Iron Chelators & HIF-1α: A New Frontier for Skin Rejuvenation

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19.1 Mechanisms of Skin-Aging

The human skin belongs to the integumentary system and represents the largest organ in the human body, comprising about 15% of the body weight. The total skin surface of an adult ranges from 12 to 20 square feet, and the skin is composed of 70% water, 25% protein and 2% lipids. Different cell types like fibroblasts, keratinocytes, and melanocytes build the external (epidermal), the middle (dermal), and the inner (subdermal) layer. The skin derives from the ectodermal tissue, interfaces with the environment, and thereby acts as the first line of defense against microbiological invasions, physical aggressions, and chemical assaults. In addition, this particular composition plays an important role in insulation, temperature regulation, sensation, and is key to the production of vitamin D [1]. All of these functions are based on physiological tissue homeostasis and require an intact epidermis, dermis, and hypodermis [2]. The thickness of skin significantly depends on body location. In humans, the skin located around the eyes and eyelids is the thinnest in the body (0.5 mm thick) and is one of the first areas to show signs of aging, colloquially known as "crow's feet". In other parts of the body like palms and soles of the feet, the skin can be up to 4 mm thick. Skin is a multifunctional organ and, like any other organ system subject to different stress factors, leading to specific impairments in its composition and functionality over time [3, 4].

In the last decade, healthy-aging principles and longevity pathways were studied [5]. At a cellular and molecular level, aging is characterized by the accumulation of damage such as DNA oxidation and progressive loss of physiological integrity, leading to impaired function and increased vulnerability, and subsequently death. At present, progressive aging in humans cannot be successfully and satisfactorily stopped. Researchers are only just beginning to understand the biological basis of aging even in relatively simple and short-lived organisms such as yeast [6]. Derived from these studies, numerous hypotheses have been formulated with the aim to explain the aging phe-

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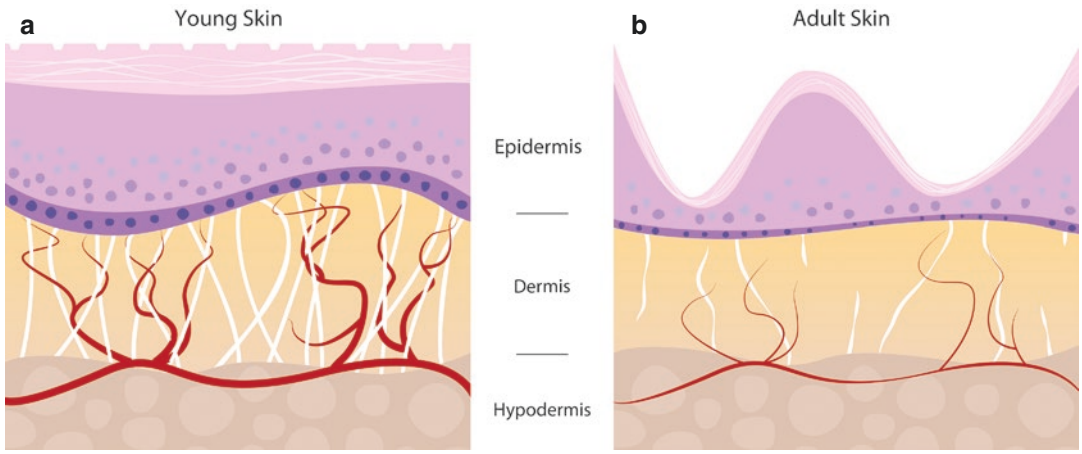

Fig. 19.1 Differences between young and aged skin. (**a**) Youthful healthy skin presents thick epidermis, a strong dermo-epidermal junction, normal collagen content, and healthy vascularity. (**b**) Aged skin contains several signs of degeneration, such as uneven epidermis, thinner dermo-epidermal junction, inadequate collagen content, and compromised vascularity [71]. Reprinted with permission

nomenon. Aging could be the natural result of entropy on the cells, tissues, and organs [7]. However, evidence is accumulating showing that aging is in part genetically regulated. In parallel, other approaches show that the regulation and the subsequent breakdown of cellular processes represent a programmatic decision by the cell to either continue or abandon maintenance procedures with age [8]. Regarding the skin, it is widely accepted that advanced age brings changes to all components of the integumentary system with consequent signs of deterioration on epidermis, dermis, and hypodermis. During the aging process, skin gets progressively thinner and the blood capillaries of the dermis become sparse and more fragile, resulting in wrinkles and a paler, translucent appearance [9] (Fig. 19.1).

Due to increasing understanding of genetic pathways and biochemical processes, research about the cutaneous aging process has experienced an unprecedented advance within the last years [10]. Generally, age-related skin changes are triggered by a combination of intrinsic factors and extrinsic ones (e.g. ultraviolet/infrared light exposure, smoking). Intrinsic or innate aging is a degenerative process, which affects the skin in the same way as it affects all other organs. Intrinsic skin aging represents the biological clock of the human body and reflects the reduction of function that is extensively described to affect internal organs [11]. This loss of function is mainly characterized by the decreased ability of response to exogenous and endogenous stress [12]. Three main protagonists of innate/intrinsic cutaneous aging are: telomere-loss, oxidative stress, and DNA-damage [13–15]. Several studies indicate that telomere length is able to modulate the pace of aging and onset of age-associated diseases [16, 17]. However, there is emerging evidence showing that lifestyle factors (obesity, smoking, and alcohol) may influence health and lifespan of an individual by directly affecting telomere length [18] demonstrating the strong interplay between the triggers of aging. Recent studies from our group and others, involving free radicals (Reactive oxygen species - ROS), suggest that oxidative stress may damage not only the lipid bi-layer in cell membranes but also connective tissue components, particularly elastin fibers and collagen [19]. Additionally, ROS 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 [20].

The second main variable of cutaneous aging, also known as "photoaging", is a result of the extrinsic capacity of the environment to damage the skin surface. This "extrinsic aging" is the result of skin exposure to external factors, most importantly ultraviolet (UV) radiation [12]. Age related changes are able to impair the two most important features of the skin: strength and elasticity [21, 22].

The so-called "elastosis" represents a progressive accumulation of elastic fibres in the upper and mid-layers of the dermis and is heavily driven by sun-exposure (therefore also named "solar elastosis") [23]. Altogether, the cutaneous aging phenomenon manifests as an observable change in the external appearance of the skin with a loss of function of cells [24]. Considering both innate and exogenous factors, aging leads to degradation and the breakage of collagen fibers, microtextural impairments, and loss of connective tissue structures.

The intracellular and the extracellular machinery is heavily impaired in aged skin [25, 26]. Aged and senescent cutaneous cells have the ability to modify their biosynthetic network by the expression of different genes such as ID3, SMAD7, and FAM83G [27]. It has been demonstrated that the rate of collagen biosynthesis is markedly lower in aged skin than in infant or foetal tissue [28]. In addition to wound healing disorders, this reduced collagen production leads to the atrophy of the dermis effecting wrinkle formation. Similarly, the rate of elastin gene expression is markedly reduced after the fourth decade of life [29]. Elastin is of paramount importance for the connective tissues by allowing the skin to return to its shape after stretching or contracting. Lack of elastin explains the impaired pliability of aged skin. An imbalance between biosynthesis and degradation of elastin fibres clinically manifests as atrophy and loss of recoil. Recent evidence further identified matrix metalloproteinases (MMPs) as important mediators of this degeneration [30]. By destroying the endogenous collagen network, proteoglycans, fibronectin, and other components of the dermis, these enzymes are leading to a rapid, but not irreversible, cutaneous aging effect [31]. Altogether, the interplay of these mechanisms affects all three layers of the skin, with its biggest influence on the dermis result in significant impairments of the regenerative capacity of aging skin [32, 33].

19.2 The Role of HIF-1 in Skin Aging

Intensive study efforts are currently undertaken to develop agents capable of mitigating or reversing the signs of cutaneous aging. Nevertheless, no single approach has been identified to address all important factors, structural and physiological components, epidermal and dermal atrophy, and loss of connective tissue structure and vascularity. While most cosmetic products only provide adequate skin hydration, they lack the ability to actively support the biological processes, which are known to be diminished in aged population.

Similar to chronic wounds, skin-aging is characterized by the dysfunction of key cellular regulatory pathways. Recent evidence suggests that the same mechanisms, which hinder the physiologic healing response in chronic wounds, are the reason for impaired tissue homeostasis in aged skin [34–39]. The Hypoxia Inducible Factor 1 alpha (HIF-1α) pathway represents one key-mechanism in both conditions [34, 39, 40]. It is widely accepted that the physiological activation of the dimeric protein HIF-1α), representing the main transcriptional factor of the HIF-1 pathway, is significantly involved in tissue homeostasis and neovascularization. Therefore, activation leads to production of new collagen strains, elastin, glycosaminoglycans, and nutritive blood vessels [41, 42]. Slight modulation of the functionality of this pathway has been clearly demonstrated to significantly enhance tissue regeneration [35, 36, 43–46]. Advanced age, similar to diabetes and other degenerative skin diseases, has been shown to correlate with attenuated HIF-1α function [34–39].

In aging, HIF-1α is destabilized by enhanced activity of the oxygen-sensitive prolyl-hydroxylases (PHD) [34, 39] resulting in impaired release of growth factors, reduced neovascularization, and inadequate tissue quality and regeneration. As mentioned above, the Hypoxia Inducible Factor (HIF-1) is a dimeric transcription factor, composed of two subunits, HIF-1α and HIF-1β. These two proteins have different molecular characteristics. While HIF-1α is an oxygen sensitive subunit, which is activated under hypoxic conditions, HIF-1β is constitutively expressed. This special geometry is essential to allow heterodimer formation between the two proteins HIF-1α and HIF-1β, such as binding to DNA on the target hypoxia response elements (HRE). In addition, the HIF-1α subunit has two different transactivation domains (TAD): NH_2-

terminal [N-TAD] and COOH-terminal [C-TAD]. These two domains are responsible for the transcriptional activity by interacting with co-activators of HRE such as p300 or the cyclic-AMP binding protein (CBP) and stabilizing HIF-1α against degradation.

In normoxia, HIF-1α protein levels are low due to constant ubiquitination-dependent degradation via the Von Hippel-Landau (VHL) E3 ligase protein [47], which recognizes proline hydroxylated (Pro-OH) HIF-1α on both transactivation domains [48–50]. These hydroxylation reactions lead to degradation of HIF-1 α and are catalyzed by the oxygen-sensitive PHD. Another level of control lies within the oxygen-sensitive asparaginyl hydroxylase FIH, a factor inhibiting HIF. The oxygen-sensitive asparaginyl hydroxylase FIH hydroxylates the HIF-1α protein and inhibits subsequently the recruitment of transcriptional co-activators p300 and CBP, thereby the HIF transcriptional activity progressively decreases [51–53]. However, in addition to the absences of oxygen, lack of local free iron is also able to inhibit of HIF-1α degradation. The consequences are decreased HIF-1α hydroxylation, decreased pVHL mediated ubiquitination, degradation and increased HIF-1α protein stability [50] (Fig. 19.2).

HIF-1 alpha is essential for skin homeostasis and is mainly expressed in the basal layer of the epidermis [54–56]. Molecular pathways between fibroblasts and keratinocytes are crucial for the skin environment, especially in the basal layers of the epidermis. Therefore, slight modulation of HIF-1 activity could be strongly involved in novel approaches for skin rejuvenation. The possibilities of a therapeutical modulation of some of these networks are prom-

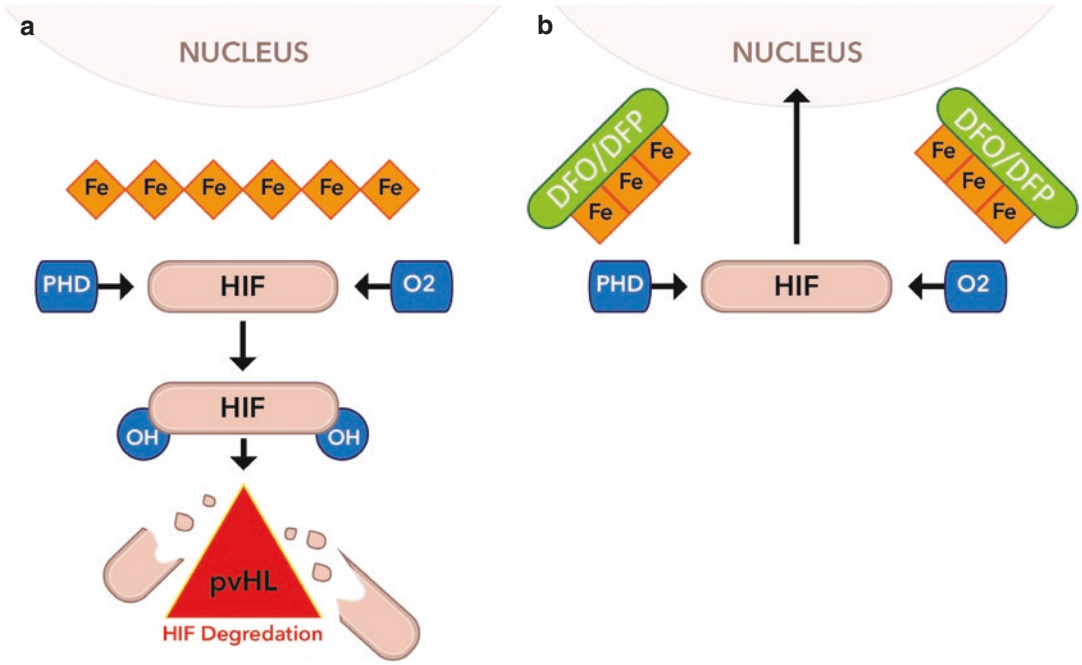

Fig. 19.2 Modulation of HIF pathway regulation. (**a**) HIF pathway activation in the presence of iron. Hydroxylation occurs by PHD, followed by ubiquitination by VHL, which facilitates enzymatic degradation of HIF1α. (**b**) Truncated HIF-1α breakdown pathway in the presence of an iron chelator. PHD is inactivated, allowing HIF1α to remain intact and free to dimerize for downstream HIF-1 pathway activation. (*DFO* Deferoxamine, *DFP* Deferiprone) [71]. Reprinted with permission

ising. The further activation of over 100 downstream genes of the HIF-1α pathway was proven to modulate angiogenesis, cell proliferation, migration, and glucose metabolism [57–59]. Regulation of HIF-1 has been demonstrated to be crucially involved in skin homeostasis [60] and wound healing [61, 62]. Therefore, localized control of tissue blood flow, or autoregulation, is a key factor in regulating tissue perfusion and oxygenation. Recently, it was demonstrated that the balance between the two transcription factor isoforms, HIF-1 alpha and HIF-2 alpha, is an essential mechanism regulating both local and systemic blood flow [63].

Recent studies using human epidermal cells showed that the controlled upregulation of HIF-1 substantially increases the growth potential of keratinocytes and fibroblasts improving the formation of viable and stratified epidermis [37, 38]. HIF-1 alpha overexpression expands dermal vasculature, suggesting a substantial influence on blood vessel formation by cutaneous cells through this pathway [64, 65]. HIF-1 has also been shown to drive the expression of Ln-332 [66], a high-molecular weight (400–900 kDa) protein of the extracellular matrix composed by an alpha-chain, a beta-chain, and a gamma-chain. Ln-332 is the major component of the basal lamina, a protein network foundation for most cells and organs, influencing cell differentiation, migration, and adhesion. The main role of Ln-332 is the maintenance of epithelial-mesenchymal cohesion in tissues that are exposed to external forces such as the skin [67]. An interaction with the heterodimeric cell surface receptors mediates adhesion of the extracellular matrix (ECM) to the cytoskeleton [68]. Notably, a diminution of keratinocyte growth potential following HIF-1 silencing was associated with a decreased expression of Ln-322 [38].

The concept of intrinsic and extrinsic damage can further be linked to age-related loss of epidermal HIF-1 expression [38]. Recent findings shown that cutaneous HIF-1 expression is modulated after UVB exposure, and that HIF-1α has an important role in the regulation of cellular responses to this type of genotoxic stress. Lastly, UVB induces ROS, which in turn influences HIF-1α expression affecting DNA repair and keratinocyte survival [69].

19.3 Iron Chelation for HIF-1 Modulation

Upregulation of HIF-1 reverses age-dependent functional impairments of the skin, and results in improved regeneration of aged tissues [70]. The biochemical reactions regulating HIF-1 signaling provide simple therapeutic strategies to promote HIF-1α stabilization and transactivation. PHD and FIH, the hydroxylases responsible for HIF-1 degradation, both belong to a family of iron-dependent dioxygenases that require iron, oxygen, and 2-oxaloglutarate (2-OG) as cofactors for the hydroxylation process [71]. Therefore, these enzymes are diminished in the absence of oxygen. Hypoxic conditions can be mimicked by the presence of iron chelators, such as deferoxamine or deferiprone, or in the presence of a 2-OG competitive inhibitor such as dimethyloxalylglycine (DMOG) [72, 73]. Our group has recently demonstrated certain advantages for utilizing iron chelators to stimulate HIF-1 and tissue regeneration [36, 70]. In this approach, the removal of iron to deprive HIF-1 degradation of a necessary co-factor is further complemented by reducing ROS stress via the binding of iron molecules. While 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 (ROS), thus stimulating oxidative damage [60]. Well known as treatment option for Beta-Thalassemia and Hemochromatosis [74, 75], iron chelating drugs have 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 [76, 77].

One of the first approaches to use iron chelation for regenerative medicine can be dated back to 1993. It was described that deferoxamine induces Erythropoietin gene expression and HIF-1 DNA-binding activity [78]. Deferoxamine

(DFO) and Deferiprone (DFP) are FDA-approved molecules with different molar masses (DFO = 560.69 g/mol and DFP = 139.152 g/mol). Because of its ability to chelate iron from ferritin and hemosiderin, hemoglobin and transferrin, Deferoxamine is already a first line therapy for Hemochromatosis. Deferiprone is an orally active agent firstly approved for use of treating thalassaemia. Due to the different molecular weight, scientists have successfully tried to exploit possible synergistic interactions to achieve a more relievable effect on iron chelation [79]. While DFO is hydrophilic, DFP belongs to the hydrophobic 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 molecule to chelate either 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 divalent metals such as Cu^{2+} and Zn^{2+}. Iron chelators like DFO have an hexadentate arrangement, allowing to bind iron in a 1:1 ratio, and therefore show the highest affinity.

All molecules of the iron chelator family have been in clinical use for decades and have favorable safety characteristics promising for therapeutic HIF-1 signaling modulation. Using an iron-chelation approach for skin rejuvenation with an appropriate monitoring of the progression of the effects on aged skin aims to become a new paradigm in anti-aging medicine. Although numerous studies are suggesting a powerful role for iron chelators in the emerging field of regenerative medicine, thorough basic science studies and a clinical proof of principle is mandatory to investigate a possible application of these molecules as cosmeceuticals.

19.4 Conclusion and Outlook

The possibilities of a therapeutic modulation of hypoxia inducible signaling pathways by repurposing iron chelators are promising. An upregulation of HIF-1 alpha mediated by iron-chelation leads to the correction of age-dependent changes of HIF-1 expression. This directly results in improved cutaneous regeneration, as well as in resistance to exogenous stressors such as radiation and infection. These benefits are mediated through positive effects on all cutaneous cell types, the upregulation of pro-regenerative cytokines, growth factors and peptides, and the recruitment of circulating regenerative cells. However, the impact of such a modulation on skin homeostasis is not fully understood. A thorough investigation of the molecular effects of HIF-1α pathway alteration by iron chelators like DFO or DFP and their influence on human keratinocytes and fibroblasts is warranted. If supported by solid clinical trial data, this approach would have the potential to become a paradigm shift in aesthetic and regenerative medicine.

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