p53* expression [40]. Furthermore, plasma 8-isoprostane, a marker of lipid peroxidation and oxidative stress, was found to be markedly reduced in mice treated with either MSE or RSV. These findings suggest the utility of MSE and RSV as anti-oxidant therapies in the context of skin aging.

Ubiquinone (coenzyme Q10), a known intracellular antioxidant and component of the mitochondrial respiratory chain, is another supplement recent research has indicated may be useful in skin aging. It has been shown to be protective against free-radical induced oxidative damage to membrane phospholipids, mitochondrial membranes, as well low-density lipoprotein-cholesterol [41]. Ubiquinone levels are known to decrease in correspondence with age and UVR [42]. Topical supplementation with it has been found to reduce the level of oxidation measured by weak photon emission, and was also able to significantly suppress the expression of dermal fibroblast collagenases following UVA irradiation [42].

Lastly, one study found that a mixture of platinum and palladium nanoparticles (PAPLAL), known to have a potent antioxidant effect, was able to resuscitate SOD and catalase functionality in *Sod1*$^{-/-}$ mice and halt the expected phenotype of skin atrophy [43]. Furthermore, PAPLAL was able to normalize the transcript levels of *Col1a1*, *Mmp2*, *Tnf-α*, amongst other genes known to be affected by free radicals. This is a promising direction for the use of nanoparticles into research of skin aging mediated by oxidative stress damage.

5. Conclusion

To some degree chronological skin aging is as of yet unavoidable. However, ongoing research has made it clear that modifiable risk factors that mediate their effect through oxidative stress are fruitful targets of further investigation, as has been observed in numerous animal and cell culture studies. Because the effects of oxidative stress are so devastating on skin structure, preventive methods are critical in management. Future research should continue to elucidate the role of antioxidants, whether topical or systemic, in anti-aging skin therapies.

Conflict of interest

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