Skin Rejuvenation through HIF-1α Modulation

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Summary: The constant intrinsic and extrinsic stress the skin is exposed to leads to significant impairments of the regenerative capacity of aging skin. Current skin rejuvenation approaches lack the ability to holistically support the biological processes that exhaust during aging skin degeneration, such as collagen production, cell migration and proliferation, and new vessel formation. Similar to chronic wounds, aged skin is characterized by dysfunction of key cellular regulatory pathways impairing regeneration. Recent evidence suggests that the same mechanisms hindering a physiologic healing response in chronic wounds are the basis of impaired tissue homeostasis in aged skin. Dysfunction of a main response-to-injury pathway, the hypoxia-inducible factor (HIF)-1α regulatory pathway, has been identified as pivotal both in chronic wounds and in aging skin degeneration. HIF-1α signaling is significantly involved in tissue homeostasis and neovascularization, resulting in the production of new collagen, elastin, and nourishing blood vessels. Modulating the functionality of this pathway has been demonstrated to significantly enhance tissue regeneration. In this review, we present an overview of the regenerative effects linked to the up-regulation of HIF-1α functionality, potentially resulting in skin rejuvenation on both the cellular level and the tissue level. (*Plast. Reconstr. Surg.* 141: 600e, 2018.)

Composed of three different layers of ectodermal tissue—the epidermis, the dermis, and the hypodermis—the human skin represents an essential barrier organ of the human body.[1] The skin interfaces with the environment and acts as the first line of defense against microbiological invasions, physical aggressions, and chemical assaults. In addition to forming a physical and immunologic barrier, the skin is key to the production of vitamin D folates and the regulation of the water and electrolyte household. All of these functions are based on physiologic skin homeostasis and require an intact epidermis, dermis, and hypodermis.[2]

Different cell types such as fibroblasts, keratinocytes, and melanocytes together form the epidermal, the dermal, and the hypodermal layers of the skin.[3] All cellular and connective tissue constituents throughout all three layers are influenced by environmental determinants, genetic predisposition, nutrition, and other factors.[4] The constant intrinsic and extrinsic stress to which the skin is exposed leads to specific changes in its composition and functionality over time.[5,6] This phenomenon of cutaneous aging is summarized in Figure 1. With aging, the skin gets thinner and the blood vessels of the dermis become sparse and more fragile, leading to wrinkles and a paler, translucent appearance.[7] Age-related changes in the connective tissue reduce the skin's strength and elasticity.[8,9] This process is known as "elastosis" and is heavily driven by sun exposure (solar elastosis).[10] All of these changes together result in a significant impairment of the regenerative capacities of aging skin.[11]

Intensive study efforts are devoted to the development of agents capable of mitigating the signs of cutaneous aging. Despite these efforts, no single approach has been developed that addresses all facets of aged skin degeneration, such as epidermal and dermal atrophy and the loss of connective tissue structure and vascularity. Although most cosmetic products provide adequate skin

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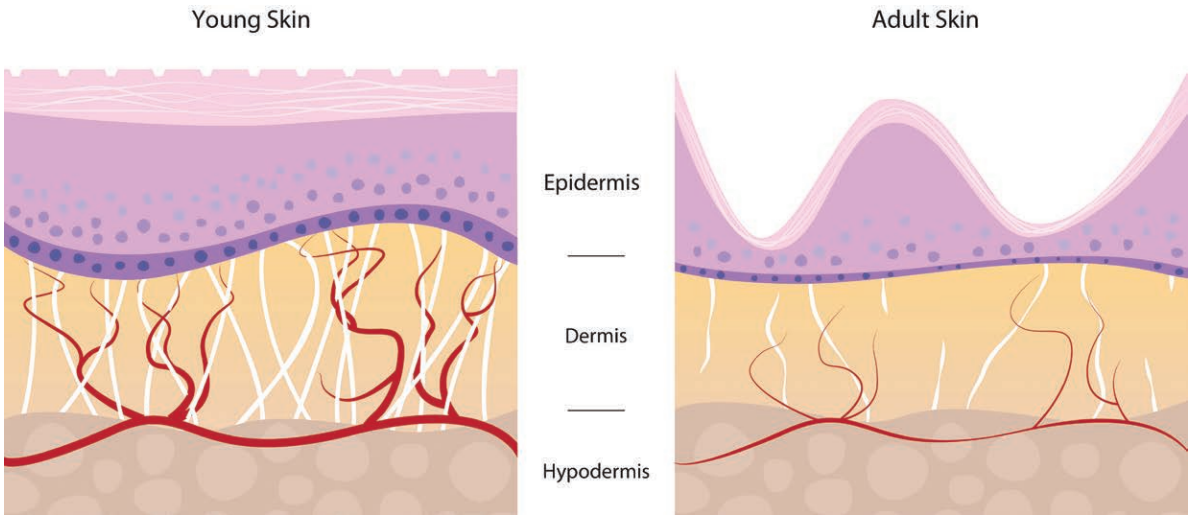

Fig. 1. Differences between young and aged skin. (*Left*) Youthful healthy skin presents thick epidermis, a strong dermoepidermal junction, normal collagen content, and healthy vascularity. (*Right*) Aged skin contains several signs of degeneration, such as uneven epidermis, thinner dermoepidermal junction, inadequate collagen content, and compromised vascularity.

hydration, they lack the ability to actively support the biological processes that are diminished by age: collagen production, cell migration and proliferation, and new vessel formation.

Similar to chronic wounds, aged skin is characterized by the dysfunction of key cellular regulatory pathways, impairing regeneration.[12–14] Recent evidence suggests that the same mechanisms that hinder the physiologic healing response in chronic wounds are the basis of impaired tissue homeostasis in aged skin.[13–18] Dysfunction of the main response-to-injury pathway, which is also activated by means of hypoxic stress, has been identified as pivotal in both chronic wounds and aging skin degeneration. Physiologic activation of this hypoxia-inducible factor (HIF)-1α regulatory pathway is significantly involved in tissue homeostasis and neovascularization, resulting in the production of new collagen, elastin, glycosaminoglycans, and nourishing blood vessels.[19,20] Modulating the functionality of this pathway has been demonstrated to significantly enhance tissue regeneration.[16,21–25] In this article, we present an overview of the regenerative effects linked to the up-regulation of HIF-1α functionality, potentially resulting in skin rejuvenation on both the cellular level and the tissue level.

MECHANISMS OF CUTANEOUS AGING

Aging is defined as morphologic changes associated with a loss of function of cells.[26] In human skin, this loss of function is characterized mainly by the decreased ability of response to exogenous and endogenous stress.[27] Intrinsic or innate aging is a degenerative process that affects the skin in the same way as it affects all other organs. The second variable of cutaneous aging, named "photoaging," is the result of skin exposure to external factors, primarily ultraviolet radiation.[27] Together, both innate and exogenous aging lead to degradation and the breakage of collagen fibers, loss of connective tissue structures, and vascularity. The interplay of these mechanisms affects all layers of the skin, with its greatest influence being on the dermis.[17]

Mechanisms of Innate Aging

Research to define the cutaneous aging process identified three protagonists of innate aging: telomeres, oxidative stress, and DNA damage.[28–30] There is emerging evidence showing that lifestyle factors may affect the health and lifespan of an individual by affecting telomere length.[31] Studies indicate that telomere length, which can be affected by various factors, can modulate the pace of aging and onset of age-associated diseases.[32,33] Other recent studies involve free radicals (reactive oxygen species), suggesting that oxidative stress may damage not only lipid bilayers in cell membranes but also connective tissue components, particularly collagen.[34] It was further demonstrated by our research group that the intracellular and extracellular machinery to tackle free radical stress is impaired in aged skin.[35,36] The resulting reactive oxygen species stress could be

linked to local dysfunction of fibroblasts, partly explaining the impaired regenerative abilities of aged skin.[35] Reactive oxygen species also interact directly with the DNA, leading to base loss, DNA modification, or breakage of strains, making DNA lesions an important factor involved in the aging process.[37]

Genetics is another factor profoundly involved in all of these complex mechanisms.[27] Aged and senescent cutaneous cells have the ability to modify their biosynthetic network by the expression of different genes (e.g., *ID3*, *SMAD7*, *FAM83G*).[38] It has been demonstrated that the rate of collagen biosynthesis is markedly lower in aged skin than in fetal tissue or during the early postnatal years.[39] This change of translation from the genetic level to the protein level may explain compromised wound healing in aged individuals. In addition, this reduced collagen production leads to atrophy of the dermis, effecting wrinkle formation. Similarly, the rate of elastin gene expression is markedly reduced after the fourth decade of life.[40] Elastin is of paramount importance in the connective tissues. Primarily, it allows skin tissue to return to its shape after stretching or contracting. An intrinsic lack of elastin explains the impaired pliability of aged skin. An imbalance between biosynthesis and degradation of elastin fibers clinically manifests as atrophy and loss of recoil.

Mechanisms of Extrinsic Aging

The mechanism of extrinsic aging is the result of continuous exposure of skin to the environment. Named after the histopathologic hallmark, this process is also called photoaging. It is represented by a progredient accumulation of elastic fibers in the upper and middle layers of the dermis, setting the scene for solar elastosis. It was shown that ultraviolet irradiation of human skin continuously increases the activity of matrix metalloproteinases.[41] At the same time, ultraviolet exposure was also shown to induce a specific tissue inhibitor of matrix metalloproteinase, the tissue inhibitor of matrix metalloproteinase inhibitor.[42] In photodamaged skin undergoing the aging process, there is a delicate imbalance between the induction of the degradative enzymes and their inhibitors.[43] In recent studies using recombinant DNA technology, it was demonstrated that ultraviolet radiation activates the human elastin promoter, resulting in subsequent activation of elastin biosynthesis.[44] This leads to a continual accumulation of elastin, which results in a leathery, inelastic, and yellowish appearance of skin, also known as cutis rhomboidalis nuchae.[45]

In addition to elastin as the main component, the elastotic material is composed of a variety of extracellular matrix components such as fibrillin and versican, a large proteoglycan.[46] This accumulation is followed by a concomitant degeneration of the surrounding physiologic network. Recent evidence identified matrix metalloproteinases as mediators of this degeneration.[47] These enzymes destroy the endogenous collagen network, proteoglycans, fibronectin, and other components of the dermis, leading to a rapid but not irreversible cutaneous aging effect.[48]

HIF-1 AND ITS ROLE IN CUTANEOUS HOMEOSTASIS

Advanced age, similar to diabetes, has been shown to correlate with attenuated HIF-1α function.[13–18] Conversely, in aging, HIF-1α is destabilized through enhanced oxygen-sensitive prolyl-hydroxylases activity,[13,14] resulting in impaired release of growth factors, reduced neovascularization, and inadequate tissue quality and regeneration. HIF-1 is a dimeric protein, composed of two subunits, HIF-1α and HIF-1β. These two factors have different molecular characteristics. Although HIF-1α is an oxygen-sensitive subunit induced under hypoxic conditions, HIF-1β is constitutively expressed. Both subunits belong to the basic helix-loop-helix–Per-Arnt-Sim protein family, as their structures are related to two nuclear proteins found in *Drosophila* (Per-Arnt-Sim) that have the basic helix-loop-helix structure.[49] This special geometry is essential to allow heterodimer formation between HIF-1α and HIF-1β, such as binding to DNA on the target genes, the so-called hypoxia response elements. In addition, the HIF-1α subunit has two transactivation domains: NH_2-terminal and COOH-terminal transactivation domains. These two domains are responsible for the transcriptional activity, interact with coactivators of hypoxia response elements such as p300 or CBP, and stabilize HIF-1α against degradation.

In normoxia, HIF-1α protein levels are low because of constant ubiquitination-dependent degradation by means of the von Hippel–Landau E3 ligase protein,[50] which recognizes proline hydroxylated HIF-1α on both transactivation domains.[51–53] These hydroxylation reactions lead to degradation of HIF-1α and are catalyzed by the oxygen-sensitive prolyl-hydroxylases (Fig. 2, *left*). Another level of control lies within the oxygen-sensitive asparaginyl hydroxylase factor inhibiting

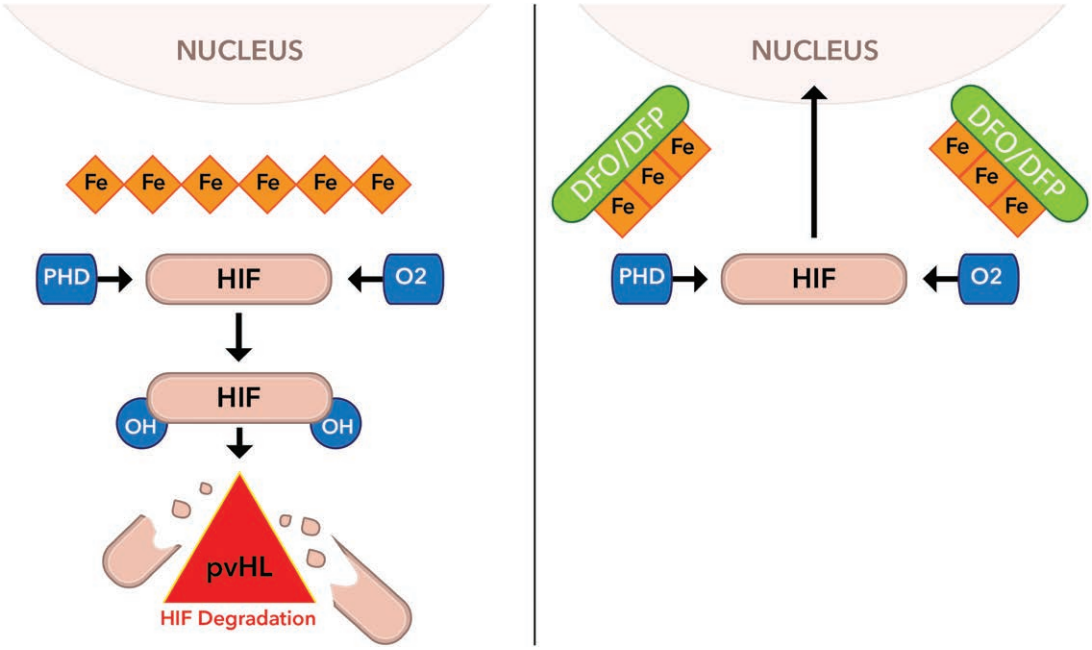

Fig. 2. Modulation of HIF pathway regulation. (*Left*) HIF pathway activation in the presence of iron (*Fe*). Hydroxylation occurs by prolyl-hydroxylase, followed by ubiquitination by von Hippel–Landau tumor suppressor (*pvHL*), which facilitates enzymatic degradation of HIF-1α. (*Right*) Truncated HIF-1α breakdown pathway in the presence of an iron chelator. Prolyl-hydroxylase (*PHD*) is inactivated, allowing HIF-1α to remain intact and free to dimerize for downstream HIF-1 pathway activation. *DFO*, deferoxamine; *DFP*, deferiprone.

HIF-1α. The oxygen-sensitive asparaginyl hydroxylase factor inhibiting HIF-1α hydroxylates the HIF-1α protein and inhibits the subsequent recruitment of transcriptional coactivators p300 and CBP, thereby progressively decreasing the HIF transcriptional activity.[54–56] However, in addition to the absences of oxygen, the lack of local free iron is also able to inhibit HIF-1α degradation. The consequences are decreased HIF-1α hydroxylation, decreased von Hippel–Lindau tumor suppressor–mediated ubiquitination, degradation, and increased HIF-1α protein stability[53] (Fig. 2, *right*).

HIF-1 is essential for skin homeostasis and is mainly expressed in the basal layer of the epidermis.[57–59] HIF-1 has been shown to modulate adhesion and migration of skin cells such as human fibroblasts[60] and keratinocytes.[61] Regulation of HIF-1 has been demonstrated as crucial for skin homeostasis,[62] important in wound healing, suggesting the involvement of HIF-1 in dermal tissue repair.[63,64] Modulation of HIF-1 activity results in many molecular processes. The further activation of over 100 downstream genes of the HIF-1α pathway is crucial for controlling angiogenesis, cell proliferation, migration, and glucose metabolism.[65–67]

In addition, recent studies using human epidermal cells showed that up-regulation of HIF-1 substantially increases the growth potential of keratinocytes, and improves the formation of viable and stratified epidermis.[17,18] HIF-1 overexpression in keratinocytes expands the dermal vasculature, suggesting a substantial influence on blood vessel formation by cutaneous cells through this pathway.[68,69] HIF-1 has also been shown to drive the expression of laminin-332,[61] an extracellular glycoprotein. The main role of laminin-332 is the maintenance of epithelial-mesenchymal cohesion in tissues exposed to external forces such as the skin.[70] Laminin-332 is involved in the attachment of the basal epidermal keratinocytes to the basement membrane. An interaction with the heterodimeric cell surface receptors mediates adhesion of the extracellular matrix to the cytoskeleton.[71] Laminin-332 has an important role in keratinocyte adhesion and migration.[72] Notably, a diminution of keratinocyte growth potential following HIF-1 silencing was associated with decreased expression of laminin-322.[18]

Loss of epidermal HIF-1 expression has further been linked to an acceleration of skin aging and affects reepithelialization.[18] In addition, it has been shown that cutaneous HIF-1 expression is modulated after type B ultraviolet exposure, and that HIF-1α has an important role in the regulation of cellular responses to this type of genotoxic

stress. Type B ultraviolet induces reactive oxygen species, which in turn influences HIF-1α expression affecting DNA repair and keratinocyte survival.[73] Lastly, HIF-1α up-regulation has been linked to the defense of the skin against infectious diseases. HIF-1α provides protection against cutaneous infections by means of up-regulation of antimicrobial peptides, which is responsible for the dermal antibacterial functionality.[74]

MODULATION OF HIF-1 FOR REGENERATION

As mentioned above, it has been demonstrated that HIF-1 is critical for cutaneous homeostasis, especially in the context of aging.[17,18] Up-regulation of HIF-1 leads to the correction of age-dependent functional impairments of the skin, and results in improved regeneration of aged tissues.[21] We and others have applied gene therapy to up-regulate HIF-1 signaling using plasmids.[25,75] However, the biochemical reactions regulating HIF-1 signaling provide simpler therapeutic strategies to promote HIF-1α stabilization and transactivation (Fig. 2, *right*). Prolyl-hydroxylases and factor inhibiting HIF-1α, the hydroxylases responsible for HIF-1 degradation, both belong to a family of iron-dependent dioxygenases that require iron, oxygen, and 2-oxaloglutarate as cofactors for the hydroxylation process. Therefore, these enzymes are inactive in the absence of oxygen. Hypoxic conditions may be replicated by the presence of iron chelators, such as deferoxamine or deferiprone, or in the presence of a 2-oxaloglutarate competitive inhibitor such as dimethyloxalylglycine.[76,77] Our group has recently demonstrated certain advantages for using iron chelators to stimulate HIF-1 and tissue regeneration.[16,21] In this approach, the removal of iron to deprive HIF-1 degradation of a necessary cofactor is further complemented by reducing reactive oxygen species stress by means of the binding of iron molecules. Although iron is essential for cellular metabolism, an excess of iron can be toxic and accelerate the aging process through catalyzing the formation of reactive oxygen species by means of the Fenton and Haber-Weiss reactions. Iron thereby potentiates the generation of highly reactive oxygen free radicals, thus stimulating oxidative damage.[59]

Iron chelators are already broadly used in different medical fields.[78] Well known as treatment options for beta-thalassemia and hemochromatosis,[79,80] iron-chelating drugs have further shown benefits in the field of plastic and reconstructive surgery. With their regenerative potential, they have the ability to increase the retention rate of fat grafts, the survival rate of free flaps, and the healing process of diabetic wounds.[81,82] Deferoxamine and deferiprone are U.S. Food and Drug Administration–approved molecules with different molar masses (i.e., deferoxamine, 560.69 g/mol; deferiprone, 139.152 g/mol). Because of the different molecular weights, scientists have successfully tried to exploit the possible interactions between deferoxamine and deferiprone to achieve a synergistic effect on iron chelation.[83] Although deferoxamine is hydrophilic, deferiprone belongs to the hydrophobic class of molecules. Despite these chemical differences, iron chelators typically contain oxygen, nitrogen, or sulfur-donor atoms that form bonds with iron. The donor atoms of the ligand affect the preference of the chelator for either the Fe(II) or Fe(III) oxidation states. Chelators that prefer Fe(II) contain "soft" donor atoms, such as nitrogen and sulfur, and consequently retain a relatively high affinity for other important divalent metals such as $Cu2^{+}$ and $Zn2^{+}$. Iron chelators such as deferoxamine have a hexadentate arrangement, binding iron in a 1:1 ratio and therefore show the highest affinity. Because of their ability to specifically chelate iron from ferritin and hemosiderin but not from cytochromes, hemoglobin, and transferrin, they have been first-line therapy for hemochromatosis since the 1970s.[84] Deferiprone, an orally administered 3:1 iron-chelating agent, was first approved as treatment for thalassemia in 1994.[85] All molecules of the iron chelator family have been in clinical use for decades and have favorable safety characteristics, making them promising leads for therapeutic HIF-1 signaling modulation to enhance regeneration.

CONCLUSIONS AND OUTLOOK

It has been demonstrated that HIF-1 is critical for cutaneous homeostasis, specifically, in the context of aging. The up-regulation of HIF-1 leads to the correction of age-dependent impairments of HIF-1 expression. This directly results in improved cutaneous regeneration and resistance to stressors such as radiation and infection. These benefits are mediated by means of positive effects on all cutaneous cell types; the up-regulation of proregenerative cytokines, growth factors, and peptides; and the recruitment of circulating regenerative cells.[17,65] The possibilities of a therapeutic modulation of hypoxia-inducible networks by repurposing iron chelators are promising. However, the

impact of such a modulation on the mechanisms of skin rejuvenation is not fully understood. A thorough investigation of the molecular effects of the HIF-1α pathway alteration by iron chelators such as deferoxamine or deferiprone and their influence on human keratinocytes and fibroblasts is warranted. However, if supported by solid clinical trial data, this approach would have the potential to become a major development area of aesthetic and regenerative medicine.

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