Stem Cells in Dermatology and Anti-aging Care of the Skin

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KEYWORDS

• Stem cells • Homeostasis • Dysregulation • Basal cells

KEY POINTS

- The activity of stem cells is stimulated by the start of tissue dysfunction.
- One important application of stem cell biology is how these cells can be used in the context of aging and age-related dysfunctions.
- A key hallmark to aging is the exhaustion or dysregulation of the endogenous stem cell population, which aids in maintaining tissue homeostasis and repair of injured tissues.

INTRODUCTION

Since the discovery of multipotent stem cells by Till and McCulloch in 1961,[1] further elucidation of stem cells' functions have been identified as both facilitating development of new cells and maintaining homeostasis of current normal cells. The activity of stem cells is stimulated by the start of tissue dysfunction. Several applications using these functions have been implemented in medicine already: reestablishing the hematopoietic lineage via bone marrow transplantation,[2] development of stem-cell based therapy for type 1 diabetes[3,4] and retinitis pigmentosa,[5] and using stem cells to advance the cure for spinal cord injury.[6] One important application of stem cell biology is how these cells can be used in the context of aging and age-related dysfunctions. During aging, DNA accumulates damage, impairing protein homeostasis, cell function and communication, as well as normal organ physiology.[7] Another key hallmark to aging is the exhaustion or dysregulation of the endogenous stem cell population, which aids in maintaining tissue homeostasis and repair of injured tissues. Because aging is so intimately tied to stem cell integrity, one of the major goals of stem cell biology and regenerative medicine is how one can use these cells to reverse aging and the associated dysfunctions that come with it.

Stem cells are undifferentiated or partially differentiated cells that are capable of dividing and generating differentiated and proliferative cells (**Fig. 1**). Stem cells range from pluripotent cells that are found in the inner cell mass of preimplantation blastocysts or isolated from other sources to unipotent progenitors such as fetal tissues, birth-associated tissues, or adult tissues. Several advances have been made to apply the unique traits of this variety of stem cell types. These include establishment of an

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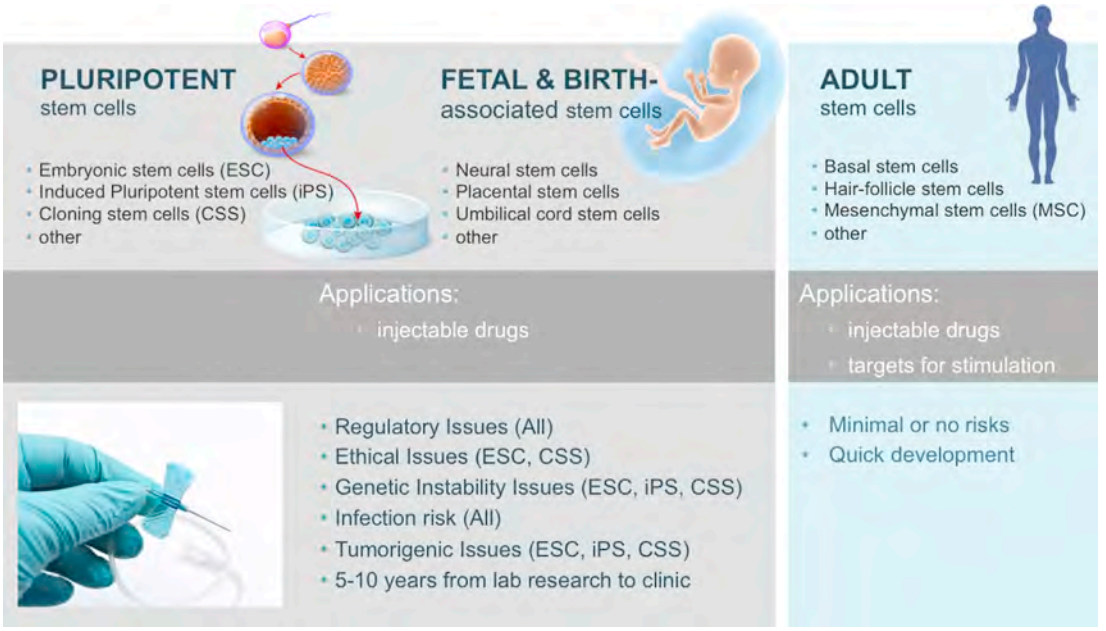

Fig. 1. Types of human stem cells.

embryonic stem cell line via in vitro fertilization, the reprogramming of differentiated adult cells to induced pluripotent stem cells (iPSC), and the generation of cloning stem cells (somatic nuclear transfer stem cells). Other strategies include the creation of parthenogenetic stem cells, isolation of stem cells from fetal tissues (including neural stem cells or retinal progenitor cells), and separation of birth-associated stem cell populations including cord blood stem cells or placental stem cells. Although these different modes of pluripotent and fetal stem cells provide great potential for treating aging and age-related diseases, there are several associated disadvantages. Pluripotent and fetal stem cells may be tumorigenic,[8] possess genetic instability,[9] and are often tied to ethical and regulatory debate.[10] Even though iPSCs bypass the ethical issues of embryonic stem cells, they still possess the same mutations and damage that the donor cells had, which can decrease its ability to proliferate and respond to its respective niche.[11] Stem cells isolated from birth-associated tissues have limited ability to proliferate and limited directions of differentiation, and therefore therapeutic potential of areas of their applications is rudimental.[12] An alternative method that is being explored is the use of pharmaceuticals to modulate endogenous stem cell populations to leverage their respective mechanism of cell signaling and communication. One such example is the use of CBP/Catenin antagonist, ICG-001, that acts more selectively than retinol by shifting the balance of cell division to asymmetric division and thus more differentiation.[13,14] With more alternative methods emerging in regenerative medicine, several other advances that target the individual's stem cells could provide the means for dealing with age-related dysfunctions such as skin aging.

DERMATOLOGIC STEM CELLS

There has been great interest in understanding the regulation and coordination of the stem cells found within the skin in order to repair aged skin (**Fig. 2**). Through wound healing and genetic knock out experiments, several stem cell populations have been elucidated in the skin that have applications to regenerative medicine.[15,16] Within the epidermis lay basal epidermal stem cells that proliferate and maintain epidermal turnover and homeostasis.[15] Other stem cells that are involved in transient repair of skin wounds (although they do not contribute skin's homeostasis on a daily basis) are hair follicle stem cells.[16] These follicular-based stem cells include *Lrig1*+ stem cells (residing in the junctional zone of the hair follicle and contributing to the infundibulum), *Gli1*+ stem cells (maintaining sebaceous glands), and *Lgr6*+ stem cells (acting as skin's master stem cells).[15,17]

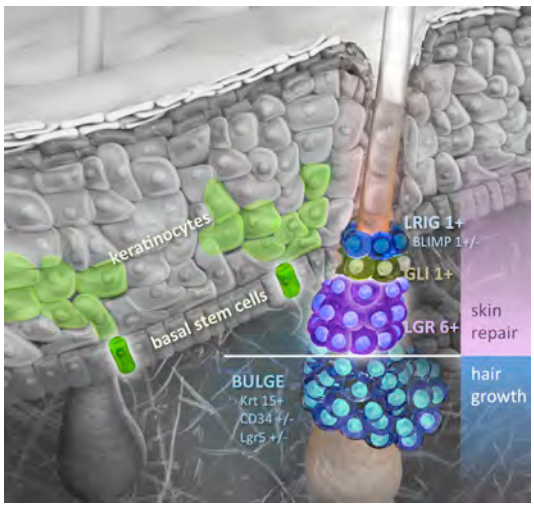

Fig. 2. Dermatologic stem cells. Basal stem cells support epidermal turnover and keratinocyte renewal. Bulge stem cells in hair follicle (Wnt-dependent cells) are primarily responsible for hair growth because of their ability to respond to Wnt, the major hair growth signal. Stem cells located above bulge of hair follicle (Wnt-independent cells) enable epidermis repair in wounds and support other functions than hair growth skin's functions; these cells can not contribute into hair growth because they have no Wnt receptors and therefore unable to respond to hair growth signal. (*From* Taub A, Bucay V, Keller G, et al. Multicenter, double-blind, vehicle-controlled clinical trial of an alpha and beta defensin-containing anti-aging skin care regimen with clinical, histopathologic, immunohistochemical, photographic, and ultrasound evaluation. J Drugs Dermatol 2018;17(4):426–41; with permission.)

The *Lgr6+* cells are termed "master" cells because they are the ones that create the entire epidermis and appendages early in utero.[18] Although these different cell types are compartmentalized in their respective niche, some are able to contribute to different tissues at different times to maintain sebaceous glands and interfollicular epidermis or aid in wound repair.[15] Moreover, these stem cells are less susceptible to damage via aging[19] (because they lay dormant for much of life until signals of distress stimulate them to reproduce), thus garnering interest in further experimentations concerning their regenerative capacity.

Epidermal Stem Cells

Within the *stratum basale* are basal cells that act as the stem cells for epidermal homeostasis. Although further understanding is needed about these cells and how they proliferate, 2 major models are proposed about the mode in which these cells divide. In the epidermal proliferative unit model,[20] a single basal cell acts as the source of self-renewal and proliferation per unit.[20] With this model, the entire epidermis can be considered a collection of epidermal proliferative units each marked by the presence of a single basal cell. On the contrary, the Committed Progenitor model proposes that basal cells divide stochastically, resulting in 2 differentiated daughter cells, more basal cells, or one of each.[21] In order to reconcile these 2 prevailing models, a third model was proposed to integrate aspects of the two. This model argues that the existence of these 2 modes of division allow the maintenance of the epidermis and also prompt wound healing when necessary.[22]

Hair Follicle Stem Cells

An important group of stem cells to the skin is found within the hair follicle. Most stem cells in the follicle express Shh with some, such as *Lgr6+* and *Lrig1+*, exhibiting Sox9 expression.[17,18,23,24] Despite a similar development, different stem cell populations are confined to a discrete section of the hair follicle, and under physiologic conditions, they have a specified regenerative function, whether it is hair cycling or sebaceous gland maintenance.[25] In addition, hair follicle stem cells do not contribute to the epidermis during normal physiologic events,[15] which could be due to minimal cross-talk between compartments as well as a gradient of responsiveness to different molecular cues. On circumstances such as wounding, certain populations are mobilized to help reepithelialize the skin or aid in hair follicle neogenesis.[26,27]

Bulge cells are slow-cycling hair follicle stem cells that are positive for *Keratin 15* (*K15*) expression.[28] Normally, these cells remain quiescent but can engage in cycling of anagen hair follicles. Bulge cells can also upregulate *Lhx2* to temporarily reconstitute interfollicular epidermis after injury.[29] Progeny of these cells provide rapid reepithelialization of the wounded skin that is later replaced by epidermally derived keratinocytes. A second stem cell population in the hair follicle is *Lrig1+* cells that exist above bulge cells in the junctional zone. These cells provide for the maintenance of the infundibulum and also sebaceous gland structures.[30] Like bulge cells, these cells can also engage in wound healing by producing epidermally fated clones. However, unlike bulge stem cells, *Lrig1+* progeny lasts longer in the epidermis and continues to proliferate postwounding.[16] Located above the hair bulge is another

stem cell population of note called *Lgr6*+ cells, which are similar to Lrig1+ stem cells. In contrast to other populations that normally maintain one aspect of dermatologic homeostasis, this population is multipotent and can provide cells to the epidermis or the sebaceous gland in the absence of skin injury.[31] In the event of wounding though, *Lgr6*+ cells also aid in long-lasting repair of the epidermis.

Sebaceous and Sweat Gland Stem Cells

Both the sebaceous and sweat glands are appendages that aid in maintenance of the skin. Sweat glands provide thermoregulation, excretion, and immune function, whereas sebaceous glands lubricate the skin. In the sweat gland are suprabasal progenitors that can create sweat-producing luminal cells.[32] Within the gland are also ductal cells that maintain ductal openings on the skin and can also regenerate the epidermis surrounding the sweat gland after tissue injury. As for sebaceous glands, *Blimp1*+ cells produce sebocyte progenitors.[32]

LGR6+ STEM CELLS: THE SKIN'S MASTER STEM CELLS

As mentioned earlier, *Lgr6*+ cells are multipotent cells found within the hair follicle above the bulge that actively cycle to contribute to the epidermis and sebaceous gland.[33] When investigating the development of these cells, Snippert and colleagues[18] observed that *Lgr6*+ is first expressed embryonically in the early placode (embryonic structures that give rise to structures such as hair follicles and teeth) and remains expressed during hair development. Eventually, expression spreads from the hair follicle to the interfollicular epidermis until it becomes later restricted to the central isthmus above the hair bulge in adult skin. Thus, *Lgr6*+ *cells* are considered primitive epidermal stem cells by establishing the epithelial placode, confirming their multipotency in adult skin.[34] This multipotency was later observed in a transplantation study in nude mice and wounded skin where *Lgr6*+ cells can form all skin lineages.[35] In addition, through lineage tracing in the wound, it was also confirmed that *Lgr6*+ cells aid in long-term wound healing.[15,18]

Lgr6+ cell's involvement in wound healing, as well as its multipotency, are 2 key interests in the study of skin aging and regeneration. It is known that *Lgr6*+ cells can migrate into the wound center to aid in reepithelialization.[34] Activation, migration, and eventual proliferation of these cells are triggered by cytokines that are secreted by neutrophils for pathogen defense.[34] Interestingly, implanted *Lgr6*+ stem cells also aided in hair follicle neogenesis within wounds 10 to 15 days postwounding and genes commonly expressed (such as vascular endothelial growth factor [VEGF], hepatocyte growth factor, and tumor necrosis factor) in wound healing were upregulated.[35] Even with these limitations, *Lgr6*+ cells remain of great interest in their role in establishing various epidermal cell lines postwounding. Elucidation of the development of these cells, how they remain localized in adult hair follicles, and wound-induced

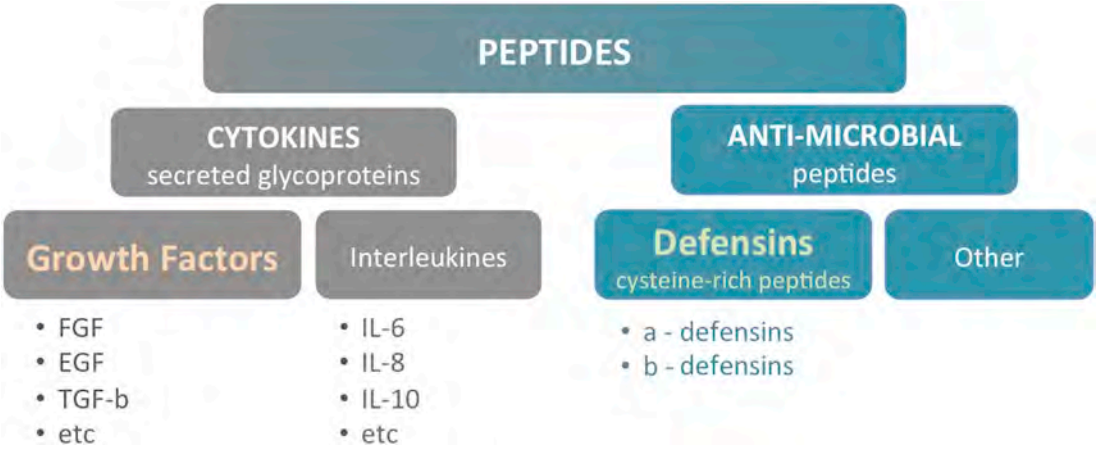

Fig. 3. Defensins are not growth factors. Defensins are a group of antimicrobial peptides that are functionally and structurally different from growth factors. (*From* Taub A, Bucay V, Keller G, et al. Multi-center, double-blind, vehicle-controlled clinical trial of an alpha and beta defensin-containing anti-aging skin care regimen with clinical, histopathologic, immunohistochemical, photographic, and ultrasound evaluation. J Drugs Dermatol 2018;17(4):426–41; with permission.)

recruitment are topics of interest for skin regeneration and aging.

DEFENSINS

Defensins are a group of antimicrobial peptides that are functionally and structurally different from growth factors (**Fig. 3**). β-defensins are peptides secreted by the skin epithelium and are of importance to *Lgr6*+-mediated skin healing. This peptide comes from a family shared with α-defensins that serves multiple functions. First, β-defensins provide innate immunity by deterring microbial colonization on the skin surface.[36] They also enhance tight junctions while bringing into tight junction structures one of their key components, claudin proteins, thereby reducing paracellular permeability of the skin epidermis (the transfer of substances across an epithelium by passing through the intercellular space between the cells) and eventually preventing transepidermal water loss.[37] A third function of β-defensin is to induce wound healing by recruiting *Lgr6*+ stem cells to create new basal stem cells in the wound and thus stimulate the creation of new keratinocytes in the wound bed[34] (**Fig. 4**).

One application proposed and studied for this peptide is the use of intestinal α-defensins on skin to stimulate *Lgr6*+ stem cells. Lough and colleagues[34] found that healing was enhanced in murine skin wounds on induction of α-defensin 5 as observed by rapid wound closure and hair follicle neogenesis within the wound bed. This enhancement of healing was mediated by the recruitment of *Lgr6*+ cells. Because of these results, use of α-defensins would be particularly useful in large-scale wounds or burns where the local stem cell niche is removed and β-defensins are no longer present on the skin surface to induce wound healing.[34] In addition, because *Lgr6*+ is involved in new keratinocyte production, α-defensins could also have applications in reversing skin aging.[34]

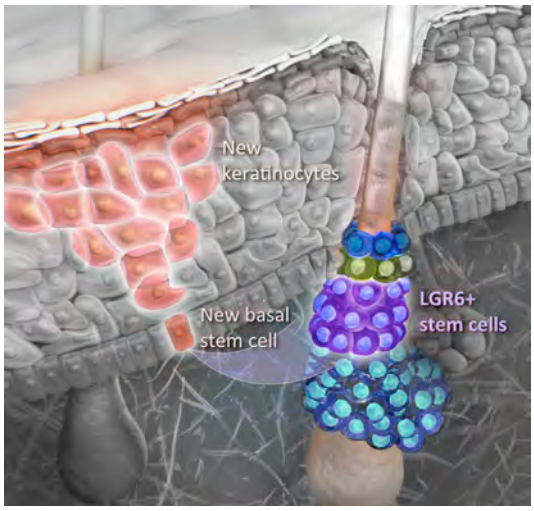

Fig. 4. Activated by defensin peptide dormant *Lgr6*+ stem cells create new basal stem cells and thus stimulate the creation of new keratinocytes. (*From* Taub A, Bucay V, Keller G, et al. Multi-center, double-blind, vehicle-controlled clinical trial of an alpha and beta defensin-containing anti-aging skin care regimen with clinical, histopathologic, immunohistochemical, photographic, and ultrasound evaluation. J Drugs Dermatol 2018;17(4):426–41; with permission.)

AGING AND APPLICATIONS

Aging is considered the decline or deterioration of physiologic functions often attributed to accumulated alterations in the genome, decreased telomere length, protein and cellular damage, increased inflammation and cell senescence, exhaustion of endogenous stem cell populations, and issues with intercellular communication.[7] Although not exhaustive, all of these molecular and cellular mechanisms can work in concert with one another to accelerate the process of aging but also attenuate aging if repaired. Moreover, these proposed molecular characteristics of aging may actually be used by the body as a form of beneficial repair that ultimately becomes detrimental and compromises the integrity of target tissues or organs.[7]

Although not comprehensive, some of the major sources that lead to skin aging include ultraviolet (UV) damage, environmental insults, inflammation, and an increase in reactive oxidative species in comparison to antioxidant.[38,39] Overall, the damage created by these different sources leads to deterioration and damage of the epidermal tissue as well as the loss of collagen and elastin in the dermis.[40] Aging is also considered the cause of a decrease in epidermal thickness and growth factors available in the skin.[40] Although they may seem as distinct events, aging and wound healing have commonalities due to similar genetic and cellular pathways, which compensate and replenish. During the initial phase of wound healing, inflammation arises via reactive oxygen species.[40] In the same manner, skin aging is often associated with the increase in the presence of reactive oxidative species.[40] Although there are several other commonalities between the two, both aging and wound healing involve a departure from fetal skin repair, where skin is scarless and maintains normal collagen integrity.

During fetal wound healing, repair of full-thickness wounds results in the full restoration of the epidermis, dermis, hair follicles, sebaceous glands, and sweat glands without scarring.[41] In contrast, adult wound healing is associated with fibrosis and an altered composition of the skin surface that is marked by dense collagen networks and loss of structures such as hair follicles. The different responses of these 2 processes have been studied, and although more needs to be understood, it seems that fetal wound healing uses different genetic and inflammatory responses along with different intercellular signaling. One example that highlights this change in responses between fetal and adult wound healing is the upregulation of fibroblast growth factor 2 during repair in fetal wounds but not in adult wounds, which inhibits the fibrosis response that is seen in adult wound healing.[42]

The insights from wounding studies demonstrate the gaps observed in adult skin healing and provide mechanisms to recapitulate the same processes seen in fetal skin regeneration. Elucidations of these different mechanisms have potential applications in the reversal and delay in skin aging. One treatment that has been proposed is the use of mesenchymal stem cells in the placenta or umbilical cord.[43] Some of the advantages for the use of these extraembryonic cells are their similarity to embryonic stem cells, multipotency, and higher efficacy in regeneration when compared with adult-derived mesenchymal stem cells. Despite these benefits, there are issues with controlling differentiation direction that these cells will take, and little information is known about how mesenchymal cells participate in fetal wound healing.[42]

Another growing field in terms of skin therapies is the use of growth factors to induce keratinocyte and collagen proliferation. Growth factors are regulatory peptides that participate in cell to cell signaling as well as intracellular signaling such as chemotaxis, division, and differentiation.[44] These proteins can be produced by fibroblasts, platelets, keratinocytes, and immunomodulatory cells. In comparison to other peptides that aid in intercellular signaling, these proteins are defined by possessing a targeted response. This is beneficial during post–skin wounding where these growth factors can diffuse into the wound bed and aid in repair by inducing collagen proliferation, promoting angiogenesis, stimulating cell migration and division, and reducing local inflammation.[45]

The understanding of growth factors in aging skin was elucidated through the studies of skin wound healing.[46] Here, growth factors were found to act in repair by mediating in the inflammatory, granulation, and remodeling stages seen after wounding. In this case, multiple growth factors such as VEGF, transforming growth factor beta (TGF-β), and interleukin 8 coordinate to resolve the wound.[46] One of the main goals seen during this event is for growth factors to reestablish the extracellular matrix and ensure collagen and elastin production is made.[47] With that in mind, the function and mechanism of growth factors in wounds can be translated in its therapeutic use to skin aging where growth factor count is diminished and the aged skin possesses a reduced collagen network. Specifically, growth factors can decelerate aging by stimulating keratinocytes to produce more growth factors that can promote collagen synthesis as well as keratinocyte division.[40]

Although growth factors have been used successfully to treat skin aging,[40] there is still a need to further understand which components are necessary for efficacy and to clarify some controversies over safety. Initial growth factors introduced into cosmeceuticals were derived from plants or plant stem cell sources. For example, kinetin has been shown to be effective in improving the appearance of aging skin due to its natural ability to prevent plant leaves from drying out and withering. Using topical 0.1% kinetin, patients saw a 26% increase in the ability of skin to retain moisture in 24 weeks.[48] Plant (including apple) stem cell extracts is a “soup” of plant-originated proteins, cytokines, and growth factors. This mixture contains undefined molecules with nonspecificity and low efficacy.[49] Because of the lack of specificity, this treatment can activate a wide array of cells, which could be deleterious if unregulated. Moreover, plant stem cells act on the host’s old basal stem cells that may still possess the genetic alterations and insults seen with the accumulative effects of internal aging and photoaging. Since then, other applications of growth factor therapy have been created, such as the use of conditioned medium growth factors.[40] The example of the secretome of cultured mesenchymal stem cells is shown in **Table 1**. Here, there is more efficacy on age reversal or deceleration compared with plant stem cells, but like its predecessor, this strategy contains undefined growth factors that are nonspecific and only target aged cells of the skin.[50] More than a decade ago, when first growth factor products were just introduced to the market it was an authentic revolutionary approach. Cancer research has unearthed concerns relating to the use of undefined compositions of growth factors (conditioned media) for skin care purposes.[51–57]

Table 1
Trophic and immunomodulatory factors secreted by cultured mesenchymal stem cells

Effect	Molecule
Antiapoptotic	VEGF HGF IGF-I Stanniocalcin-1 TGF-b bFGF GM-CSF
Immunomodulatory	PGE-2 TGF-b HGF mpCCL2 IDO iNOS HLA-G5 LIF
Antiscarring	bFGF HGF Adrenomedullin (?)
Supportive	SCF LIF IL-6 M-CSF SDF-1 Angiopoietin-1
Angiogenic	bFGF VEGF PlGF MCP-1 IL-6 Extracellular matrix molecules
Chemoattractant	CCL2 (MCP-1) CCL3 (MIP-1a) CCL4 (MIP-1b) CCL5 (RANTES) CCL7 (MCP-3) CCL20 (MIP-3a) CCL26 (eotaxin-3) CX3CL1 (fractalkine) CXCL5 (ENA-78) CXCL11 (i-TAC) CXCL1 (GROa) CXCL2 (GROb) CXCL8 (IL-8) CCL10 (IP-10) CXCL12 (SDF-1)

Abbreviations: bFGF, basic fibroblast growth factor; ENA-78, epithelial-derived neutrophil-activating peptide 78; GM-CSF, granulocyte-macrophage colony-stimulating factor; HGF, hepatocyte growth factor; HLA-G5, human leukocyte antigen-G; IDO, indoleamine 2,3-deoxygenase; IGF, insulin-like growth factor; IL-6, interleukin 6; iNOS, induced nitric oxide synthase; IP-10, interferon γ-induced protein 10; i-TAC, interferon-inducible T-cell alpha chemoattractant; LIF, leukemia inhibitory factor; MCP-1, monocyte chemoattractant protein-1; MIP-3a, macrophage inflammatory protein; PGE-2, prostaglandin E2; PlGF, placental growth factor; SDF-1, stromal cell-derived factor 1; SCF, stem cell factor.

From Meirelles Lda S, Fontes AM, Covas DT, et al. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. Cytokine Growth Factor Rev 2009;20(5–6):419–27; with permission.

For example, TGF-β is present in most of the conditioned media.[51,52] Multiple cancer research studies show that TGF-β is a very potent trigger of the cancer-related pathways.[53,54,57] For example, TGF-β overproduction, as a driver of the fibrotic process of chronic phases of inflammatory diseases, precedes tumor formation and prepares a favorable microenvironment for cancer cells.[55,56]

Because of these drawbacks, more well-defined growth factors were the next step in skin aging therapy. In comparison to the preceding 2 treatments, there is a defined growth factor that is given for treatment, leading to greater control of application and results.[58] However, there is still nonspecificity involved with using this approach, and again, these growth factors only activate on aged skin cells (**Table 2**).

Although these 3 treatments have been considered for its role in decelerating skin aging, there are still disadvantages involved with their use. The most popular products are derived from the supernatant of cell cultures or the cytoplasmic contents of fetal epithelial cells. These products contain a great many biologically active substances and it is not known which contribute to the desired effect or even an undesired effect, such as tumorigenesis (**Fig. 5**A). Another drawback is the lack of standardization seen in what growth factors and proteins are being made and applied to.[40]

APPLICATIONS OF *LGR6*+ STEM CELLS AND DEFENSINS

A new approach to aid in skin aging could be the use of defensins to activate *Lgr6*+ stem cells (see **Table 1**). Unlike past treatments, defensins would only target *Lgr6*+ cells, as opposed to many potential targets that may not only be helpful but also be deleterious or even tumorigenic in skin tissue (**Fig. 5**B); the investigators were not able to find any publications with respect to involvement of defensins into cancer-related pathways. Moreover, some tissues respond to tumor growth by enhanced expression of defensins as a natural protective immune response.[59,60] Studies also show the ability of defensins to suppress tumor growth both in vitro and in vivo.[60–63] In addition,

Table 2
Generations in stem cell skin care

What Is This?	1 Plant Stem Cells	2 Growth Factors from Conditioned Medium	3 Growth Factors (Defined)	4 Target-specific Stem Cell Activators
Formulation	Undefined: mixture of active molecules	Undefined: mixture of growth factors—everything that cells release during in vitro culture	Defined: predefined composition of growth factors	Defined: predefined composition of natural peptides
Type of activation	Nonspecific: "switch-on of everything" mechanism Low efficacy	Nonspecific: "switch-on of everything" mechanism Contains TGF-β, potent cancer trigger	Nonspecific: "switch-on of everything"-mechanism	Highly specific: peptide (defensins) activate specific stem cells (*Lgr6*+ stem cells)
What it does?	Forces "Old" and "exhausted" skin cells work even harder than before	Forces "Old" and " exhausted" skin cells work even harder than before	Forces "Old" and "exhausted" skin cells work even harder than before	Creates "New" and "fresh" skin cells using resource of our own body

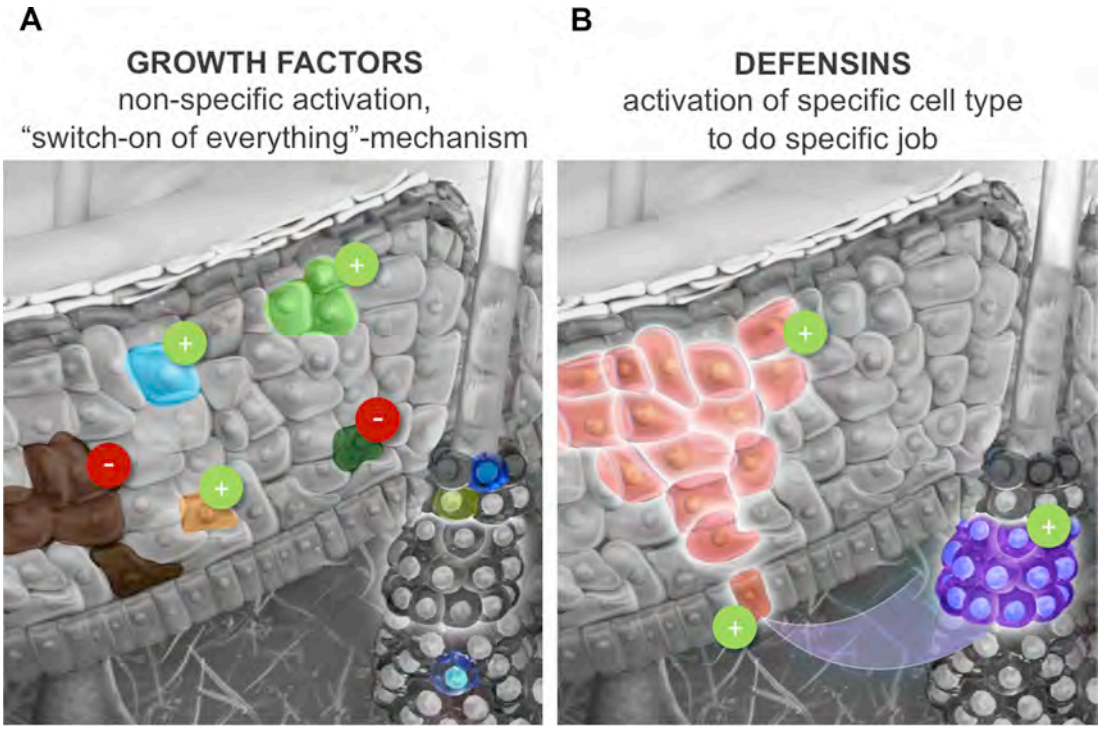

Fig. 5. Nonspecific versus specific targeting. (*A*) Because of the variety of functions, growth factors have an ability to activate and stimulate the different cells in skin including potentially "unstable" tumorigenic cells; therefore, the activation by growth factors is nonspecific and is based on a "switch-on of everything" mechanism. (*B*) Defensins activate only 1 specific cell type in skin, *Lgr6*+ stem cells, thus representing a target-specific activation.

Lgr6+ cells are quiescent when compared with basal stem cells and reside in the isthmus, which is not as directly exposed to UV radiation, thus *Lgr6*+ cells would have accumulated less mutations and damage than basal stem cells.[19] Thus, by activating these cells, there would be differentiation and proliferation of less damaged keratinocytes.

In a 6-week pilot study,[64] it was observed that there was a global improvement in wrinkle reduction and decreased skin oil production in the 22 subjects who used synthetic α-defensin 5– and β-defensin 3–based skin care regimen. To affirm these findings, a placebo-controlled, double-blind study across multiple medical centers was carried out with 45 subjects for 12 weeks. The results of this study followed those from the pilot, suggesting some potential for the use of defensins as a skin therapy.

In a randomized, double-blind, placebo multicenter controlled study of 45 patients, the same defensin-based 3-product skin care regimen was shown to be effective for global signs of skin aging on the face and neck (in press). The full formula regimen caused a significant (P = .027) increased thickness of the epidermis as seen in histology, not seen in the placebo group, with no signs of inflammation (**Fig. 6**). No excessive cell proliferation was detected in either group as measured by Ki67-immunohistochemistry. Reduction in visible pores, superficial wrinkles, oiliness, pigmentation, and improvement of skin evenness were statistically significant (**Fig. 7**). A trend for improvement was also observed in skin elasticity, transepidermal water loss, and hydration; these did not achieve statistical significance. Ultrasound and histopathology demonstrated increases in dermal thickness in individual patients, without statistical significance (**Fig. 8**). Comprehensive improvement in all 5 parameters, including visible pores, hyperpigmentation, superficial and deep wrinkles, and epidermal thickness, was statistically significant when the subset of participants assigned for histology in full formula group was compared with the placebo group participants.

Although further investigation must be done to fully understand the mechanisms behind defensins and skin repair, this therapy provides a new avenue for a more targeted treatment in skin aging.

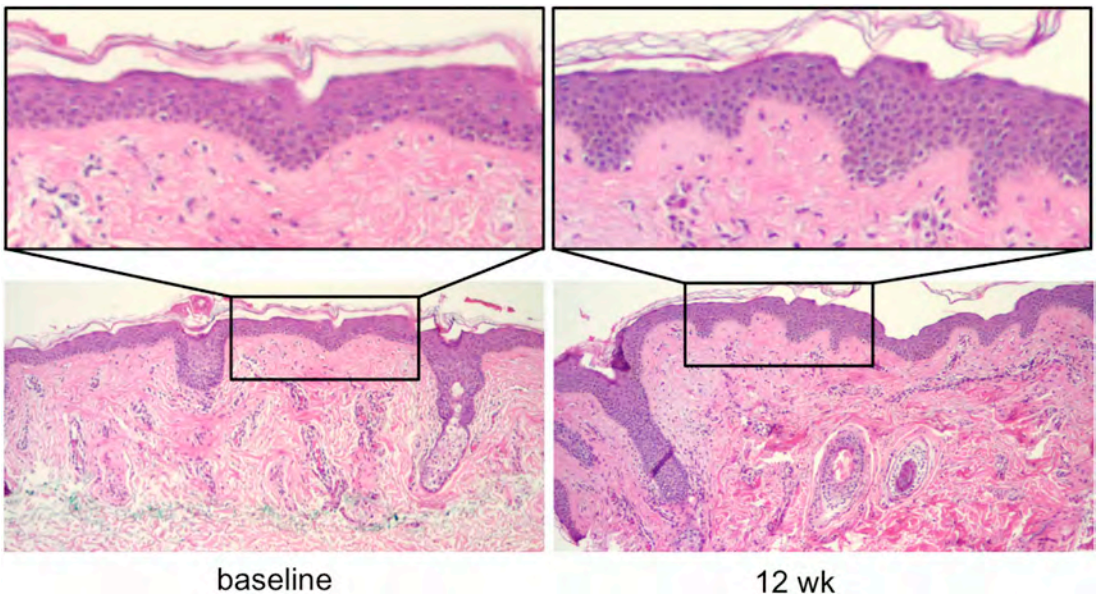

Fig. 6. The defensin-based full formula causes thickening of the epidermis and dermis without visible signs of inflammation. Hematoxylin and eosin staining of skin biopsy samples collected from participants of the full formula group. The images indicate an increased number of keratinocytes in the epidermis and the thickening of the epidermis, observed in all participants of the full formula group assigned for histopathologic evaluation. Photos were taken at 10x magnification. (*From* Taub A, Bucay V, Keller G, et al. Multi-center, double-blind, vehicle-controlled clinical trial of an alpha and beta defensin-containing anti-aging skin care regimen with clinical, histopathologic, immunohistochemical, photographic, and ultrasound evaluation. J Drugs Dermatol 2018;17(4):426–41; with permission.)

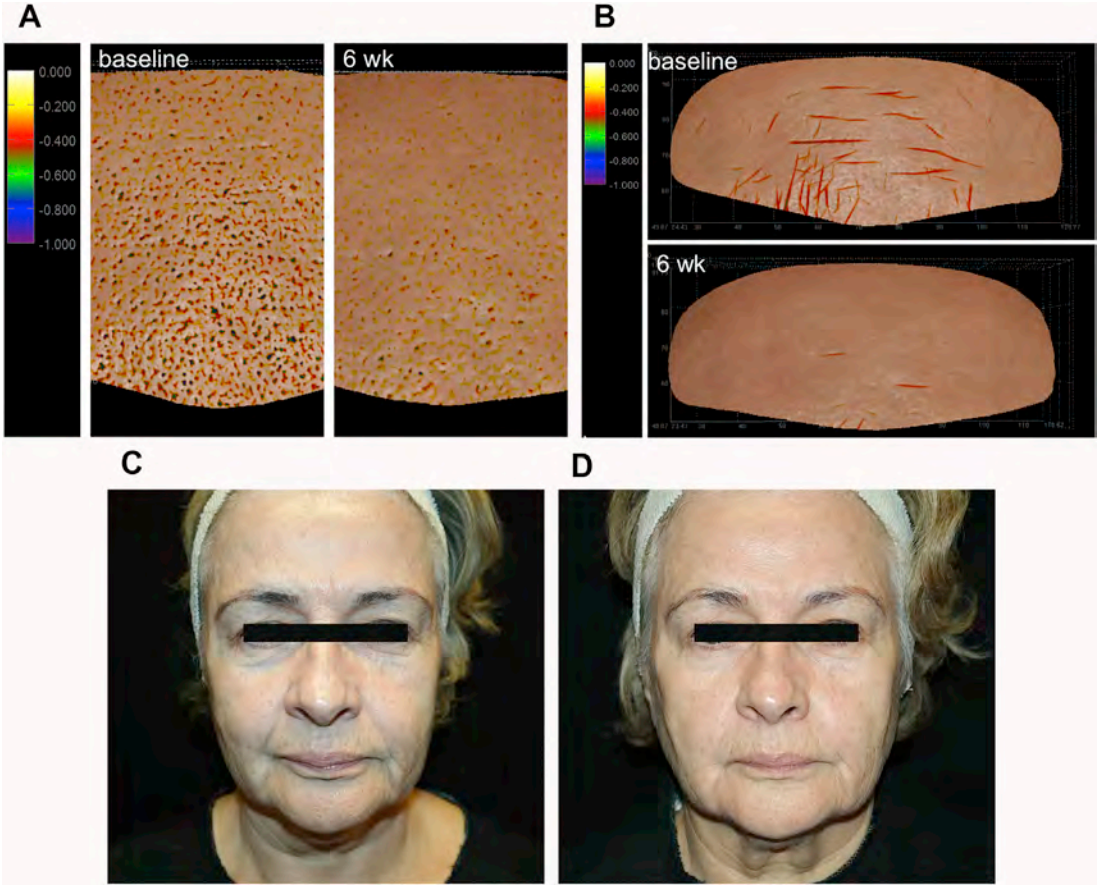

Fig. 7. Comprehensive improvement of the signs of skin aging with the defensin-based full formula. (*A*) Three-dimensional (3D) images of patient's skin (forehead) demonstrating the reduction of visible pores. The deepest areas of pores are shown in blue; the low-profile pores are shown in red-yellow. (*B*) 3D images of participant's skin (forehead). The deepest areas of wrinkles are shown in blue; the low-profile wrinkles are shown in red-yellow. (*C*, *D*) High-resolution photograph of a participant in the full formula group at baseline (*C*) and after 12 weeks (*D*). (*From* [*A, B*] Taub A, Bucay V, Keller G, et al. Multi-center, double-blind, vehicle-controlled clinical trial of an alpha and beta defensin-containing anti-aging skin care regimen with clinical, histopathologic, immunohistochemical, photographic, and ultrasound evaluation. J Drugs Dermatol 2018;17(4):426–41; with permission.)

SUMMARY

Currently, different skin therapies are emerging to treat and reverse the signs of aging. One approach is the utilization of growth factors to activate cell populations in the skin.[40] Initially starting with plant stem cells, to conditioned medium growth factors, and finally to defined growth factors, there is increasing specificity in the growth factors being applied but there are several disadvantages to these 3 treatments. First is the lack of specificity to target cells such that these stem cells and growth factors can activate cells that are not normally involved in skin rejuvenation and be deleterious or tumorigenic. In addition, there are concerns with the efficacy and safety of these treatments as the composition of growth factors are not fully defined and there is a dearth of clinical research to affirm how effective these treatments are. Another aspect to their disadvantage is that all 3 activate aged basal stem cells that have accumulated photodamage, genetic mutations, and epigenetic alterations. By activating these cells, the differentiated keratinocytes will still possess these damages, thus not decelerating aging at an optimal rate.

Nevertheless, new findings demonstrate that certain stem cell populations in the hair follicle can facilitate in wound healing by creating long-term keratinocyte progenitors as well as appendages such as the hair follicle and sebaceous gland. One population of note is *Lgr6*+stem cell located in the hair follicle isthmus. This multipotent stem cells act as skin's master stem cells and in cases where there is wounding or other insults, these

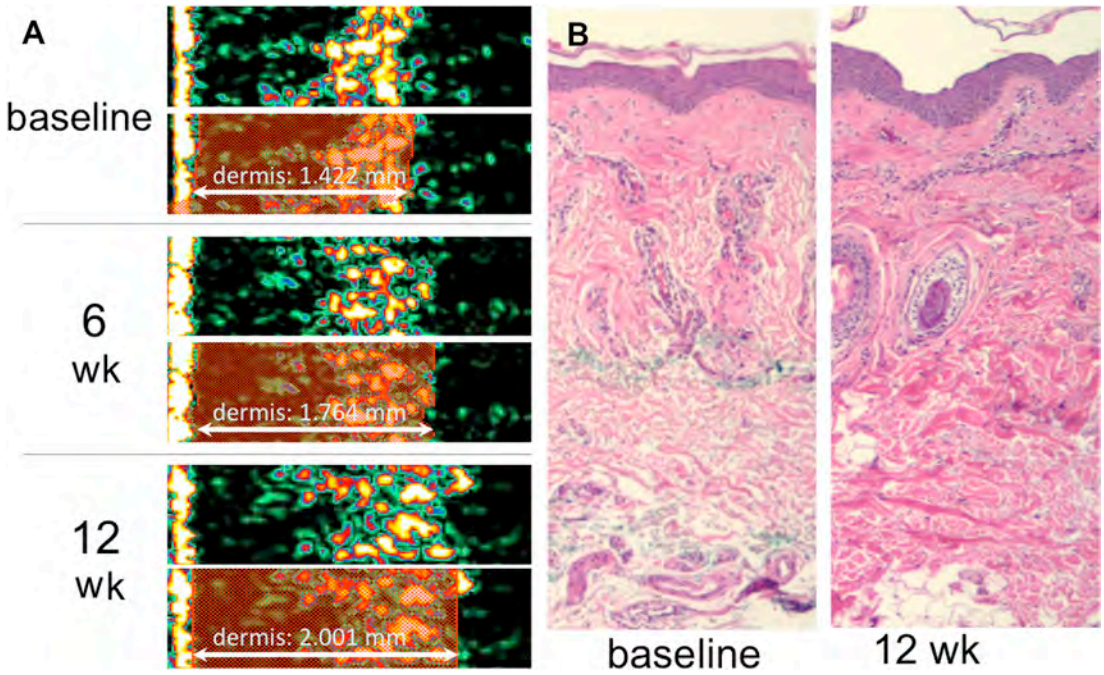

Fig. 8. Participants of the defensin-based full formula group demonstrated increase in dermal thickness. (*A*) High-resolution skin ultrasound demonstrates the increase in dermal thickness in the best participant. Dermis area is highlighted in red. Original US image is shown above the red-highlighted image. (*B*) Hematoxylin and eosin staining of skin biopsy samples showing increased dermal thickness. Photos were taken at 10x magnification. (*From* Taub A, Bucay V, Keller G, et al. Multi-center, double-blind, vehicle-controlled clinical trial of an alpha and beta defensin-containing anti-aging skin care regimen with clinical, histopathologic, immunohistochemical, photographic, and ultrasound evaluation. J Drugs Dermatol 2018;17(4):426–41; with permission.)

cells can proliferate and reprogram to epidermal fates and create new basal stem cells and, eventually, new keratinocytes.[34] In order for *Lgr6*+ cells to migrate into the wound bed, defensins must be present to target and activate these cells. β-defensin peptides are produced by the skin in cases where innate immunity is needed. Not only does it have immunomodulatory qualities but it also can specifically act on *Lgr6*+ cells for migration and proliferation onto the wound bed.[34]

Using this mechanism, further applications can be done in terms of skin aging therapy. Synthetic B-defensin 3 or A-defensin 5 has some advantages over previous growth factor treatments.[34] Each application will have a known composition because only defensins and a vehicle are necessary. Because defensins specifically target *Lgr6*+ cells, there will not be issues of inappropriate activation of other cell types. This approach would also activate a stem cell population that can produce basal stem cells and keratinocytes with less genetic damage and more signaling responsiveness in comparison to the keratinocytes that were derived from aged basal cells. Pilot studies have demonstrated that a composition of defensins, topically applied on intact skin, dramatically improve overall quality of epidermis and comprehensively address the visible signs of aging skin. The observing effect may be caused by defensin-activated repopulation of epidermis with new and "healthy" basal cells following the increase of epidermal mass. Normalized/refreshed epidermis may enhance the performance of dermis renewal and function.

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