**Title**

Advances in Pigmentation Management: A Multipronged Approach.

**Permalink**

https://escholarship.org/uc/item/7v36x54d

**Journal**

Journal of Drugs in Dermatology, 21(11)

**ISSN**

1545-9616

**Authors**

Widgerow, Alan
Wang, Jordan
Ziegler, Mary
et al.

**Publication Date**

2022-11-01

**DOI**

10.36849/jdd.7013

Peer reviewed

# Advances in Pigmentation Management: A Multipronged Approach

Alan D. Widgerow MBBCh MMed FCS FACS,[a,b] Jordan V. Wang MD MBE MBA,[c]
Mary E. Ziegler PhD,[b] Sabrina G. Fabi FAAD FAACS,[a,d] John A. Garruto BS VP,[b]
Deanne Mraz Robinson MD FAAD CMO,[f,g] Michaela Bell BS MBA,[b]

[a]University of California, Irvine, CA
[b]Alastin Skincare, a Galderma company
[c]Laser & Skin Surgery Center of New York, New York, NY
[d]Goldman Butterwick Fitzpatrick Groff & Fabi Cosmetic Laser Dermatology, San Diego, CA
[f]Modern Dermatology, Westport, CT
[g]Yale University of Medicine, New Haven, CT

## ABSTRACT

**Background:** Key cellular players regulating human skin pigmentation include melanocytes in the epidermis that synthesize melanin, neighboring keratinocytes that receive and distribute melanin in the upper layers, and fibroblasts in the dermis that affect overlying melanocytes and keratinocytes. In addition, endocrine factors from the blood supply (endothelial cells) and inflammation-related factors play a role. Thus, new strategies for affecting pigmentation need to consider these multiple cell lines to adequately cover various causes and disease processes associated with hyperpigmentation.

**Methods:** Pathophysiologic mechanisms and cellular pathways involved in melanogenesis were thoroughly reviewed with particular emphasis on the cellular interplay involved in the process. A complex system of interlinking and independent pathways was defined and described demonstrating differing pathways for altered pigmentary disorders – melasma associated with endothelial cell interactions; post inflammatory hyperpigmentation associated with keratinocyte inflammatory mediators (PGE2 in particular); and photodamage involving all 4 cell types. In vitro validation studies were then undertaken to define differing cell group gene expression profiles with selected peptides and other active agents. Melanocytic production of pigment was then tested with these agents to identify key potential players capable of limiting pigmentation.

**Results:** Hexapeptide-12 and lactoferrin (melanocytes), Hexapeptide-11 (in keratinocytes), and phosphatidylserine (endothelial cells) were identified as major inhibitors of melanogenesis based on their gene expression profiles. This was confirmed by secondary melanin production tests performed on melanocytic lines. Additional active agents were also identified as inhibitors of melanocytic production of melanin, and together, these constituents formed the basis for a novel formulation for use in pigmentary disorders.

**Conclusion:** A comprehensive scientific narrative of the various facets relating to pigmentation has been presented including differing pathways affecting varied cell lines that effect pigment production. Based on this concept, actives were tested using gene expression studies as well as in vitro melanogenic model testing in different cell lines. Using this novel multi-faceted approach, we have selected and validated a series of active agents to be used in a formulation targeting the complex problem of hyperpigmentation.

*J Drugs Dermatol.* 2022;21(11):1206-1220. doi:10.36849/JDD.7013

## INTRODUCTION

Skin, hair, and eye color are derived from melanin produced within melanosomes through the process of melanogenesis. The amount of melanin produced depends on genetic, local regulatory, and environmental factors, particularly exposure to ultraviolet (UV) light. Human skin pigmentation may be constitutive or facultative. Facultative pigmentation relates to extrinsic effects on pigmentation, such as UV radiation (UVR), drugs, and hormones, which may lead to abnormal skin hyperpigmentation.[2] In contrast, constitutive pigmentation is predetermined intrinsically by gene status, which directs the size, density, and shape of melanosomes, and these can vary significantly among European, African, and Asian populations.[2] One of the important roles of melanin is to protect the skin, tissues, and genes from UV-induced skin injury.

## MATERIALS AND METHODS

UVA and UVB radiation can result in skin damage with darkening and pigmentation secondary to free oxygen radical formation. These radicals stimulate tyrosine synthesis, which increases the level of melanin. This melanin protects the skin from UVR.
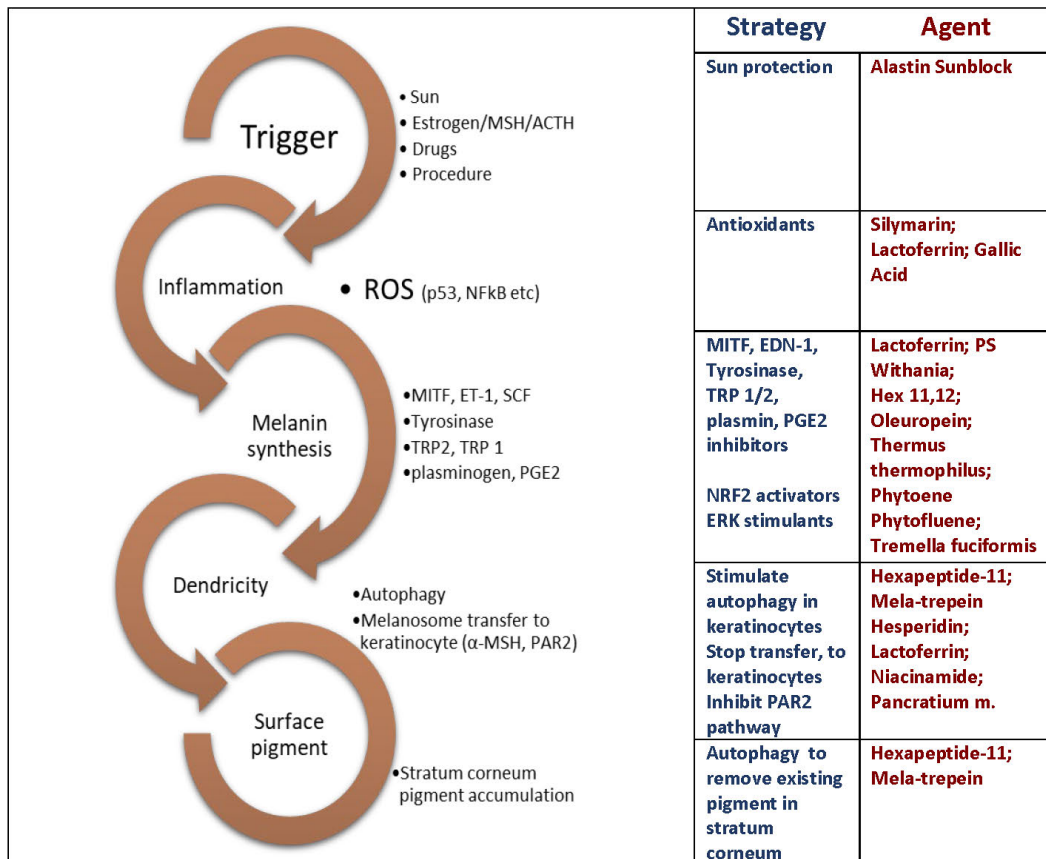
More specifically, melanogenesis begins with tyrosinase (TYR) oxidation of tyrosine (derived from phenylalanine) to dihydroxyphenylalanine (DOPA) and then to dopaquinone.[3] A second enzyme in the melanin synthesis pathway, tyrosinase-related protein 2 (TRP-2), converts dopaquinone to dopachrome and subsequently converts dopachrome to 5,6-dihydroxyindole (DHI) or indole 5,6-quinone 2-carboxylic acid (DHICA). The last enzyme involved in melanin synthesis, tyrosinase-related protein 1 (TRP-1), catalyzes the oxidation of DHICA and forms eumelanins.[3] In human skin, this pigmentation process undergoes multiple steps of conversion to ultimately give rise to yellow-red pheomelanin and black eumelanin.[2] Finally, the melanin is transferred from melanosomes to keratinocytes, where it caps the nucleus in an effort to protect the cell from further UV injury. Melanosomes in melanocytes are intracellularly transported from their origin to the dendritic tips via cytoskeletal components before being transferred to keratinocytes.[2] Each melanocyte typically supplies 36 keratinocytes with melanin, forming the epidermal-melanin unit. Excessive melanin in the skin results in hyperpigmentation, which can contribute clinically to melasma, freckles, and lentigines. Studies regarding the regulation and mechanism of melanogenesis are important to identify targets for the prevention and treatment of pigmentary disorders.

The broad sequence of melanogenesis is as follows (Figure 1):

1. The melanocyte can be activated via cell surface receptors by various stimuli. These include hormones (eg, estrogen, melanocyte-stimulating hormone [MSH], adrenocorticotropic hormone [ACTH]), systemic or local inflammation (eg, procedural, acne), and external environmental insults (eg, UVR producing free radicals and prostaglandins).

**FIGURE 1.** Pigmentation sequences with strategies and relevant active agents. Melanogenesis in the skin is regulated by various factors, among which tyrosinase is a key enzyme. Upon exposure to a trigger, such as UVB radiation, inflammatory responses produce ROS, and keratinocytes potently induce or secrete various cytokines. These peptides and growth factors include αMSH and its receptor, MC1R, EDN1 and its receptor, EDNBR, IL-2, bFGF, SCF, nitric oxide, ACTH, prostaglandins, leukotrienes, and histamine. These various factors through paracrine action on melanocytes can trigger or inhibit melanogenesis. The expression of tyrosinase is induced by the main regulator, MITF. p53 also plays a crucial role in melanogenesis, not only through ultraviolet (UV)-induced pigmentation via ROS, such as $H_2O_2$ that induces p53 via the NF-κB pathway, but also through non-UV-induced pigmentation, such as inflammatory or post-inflammatory hyperpigmentation.[3-5] Throughout these sequences, strategies to impact various steps can be undertaken using active agents listed.



| Strategy | Agent |
|---|---|
| Sun protection | Alastin Sunblock |
| Antioxidants | Silymarin; Lactoferrin; Gallic Acid |
| MITF, EDN-1, Tyrosinase, TRP 1/2, plasmin, PGE2 inhibitors<br><br>NRF2 activators ERK stimulants | Lactoferrin; PS Withania; Hex 11,12; Oleuropein; Thermus thermophilus; Phytoene Phytofluene; Tremella fuciformis |
| Stimulate autophagy in keratinocytes Stop transfer, to keratinocytes Inhibit PAR2 pathway | Hexapeptide-11; Mela-trepein Hesperidin; Lactoferrin; Niacinamide; Pancratium m. |
| Autophagy to remove existing pigment in stratum corneum | Hexapeptide-11; Mela-trepein |

Figure diagram labels:
- Trigger: Sun; Estrogen/MSH/ACTH; Drugs; Procedure
- Inflammation: ROS (p53, NFkB etc)
- Melanin synthesis: MITF, ET-1, SCF; Tyrosinase; TRP2, TRP 1; plasminogen, PGE2
- Dendricity: Autophagy; Melanosome transfer to keratinocyte (α-MSH, PAR2)
- Surface pigment: Stratum corneum pigment accumulation

Abbreviations: ROS: reactive oxygen species; αMSH: α-melanocyte stimulating hormone; MC1R: melanocortin 1 receptor; EDN1: endothelin-1; EDNBR: endothelin B receptor; IL-2: interleukin 2; bFGF: basic fibroblast growth factor; SCF: stem cell factors; ACTH: adrenocorticotropic hormone; MITF: microphthalmia associated transcription factor; PS: Phosphatidylserine

**FIGURE 2.** Extrinsic influences triggering various pigmentation pathways.
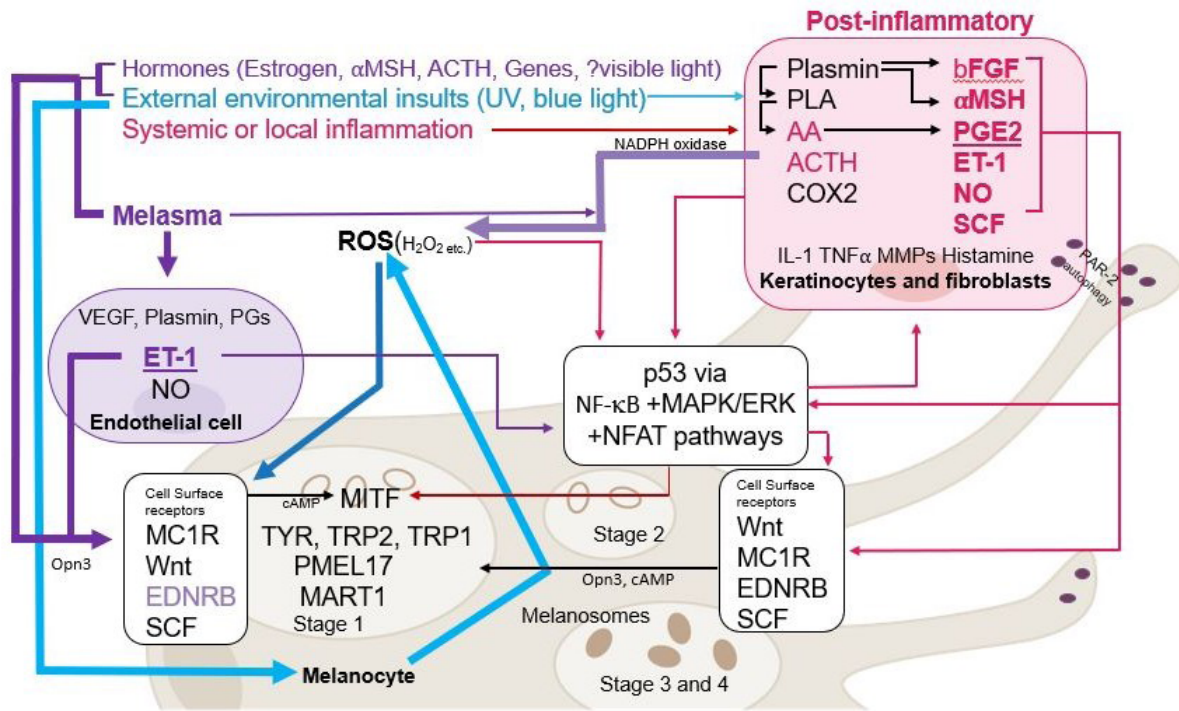


Key:
1. Surface receptors on melanocyte: EDNRB: Endothelin receptor B; MC1R: melanocortin-1 receptor – agonist is αMSH; Wnt pathway; SCF: Stem Cell Factor
2. Cytokines and mediators in Keratinocytes and fibroblasts: PLA: phospholipase A; AA: Arachidonic acid; PGE-2: Prostaglandin E2; bFGF: Basic fibroblast Growth Factor; EDN1: Endothelin-1; αMSH: Alpha Melanocyte Stimulating Hormone; NO: Nitric oxide; Plasmin, COX2, IL-1, Histamine, MMPs, etc.
3. Enzymes and transcription factors in the melanocyte: MITF: microphthalmia associated transcription factor; TYR: tyrosinase; TRP-2: tyrosinase-related protein 2; TRP-1: tyrosinase-related protein 1; cAMP: cyclic adenosine monophosphate

2. These stimuli induce the upregulation of the tyrosinase gene via reactive oxygen species (ROS), p53 and prime regulator microphthalmia-associated transcription factor (MITF), mitogen-activated protein kinase (MAPK) signaling, etc, inducing tyrosinase gene transcription. Activated tyrosine is then capable of converting tyrosine into DOPA, which is subsequently converted into dopaquinone, and then into melanin.
3. Finally, melanin is packaged into melanosomes and transferred via dendrites to keratinocytes.

To effectively reduce melanogenesis, the pathway must be simultaneously blocked at multiple levels. For simplification, the blocking of melanogenesis has traditionally been divided into 4 different steps:
1. reduction of melanocyte activation;
2. inhibition of melanin synthesis;
3. reduction of melanin transfer; and
4. exfoliation of keratinocytes containing melanosomes or autophagy of melanosomes.

During the first 2 steps, a complicated network composed of paracrine and autocrine cytokines secreted by keratinocytes, melanocytes, fibroblasts, and endothelial cells all play important

roles in regulating melanogenesis in tandem with their corresponding receptors, whose expression is also regulated by various cytokines.[4] Current research is focused on depigmenting compounds, which selectively inhibit TYR activity and reduce hyperpigmentation in affected melanocytes without cytotoxic effects to normal melanocytes (as opposed to hydroquinones).[2] More particularly, efforts are directed at the melanogenesis-regulatory enzymes and proteins named above, such as MITF, TYR, TRP-1, TRP-2, extracellular signal-regulated kinase (ERK), N-terminal kinases (JNK), and p38 MAPK.[3,5]

Key cellular players that regulate human skin pigmentation include melanocytes in the epidermis that synthesize the melanin and neighboring keratinocytes that receive and distribute it in the upper layers of the skin. Other intrinsic factors that help regulate skin pigmentation include the fibroblast action in the dermis that affects the overlying melanocytes and keratinocytes, endocrine factors from the blood supply (endothelial cells), as well as neural factors and inflammation-related factors (Figure 2).[6] Thus, new strategies for affecting pigmentation need to consider multiple cell lines (eg, melanocytes, keratinocytes, fibroblasts, endothelial cells) to adequately cover various causes and disease processes associated with hyperpigmentation (Figure 2).

## Cellular Interplay Impacting Melanogenesis

Melanin formation is a protective mechanism initiated by the body to protect cells (primarily keratinocytes) from harmful DNA damage that can occur following extrinsic stimuli, such as UVR. Melanocytes are stimulated to produce melanosomes, which gradually work their way into the dendritic extension of the melanocytes that pass between the keratinocytes. There, they unload their melanin packages, which are distributed as nuclear caps in the keratinocytes to protect these cells from further damage.

Melanin is synthesized within the melanosome and proceeds through four stages (Figure 2). In stage I melanosome vesicles are derived from early endosomal membranes and contain the amyloid protein Pmel17 and MART-1.[7] As the stage I melanosome matures, Pmel17 forms fibrillar striations that characterize stage II melanosomes. The resulting premelanosomes mature to stage III and IV melanosomes after the delivery of melanogenic proteins that stimulate melanin pigment synthesis and deposition onto the Pmel17 striations (stage III melanosome), eventually giving rise to the stage IV melanosome, which appears opaque by electron microscopy.[7] MITF is known to be the master regulator for the transcription of enzymes TYR (the rate-limiting step in melanin synthesis), TYRP1, Pmel17, and MART-1.

Although overlap of pigmentary pathways is inevitable, multiple pathways can be involved in contributing to pigmentation. The most recognized pathway involves common stimuli (eg, hormones, inflammation, UVR) that can induce the formation of ROS, including $H_2O_2$, and inflammatory mediators mainly by keratinocytes and melanocytes. These species interact with surface receptors (eg, Wnt, endothelin receptor type B [EDNRB], stem cell factor, melanocortin 1 receptor [MC1R]) on the melanocyte to initiate the intracellular stimulation of melanin formation by melanosomes, all under the regulation of MITF and subsequent influence of TYR, TRP-2, and TRP-1 enzymes. In addition, ROS can affect the MAPK, p53/nuclear factor kappa B (NF-κB), nuclear factor of activated T-cells (NFAT), protease-activated receptor (PAR-2) pathways to directly impact MITF, and regulation of the melanin formation cascade.

An additional receptor expressed on keratinocytes that seems to be closely involved with melanosome transfer is PAR-2, which mediates the phagocytosis of melanosomes.[6] Keratinocytes and fibroblasts secrete a large amount of a cytokine, called stem cell factor, which plays a critical role in regulating the life cycle of human melanocytes.[8] PAR-2 and endothelin 1 (EDN1) are reported to be involved in the expression of stem cell factor.[9] The interaction of EDN1 with its receptor EDNRB is one of the key paracrine connections between keratinocytes and melanocytes and was first isolated from vascular endothelial cells. EDN1 was reported to induce melanogenesis in human melanocytes.[9] Wnt signaling also plays an important role in melanocyte development. Wnt receptor activation enhances MITF expression, which stimulates melanogenesis.[9] Nitric oxide is one of the paracrine factors produced by keratinocytes in response to UVB irradiation inducing increased MITF expression and melanogenesis.

Thus, it is apparent that specific pathways and cell lines may be relevant to certain pigmentary disorders, and although there is much overlap in general pathways, post-inflammatory pigmentation and melasma are associated with unique cellular and signaling mechanisms when it comes to pigment stimulation (Figure 2).

## Nuances Related to Hyperpigmentary Conditions

Post-inflammatory hyperpigmentation (PIH) is a condition in which an injury or inflammation to the skin causes increased pigment production. There are 2 clinical forms of PIH: epidermal and dermal. Epidermal hyperpigmentation tends to be light to dark brown, whereas dermal hyperpigmentation tends to have a blue-gray coloration. Inflammation starts in the epidermis and manifests as pigmented keratinocytes superficially, but as the process continues, the dermoepidermal junction may be affected, encouraging deeper dyspigmentation. Epidermal UV-protective melanin can enter the dermis via damaged basal cells and is phagocytized by macrophages, forming dermal melanophages.[10] Epidermal PIH usually disappears spontaneously within months or years, whereas dermal PIH has a longer course and may be permanent. Intense, repetitive inflammation tends to produce a long-lasting PIH with a dark color.[10]

In extrinsic aging, exposure of skin to both UVA and UVB radiation causes a rapid increase in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which then activates signaling pathways in keratinocytes in the epidermis and fibroblasts in the dermis, leading to the activation of inflammatory genes. It has been proposed that NADPH oxidase induces $H_2O_2$, which subsequently activates p53 expression via NF-κB signaling pathway. It may also involve several inflammatory mediators, such as interleukin-1α, ET-1, arachidonic acid, prostaglandins, and histamine, stimulating melanogenesis. In a similar manner, PIH involves an inflammatory response within keratinocytes. The epidermal inflammatory response (eg, dermatitis) results in the release and oxidation of arachidonic acid to prostaglandins (eg, prostaglandin E2 [PGE-2]), leukotrienes, and other products. Levels of PGE-2, a hormone-like mediator, increase quickly when skin is exposed to UVR and inflammatory stimuli, and this smoldering inflammation causes continuous damage to the dermal matrix.[11] There are 3 main signaling pathways stimulated by UVR and ROS that are responsible for the activation of many inflammatory genes and also those involved in skin aging. These are the MAP kinase pathway (and related kinases, MAPK, ERK), the NF-κB pathway, and the NFAT pathway.[11] NF-κB

1210

A.D. Widgerow, J.V. Wang, M.E. Ziegler, et al

activation, via basic fibroblast growth factor (bFGF), is involved in melanocyte proliferation.[12] Several depigmenting agents work by preventing the development of such mediators from the inflammation-related pathway. In addition, recent findings point to the dysregulation of nuclear factor erythroid 2-related factor 2 (NRF2) signaling being involved in the pathogenesis of UV-induced skin pigmentation.[13]

MSH and PGE-2 stimulate melanogenesis in melanocytes.[11] PGE-2 in photodamaged, inflamed, and chronologically aged skin is produced by keratinocytes, fibroblasts, and infiltrating immune cells. The cytokines and PGE-2 produced by these cells act through both paracrine and autocrine events and use multiple signaling pathways to further increase PGE-2 production. Increases in PGE-2 production are the result of UVR, ROS, and inflammatory cytokines, which increase the gene expression and activity of cyclooxygenase-2 (COX-2), PGE synthases, and phospholipase A2. Although there are several UVR-induced hormone-like factors, including MSH, ACTH, and EDN1, that can stimulate melanin production in melanocytes, PGE-2 is one of the most potent stimulators of pigmentation in human melanocytes.[14] Therefore, the increase in the synthesis of PGE-2 and its secretion from keratinocytes and fibroblasts as a result of UVR or inflammation plays a significant role in the formation of solar lentigines and inflammatory hyperpigmentation. PGE-2 not only increases melanin production, but it also increases dendrite formation and enhances melanosome transfer from melanocytes to keratinocytes. Additionally, UVR activates PAR-2 on keratinocytes, and this activation results in 2 events: First, there is increased keratinocyte internalization of melanosomes, and as the keratinocytes reach the surface, the skin becomes visibly pigmented. Second, PAR-2 stimulates PGE-2 production in keratinocytes, thereby further enhancing melanogenesis.[11] The direct involvement of PAR-2 receptor in controlling melanogenesis was demonstrated in a 3D human explant model; therefore, targeting PAR-2 is a novel approach to the treatment of pigmentary disorders.[9]

### Melasma

Melasma is a common acquired hyperpigmentation found predominantly in females with Fitzpatrick skin types III to IV. It usually presents as tan to brownish patches with an irregular border on sun-exposed areas of the face, especially the forehead, malar areas, and perioral area; however, it may also be found on the neck and arms. UVR induces melanocyte proliferation and migration and can lead to the production of multiple cytokines, including interleukin 1 (IL-1), EDN1, α-MSH, and ACTH from keratinocytes, which in turn upregulate melanogenesis.[15] Melasma is not usually associated with inflammation.[15] Risk factors include genetic predisposition, exposure to UV light, pregnancy, and exogenous hormones (eg, oral contraceptives and hormone replacement therapy).

Sanchez et al. described two basic patterns: an epidermal form with melanin deposition mainly in the basal and suprabasal layers with melanocytes highly dendritic and full of pigment, and a dermal form with superficial and deep perivascular melanophages in the dermis with noticeably less prominent epidermal pigmentation.[16] These 2 patterns may clinically overlap. Melanocytes in melasma skin had more dendrites, mitochondria, golgi, and rough endoplasmic reticulum, suggesting that they were more biologically active than their counterparts in normal skin.[16]

Although melasma is characterized by epidermal pigmentation, dermal extracellular matrix (ECM) abnormalities with basement membrane disruption are commonly observed.[17] Solar elastosis, an abnormal collection of fragmented elastic tissue, is commonly seen in the dermis of 83% to 93% of melasma patients.[17] Basement membrane disruption allows for the descent of melanocytes and melanin into the dermis, appearing as free melanin or melanophages. As a result of this disruption, melasma is difficult to treat with high recurrence rates, and restoration of the basement membrane is essential to long-term management of melasma.[17] Mast cells are more frequently observed in melasma skin than in non-lesional skin, especially in the dermal elastotic areas, likely related to repetitive UVR exposure. Mast cell tryptase activates pro-matrix metalloproteinases-9 (MMP-9) and degrades type IV collagen, which could weaken the basement membrane in melasma skin.[17] In addition, PGE-2 levels in the skin gradually increase with age, and this ongoing inflammation causes continuous damage to the dermal matrix.[11] Thus, even though topical treatment can decrease melanogenesis, it still needs to be combined with an anti-aging approach, such as topical tretinoin, a proprietary topical combining tripeptide and hexapeptide (TriHexTechnology, Alastin), or laser or light therapy to decrease the aging effects that accompany melasma.[18]

In addition to keratinocytes and fibroblasts, growing evidence also implicates endothelial cells in pigmentation. Indeed, melanocytes express receptors that can potentially be regulated by several factors secreted by endothelial cells, such as vascular endothelial growth factor (VEGF), EDN1, nitric oxide, and leukotrienes. In addition, histologic studies have clearly shown a significant increase in vascularization within melasma lesions compared to surrounding skin.[19] Clinical reports have detailed acquired telangiectasias with hyperpigmented macules suggesting a close relationship between melanocytes and endothelial cells possibly related to released endothelin.[19] The association of pigmentation with vascularization involves EDNRB. In a co-culture model of endothelial cells and melanocytes, EDN1 released by endothelial cells induced increased melanogenesis signaling in melanocytes (MAPK and p38), characterized by MITF stimulation and increased TYR and DOTA levels.[19,20] In addition, EDN1 also increased melanosome formation and transport in melanocytes and

increased melanin transfer to keratinocytes, while inhibition of EDN1 function substantially depressed these effects.[19,21] Finally, culturing reconstructed skin with microvascular endothelial cells led to increased skin pigmentation that was prevented by inhibiting EDNRB.[19] This confirms the role of vascularization in skin pigmentation, a finding that could open new fields of research for treating pigmentary disorders, such as melasma.[19] Developing topical agents to inhibit ET-1 or ENDRB activation on melanocytes may limit the impact of the vascular endothelial cells and prove an important addition to classic depigmenting agents to treat melasma and prevent relapse.[19,21] In addition, tranexamic acid, an anti-fibrinolytic plasmin inhibitor used to prevent bleeding, has been used in melasma treatment. The combined use of this agent topically and orally for 8 weeks decreased hyperpigmentation in melasma lesions, and histologic examinations confirmed a decrease in melanin content and vascularization.[19] These pilot studies underline the potential interest in targeting the vascular component when treating melasma. However, using high-powered laser approaches with significant thermal side effects to remove the vessels may promote PIH, especially in darker skin types. Thus, endothelial cells play a role, through the secretion of endothelin, in upregulating key gene regulators of melanogenesis, including MITF, tyrosinase, and dopachrome tautomerase, without any UV stimulation. However, endothelial cell activation of melanogenesis might be even stronger after UVR.[19] Ultraviolet radiation is considered the main causative factor of relapses in melasma, and strict avoidance of sun exposure is recommended. Despite the use of effective sunscreens, many patients have relapses of the hyperpigmented lesions after the summer period. Recently, it was demonstrated that visible light induced an increase of skin pigmentation, at least in dark skin types. In dark skin patients (Fitzpatrick skin types IV to VI), both UVA and visible light increased pigmentation, but the pigmentation was more intense and stable after visible light, possibly blue light exposure as compared to UVA.[22,23] It appears that visible light acts directly on melanocytes to stimulate melanogenesis and that opsin 3 (light-activated receptors that normally mediate photoreception in the eye) serves as the sensor for blue light in melanocytes.[22] One set of investigators reported that after stimulation of opsin 3 by blue light, a calcium flux activates calmodulin-dependent protein kinase II (CaMKII), then CREB, ERK, and p38, leading to the phosphorylation of MITF, increased tyrosinase, and dopachrome tautomerase, and finally the increase of melanin in cells.[22] In stark contrast, more recently, other authors have shown that in melanocytes, opsin 3 acts as a negative regulator of melanin production by modulating MC1R signaling, and they did not detect opsin 3 sensitivity to blue light by either spectrophotometric analysis or monitoring Ca2+ levels in melanocytes.[23] Opsin 3 regulates melanin by decreasing the levels of cyclic adenosine monophosphate produced by MC1R. The light-independent function of opsin 3 in regulating melanin levels in human melanocytes with opsin 3 is suggested to yield

a novel therapeutic target for skin pigmentation disorders, such as melasma.[23] Either way it appears that opsin 3 may be a novel regulator of pigmentation, although its exact mode of action still needs defining. So far, only physical shield (mainly iron oxide) can be used to protect skin from visible light irradiation. Thus, the use of tinted mineral sunscreens could protect against both UV and visible light and may be more effective for preventing melasma relapses.[24]

*Autophagy*
Recent studies show that the autophagy system might also be involved in the initial stages of melanosome formation and its degradation in keratinocytes.[25] This process varies depending on ethnicity and skin color.[26] For instance, decreased pigmentation is accompanied by significantly higher autophagic activity in the keratinocytes of White skin compared to those in Black skin. The inhibition or activation of autophagy in darkened or lightened skin color, respectively, occurs via regulating melanosome degradation in keratinocytes.[26] Ordinary epidermal melanosome turnover with desquamation takes about 4 weeks. Thus, it has been suggested that the regulation of autophagic processes could more efficiently and rapidly lighten skin by focusing on the existing melanosomes in the keratinocytes rather than targeting melanin synthesis or transfer.[26] Additionally, several regulators of autophagy, including genes that regulate the initial stages of melanosome formation (eg, beclin 1) and genes that regulate the turnover of autophagic vesicles (eg, WIPI1), were identified as potent regulators of melanogenesis.[13] These studies provide compelling evidence that autophagy is involved in the regulation of melanin. Similarly, autophagy likely plays a role in the selective removal of defective melanosomes.[7]

Targeting the autophagy mechanism is considered an important approach, because autophagy can degrade already formed melanin, whereas targeting other melanogenesis proteins only prevents de novo melanin formation.[9] Therefore, the autophagy-mediated depigmentation process may be considered an important approach for treating various skin problems.[9]

Finally, air pollution, particularly polycyclic aromatic hydrocarbons, have been implicated in the development of melasma. These chemicals are thought to enter the skin via nanoparticles and generate quinones that produce ROS.[27] This is particularly relevant to persons of Fitzpatrick skin types III to VI living in India and South East Asia, which are also geographic regions with very heavy pollution. India, South East Asia, China, and United States lead the world in air pollution.[27]

**Strategies for Managing Pigmentation in All Its Forms**
Delineation of the pigmentation pathways from different causes presents opportunities for therapies to target these unique sequences. In addition, the transfer of melanosomes to keratinocytes and the effect on this function may also

be targeted. Thus, a comprehensive strategic approach is presented (Figure 1). Prior to active ingredient selection, two comprehensive in vitro studies were completed for validation of component selection.

**Validation Studies**

*RNA sequencing (RNA-seq)*

As detailed above, 4 cell types are intimately associated with pigmentation pathways: melanocytes, keratinocytes, fibroblasts, and endothelial cells. Thus, in vitro studies examining these pathways should include all these cell types to make better predictions of the in vivo response. Here, we sought to evaluate the response of these cells to various compounds related to pigmentation. First, to simulate the UV-induced pigmentation pathway process, melanocytes were pre-treated with PGE-2 (10 µM), a normal direct consequence of UVR usually emanating from exposed keratinocytes, for 48 hours. In addition, primary human adult dermal fibroblasts and keratinocytes, and human umbilical vein endothelial cells (HUVEC) were cultured. After 48 hours of culture and/or PGE-2 treatment, all 4 cell types were treated with the following test compounds:

1. Lactoferrin
2. TCVRRAF peptide (Lf derivative)
3. Tri-peptide-1
4. Hexapeptide-12
5. TriHex combination
6. Hexapeptide-11
7. Tranexamic acid 5%
8. Octapeptide (proprietary)
9. Phosphatidylserine
10. Cannabidiol (CBD)
11. Entire formulation
12. No treatment control

After 24 hours of treatment, RNA lysates were prepared. The samples were shipped to MedGenome (Foster City, CA) for RNA extraction, library construction, and sequencing to 25M paired-end 100bp reads per sample. Library prep and sequencing were completed at MedGenome.

Out of the 4 cell lines, it was possible to identify 'hero' actives with significant melanogenesis activity in 3 cell lines: melanocytes, keratinocytes, and HUVEC. The fibroblasts showed activation of many alternate functions (predominantly wound healing), but unsurprisingly, little relation to melanogenesis activity.

Thus, using bioinformatics, stand-out performers were identified within the general melanocytic activity pathways; Figure 3 demonstrates the dominance of Hexapeptide-12 and lactoferrin in melanogenic activity, and Figures 4 and 5 demonstrate the downregulation of melanogenic genes after treatment with these two compounds. The *POMC* gene provides instructions for making a large protein called proopiomelanocortin (POMC), which is cut (cleaved) into smaller peptides with different functions, including ACTH, aMSH, etc.

**FIGURE 3.** Gene expression studies in melanocyte cell line demonstrating significant downregulation of melanogenesis pathways by hexapeptide-12 and lactoferrin among several agents tested.

A.D. Widgerow, J.V. Wang, M.E. Ziegler, et al
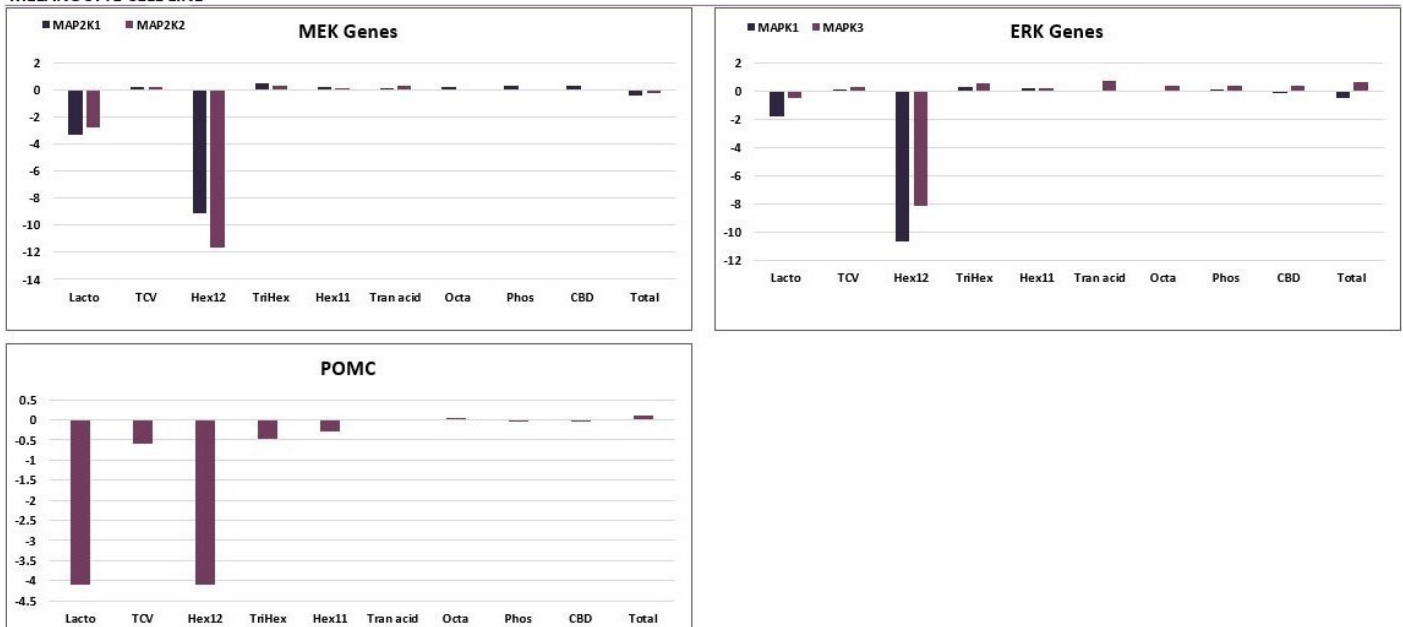
Similarly, after the treatments, the keratinocytes revealed that Hexapeptide-11 was the most active regarding melanogenesis activity (Figure 6), while Figure 7 demonstrates the downregulation of a variety of important genes in the keratinocytes after treatment with Hexapeptide-11.[28] The downregulated genes highlighted in Figure 7 nearly perfectly echo those described by Fu et al. in an investigation of keratinocyte inflammatory mediator elaboration and the effect on melanocytes and pigmentation.[28]

For the HUVEC, we searched for agents active against EDN1 and SCF (Figure 8), revealing phosphatidylserine as the most active agent. Figure 9 demonstrates the potent activity of phosphatidylserine on EDN1 and other melanogenic genes.

Thus, cumulatively, the cells tested revealed that melanogenic gene activity was most affected by lactoferrin, Hexapeptide-11, Hexapeptide-12, and phosphatidylserine, which served as our first candidates for inclusion in a formulation. Next, a melanocyte pigmentation model was designed to test the agents and demonstrate whether they had a direct effect on the melanocyte cell and melanin production.

**Melanocyte pigmentation model: Melanin comparative analysis via absorbance readings**

This model aimed to analyze melanin production from compound-treated melanocyte samples via absorbance. Melanocytes were cultured. Once confluent, they were stimulated with 2 concentrations of each of the following compounds for 4 days:

1. Lactoferrin (1000 ug/mL)
2. Hexapeptide-12 (4.9 ug/mL)
3. Hexapeptide-11 (200 ug/mL)
4. Phosphatidylserine (500 ug/mL)
5. Silymarin 0.7% (20 ug/mL)
6. Sesamol (100 ug/mL)
7. Tranexamic acid (1000 ug/mL)
8. Phytoene, phytofluene 5%
9. MSH (10mM)

Stimulation with MSH was used as a positive control to validate the absorbance reading. Figure 10 demonstrates results from 3 absorption wavelengths at 2 concentrations of the selected compounds (only one for MSH). The findings confirmed the efficacy of the selected agents for reducing melanogenesis (percent decrease absorption denoting decreased melanin formation) in melanocytes. Once again, regulation of melanogenesis was confirmed for the 4 agents identified in study 1 (lactoferrin, Hexapeptide-11, Hexapeptide-12, and phosphatidylserine). In addition, 4 more candidates were selected (concentrations in green) to add to the formulation.

## DISCUSSION

The delineation of the melanogenic pathways related to different pigmentation problems described above provides a framework for tackling this complex problem. In addition, validation studies led to the selection of targeted active agents based on RNA-sequencing studies and an in vitro melanogenic model analysis. Overall, these studies allow us to select agents in a cell-specific manner (eg, melanocyte, keratinocyte, and endothelial cell) for their effect on melanogenic function and effectively target a reduction of melanocyte synthesis and activation through antioxidant activity and enzyme/pathway regulation. This is then followed by targeted approaches to reduce melanin transfer and autophagic stimulation and exfoliation of existing melanosomes.

Hydroquinone ($C_6H_6O_2$) (HQ) has intracellular cytotoxic effects that reduce pigmentation levels by non-selectively destroying the epidermal melanocytes and keratinocytes. Due to the aggressive mode of action of HQ in reducing the level of pigmentation, it has been associated with various significant side effects, such as ochronosis, and animal studies even suggest possible skin cancer connections.[2] Recently, HQ products have been banned in many countries, but in some, HQ can be used for medical purposes under physician guidance. Although HQ is a powerful agent and may be efficacious in dealing with hyperpigmentation in some patients, the potential for adverse events and its limitation in being able to be used long term without a break, necessitate the quest to find a less toxic alternative that can be used long term and is efficacious in that long-term period to avoid the potential rebound that short-term HQ use may induce.

Thus, concentrating on the steps outlined in Figure 1, the following agents are included in these categories:
**Antioxidants, MITF, Tyrosinase, TRP-1/2, plasmin PGE2, EDN1 inhibitors, and ERK stimulants**

*Lactoferrin*
Lactoferrin (Lf) is an 80 kDa iron-binding glycoprotein of the transferrin family that is found in exocrine secretions (eg, tears, saliva, milk, nasal and bronchial secretions, and gastrointestinal fluids). The effects of Lf range from antimicrobial to anti-inflammatory, antioxidant, and immune-modulating activities with high iron-binding affinity.[29] Lactoferrin downregulates TNFα and other cytokine production (IL-1) by local skin cells and is useful for bruising resolution and the prevention of post-inflammatory pigmentation.[30,31] Lactoferrin is also demonstrated to positively affect wound healing.[32] Concerning pigmentation, studies on bovine Lf at 5ppm in a 3D skin model demonstrate reduced melanin production by about 80% compared to control, which suggests that Lf is transdermally absorbed and suppresses

melanin production. Lf interacts with both keratinocytes and melanocytes, thereby interfering with melanosome transfer from melanocytes to keratinocytes in the 3D skin model.[30] Treatment of human melanoma cells with Lf results in significant dose-dependent suppression of melanin production.[30] The key feature behind the anti-melanogenic effect of Lf appears to be the downregulation of MITF, leading to decreased tyrosinase activity.[29] This occurs through the phosphorylation of ERK, which leads to the phosphorylation and degradation of MITF, suppressing melanin production in melanocytes—thus acting as a skin-lightening agent.[29] Lf at 1ppm and 10 ppm achieved 20% inhibition, which was almost identical to the inhibitory activity exhibited by tranexamic acid.[30] Lf also blocks plasminogen activation on the cell surface by directly binding to human plasminogen, which results in similar suppression of PGE-2 and angiogenesis described above for tranexamic acid.[33,34] In addition, recent findings point to the dysregulation of NRF2 signaling being involved in the pathogenesis of UV-induced skin pigmentation, and pharmacologic activation of NRF2 may represent a potential therapeutic target in photoaging.[13] Lf is a significant activator of NRF2.[35]

### Tranexamic Acid

Tranexamic acid is a plasmin inhibitor used to prevent fibrinolysis and reduce blood loss. Its use in melasma treatment was described for the first time by Nijor in 1979 in Japan. It is a synthetic derivative of lysine and exerts its effect by reversibly blocking the lysine binding sites on the plasminogen molecule to prevent plasminogen from binding to basal keratinocytes; thereby inhibiting the conversion of plasminogen to plasmin and thus decreasing the production of prostaglandins (particularly PGE-2 via arachidonic acid, PLA).[34,36-38] Moreover, the release of arachidonic acid is increased by plasmin in endothelial cells. Increased plasmin itself elevates α-MSH, which activates melanin synthesis in melanocytes. Plasmin also increases the release of bFGF, which is a potent melanocyte growth factor. All of these processes result in increased melanin production in the skin. Apart from the effect on melanocytes, plasmin also plays an important role in angiogenesis, by suppressing angiogenesis and inhibiting bFGF-induced neovascularization.[38] In addition, tranexamic acid is similar to tyrosine in its structure, which means that it can competitively inhibit the enzymatic activity of tyrosinase.[38] A clinical study using a topical facial serum containing 3% tranexamic acid, 1% kojic acid, and 5% niacinamide, was shown to be a safe and effective treatment for melasma, hyperpigmentation, and PIH.[37] Another study demonstrated equivalency when comparing a topical 5% tranexamic acid solution to HQ 3%.[39] Finally, tranexamic acid is shown to improve the production of autophagy-related proteins and the formation of autophagosomes; thus inhibiting melanogenesis by activating the autophagy system.[25]

### Phytoene, Phytofluene

Although much research has been conducted on the UVR-absorbing activity of the colorless carotenoids phytoene and phytofluene,[40-42] we are the first to report the potent activity of these compounds in reducing melanogenesis in melanocytes. This finding is not surprising as previous studies have demonstrated that this carotenoid treatment led to a 47% decrease in PGE-2 levels in cells compared to control.[43] As noted above, PGE-2 is a major instigator in inflammatory-primed pigmentation, and our study included melanocyte PGE-2 priming, which demonstrates the appealing efficacy of these agents for this indication.

**FIGURE 4.** Examination of melanocyte gene expression related to hexapeptide-12 activity alone on melanogenesis pathways demonstrating powerful downregulation across the range.
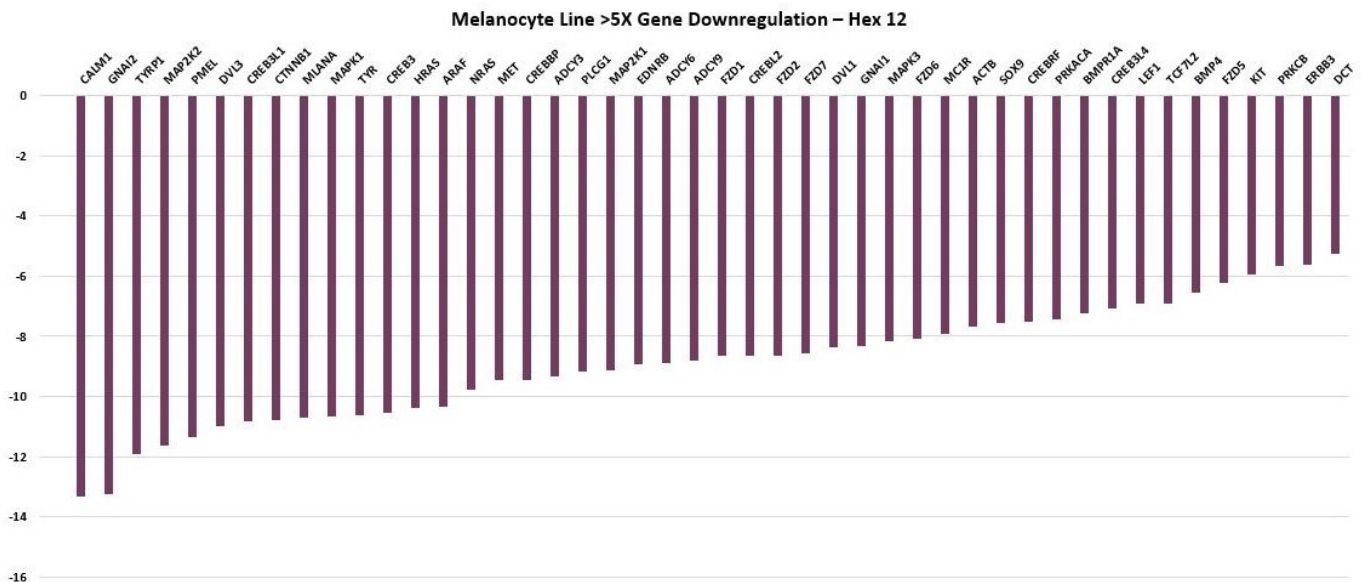
### Silymarin

Silymarin is derived from the milk thistle plant, *Silybum marianum*. Silibinin, the main component of silymarin, demonstrates powerful antioxidant and photoprotective effects. It minimizes UVR effects, such as oxidative stress, inflammation, erythema, and DNA damage. It prevents melanin production without affecting cell viability and decreases the expression of tyrosinase protein.[44] Silymarin cream was more effective than intradermal tranexamic acid in a study on patients with melasma.[44] It was also reported that silymarin suppressed the production of IL-1b and PGE-2 produced by COX-2 in keratinocytes and macrophages, and silibinin decreased inducible nitric oxide synthase and COX-2, as well as NF-κB. Thus, it would appear that the anti-melanogenesis activity may be related to its anti-inflammatory effect.[45,46]

### Hexapeptide-12

Traditionally, Hexapeptide-12 is a peptide recognized as an elastin-binding protein that stimulates elastin and collagen increasing fibronectin and glycosaminoglycans.[47] In addition, our gene studies have also demonstrated a significant effect on a multitude of melanogenic genes showing potent downregulation of these genes (Figure 4). This was again confirmed in the melanogenic model, where a significant decrease in melanin production was demonstrated (Figure 10).

### Phosphatidylserine

Phosphatidylserine treatment has been demonstrated to prevent UV-induced reduction in procollagen expression and inhibit UV-induced MMP-1, and it is thus used as an anti-aging agent.[48] Phosphatidylserine is normally confined to the inner leaflet of the cell membrane facing the cytoplasm and is extremely sensitive to ischemic conditions.[48] EDN1 is a potent endogenous vascular regulator, mainly secreted by endothelial cells. Apart from its regulatory function, EDN1 causes dysfunction of the vascular cells and stimulates ROS production.[49] Nitrous oxide synthesized by nitric oxide synthase 3 in the endothelium is considered to be the major source of nitric oxide for regulating vasoactivity.[50] As far as pigmentation is concerned, the phosphatidylserine effect of decreasing nitric oxide synthase 3, EDN1, SCF, and VEGF, as demonstrated in Figure 9, was very focused on the desired anti-melanogenic effects. This was again reflected in the melanogenesis model, where phosphatidylserine significantly decreased melanin formation.

### Withania Somnifera Extract

Apart from its apparent hydrating abilities, *Tremella fuciformis* has also been demonstrated to inhibit melanin production. Its extracts markedly decrease melanin content and tyrosinase activity, as well as to down-regulate MITF and both TRP-1 and 2 levels in α-MSH-stimulated melanocytes.[51]

### Gallic Acid

Gallic acid, a dietary phenolic present in plants and fruits, provides beneficial effects against hyperpigmentation. This is potentially through its antioxidant properties and its ability to suppress melanogenesis by down-regulating various melanogenic regulatory genes, including MITF, TYR, TRP-1, and dopachrome tautomerase.[52,53]

### Additional PGE2 Inhibitors

Thermus thermophilus ferment is a microorganism that comes

**FIGURE 5.** Examination of melanocyte gene expression related to lactoferrin activity alone on melanogenesis pathways demonstrating powerful downregulation across the range.

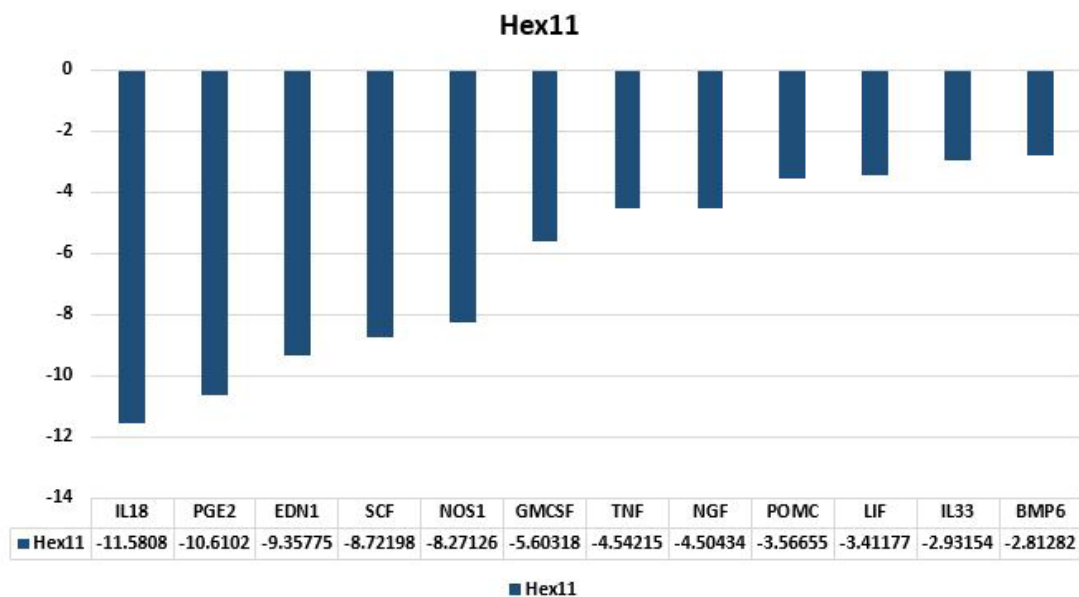Melanocyte Line >2X Gene Downregulation - Lactoferrin
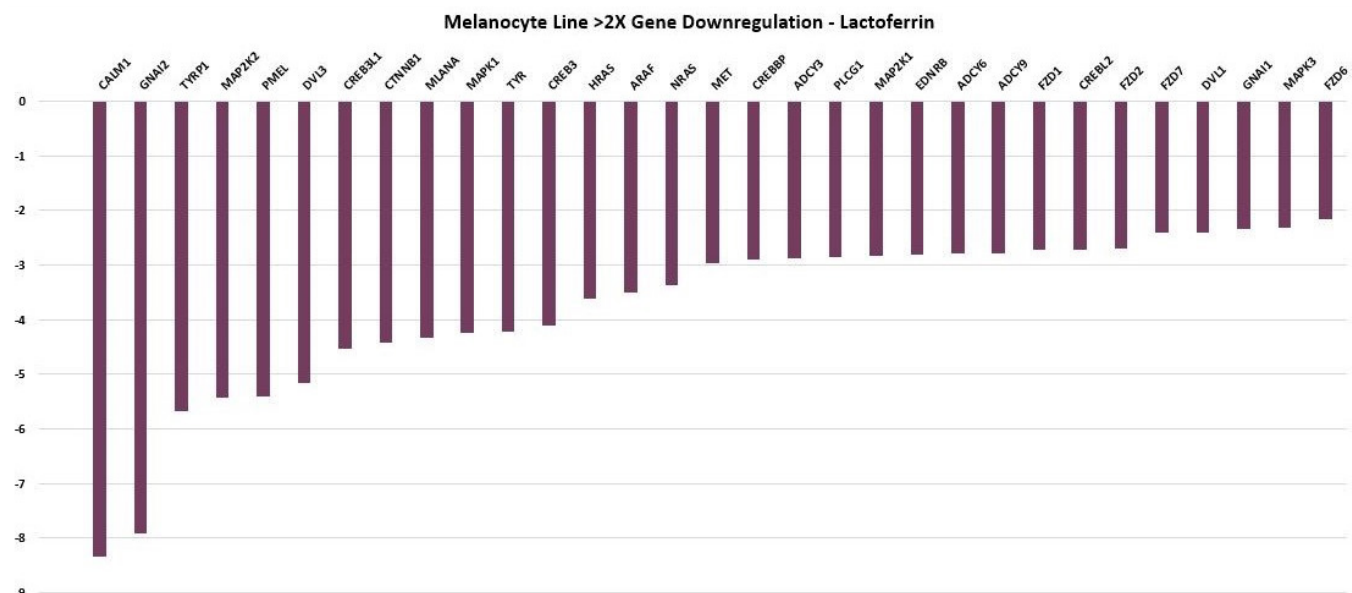
**FIGURE 6.** Gene expression studies in keratinocyte cell line demonstrating significant downregulation of melanogenesis pathways by hexapeptide-11 among several agents tested.
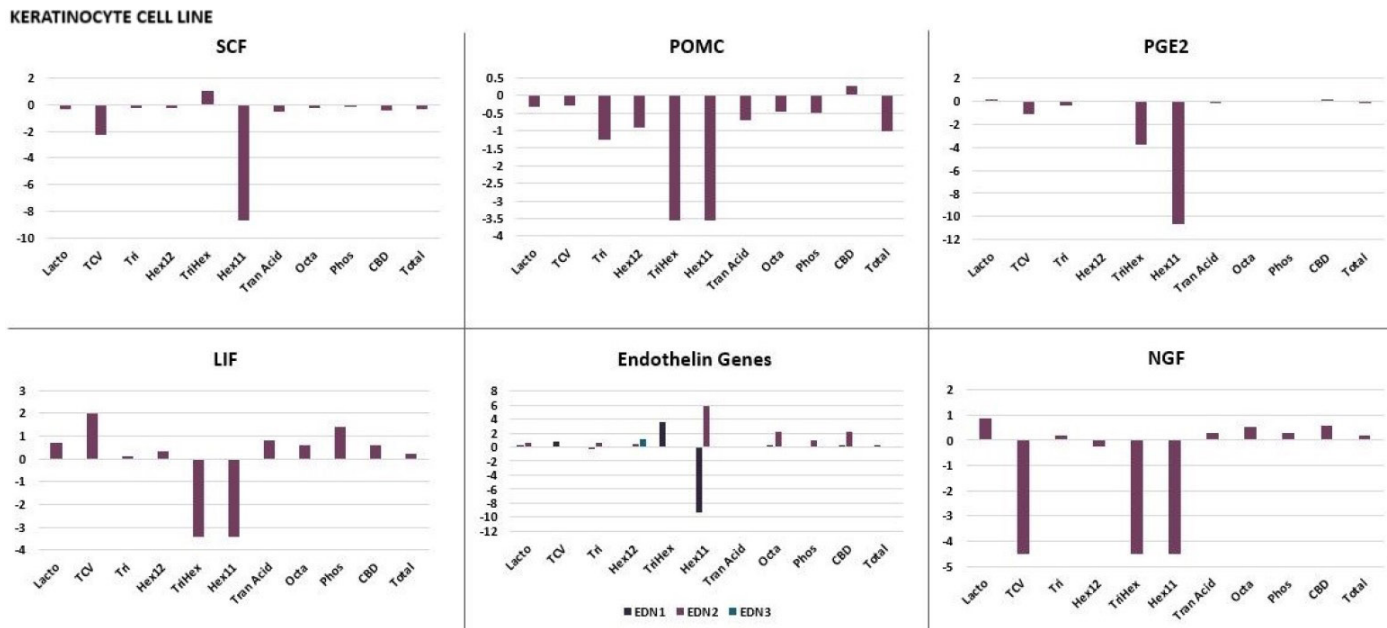


from marine waters located near very deep hydrothermal vents. It is thought to limit the increase of pro-inflammatory mediators (eg, PGE-2, IL-6, and IL-8) due to UV irradiation.[54] Oleuropein, a potent anti-inflammatory antioxidant derived from olive trees, also demonstrates tyrosinase inhibitory activity.[55]

The remaining categories for melanogenic regulation involve the transfer of melanosome/melanin to the keratinocyte and autophagy regulation.

**FIGURE 7.** Examination of keratinocyte gene expression (particularly involving inflammatory pathways related to PIH) reflecting hexapeptide-11 activity alone on melanogenesis pathways demonstrating powerful downregulation across the range.



| | IL18 | PGE2 | EDN1 | SCF | NOS1 | GMCSF | TNF | NGF | POMC | LIF | IL33 | BMP6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ■ Hex11 | -11.5808 | -10.6102 | -9.35775 | -8.72198 | -8.27126 | -5.60318 | -4.54215 | -4.50434 | -3.56655 | -3.41177 | -2.93154 | -2.81282 |

■ Hex11

**FIGURE 8.** Gene expression studies in endothelial cell line demonstrating significant downregulation of melanogenesis pathways by phosphatidylserine among the limited melanogenesis pathways apparent in endothelial cells.
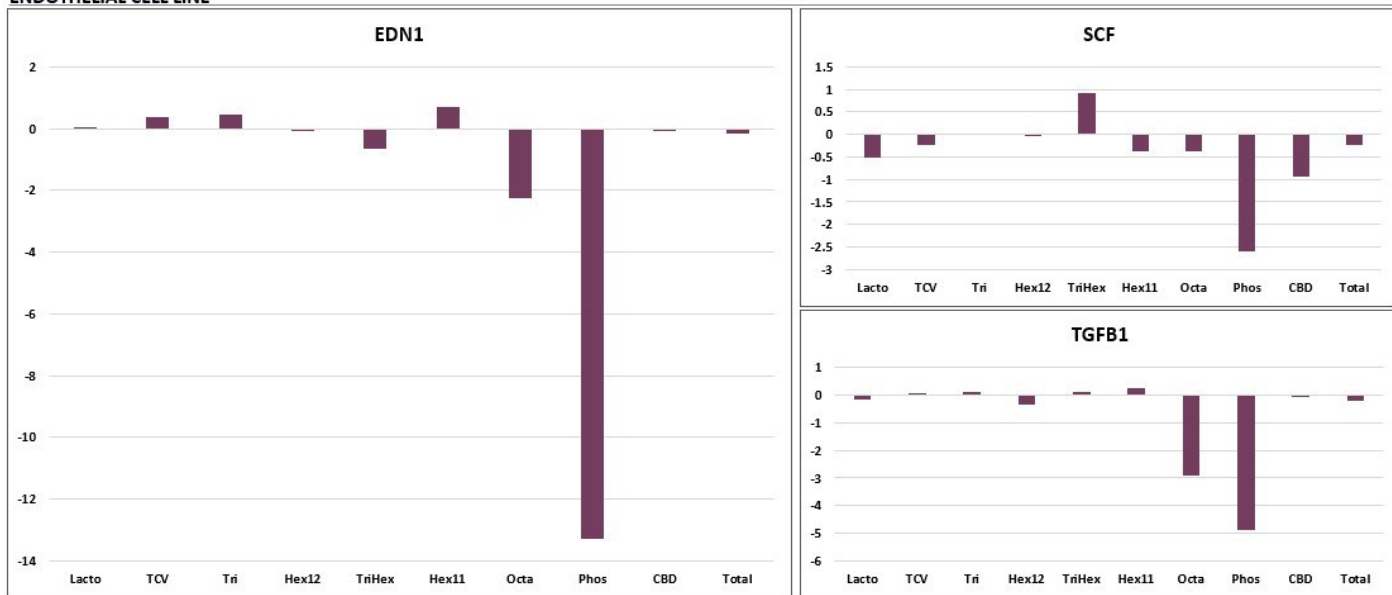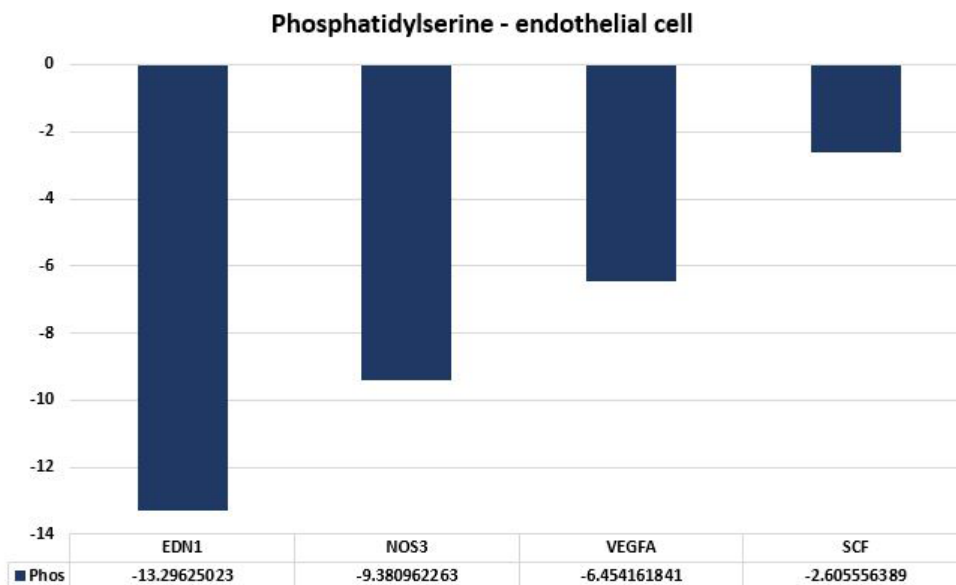


## Melanosome Transfer

### Hesperidin

Hesperidin is a citrus flavonoid that inhibits melanosome transport in melanocytes and has demonstrated skin-lightening effects in a pigmented reconstructed epidermis model.[56] Hesperidin exerts a depigmenting effect by blocking the Rab27A protein-melanophilin interaction, which prevents the docking of melanosomes to the plasma membrane.[56,57]

### Pancratium Maritimum

Melanin release by melanocytes to keratinocytes is stimulated by neuropeptides released by nerve fibers present in the epidermis, particularly substance P. Pancratium inhibits melanin transfer via its action on the substance P receptor, which is present on melanocyte dendrites.[58]

**FIGURE 9.** Gene expression studies demonstrating significant downregulation of the important melanogenesis pathways in endothelial cell lines by phosphatidylserine alone.



| | EDN1 | NOS3 | VEGFA | SCF |
|---|---|---|---|---|
| ■ Phos | -13.29625023 | -9.380962263 | -6.454161841 | -2.605556389 |

**FIGURE 10.** Melanocyte pigmentation model results — comparative analysis of melanin production via absorbance readings. Stimulation with MSH (orange) was used as a positive control to validate the absorbance reading. Three absorption wavelengths were assessed at 2 concentrations of the selected compounds (only one for MSH). The findings confirmed the efficacy of the selected agents for reducing melanogenesis (% decrease absorption denoting decreased melanin formation) in melanocytes.

| Compound | Concentration (ug/ml) | Absorbance 405nm | %decrease at 405nm | Absorbance 490nm | % decrease at 490nm | Absorbance 492nm | % decrease at 492nn |
|---|---|---|---|---|---|---|---|
| Lactoferrin | 0 | 0.887 | 0 | 0.576 | 0 | 0.411 | 0 |
| Lactoferrin | 500 | 0.509 | 44% | 0.334 | 42% | 0.217 | 47% |
| Lactoferrin | 1000 | 0.519 | 43% | 0.312 | 46% | 0.226 | 45% |
| HEX-11 | 0 | 0.91 | 0 | 0.576 | 0 | 0.411 | 0 |
| HEX-11 | 100 | 0.461 | 49% | 0.288 | 50% | 0.209 | 49% |
| HEX-11 | 200 | 0.338 | 63% | 0.227 | 61% | 0.163 | 60% |
| Phosphatidylserine | 0 | 0.779 | 0 | 0.501 | 0 | 0.35 | 0 |
| Phosphatidylserine | 250 | 0.686 | 23% | 0.438 | 24% | 0.338 | 18% |
| Phosphatidylserine | 500 | 0.61 | 31% | 0.38 | 34% | 0.288 | 30% |
| Silymarin 0.7% | 0 | 0.887 | 0 | 0.576 | 0 | 0.411 | 0 |
| Silymarin 0.7% | 10 | 1.634 | Error | 0.513 | 11% | 0.344 | 16% |
| Silymarin 0.7% | 20 | 0.592 | 35% | 0.341 | 41% | 0.246 | 40% |
| Tranexamic Acid | 0 | 0.887 | 0 | 0.576 | 0 | 0.411 | 0 |
| Tranexamic Acid | 500 | 0.395 | 57% | 0.254 | 56% | 0.187 | 55% |
| Tranexamic Acid | 1000 | 0.406 | 55% | 0.243 | 58% | 0.176 | 57% |
| Phytoene/Phytofluene | 0.00% | 0.887 | 0 | 0.576 | 0 | 0.411 | 0 |
| Phytoene/Phytofluene | 2.50% | 0.726 | 20% | 0.453 | 21% | 0.322 | 22% |
| Phytoene/Phytofluene | 5.00% | 0.428 | 53% | 0.275 | 52% | 0.2 | 51% |
| Hex-12 | 0 | 1.67 | 0 | 1.12 | 0 | 0.74 | 0 |
| Hex-12 | 2.9 | 1.32 | 21% | 0.94 | 16% | 0.64 | 14% |
| Hex-12 | 4.9 | 0.96 | 43% | 0.64 | 43% | 0.46 | 38% |
| MSH | 0 | 1.649 | 0 | 0.971 | 0 | 0.501 | 0 |
| MSH | 10nm media | 1.921 | -17% | 1.155 | -19% | 0.573 | -14% |

### Niacinamide: (General)

Niacinamide has a depigmentation effect by reducing melanosome transfer between melanocytes and surrounding keratinocytes.[59] In addition, daily use of a niacinamide moisturizer was shown to be effective in reducing hyperpigmentation and in increasing lightness of basal skin color compared to control moisturizer.[59]

### Autophagy

#### Hexapeptide-11

Hexapeptide-11 promotes activation of the proteasome-, autophagy-, chaperones-, and antioxidant- related-gene responses.[1] This stimulation of autophagy is also important in the transformation of monocytes to macrophages[60] enabling the engulfment and digestion of extracellular fragments. Hexapeptide-11 is a potent inducer of cell component digestion.[1] One of those components is the melanosome, and the delivery of melanin from the melanocyte to the keratinocyte is thought to be influenced by autophagy as detailed above. In addition, recent findings point to dysregulated NRF2 signaling being involved in the pathogenesis of UV-induced skin pigmentation. The pharmacological activation of NRF2 may represent a potential therapeutic target in photoaging.[13] Hexapeptide-11 is demonstrated to be a significant activator of NRF2.[1]

#### Heptasodium Hexacarboxymethyl Dipeptide-12 (HHD12)

Melanosomes are transferred to neighboring keratinocytes and then naturally degraded by autophagy. However, when the skin is constantly exposed to UVR, pathogens, and hormonal changes, the autophagic processes are disturbed, and melanosomes cannot be degraded. Accumulation of undegraded melanosomes in keratinocytes results in skin hyperpigmentation. HHD12 activates autophagy to break down melanosomes in keratinocytes and inhibits melanosome uptake into keratinocytes at the same time.[61]

### Liposome Delivery

Finally, the question of topical agent absorption remains an important issue. In most cases, the peptides used in the formulation are within 500-800 Da, which allows efficient interfollicular transdermal delivery. However, to ensure the delivery of certain actives and to aid in further protection against UV-induced changes, a multi liposome combination is introduced. Photosomes are liposomes endowed with DNA repair technology, which can repair broken UV-induced dimers using the DNA repair enzyme photolyase.[62] The topical application of photolyase is effective in dimer reversal and leads to immunoprotection, which is an added bonus to a pigmentation prevention program.[62] Added to the photosome is an additional tranexamic acid liposome and our proprietary liposome that allows for non-palmitoylated hexapeptide and lactoferrin to be encapsulated in a liposome, wherein the average particle size of the composition is no more than 220 nm.

## CONCLUSION

A comprehensive background scientific narrative of the various facets related to pigmentation has been presented. This highlighted the potential nuanced differences that routine photodamage, melasma, and PIH present. In particular, melasma appears to involve EDN1 in addition to the normal photodamage pathway, while PIH revolves around a potent PGE-2 messenger among other inflammatory mediators. When designing a

formulation that impacts all of these nuanced facets of different causes of pigmentation, it is important to accommodate actives that impact various steps along this complex pathway taking cognizance of the different cell types involved. The actives chosen here are based on comprehensive RNA-seq studies as well as in vitro melanogenic model testing. Using this approach, we selected and validated a series of active agents that regulate melanocyte, keratinocyte, and endothelial cell involvement in melanin stimulation and synthesis, have added those that affect melanin transfer, and have introduced autophagic stimulatory agents to deal with already formed melanosomes and melanin. Additionally, ongoing photoprotection with recognized broad-spectrum mineral sunscreens is vital. It is believed that this combination offers a novel multi-faceted approach to the complex problem of hyperpigmentation.

## DISCLOSURES

## ACKNOWLEDGMENT

## REFERENCES

1. Sklirou AD, Ralli M, Dominguez M, Papassideri I, Skaltsounis AL, Trougakos IP. Hexapeptide-11 is a novel modulator of the proteostasis network in human diploid fibroblasts. *Redox Biol.* 2015;5:205-215. doi: 10.1016/j.redox.2015.04.010

2. Lajis AFB, Ariff AB. Discovery of new depigmenting compounds and their efficacy to treat hyperpigmentation: Evidence from in vitro study. *J Cosmet Dermatol.* 2019;18(3):703-727. doi: 10.1111/jocd.12900.

3. Baek SH, Lee SH. Sesamol decreases melanin biosynthesis in melanocyte cells and zebrafish: Possible involvement of MITF via the intracellular cAMP and p38/JNK signalling pathways. *Exp Dermatol.* 2015;24(10):761-766. doi: 10.1111/exd.12765

4. Murase D, Hachiya A, Amano Y, Ohuchi A, Kitahara T, Takema Y. The essential role of p53 in hyperpigmentation of the skin via regulation of paracrine melanogenic cytokine receptor signaling. *J Biol Chem.* 2009;284(7):4343-4353. doi: 10.1074/jbc.M805570200.

5. Brenner M, Hearing VJ. Modifying skin pigmentation - approaches through intrinsic biochemistry and exogenous agents. *Drug Discov Today Dis Mech.* 2008;5(2):e189-e199. doi: 10.1016/j.ddmec.2008.02.001.

6. Yamaguchi Y, Hearing VJ. Physiological factors that regulate skin pigmentation. *Biofactors.* 2009;35(2):193-199. doi: 10.1002/biof.29.

7. Ho H, Ganesan AK. The pleiotropic roles of autophagy regulators in melanogenesis. *Pigment Cell Melanoma Res.* 2011;24(4):595-604. doi: 10.1111/j.1755-148X.2011.00889.x.

8. Li P-H, Liu L-H, Chang C-C, et al. Silencing Stem Cell Factor Gene in Fibroblasts to Regulate Paracrine Factor Productions and Enhance c-Kit Expression in Melanocytes on Melanogenesis. *International Journal of Molecular Sciences.* 2018;19(5). doi: 10.3390/ijms19051475.

9. Pillaiyar T, Manickam M, Jung SH. Recent development of signaling pathways inhibitors of melanogenesis. *Cell Signal.* 2017;40:99-115. doi: 10.1016/j.cellsig.2017.09.004.

10. Silpa-Archa N, Kohli I, Chaowattanapanit S, Lim HW, Hamzavi I. Postinflammatory hyperpigmentation: A comprehensive overview: Epidemiology, pathogenesis, clinical presentation, and noninvasive assessment technique. *J Am Acad Dermatol.* 2017;77(4):591-605. doi: 10.1016/j.jaad.2017.01.035.

11. Fuller B. Role of PGE-2 and Other Inflammatory Mediators in Skin Aging and Their Inhibition by Topical Natural Anti-Inflammatories. *Cosmetics.* 2019;6(1). doi: 10.3390/cosmetics6010006.

12. Tanaka K, Hasegawa J, Asamitsu K, Okamoto T. Prevention of the ultraviolet B-mediated skin photoaging by a nuclear factor kappaB inhibitor, parthenolide. *J Pharmacol Exp Ther.* 2005;315(2):624-630. doi: 10.1124/jpet.105.088674.

13. Kerns ML, Chien AL, Kang S. A Role for NRF2-Signaling in the Treatment and Prevention of Solar Lentigines. *Plast Reconstr Surg.* 2021;148(6S):27S-31S. doi: 10.1097/PRS.0000000000008783.

14. Starner RJ, McClelland L, Abdel-Malek Z, Fricke A, Scott G. PGE(2) is a UVR-inducible autocrine factor for human melanocytes that stimulates tyrosinase activation. *Exp Dermatol.* 2010;19(7):682-684. doi: 10.1111/j.1600-0625.2010.01074.x.

15. Sheth VM, Pandya AG. Melasma: a comprehensive update: part I. *J Am Acad Dermatol.* 2011;65(4):689-697. doi: 10.1016/j.jaad.2010.12.046.

16. Sanchez NP, Pathak MA, Sato S, Fitzpatrick TB, Sanchez JL, Mihm MC, Jr. Melasma: A clinical, light microscopic, ultrastructural, and immunofluorescence study. *Journal of the American Academy of Dermatology.* 1981;4(6):698-710. doi: 10.1016/s0190-9622(81)70071-9.

17. Kwon SH, Hwang YJ, Lee SK, Park KC. Heterogeneous Pathology of Melasma and Its Clinical Implications. *Int J Mol Sci.* 2016;17(6):824. doi: 10.3390/ijms17060824.

18. Widgerow AD, Jiang LI, Calame A. A single-center clinical trial to evaluate the efficacy of a tripeptide/hexapeptide antiaging regimen. *J Cosmet Dermatol.* 2018;18(1):176-182. doi: 10.1111/jocd.12507.

19. Regazzetti C, De Donatis GM, Ghorbel HH, et al. Endothelial Cells Promote Pigmentation through Endothelin Receptor B Activation. *J Invest Dermatol.* 2015;135(12):3096-3104. doi: 10.1038/jid.2015.332.

20. Hyter S, Coleman DJ, Ganguli-Indra G, et al. Endothelin-1 is a transcriptional target of p53 in epidermal keratinocytes and regulates ultraviolet-induced melanocyte homeostasis. *Pigment Cell Melanoma Res.* 2013;26(2):247-258. doi: 10.1111/pcmr.12063.

21. Murase D, Hachiya A, Kikuchi-Onoe M, et al. Cooperation of endothelin-1 signaling with melanosomes plays a role in developing and/or maintaining human skin hyperpigmentation. *Biol Open.* 2015;4(10):1213-1221. doi: 10.1242/bio.011973.

22. Regazzetti C, Sormani L, Debayle D, et al. Melanocytes Sense Blue Light and Regulate Pigmentation through Opsin-3. *J Invest Dermatol.* 2018;138(1):171-178. doi: 10.1016/j.jid.2017.07.833.

23. Ozdeslik RN, Olinski LE, Trieu MM, Oprian DD, Oancea E. Human nonvisual opsin 3 regulates pigmentation of epidermal melanocytes through functional interaction with melanocortin 1 receptor. *Proc Natl Acad Sci U S A.* 2019;116(23):11508-11517. doi: 10.1073/pnas.1902825116.

24. Passeron T. Melasma pathogenesis and influencing factors – an overview of the latest research. *Journal of the European Academy of Dermatology and Venereology.* 2013;27(suppl 1):5-6. doi: 10.1111/jdv.12049.

25. Cho YH, Park JE, Lim DS, Lee JS. Tranexamic acid inhibits melanogenesis by activating the autophagy system in cultured melanoma cells. *Journal of Dermatological Science.* 2017;88(1):96-102. doi: 10.1016/j.jdermsci.2017.05.019.

26. Murase D, Hachiya A, Takano K, et al. Autophagy Has a Significant Role in Determining Skin Color by Regulating Melanosome Degradation in Keratinocytes. *Journal of Investigative Dermatology.* 2013;133(10):2416-2424. doi: 10.1038/jid.2013.165.

27. Roberts WE. Pollution as a risk factor for the development of melasma and other skin disorders of facial hyperpigmentation is there a case to be made? (1545-9616 (Print)).

28. Fu C, Chen J, Lu J, et al. Roles of inflammation factors in melanogenesis (Review). *Mol Med Rep.* 2020;21(3):1421-1430. doi: 10.3892/mmr.2020.10950.

29. García-Montoya IA, Cendón TS, Arévalo-Gallegos S, Rascón-Cruz Q. Lactoferrin a multiple bioactive protein: An overview. *Biochimica et Biophysica Acta (BBA) - General Subjects.* 2012;1820(3):226-236. doi: 10.1016/j.bbagen.2011.06.018.

30. Ishii N, Ryu M, Suzuki YA. Lactoferrin inhibits melanogenesis by down-

regulating MITF in melanoma cells and normal melanocytes. *Biochem Cell Biol.* 2017;95(1):119-125. doi: 10.1139/bcb-2016-0053.

31. Hassoun LA, Sivamani RK. A systematic review of lactoferrin use in dermatology. *Crit Rev Food Sci Nutr.* 2017;57(17):3632-3639. doi: 10.1080/10408398.2015.1137859.

32. Vogel HJ. Lactoferrin, a bird's eye view. *Biochem Cell Biol.* 2012;90(3):233-244. doi: 10.1139/o2012-016.

33. Zwirzitz A, Reiter M, Skrabana R, et al. Lactoferrin is a natural inhibitor of plasminogen activation. *Journal of Biological Chemistry.* 2018;293(22):8600-8613. doi: 10.1074/jbc.RA118.003145.

34. Dashore S, Mishra K. Tranexamic acid in melasma: Why and how? *Indian Journal of Drugs in Dermatology.* 2017;3(2):61-63. 10.4103/ijdd.ijdd_37_17

35. Zakharova ET, Sokolov AV, Pavlichenko NN, et al. Erythropoietin and Nrf2: key factors in the neuroprotection provided by apo-lactoferrin. *Biometals.* 2018;31(3):425-443. doi: 10.1007/s10534-018-0111-9.

36. Colferai MMT, Miquelin GM, Steiner D. Evaluation of oral tranexamic acid in the treatment of melasma. *J Cosmet Dermatol.* 2018. doi: 10.1111/jocd.12830.

37. Desai S, Ayres E, Bak H, et al. Effect of a Tranexamic Acid, Kojic Acid, and Niacinamide Containing Serum on Facial Dyschromia: A Clinical Evaluation. *Journal of Drugs in Dermatology.* 2019;18(5):454-461.

38. Poojary S, Minni K. Tranexamic Acid in Melasma: A Review. *Journal of Pigmentary Disorders.* 2015;2(12). doi:10.4172/2376-0427.1000228.

39. Janney MS, Subramaniyan R, Dabas R, Lal S, Das NM, Godara SK. A Randomized Controlled Study Comparing the Efficacy of Topical 5% Tranexamic Acid Solution versus 3% Hydroquinone Cream in Melasma. *J Cutan Aesthet Surg.* 2019;12(1):63-67. doi: 10.4103/JCAS.JCAS_40_18.

40. Melendez-Martinez AJ, Stinco CM, Mapelli-Brahm P. Skin Carotenoids in Public Health and Nutricosmetics: The Emerging Roles and Applications of the UV Radiation-Absorbing Colourless Carotenoids Phytoene and Phytofluene. *Nutrients.* 2019;11(5):1093. doi: 10.3390/nu11051093.

41. Melendez-Martinez AJ, Mapelli-Brahm P, Benitez-Gonzalez A, Stinco CM. A comprehensive review on the colorless carotenoids phytoene and phytofluene. *Arch Biochem Biophys.* 2015;572:188-200. doi: 10.1016/j.abb.2015.01.003.

42. Havas F, Krispin S, Melendez-Martinez AJ, von Oppen-Bezalel L. Preliminary Data on the Safety of Phytoene- and Phytofluene-Rich Products for Human Use including Topical Application. *J Toxicol.* 2018;2018:5475784. doi: 10.1155/2018/5475784.

43. Fuller B, Smith D Fau - Howerton A, Howerton A Fau - Kern D, Kern D. Anti-inflammatory effects of CoQ10 and colorless carotenoids. *J Cosmet Dermatol.* 2006;5():30-38. doi: 10.1111/j.1473-2165.2006.

44. Nofal A, Ibrahim AM, Nofal E, Gamal N, Osman S. Topical silymarin versus hydroquinone in the treatment of melasma: A comparative study. *J Cosmet Dermatol.* 2018;18(1):263-270. doi: 10.1111/jocd.12769.

45. Choo SJ, Ryoo IJ, Kim YH, et al. Silymarin inhibits melanin synthesis in melanocyte cells. *J Pharm Pharmacol.* 2009;61(5):663-667. doi: 10.1211/jpp.61.05.0016.

46. Kang JS, Jeon YJ, Park S-K, Yang K-H, Kim HM. Protection against lipopolysaccharide-induced sepsis and inhibition of interleukin-1β and prostaglandin E2 synthesis by silymarin. *Biochemical Pharmacology.* 2004;67(1):175-181. doi: 10.1016/j.bcp.2003.08.032.

47. Schagen S. Topical Peptide Treatments with Effective Anti-Aging Results. *Cosmetics.* 2017;4(2):16. doi: 10.3390/cosmetics4020016

48. Widgerow AD. Ischemia-reperfusion injury: influencing the microcirculatory and cellular environment. *Annals of plastic surgery.* 2014;72(2):253-260. doi: 10.1097/SAP.0b013e31825c089c.

49. Kowalczyk A, Kleniewska P, Kolodziejczyk M, Skibska B, Goraca A. The role of endothelin-1 and endothelin receptor antagonists in inflammatory response and sepsis. *Arch Immunol Ther Exp (Warsz).* 2015;63(1):41-52. doi: 10.1007/s00005-014-0310-1.

50. Chen K, Pittman RN, Popel AS. Nitric oxide in the vasculature: where does it come from and where does it go? A quantitative perspective. *Antioxid Redox Signal.* 2008;10(7):1185-1198. doi: 10.1089/ars.2007.1959.

51. Lee K, Park H, Yoon I, et al. Whitening and Anti-Wrinkle Effects of Tremella fuciformis Extracts. *Korean Journal of Medicinal Crop Science.* 2016;24(1):38-46.

52. Panich U, Onkoksoong T, Limsaengurai S, Akarasereenont P, Wongkajornsilp A. UVA-induced melanogenesis and modulation of glutathione redox system in different melanoma cell lines: the protective effect of gallic acid. *J Photochem Photobiol B.* 2012;108:16-22. doi: 10.1016/j.jphotobiol.2011.12.004.

53. Kumar KJ, Vani MG, Wang SY, et al. In vitro and in vivo studies disclosed the depigmenting effects of gallic acid: a novel skin lightening agent for hyperpigmentary skin diseases. *Biofactors.* 2013;39(3):259-270. doi: 10.1002/biof.1064.

54. Armstrong G. Chapter 2.12: Carotenoid Genetics and Biochemistry. In: Barton SD, Nakanishi K, Meth-Cohn O, eds. *Comprehensive Natural Products Chemistry.* Elsevier Science; 1999:321-352.

55. Yang D-M, Ouyang M-A. Antioxidant and Anti-Tyrosinase Activity from Olea Leaf Extract Depended on Seasonal Variations and Chromagraphy Treatment. *International Journal of Organic Chemistry.* 2012;02(04):391-397. doi: 10.4236/ijoc.2012.24054.

56. Kim B, Lee J-Y, Lee H-Y, et al. Hesperidin Suppresses Melanosome Transport by Blocking the Interaction of Rab27A-Melanophilin. *Biomolecules and Therapeutics.* 2013;21(5):343-348. doi: 10.4062/biomolther.2013.032.

57. Hou M, Man M, Man W, et al. Topical hesperidin improves epidermal permeability barrier function and epidermal differentiation in normal murine skin. *Exp Dermatol.* 2012;21(5):337-340. doi: 10.1111/j.1600-0625.2012.01455.x.

58. Morvan P-y, Pentecouteau L, Vallée R. *Effect of a marine plant extract on skin melanogenesis.* Poster presented at: 21st IFSCC Conferences; December 2011; Bangkok, Thailand

59. Hakozaki T, Minwalla L, Zhuang J, et al. The effect of niacinamide on reducing cutaneous pigmentation and suppression of melanosome transfer. *British Journal of Dermatology.* 2002;147(1):20-31. doi: 10.1046/j.1365-2133.2002.04834.x.

60. Zhang Y, Morgan MJ, Chen K, Choksi S, Liu ZG. Induction of autophagy is essential for monocyte-macrophage differentiation. *Blood.* 2012;119(12):2895-2905. doi: 10.1182/blood-2011-08-372383.

61. Kim JY, Kim J, Ahn Y, et al. Autophagy induction can regulate skin pigmentation by causing melanosome degradation in keratinocytes and melanocytes. Pigment Cell Melanoma Res. 2020;33(3):403-415. doi: 10.1111/pcmr.12838.

62. Stege H, Roza L, Vink AA, et al. Enzyme plus light therapy to repair DNA damage in ultraviolet-B-irradiated human skin. *Proc Natl Acad Sci U S A.* 2000;97(4):1790-1795. doi: 10.1073/pnas.030528897.

## AUTHOR CORRESPONDENCE

**Alan D. Widgerow**
E-mail:...................……................... alan.widgerow@galderma.com