

## Topical Agents for Melasma: A Perspective on Therapeutic Approaches and their Molecular Bases

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### Abstract

Melasma is a common skin disease involving changes in normal skin pigmentation. It results from epidermal melanocyte hyperactivity that causes increased production and accumulation of melanin. The onset of the disease has been associated with sun exposure, drugs, genetic and hormonal factors. The primary focus of this review was to provide an updated overview of the main biological aspects behind skin pigmentation and melasma development.

As a second aim of this review, the main mechanisms by which different compounds could reduce melanogenesis were also discussed. Common hypo-pigmenting agents act by reducing the melanogenesis through several mechanisms; they can affect melanin transcription and glycosylation, inhibit tyrosinase (a pivotal enzyme in melanin synthesis), slow the melanosome transfer or increase the skin turnover. Although a number of skin-lightening agents were proposed for treatment of hyperpigmentary disorders, none of these has achieved satisfactory effects. In this light, the most recent therapeutic strategies for melasma, and emerging molecular targets to control skin pigmentation, such as MITF, Wnt and mTOR, were herein described.

**Keywords:** Melanin; Melanogenesis; Tyrosinase; Hyperpigmentation disorders; Hypo-pigmenting agents

**Abbreviations:** UV-R: UV Radiation; SCF: Stem Cell Factors; FGF<sub>2</sub>: Fibroblast Growth Factor; PAH: Phenylalanine Hydroxylase; TH-1: Tyrosinase Hydroxylase 1; TRP-2: DOPA-Chrome Tautomerase; TRP-1: DHICA Oxidase; DHI: 5,6-Dihydroxyindole; DHICA: 5,6-Dihydroxyindole-2-Carboxylic Acid; Indole-5,6-Quinone I-Q; I-QCA: Indole-5,6-Quinone Carboxylic Acid; MSH: Melanocyte-Stimulating Hormone; TYR: Tyrosinase; MITF: Microphthalmia-Associated Transcription Factor; POMC: Proopiomelanocortin; MC1-R: Melanocortin 1 Receptor; PKC: Protein Kinase C; ET-1: Endothelin-1; Bfgf: Basic Fibroblast Growth Factor; NGF: Nerve Growth Factor; GM-CSF: Granulocyte-Macrophage Colony-Stimulating Factor; LIF: Leukemia Inhibitory Factor; HGF: Hepatocyte Growth Factor; PKA: Protein Kinase A; CREB: Camp Response Element Binding Protein; CRE: Camp Response Element; ASP: Agouti Signalling Peptide; Pges: Prostaglandins; ETBR: ET-1 G Protein-Coupled Receptor; MAPK: Mitogen-Activated Protein Kinase; IL-1: Interleukin-1; PAR-2: Proteinase-Activated Receptor 2; Inos: Inducible Nitric Oxide Synthase

### Skin Structures

The skin, in addition to provide a vast barrier against external physical threats, acts as a defence system against UV radiation (UV-R), through the high-molecular-weight brown pigment melanin [1,2]. The three skin's layers are epidermis, dermis, and hypodermis [3].

The epidermis is the outermost layer, providing a waterproof barrier; it is a stratified epithelium devoid of blood or nerve supplies, and composed of several distinct cell populations, above all keratinocytes and melanocytes [4]. It is arranged in four layers, as follows (Table 1):

The dermis is beneath the epidermis and forms the neural, vascular, lymphatic, and secretory apparatus of the skin. It contains connective tissue, fibroblasts (required for synthesis and degradation of the extracellular matrix), macrophages and mast cells (able to trigger allergic reactions by secreting bioactive mediators), hair follicles (providing a protective niche to several stem cell populations required during wound healing), nails, excretory and secretory glands [1,3,5].

The hypodermis is the deeper subcutaneous tissue. It contains fat and connective tissue; typical cells found in this layer are fibroblasts,

adipose cells, and macrophages. It is used mainly for fat storage.

### Melanocytes, Melanosomes and Melanogenesis

Normal human skin colour is mainly influenced by the production of the brown pigment melanin that also protects skin against ultraviolet light, and determines several aspects of the phenotypic appearance. The exogenous yellow pigment carotenoids and the endogenous red-oxygenated or blue-reduced haemoglobins contribute to skin colour, too [6]. However, skin and hair pigmentations depend mostly on size, number, composition and distribution of melanosomes, the melanocyte cytoplasmic particles containing melanin. In addition, human pigmentation may be determined by the melanogenic activity inside melanocytes and by the melanin synthesis rate [6,7].

Melanocytes are dendritic cells, embryologically derived from the melanocyte precursor cells melanoblasts. The development of melanoblasts and their migration from the neural crest to peripheral sites are the first important steps of melanogenesis. Melanoblasts migrate from the neural crest throughout the embryo mesenchyme reaching specific target sites, mainly dermis (between the 10<sup>th</sup> and the 12<sup>th</sup> week of embryonic life), epidermis (between the 12<sup>th</sup> and the 14<sup>th</sup> week of development), and hair follicles, but also the eyes (retina pigment epithelium, iris and choroid), ears (vascular strias), and central nervous system (leptomeninges) [7,8].

During embryogenesis, the proper migration of neural crest-derived cells is greatly dependent on interactions between specific receptors and their extracellular ligands. For example, mast cell growth factor or stem cell factors (SCF) bind specific receptors on melanocytes and melanoblasts. Genetic mutations affecting the SCF pattern genes

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Stratum	Characteristics
<b>S. basale</b> (or germinativum)	- a single layer of cells attached to a non-cellular basement membrane separating the epidermis from the dermis; - basal <b>keratinocytes</b> with stem cell-like properties, Merkel cells (for the transmission of touch sensation) and <b>melanocytes</b> .
<b>S. spinosum</b>	- a pivotal role in immunological reactions - irregular polyhedral keratinocytes, Langerhans' cells (bone marrow-derived sentinel cells of the immune system).
<b>S. granulosum</b>	- flattened, polyhedral non-dividing keratinocytes (producing granules of keratinohyalin). The dividing cells underneath them progressively push non-dividing keratinocytes toward the skin surface.
<b>S. corneum</b>	- non-viable, but biochemically active corneocytes. - keratinocytes continue to differentiate as they move from the basal layer to the stratum corneum, resulting in cornified cells with abundant keratin and lack cytoplasmic organelles. - a barrier against the physical and chemical agents, able also to reduce transepidermal water loss from within.

**Table 1:** Layers of the epidermis

decrease are responsible for certain pigmentation disorders [1]; in example, the Albinism Database (<http://www.ifpcs.org/albinism/>) collects genetic mutations, which had been associated with all major known forms of pigmentary disorders.

Adult melanocytes are located on the basal layer of the epidermis and, occasionally, on the dermis. The melanocyte density varies in different parts of the body: over 2,000 epidermal melanocytes/mm<sup>2</sup> of skin are in the head and forearm, and about the half in the rest of the body. This rigorous regulation of melanocyte density on epidermis seems to be mediated by specific mediators, such as the fibroblast growth factor (FGF<sub>2</sub>) [9]. Furthermore, the number of melanocytes is reduced with age in areas not exposed to light, at a constant proportion per decade [8].

The pivotal function of melanocytes is the production of melanin and its storage in the melanosomes, specific intracytoplasmatic structures. Then, the dendrites of melanocytes, crossing over *basale* and *spinosus strata* (Malpighian *stratum*), transfer the melanosomes to keratinocytes (Figure 1). This melanocyte-keratinocyte association is the epidermal melanin unit; in human, it has been estimated that each melanocyte is in contact with ~40 keratinocytes [10].

Melanosomes are highly specialized elliptical organelles, where there is the synthesis and the deposition of melanin, and storage of the tyrosinase (TYR) enzymes. Melanosomes mature in four morphologically defined stages, from no pigmented (stage I) to melanin filled (stage IV) organelles [1,11].

The major phenotypical difference between the more pigmented and less pigmented skins resides in the quality of the melanosomes; they are larger and more mature in hyper-pigmented than hypo-pigmented skins, and are stored more as units than in clusters. The higher levels of skin pigmentation are also maintained by a delay in melanosome degradation in the keratinocytes [7].

In normal melanosomes, melanin is extremely dense. It is an insoluble high-molecular-weight nitrogenized polymer forming a pigment, which plays an important UVR damage protection role filtering and absorbing UV-R. An inverse correlation between the melanin content and the incidence of skin tumours was reported in literature [12].

The process of melanin synthesis and distribution is called melanogenesis. It takes place exclusively in melanosomes and depends on many genes. The melanin synthetic pathway is schematized in Figure 2.

The synthesis of melanin initiates with the transformation of L-phenylalanine into L-tyrosine and in turn to produce L-DOPA and DOPA-quinone, via phenylalanine hydroxylase (PAH), tyrosinase (TYR) and partly tyrosinase hydroxylase 1 (TH-1). From DOPA-

quinone, the pathways are then divided into eumelanogenesis or pheomelanogenesis. The other melanogenic enzymes are TRP-2 (DOPA-chrome tautomerase) and TRP-1 (DHICA Oxidase) for eumelanogenesis. (DHI: 5,6-Dihydroxyindole; DHICA: 5,6-Dihydroxyindole-2-carboxylic acid; I-Q: Indole-5,6-quinone; I-QCA: Indole-5,6-quinone carboxylic acid) [13].

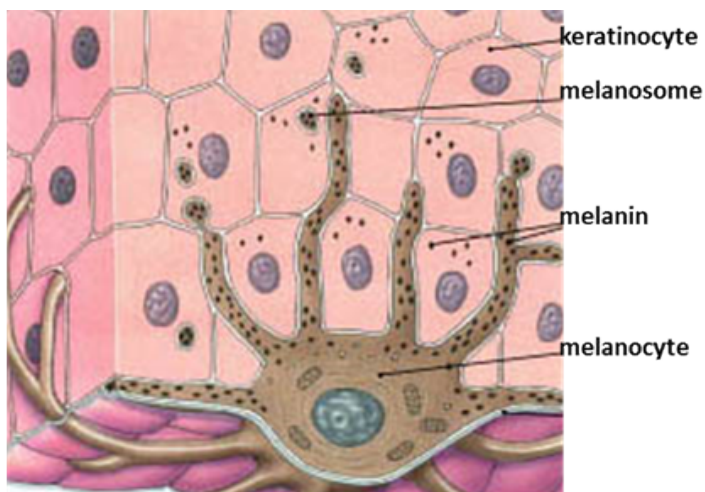
The initial elements of melanogenesis are tyrosine, an essential amino acid, and tyrosinase, a copper protein enzyme complex. Tyrosinase is a glycoprotein located in the membrane of the melanosome; it has an inner melanosomal domain containing the catalytic region, a short transmembrane domain and a cytoplasmic domain composed of approximately 30 amino acids [14]. Histidine residues are present in the catalytic portion of tyrosinase and bind copper ions required for tyrosinase activity [15]. Other two members of the tyrosinase-related enzyme family are involved in the melanogenesis: tyrosinase-related protein 1 (TRP-1), and DOPA-chrome tautomerase (TRP-2) [16].

Two types of melanin are synthesized within melanosomes, eumelanin and pheomelanin; eumelanin is a dark brown-black insoluble polymer, whereas pheomelanin is a light red-yellow sulphur-containing soluble polymer [17]. In the presence of molecular oxygen, tyrosinase oxidizes tyrosine into DOPA and this into DOPA-quinone. From then on, the content in cysteine determines the progression of pathway through eumelanin or pheomelanin [18]. Indeed, in the absence of cysteine, DOPA-quinone is converted into DOPA-chrome and then into DHI (dopa-5,6-dihydroxyindole), mostly, or DHICA (5,6-dihydroxyindole-2-carboxylic acid). This process is catalyzed by TRP-2. Finally, the dihydroxyindoles are oxidized into eumelanin by TRP-1 [18].

On the contrary, in the presence of cysteine, DOPA-quinone quickly reacts with cysteine to generate 5-S-cysteinyl-dopa and 2-S-cysteinyl-dopa, which are oxidized into intermediates to produce pheomelanin (Figure 2).

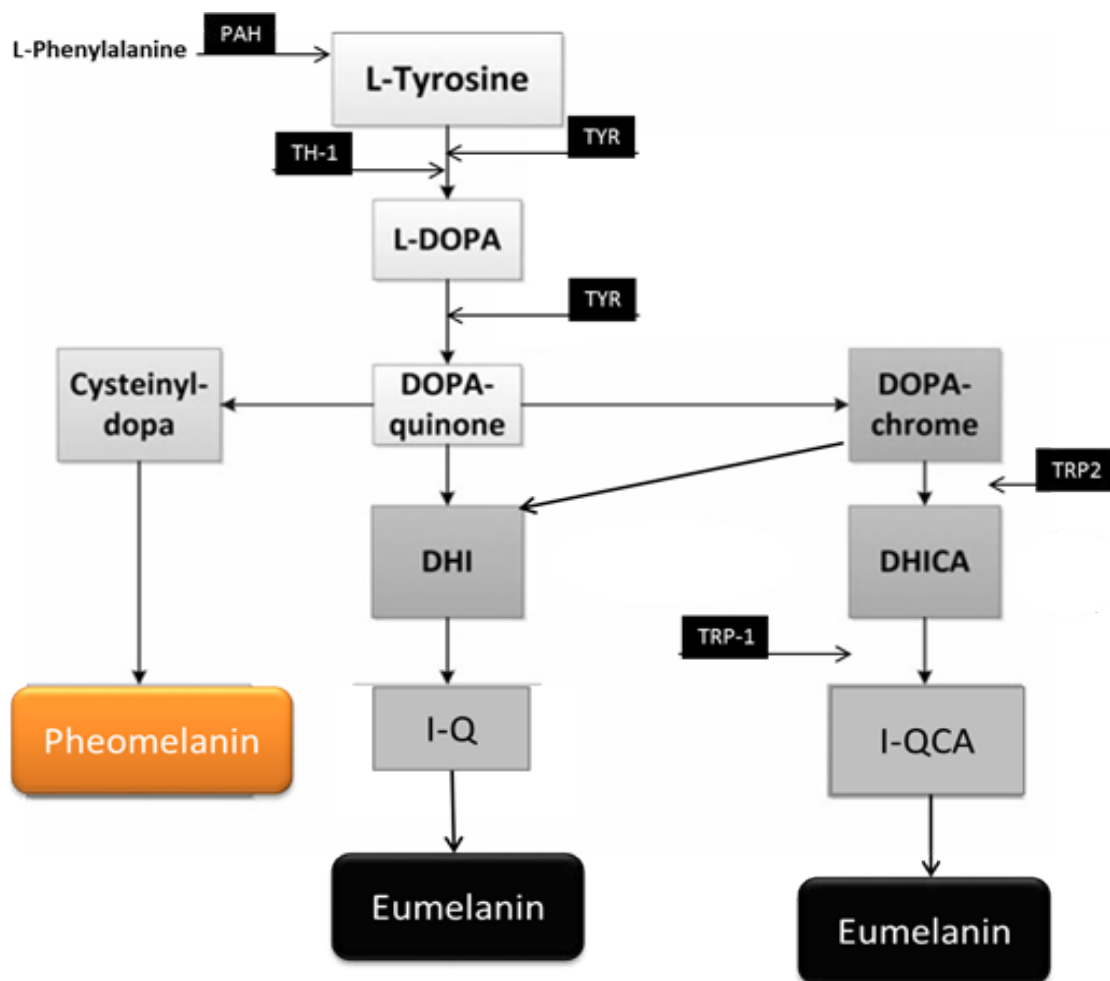
Eumelanin absorbs and disperses ultraviolet light, attenuating its penetration on the skin and reducing the harmful effects of the sun. On the other hand, pheomelanin has a great potential to generate free radicals in response to UV-R, which are capable of causing damage to DNA, and, in this manner, may contribute to the phototoxic effects of UV-R [19].

The second step of melanogenesis is the melanin distribution that uses the cytocin activity of melanocytes. Indeed, following the synthesis of melanins, filled melanosomes are introduced in the keratinocytes in the corresponding epidermal melanin unit, through the melanocyte dendritic extensions. Once inside keratinocytes, the melanosomes tend to spread through the cytoplasm, over the nucleus, to protect it from ultraviolet radiations [9,20,21].



Modified from <http://droualb.faculty.mjc.edu> - Chapter 4 - Integumentary System-

Figure 1: Integumentary System



Adapted from ref 13

Figure 2: Melanin synthesis

## Melanogenic Regulatory Proteins

The melanocyte-keratinocyte complex responds quickly to a wide range of environmental stimuli, often in paracrine and/or autocrine manners. Thus, melanocytes respond to UV-R, signaling proteins, melanocyte-stimulating hormone (MSH), endothelins, growth factors, cytokines, etc. [1,13,22] (Figure 3).

The gene encoding the basic helix-loop-helix leucine zipper Microphthalmia-Associated Transcription Factor (MITF) [23,24] appears to be fundamental for the regulatory network of signalling pathways controlling the survival, proliferation and differentiation of melanocyte lineage [25]. Melanocyte development and pigmentation are affected by MITF via its transcriptional regulatory effect on tyrosinase, TRP-1 and TRP-2 [26], and on Rab27A, a protein important for melanosome transport [27].

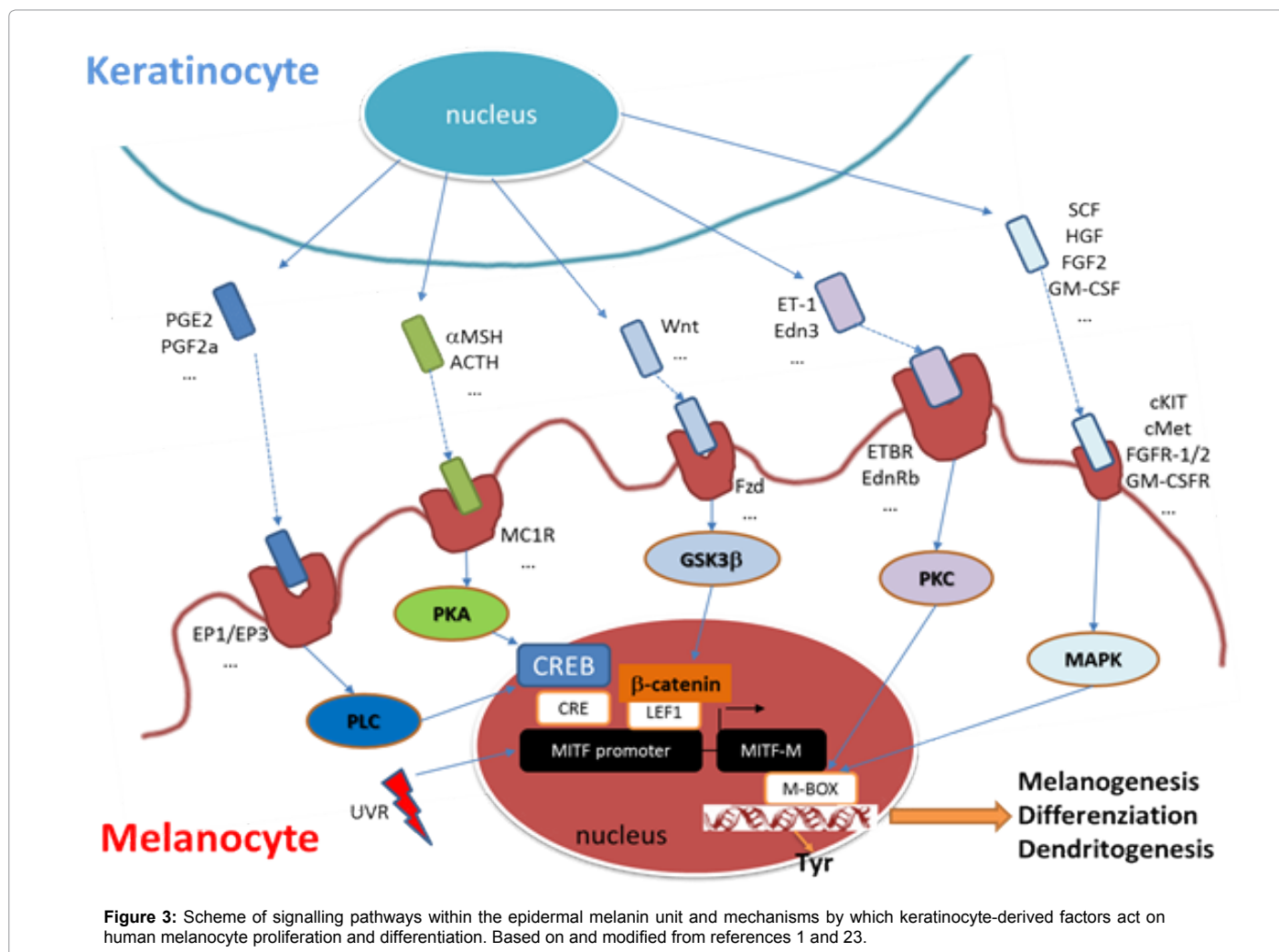
UV radiation stimulates the melanocyte expression of proopiomelanocortin (POMC, the precursor of MSH) and its receptor melanocortin 1 receptor (MC1-R), TYR and TYRP1, protein kinase C (PKC), and other signalling factors [28,29], and increases also the production of endothelin-1 (ET-1) and POMC by keratinocytes [30,31] and those peptides can then act in a paracrine manner to stimulate melanocytes.

In addition, keratinocytes and fibroblasts produce cytokines,

growth factors, and inflammatory mediators that can increase melanin production and/or stimulate melanin transfer to keratinocytes by melanocytes.  $\alpha$ -MSH, ACTH, basic Fibroblast Growth Factor (bFGF), Nerve Growth Factor (NGF), endothelins, Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), stem cell factors, Leukemia Inhibitory Factor (LIF), and Hepatocyte Growth Factor (HGF) are keratinocyte-derived factors involved in the regulation of the proliferation and/or differentiation of melanocytes [32] (Figure 3).

The main factors that regulate the quantity and quality of the melanin produced by melanocytes include  $\alpha$ -MSH, MC1-R, Agouti signalling peptide (ASP), ET-1, prostaglandins (PGEs), bFGF, SCF and HGF [1,6].

$\alpha$ -MSH is a tridecapeptide with a sequence identical to the first 13 amino acids of ACTH. The proteolytic cleavage of proopiomelanocortin, on the pituitary gland, is responsible for the origin of  $\alpha$ -MSH [18]. Human keratinocytes and melanocytes are capable of synthesizing  $\alpha$ -MSH at physiological quantities [6,19,31,33].  $\alpha$ -MSH and ACTH are produced in and released by keratinocytes and are involved in regulating melanogenesis and dendrite formation. They bind to a melanocyte-specific receptor, MC1-R [34], which activates adenylate cyclase through a G protein, which then elevates cAMP from adenosine triphosphate. Cyclic AMP exerts its effect in part through Protein Kinase A (PKA), which phosphorylates and activates the cAMP



Response Element Binding Protein (CREB) that binds to the cAMP Response Element (CRE) present in the M promoter of the MITF gene [35,36]. The increase in MITF-M expression induces the up-regulation of TYR, TYRP1, and TRP-2 leading to melanin synthesis. Notably, it has been well established the activation of MC1-R influences the relative quantities of pheomelanin and eumelanin produced, and its activity loss is associated to red or yellow hair; variants of MC1-R have been associated with red hair inheritance, in which more yellow-reddish pheomelanin pigment is produced and they present very small tanning capacity [19,37,38]. In 1994, the discovery of a peptide consisting of 131 amino acids acting as an inverse agonist at MC1 was reported, the ASP [39]. In mice, the agouti gene encodes a paracrine signalling molecule that causes hair follicle melanocytes to synthesize the yellow pigment pheomelanin instead of the black or brown pigment eumelanin.

Endothelin-1 is a 21 amino acid peptide with vasoactive properties synthesized and secreted by keratinocytes after UV-R exposure [1]. Binding of ET-1 to its G protein-coupled receptor (ETBR) on melanocytes activates a cascade of signaling pathways, resulting in calcium mobilization, PKC activation, raise of cAMP levels, and activation of mitogen-activated protein kinase (MAPK). The ET-1 effect is the increase of melanocyte dendricity and the enhancement of melanocyte migration and melanisation [40]. Interestingly, UV-R stimulates keratinocytes to produce interleukin-1 (IL-1) that induce ET-1 expression in keratinocytes in an autocrine manner. These intracellular events in keratinocytes lead to increased TYR mRNA, protein, and enzymatic activity in neighboring melanocytes as well as to an increase in melanocyte number [41].

Prostaglandins PGE2 and PGF2 $\alpha$  are known to be produced/released from keratinocytes by the stimulation of proteinase-activated receptor 2 (PAR-2). PGE2 and PGF2 $\alpha$  stimulate the cAMP-independent dendritogenesis, through EP1, EP3, and FP receptors [42].

SCF and FGF are expressed by keratinocytes and are involved in proliferation and melanogenesis/dendritogenesis of melanocytes [43-46].

HGF binds to its specific receptor, c-Met, activates MAPK, eliciting the up-regulation of proteins required for melanocyte proliferation [47-49].

GM-CSF binds to its specific receptor, GM-CSFR, activates the signal transducer and activator of transcription (STAT-1, STAT-3, and STAT-5) or MAPK, inducing the up-regulation of proteins required for the proliferation of melanocytes, and TYR, TYRP1 [50-53].

Finally, the molecular and cellular mechanisms involved in melanosome transfer to keratinocytes are not completely understood yet. Studies of the keratinocyte receptor PAR-2 suggested it controls the melanosome ingestion and phagocytosis by keratinocytes. Moreover, PAR-2 is induced by UV irradiation and inhibition of PAR-2 activation results in the prevention of UVB-induced tanning [54].

In summary, the epidermis has a complex network that secretes as well as responds to autocrine and paracrine factors produced by keratinocytes and melanocytes. It is likely that the melanocyte proliferation requires the cross talking of several signaling pathways (including the cAMP/PKA, PKC, and tyrosine kinase pathways), and the mechanisms by which various factors increase skin pigmentation are closely inter-related.

## Melasma Pathogenesis

Up or down regulation of the interconnected network so far described is intrinsically involved in the alteration of melanocytic

functions occurring in many epidermal pigmentation disorders [55]. In literature, it has been evidenced that in most hyperpigmentation syndromes multiple pathways regulating melanoblast differentiation/migration, melanogenesis and melanocyte proliferation are simultaneously affected.

Among skin pigmentation disorders, a typical melanogenesis dysfunction characterized melasma, a chronic acquired hypermelanosis of the skin [56,57]. Melasma common presentation consists of facial hyperpigmented macules, which become more evident after sun exposure. It may affect both sexes and all races, but it occurs more often in Asian or Hispanic people with intermediate phototypes. It is more common in adult women in childbearing age, but its onset can also be after menopause. The age of onset is usually between 30-55 years and men account for 10% of cases [58-60].

In melasma, the melanocytes are enlarged and highly dendritic, as in a hypermetabolic state, and an increase in melanin deposition in epidermis and dermis is evidenced [61,62].

There are numerous factors involved in the aetiology of the disease, including genetic influences, endocrinopathies, pregnancy, exposure to UV-R, distress, hormone therapy, drugs and cosmetics; among all these, it seems that genetic predisposition and exposure to sun radiation play the pivotal role.

During pregnancy, increased levels of estrogen, progesterone and MSH have been associated with melasma. In addition, oral contraceptives have been linked to skin hyperpigmentation; it has been speculated that increased levels of estrogens may stimulate the activity of melanocytes [63]. Indeed, melanocytes express estrogen receptors and estradiol stimulates melanogenesis enzymes, such as TYR, TRP1, and TRP2 [64]. Moreover,  $\beta$ -estradiol increases the expression of  $\alpha$ -MSH and MC1-R in melanocytes [65]. In addition, a case report study demonstrated an increased expression of estrogen receptors on skin in two patients with melasma [66].

A strong  $\alpha$ -MSH immunoreactivity on skin with melasma was suggested by immunohistochemical findings. A strong expression of  $\alpha$ -MSH antigen in keratinocytes of melasma-affected skin suggested that  $\alpha$ -MSH plays a key role in the hyperpigmentation [58,67]. Probably, persistent overexpression of  $\alpha$ -MSH following UV exposure contributes to the development of melasma [67]. Nonetheless, the exact pathogenesis remains to be elucidated.

Other hypotheses on melasma pathogenesis include a) an up-regulation of genes modulating Wnt and prostaglandin pathways [68]; b) the involvement of non-coding RNA (H19 gene) [69]; c) the UV-mediated increase in inducible nitric oxide synthase (iNOS) levels, which can activate the AKT-NF $\kappa$ B pathway [70,71]. Finally, a genetic predisposition has been suggested in melasma development by reports of family occurrence [72].

## Topical treatments for melasma and drugs affecting melanogenesis

Open clinical trials, randomized controlled and non-randomized trials about the interventions in the treatment of melasma evidenced that the conventional treatments for melasma include sunscreens, cosmetic camouflage, bleaching creams, acne creams, topical retinoids, chemical peels and laser therapy [73]. Furthermore, some treatments incorporate a combination approach; the most popular combination is a triple-combination cream consisting of hydroquinone, tretinoin, and steroid [74].

DRUG (name)	Biochemical Effects	Ref
<b>Hydroquinone (HQ, 1,4-dihydroxybenzene)</b>	HQ has been used for more than 50 years and is the standard drug for the treatment of facial hyperpigmentation. It acts by inhibition of tyrosinase, thus arrests the conversion of DOPA to melanin. Other proposed mechanisms of action are degradation of melanosomes and inhibition of DNA and RNA synthesis. The efficacy of hydroquinone depends on several factors, such as location of pigment and vehicle of administration.	77-79
<b>Mequinol (4-hydroxyanisole, hydroquinone monomethyl ether)</b>	Mequinol is a derivative of hydroquinone. It is thought to be a substrate of tyrosinase, and acts as a competitive inhibitor of the enzyme formation of melanin precursors.	80
<b>Retinoids</b>	Retinoids reduce hyperpigmentation through many mechanisms, such as stimulation of keratinocyte turnover and reduction of melanosome transfer. Retinoids inhibit tyrosinase transcription, interfere with melanin synthesis and inhibit tyrosinase-related proteins 1 and 2. <b>Tretinoin</b> (retinoic acid, RA, vitamin A acid) is thought to have an effect on tyrosinase by inhibiting the enzyme's transcription, as well as on dopachrome conversion factor, with a resulting interruption of melanin synthesis. RA reduces hyperpigmentation also through the induction of skin desquamation. <b>Adapalene</b> is a naphthoic acid derivative with retinoid activity, controlling cell proliferation/differentiation. It has also significant anti-inflammatory actions.	81-84
<b>Azelaic acid (9-carbon dicarboxylic acid)</b>	Azelaic acid is a compound derived from <i>Pityrosporum ovale</i> . It acts as a weak, reversible, competitive inhibitor of tyrosinase in vitro. Moreover, it has antiproliferative and cytotoxic effects on melanocytes, via inhibition of mitochondrial oxidoreductase activity and DNA synthesis.	85
<b>N-acetyl-4-S-cysteaminylphenol (NCAP)</b>	NCAP is a synthetic compound bearing phenol, catechol, and sulphur moieties. It acts as inhibiting the tyrosinase's activity as alternative substrate for it. It is more stable and causes less irritation than HQ.	86
<b>Kojic acid (5-hydroxy-2-hydroxymethyl-4H-pyran-4-one)</b>	Kojic acid is an antibiotic generated by many species of <i>Aspergillus</i> , <i>Acetobacter</i> and <i>Penicillium</i> . It inhibits tyrosinase through chelation of copper at the enzyme's active site. Moreover, it has NFκB activation-inhibitory effects in keratinocytes and is a potent antioxidant.	87-92
<b>Topical steroids</b>	It is well known that reversible hypopigmentation of normal skin is an untoward effect of prolonged potent steroid application, but the mechanism of this effect is still to clarify. Corticosteroids show inhibitory effects on the synthesis of prostaglandin and leukotriens and this action may partly explain their effects on melanogenesis.	93-95
<b>Glycolic acid</b>	Glycolic acid is an alpha-hydroxy acid that directly inhibits tyrosinase. In addition, it acts on epidermal remodeling and accelerated skin desquamation.	96
<b>Ascorbic acid (Vitamin C)</b>	Vitamin C has antioxidant properties and reduces melanogenesis by interacting with copper at the active site of tyrosinase. It also reduces DOPAquinone by blocking dihydrochindol-2-carboxyl acid oxidation. Because of its instability in aqueous solution, the magnesium ascorbyl-2-phosphate (MAP) ester has been used. Often acid ascorbic is in association with Iontophoresis in order to increase the penetration of vitamin C into the skin.	97-100
<b>Liquorice derivatives</b>	Liquorice is the root of the <i>Glycyrrhiza glabra</i> . Active drugs are glabridin, which inhibits tyrosinase in vitro, liquiritin, which disperse melanin, and isoliquiritin containing flavonoids. Liquorice extract has also anti-inflammatory properties in experimental studies.	101, 102
<b>Soy</b>	Soybean trypsin inhibitor reversibly inhibits the protease-activated receptor-2 pathway. Impaired activation of this receptor in keratinocytes, resulting in the accumulation of melanosomes within melanocytes. Inhibition of this receptor therefore blocks melanosome transfer between these cells, thus also blocking the dispersion of pigment to keratinocytes.	103, 104
<b>Niacinamide (nicotinamide, vitamin B3).</b>	Niacinamide reduces pigmentation by reversibly preventing the transfer of melanosomes from melanocytes to the keratinocytes, without effect on tyrosinase activity.	105, 106
<b>N-Acetylglucosamine (NAG)</b>	The carbohydrate NAG represents the monomeric unit of chitin. It acts by inhibiting the conversion of protyrosinase to tyrosinase. NAG decreases melanin synthesis and downregulate the pigmentation-related gene expression.	107
<b>Lignin peroxidase</b>	Lignin peroxidase is a novel method of skin lightening and acts by targeting, enzymatically oxidizing and breaking down melanin in the skin. It acts with efficacy parity to hydroquinone.	108

**Table 2:** Classical agents commonly proposed in melasma treatment

Many known substances can reduce the level of skin pigmentation, mostly having a tyrosinase-inhibiting effect that lead to reduced total melanin production (e.g. hydroquinone, kojic acid). Other drugs show an effect on the melanin transfer from melanocytes to keratinocytes, causing an overall lighter skin colour (e.g. nicotinamide and soybean). The increase in the desquamation of the skin is also commonly used to remove excessive melanin content within the skin (e.g. retinoic acid). Other agents act as inhibitors of the inflammation-induced melanogenic response mechanisms [75]. A recent review of randomized controlled trials on interventions for melasma evidenced that, although there was poor methodology, a lack of standardized outcome assessments and short duration of studies, the current limited evidence supports the efficacy of multiple interventions [76].

Although melasma can be difficult to treat and the prophylactic management is often the most effective means of prevention, some of the most important agents, commonly used against melasma, are reported in Table 2.

Despite the wide availability of classical agents currently used in melasma, the treatment of this skin disease is usually dissatisfactory,

above all due to the great recurrence of lesions and due to the absence of a definitive whitening alternative.

In the light of unsuccessful action of current therapies, a number of agents, both synthetic and derived from natural sources, have been investigated for their potential role in reducing melanin pigmentation. Other agents either or combined with other products are currently under investigations to enhance skin-lightening effects. Although earlier experimental evidence suggests possible benefits, controlled clinical trials are mostly lacking. Some of these compounds are reported in Table 3.

## Final Considerations

Skin-color is due to complex processes including tyrosinase reactions, formation of melanosomes in melanocytes, transfer and organization in the keratinocytes. Although the knowledge of melanocyte biology has made significant advancement, the pathogenic mechanisms underlying acquired hyperpigmentation, such as melasma, have to be fully elucidated yet. However, the research has led to development of safer and enough effective skin-lightening drugs, mainly targeting the rate-limiting enzyme of melanogenesis,

Biochemical Effect	Molecules under investigation	Ref
Regulation of tyrosinase and related enzymes		
Inhibition of tyrosinase activity	<p><b>Gentisic acid</b> and its methyl ester (2,5-dihydroxybenzoic acid) are natural products of Gentiana root. They inhibit melanin synthesis in melanocytes.</p> <p><b>4-n-butylresorcinol</b> effectively inhibited tyrosinase activity in a cell-free system, as an effective direct tyrosinase inhibitor in a mouse melanocytic cell line.</p> <p><b>p-coumaric acid</b> did not directly inhibit tyrosinase activity, but a competitive inhibition was demonstrated between p-coumaric acid and tyrosine, indicating that an alternative substrate of tyrosine can be used to induce hypopigmenting effects in cells.</p> <p><b>Arbutin</b>, derived from the bearberry plant, is a D-glucopyranoside derivative of hydroquinone. Arbutin is hydrolyzed by the normal skin microflora to hydroquinone; it produces skin lightening by direct, dose-dependent inhibition of tyrosinase.</p>	109-115
Decreased tyrosinase production	<p><b>Sphingosine-1-phosphate (sp-1)</b> caused the sustained ERK activation, resulting in MITF phosphorylation and degradation, which are in turn responsible for reduced melanin synthesis.</p> <p><b>Transforming growth factor-β1 (TGF-β1)</b> induced a significant delay in ERK activation and ERK-induced MITF downregulation, which could contribute to hypopigmentation.</p> <p>Lysophosphatidic acid and C2 ceramides modulated AKT/protein kinase B or ERK, and were able to induced MITF degradation and blocked MITF expression, respectively by</p> <p><b>Sphingosylphosphorylcholine</b> inhibited melanogenesis via ERK-dependent transcriptional regulation of the tyrosinase gene.</p>	116-121
Increased tyrosinase degradation	<p><b>Fatty acids</b> have been demonstrated to affect melanogenesis. The mechanism is complex, as unsaturated linolenic, linoleic and oleic acids reduce tyrosinase activity, while saturated palmitic or stearic acids increase it. The number of melanosomes and the level of tyrosinase mRNA did not appear to be influenced, suggesting hypopigmenting effect due to a reduction in the amount of tyrosinase, probably caused by stimulation of tyrosinase ubiquitination and proteasomal degradation.</p> <p><b>Phospholipase D2</b> also reduces melanogenesis via the mechanism of ubiquitin-mediated degradation of tyrosinase.</p>	122-125
Modification of tyrosinase proteins	<p><b>Glucosamine</b> and <b>unicamycin</b> are specific inhibitors of lipid carrier-dependent glycosylation and induce marked hypopigmentation, altering tyrosinases glycosylation. Calcium D-pantetheine- S-sulfonate, probably generated via the alteration of tyrosinase and TRP-1 glycosylation, exerts an inhibitory effect on melanogenic enzymes, without affecting their expression, and causes reversible hypopigmentation in normal human melanocytes.</p>	126, 127
Multi-actions	<p><b>Terrein</b>, a fungal metabolite isolated from a Penicillium species, is a hypopigmenting agent that inhibits melanogenesis by dual actions, including the downregulation of tyrosinase transcription (via ERK inhibition) and the upregulation of degradation (via ubiquitin- dependent proteasomal degradation induction).</p> <p>α-MSH can increase melanin synthesis by binding to 6(R)-L-erythro-5,6,7,8-tetrahydrobiopterin (<b>6BH4</b>), a competitive inhibitor of tyrosinase. 6BH4 analogues such as 6,7-(R,S)-dimethyl-tetrahydropterine and 6-(R,S)-tetrahydromonapterine have been studied as possible tyrosinase inhibitors, and it has been suggested that these compounds, like 6BH4, can act through an uncompetitive allosteric mechanism. It has been demonstrated that 6BH4 (and their analogues) also reduces o-dopaquinone non-enzymatically.</p>	128-134
Regulation of melanosome formation		
Interference with melanosome maturation	<p><b>TGF-β1</b> added to melanocytes arrested the melanosome maturation to stage III.</p> <p>A decreased number of pigmented melanosomes was detected in <b>sp-1</b>-treated melanocytes and the presence of undifferentiated earlystage melanosomes, whereas the control cells produced melanosomes with internal fibrils and dense pigmentation.</p>	135, 136
Peroxidase inhibitors	<p><b>Methimazole</b> is an antithyroid agent belonging to the thionamide group, which inhibits both mushroom tyrosinase and peroxidase. In the melanogenic intermediate polymerization, peroxidase is involved; peroxidase inhibition has been shown to inhibit melanization.</p>	136-138
Interference with melanosome transfer	<p><b>Centaureidine</b> is a flavonoid glucoside isolated from yarrow able to inhibit protease-activated receptor 2 in keratinocyte. It reduces dendritic growth and the transfer of melanosomes to keratinocytes. The inhibition of serine protease has been shown to result in impaired activation of protease-activated receptor 2 in keratinocytes, resulting in the accumulation of melanosomes within melanocytes that therefore blocks melanosome transfer.</p>	103, 104, 139
Other mechanisms		
Diverse antioxidants and various mechanisms	<p>Compounds with antioxidant properties exert hypopigmenting effects by interacting with copper at the active site of tyrosinase, or avoiding the oxidative polymerization of melanin intermediates, or inhibit the signaling process, enabling the stimulation of melanogenesis by ROS after sun exposure.</p> <p><b>α-Tocopherol</b> (α-Toc) interferes with the membrane lipid peroxidation and increases intracellular glutathione content. It inhibits tyrosinase and melanogenesis in melanocytes. The alpha-tocopheryl ferulate, a compound consisting of alpha-tocopherol and ferulic acid, can absorb ultraviolet radiation was found to have significant effect in the retardation of melanogenesis, possibly by inhibiting tyrosine hydroxylase activity in an indirect manner.</p> <p><b>6-Hydroxy-3,4-dihydrocoumarins</b> are antioxidants with α-Toc-like chemical structures that have recently been reported to exert anti-melanogenic effects in cultured normal human melanocytes at non-cytotoxic concentrations, without interfering with tyrosinase activity.</p> <p>These agents might act via acceleration of glutathione synthesis and inhibition of tyrosinase transfer.</p> <p><b>Thioctic acid</b> (α-lipoic acid) prevents UV-induced oxidative damage, principally via the down-modulation of NF-κB activation, and inhibit tyrosinase activity, probably by chelating its copper ions.</p> <p><b>Flavonoids</b> are natural polyphenolic compounds characterized by ROS-scavenging properties and ability to chelate metals at the metalloenzyme active site. These polyphenols have well-known anti-inflammatory, antioxidant, antiviral, and anticarcinogenic properties. A number of flavonoids are frequently used in skin-lightening preparations. <b>Aloesin</b> has been proven to competitively inhibit tyrosinase but also been shown to inhibit TH and DOPA oxidase activities. Some of the more efficient pigment-lightening flavonoid subcategories are the <b>hydroxystilbene</b> compounds, of which resveratrol is one common example. Resveratrol is found in red wine and has been shown to reduce not only tyrosinase activity but also MITF expression in B16 mouse melanoma cells.</p> <p>Other plant-derived flavonoid compounds are still under investigation, such as catechin conjugated with <b>gallic acid</b>, and <b>ellagic acid</b>. <b>Taxifolin</b> and <b>luteolin</b> were shown to inhibit effectively tyrosinase-catalysed oxidation of L-dihydroxyphenylalanine and thereby reducing melanogenesis.</p> <p>Anyway, there are some controversies however regarding the use of flavonoids in skin-lightening preparations, as some flavonoids are known to increase melanogenesis, such as the citrus flavonoid naringenin or quercetin.</p>	99, 140-152

<b>Inhibitors of inflammation-induced melanogenic response</b>	<i>Matricaria chamomilla</i> extract inhibited UV-induced pigmentation by avoiding ET-1-induced DNA synthesis but not interleukin- $\alpha$ -induced ET-1 production and tyrosinase activation. <b>Glabridin</b> , from licorice extracts, inhibits cyclooxygenase activity and superoxide anion production suggesting that its anti-inflammatory effect involves interference with the arachidonic acid cascade, and that protection against oxidative stress performs a key role in modulating melanogenesis.	101, 153
<b>Modulators of <math>\beta</math>2-adrenoreceptors and catecholamines.</b>	From animal studies on proopiomelanocortin-deficient mice, it has been proposed an alternative cAMP-dependent pathway turning on melanogenesis: the adrenergic system. Human epidermal melanocytes express $\beta$ 2-adrenergic receptors ( $\beta$ 2-AR) that when activated increase melanin synthesis. <b><math>\beta</math>2-AR antagonists</b> blocked UV-induced melanogenesis.	154-157
<b>Modulation of sex hormones</b>	Sex hormones affect several functions of human skin; <b>oestrogens</b> have been implicated in skin aging, pigmentation, hair growth, sebum production and skin cancer, while <b>androgens</b> affect sebaceous gland growth and differentiation, hair growth, epidermal barrier homeostasis and wound healing. Epidermal melanocytes are oestrogen responsive, but there are conflicting reports in the literature concerning their effect on pigmentation. A large series of case studies has shown that pregnant women and women on hormonal contraception have increased prevalence of melasma. The androgen precursor dehydroepiandrosterone was shown to reduce skin pigmentation when taken orally.	158-162
<b>Other modulators</b>	As transcriptional regulator of tyrosinase, MITF plays a critical role in the regulation of melanogenesis. A negative regulator of the Wnt signaling pathway, the protein <b>DKK1</b> , decreased the levels of MITF, and therefore inhibited melanocyte growth and pigment production. <b>Calpain inhibitors</b> have been shown to cause marked reductions in both tyrosinase and its mRNA levels in B16 cells. <b>Glutamatergic receptors</b> have been shown to affect specifically MITF expression, and blockage of the ionotropic glutamatergic receptors resulted in a sharp reduction in the MITF expression. Inhibition of these receptors caused rapid morphological changes in melanocyte associated with microfilament disorganization.	131, 163-166

**Table 3:** Investigated compounds and their possible benefits

tyrosinase. Different hypopigmenting agents have been discussed based on a review of the literature. Moreover, other potential targets for control of human pigmentation have been described and new drugs under investigation were reported. Nevertheless, there are currently no guidelines for the management of melasma and the comparisons among outcomes are difficult.

## References

- Costin GE, Hearing VJ (2007) Human skin pigmentation: melanocytes modulate skin color in response to stress. *FASEB J* 21: 976-994.
- Jablonski NG, Chaplin G (2000) The evolution of human skin coloration. *J Hum Evol* 39: 57-106.
- Tobin DJ (2006) Biochemistry of human skin—our brain on the outside. *Chem Soc Rev* 35: 52-67.
- Robins AH (1991) *Biological Perspectives on Human Pigmentation*, Cambridge University Press, Cambridge, UK.
- Ito M, Liu Y, Yang Z, Nguyen J, Liang F, et al. (2005) Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. *Nat Med* 1: 1351-1354.
- Lin JY, Fisher DE (2007) Melanocyte biology and skin pigmentation. *Nature* 445: 843-850.
- Sulaimon SS, Kitchell BE (2003) The biology of melanocytes. *Vet Dermatol* 14: 57-65.
- Boissy RE (1988) The melanocyte. Its structure, function, and subpopulations in skin, eyes, and hair. *Dermatol Clin* 6: 161-173.
- Rousseau B, Dubayle D, Sennlaub F, Jeanny JC, Costet P, et al. (2000) Neural and angiogenic defects in eyes of transgenic mice expressing a dominant-negative FGF receptor in the pigmented cells. *Exp Eye Res* 71: 395-404.
- Fitzpatrick TB, Breathnach AS (1963) The epidermal melanin unit system. *Dermatol Wochenschr* 147: 481-489.
- Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF (1999) *Dermatology in General Medicine*. McGraw-Hill, New York, USA.
- Rouzaud F, Kadekaro AL, Abdel-Malek ZA, Hearing VJ (2005) MC1R and the response of melanocytes to ultraviolet radiation. *Mutat Res* 571: 133-152.
- Gillbro JM, Olsson MJ (2011) The melanogenesis and mechanisms of skin-lightening agents—existing and new approaches. *Int J Cosmet Sci* 33: 210-221.
- Kwon BS, Haq AK, Pomerantz SH, Halaban R (1987) Isolation and sequence of a cDNA clone for human tyrosinase that maps at the mouse c-albino locus. *Proc Natl Acad Sci USA* 84: 7473-7477.
- Hearing VJ, Jimenez M (1987) Mammalian tyrosinase—the critical regulatory control point in melanocyte pigmentation. *Int J Biochem* 19: 1141-1147.
- Murisier F, Beermann F (2006) Genetics of pigment cells: lessons from the tyrosinase gene family. *Histol Histopathol* 21: 567-578.
- Ito S, Wakamatsu K (2003) Quantitative analysis of eumelanin and pheomelanin in humans, mice, and other animals: a comparative review. *Pigment Cell Res* 16: 523-531.
- Ito S (2003) A chemist's view of melanogenesis. *Pigment Cell Res* 16: 230-236.
- Thody AJ, Graham A (1998) Does alpha-MSH have a role in regulating skin pigmentation in humans? *Pigment Cell Res* 11: 265-274.
- Hearing VJ (2005) Biogenesis of pigment granules: a sensitive way to regulate melanocyte function. *J Dermatol Sci* 37: 3-14.
- Boissy RE (2003) Melanosome transfer to and translocation in the keratinocyte. *Exp Dermatol* 12: 5-12.
- Osawa M (2009) Melanocyte stem cells. *StemBook*, ed. The Stem Cell Research Community, StemBook.
- Hodgkinson CA, Moore KJ, Nakayama A, Steingrimsson E, Copeland NG, et al. (1993) Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. *Cell* 74: 395-404.
- Hughes MJ, Lingrel JB, Krakowsky JM, Anderson KP (1993) A helix-loop-helix transcription factor-like gene is located at the mi locus. *J Biol Chem* 268: 20687-20690.
- Vance KW, Goding CR (2004) The transcription network regulating melanocyte development and melanoma. *Pigment Cell Res* 17: 318-325.
- Goding CR (2000) Mitf from neural crest to melanoma: signal transduction and transcription in the melanocyte lineage. *Genes Dev* 14: 1712-1728.
- Chiaverini C, Beuret L, Flori E, Busca R, Abbe P, et al. (2008) Microphthalmia-associated transcription factor regulates RAB27A gene expression and controls melanosome transport. *J Biol Chem* 283: 12635-12642.
- Chakraborty AK, Funasaka Y, Slominski A, Ermak G, Hwang J, et al. (1996) Production and release of proopiomelanocortin (POMC) derived peptides by human melanocytes and keratinocytes in culture: regulation by ultraviolet B. *Biochim Biophys Acta* 1313: 130-138.
- Funasaka Y, Chakraborty A K, Hayashi Y, Komoto M, Ohashi A, et al. (1998) Modulation of melanocyte-stimulating hormone receptor expression on normal human melanocytes: evidence for a regulatory role of ultraviolet B, interleukin-1 alpha, interleukin-1 beta, endothelin-1 and tumor necrosis factor-alpha. *Br J Dermatol* 139: 216-224.
- Tada A, Suzuki I, Im S, Davis M B, Cornelius J, et al. (1998) Endothelin-1 is a paracrine growth factor that modulates melanogenesis on human melanocytes and participates in their responses to ultraviolet radiation. *Cell Growth Differ* 9: 575-584.



31. Abdel-Malek Z, Scott MC, Suzuki I, Tada A, Im S, et al. (2000) The melanocortin-1 receptor is a key regulator of human cutaneous pigmentation. *Pigment Cell Res* 8: 156–162.
32. Hirobe T (2005) Role of keratinocyte-derived factors involved in regulating the proliferation and differentiation of mammalian epidermal melanocytes. *Pigment Cell Res* 18: 2–12.
33. Thody AJ. (1999) alpha-MSH and the regulation of melanocyte function. *Ann N Y Acad Sci* 885: 217–29.
34. Cone RD, Lu D, Koppula S, Vage DI, Klunghand H, et al. (1996) The melanocortin receptors: agonists, antagonists, and the hormonal control of pigmentation. *Recent Prog Horm Res* 5: 287–317.
35. Busca R, Ballotti R (2000) Cyclic AMP a key messenger in the regulation of skin pigmentation. *Pigment Cell Res* 13: 60–69.
36. Tachibana M (2000) MITF: a stream flowing for pigment cell. *Pigment Cell Res* 13: 230–240.
37. Abdel-Malek ZA, Scott MC, Furumura M, Lamoreux ML, Ollmann M, et al. (2011) The melanocortin 1 receptor is the principal mediator of the effects of agouti signaling protein on mammalian melanocytes. *J Cell Sci* 114: 1019–1024.
38. Voisey J, Carroll L, van Daal A (2003) Melanocortins and their receptors and antagonists. *Curr Drug Targets*. 4: 586–597.
39. Lu D, Willard D, Patel IR, Kadwell S, Overton L, et al. (1994) Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. *Nature* 37: 709–802.
40. Hara M, Yaar M, Gilchrist BA (1995) Endothelin-1 of keratinocyte origin is a mediator of melanocyte dendricity. *J Invest Dermatol* 105: 744–748.
41. Imokawa G, Miyagishi M, Yada Y (1995) Endothelin-1 as a new melanogen: coordinated expression of its gene and the tyrosinase gene in UVB-exposed human epidermis. *J Invest Dermatol* 105: 32–37.
42. Scott G, Leopardi S, Printup S, Malhi M, Seiberg M, et al. (2004) Proteinase-activated receptor-2 stimulates prostaglandin production in keratinocytes: analysis of prostaglandin receptors on human melanocytes and effects on PGE2 and PGF2alpha on melanocyte dendricity. *J Invest Dermatol* 122: 1214–1224.
43. Hachiya A, Kobayashi A, Ohuchi A, Takema Y, Imokawa G (2001) The paracrine role of stem cell factor/c-kit signaling in the activation of human melanocytes in ultraviolet- B-induced pigmentation. *J Invest Dermatol* 116: 578–586.
44. Halaban R, Langdon R, Birchall N, Cuono C, Baird A, et al. (1988) Basic fibroblast growth factor from human keratinocytes is a natural mitogen for melanocytes. *J Cell Biol* 107: 1611–1619.
45. Schauer E, Trautinger F, Köck A, Schwarz A, Bhardwaj R, et al. (1994) Proopiomelanocortin-derived peptides are synthesized and released by human keratinocytes. *J Clin Invest* 93: 2258–2262.
46. Imokawa G, Yada Y, Kimura M, Morisaki N (1996) Granulocyte/macrophage colony-stimulating factor is an intrinsic keratinocyte-derived growth factor for human melanocytes in UVA-induced melanosis. *Biochem J* 313: 625–631.
47. Bottaro DP, Rubin JS, Faletto DL, Chan AM, Kmiecik TE, et al. (1991) Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. *Science* 25: 802–804.
48. Halaban R, Tyrrell L, Longley J, Yarden Y, Rubin J (1993) Pigmentation and proliferation of human melanocytes and the effects of melanocyte-stimulating hormone and ultraviolet B light. *Ann N Y Acad Sci* 680: 290–301.
49. Matsumoto K, Tajima H, Nakamura T (1991) Hepatocyte growth factor is a potent stimulator of human melanocyte DNA synthesis and growth. *Biochem Biophys Res Commun* 176: 45–51.
50. Chiba S, Shibuya K, Miyazono K, Tojo A, Oka Y, et al. (1990) Affinity purification of human granulocyte macrophage colony-stimulating factor receptor alpha-chain. Demonstration of binding by photoaffinity labeling. *J Biol Chem* 265: 19777–19781.
51. Mui AL, Wakao H, O'Farrell AM, Harada N, Miyajima A (1995) Interleukin-3, granulocyte-macrophage colony stimulating factor and interleukin-5 transduce signals through two STAT5 homologs. *EMBO J* 14: 1166–1175.
52. Wang Y, Morrella KK, Ripperger J, Lai CF, Gearing DP, et al. (1995) Receptors for interleukin-3 (IL-3) and growth hormone mediate an IL-6-type transcriptional induction in the presence of JAK2 or STAT3. *Blood* 86: 1671–1679.
53. Okuda K, Sanghera JS, Pelech SL, Kanakura Y, Hallek M, et al. (1992) Granulocyte macrophage colony-stimulating factor, interleukin-3, and steel factor induce rapid tyrosine phosphorylation of p42 and p44 MAP kinase. *Blood* 79: 2880–2887.
54. Seiberg M (2001) Keratinocyte-melanocyte interactions during melanosome transfer. *Pigment Cell Res* 14: 236–242.
55. Speeckaert R, Van Gele M, Speeckaert MM, Lambert J, van Geel N (2014) The biology of hyperpigmentation syndromes *Pigment Cell Melanoma Res* 27: 512–524.
56. Sheth VM, Pandya AG (2011) Melasma: a comprehensive update: part I. *J Am Acad Dermatol* 65: 689–697.
57. Sheth VM, Pandya AG (2011) Melasma: a comprehensive update: part II. *J Am Acad Dermatol* 65: 699–714.
58. Kang WH, Yoon KH, Lee ES, Kim J, Lee KB, et al. (2002) Melasma: histopathological characteristics in 56 Korean patients. *Br J Dermatol* 146: 228–237.
59. Piamphongsant T (1998) Treatment of melasma: a review with personal experience. *Int J Dermatol* 3: 897–903.
60. Guevara IL, Pandya AG (2001) Melasma treated with hydroquinone, tretinoin, and a fluorinated steroid. *Int J Dermatol* 40: 212–215.
61. Grimes PE, Yamada N, Bhawan J (2005) Light microscopic, immunohistochemical, and ultrastructural alterations in patients with melasma. *Am J Dermatopathol* 27: 96–101.
62. Kosmadaki M, Kontochristopoulos G (2011). Disorders of cutaneous pigmentation. In *Manual of Gender Dermatology*, Parish, Brenner, Ramos-e-Silva, Parish, eds. Jones & Bartlett Learning, London, UK.
63. Wong RC, Ellis CN (1984) Physiologic skin changes in pregnancy. *J Am Acad Dermatol* 10: 929–940.
64. Kippenberger S, Loitsch S, Solano F, Bernd A, Kaufmann R (1998) Quantification of tyrosinase, TRP-1 and Trp-2 transcripts in human melanocytes by reverse transcriptase-competitive multiplex PCR-regulation by steroid hormones. *J Invest Dermatol* 110: 364–367.
65. Scott MC, Suzuki I, Abdel-Malek ZA (2002) Regulation of the human melanocortin 1 receptor expression in epidermal melanocytes by paracrine and endocrine factors and by ultraviolet radiation. *Pigment Cell Res* 15: 433–439.
66. Lieberman R, Moy L (2008) Estrogen receptor expression in melasma: results from facial skin of affected patients. *J Drugs Dermatol* 7: 463–465.
67. Im S, Kim J, On W, Kang WH (2007) Increased expression of alpha-melanocyte-stimulating hormone in the lesional skin of melasma. *Br J Dermatol* 146: 165–167.
68. Kang HY, Suzuki I, Lee DJ, Ha J, Reiniche P, et al. (2011). Transcriptional profiling shows altered expression of wnt pathway and lipid metabolism-related genes as well as melanogenesis-related genes in melasma. *J Invest Dermatol* 13: 1692–1700.
69. Kim NH, Lee CH, Lee AY (2010) H19 RNA downregulation stimulated melanogenesis in melasma. *Pigment Cell Melanoma Res* 23: 84–92.
70. Jo HY, Kim CK, Suh IB, Ryu SW, Ha KS, et al. (2009) Co-localization of inducible nitric oxide synthase and phosphorylated Akt in the lesional skins of patients with melasma. *J Dermatol* 36: 10–16.
71. Passeron T (2013) Melasma pathogenesis and influencing factors - an overview of the latest research. *J Eur Acad Dermatol Venereol* 27 Suppl 1: 5–6.
72. Sanchez NP, Pathak MA, Sato S, Fitzpatrick TB, Sanchez JL, et al. (1981) Melasma: a clinical, light microscopic, ultrastructural, and immunofluorescence study. *J Am Acad Dermatol* 4: 698–710.
73. Rajaratnam R, Halpern J, Salim A, Emmett C (2010) Interventions for melasma. *Cochrane Database Syst Rev* : CD003583.
74. Gupta AK, Gover MD, Nouri K, Taylor S (2006) The treatment of melasma: a review of clinical trials. *J Am Acad Dermatol* 55: 1048–1065.
75. Park KC, Huh SY, Choi HR, Kim DS. (2010) Biology of melanogenesis and the search for hypopigmenting agents *Dermatol Sinica* 28: 53–58.
76. Jutley GS, Rajaratnam R2, Halpern J3, Salim A4, Emmett C5 (2014) Systematic review of randomized controlled trials on interventions for melasma: an abridged Cochrane review. *J Am Acad Dermatol* 70: 369–373.

77. Palumbo A d'Ischia M, Misuraca G, Prota G (1991) Mechanism of inhibition of melanogenesis by hydroquinone. *Biochim Biophys Acta* 1073: 85-90.
78. Jimbow K, Obata H, Pathak MA, Fitzpatrick TB (1974) Mechanism of depigmentation by hydroquinone. *J Invest Dermatol* 62: 436-449.
79. Briganti S, Camera E, Picardo M (2003) Chemical and instrumental approaches to treat hyperpigmentation. *Pigment Cell Res* 16: 101-110.
80. Draelos ZD (2007) Cosmetic therapy. In *Comprehensive Dermatologic Drug Therapy*. (2nd edn) Philadelphia, Saunders.
81. Ortonne JP (2006) Retinoid therapy of pigmentary disorders. *Dermatol Ther* 19: 280-288.
82. Roméro C, Aberdam E, Larnier C, Ortonne JP (1994) Retinoic acid as modulator of UVB-induced melanocyte differentiation. Involvement of the melanogenic enzymes expression. *J Cell Sci* 107: 1095-1103.
83. Leenutaphong V, Nettakul A, Rattanasuwon P (1999) Topical isotretinoin for melasma in Thai patients: a vehicle-controlled clinical trial. *J Med Assoc Thai* 82: 868-875.
84. Shroot B (1998) Pharmacodynamics and pharmacokinetics of topical adapalene. *J Am Acad Dermatol* 39: S17-24.
85. Fitton A, Goa KL (1991) Azelaic acid. A review of its pharmacological properties and therapeutic efficacy in acne and hyperpigmentary skin disorders. *Drugs* 41: 780-798.
86. Jimbow K (1991) N-acetyl-4-S-cysteaminylphenol as a new type of depigmenting agent for the melanoepidermis of patients with melasma. *Arch Dermatol* 127: 1528-1534.
87. Halder RM, Richards GM (2004) Topical agents used in the management of hyperpigmentation. *Skin Therapy Lett* 9: 1-3.
88. Kahn V. (1995) Effect of kojic acid on the oxidation of KL-DOPA. Norepinephrine and dopamine by mushroom tyrosinase. *Pigment Cell Res* 8: 234-240.
89. Kim YJ, Uyama H (2005) Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future. *Cell Mol Life Sci* 62: 1707-1723.
90. Lim JT (1999) Treatment of melasma using kojic acid in a gel containing hydroquinone and glycolic acid. *Dermatol Surg* 25: 282-284.
91. Battaini G, Monzani E, Casella L, Santagostini L, Pagliarini R (2000) Inhibition of the catecholase activity of biomimetic dinuclear copper complexes by kojic acid. *J Biol Inorg Chem* 5: 262-268.
92. Moon KY, Ahn KS, Lee J, Kim YS (2001) Kojic acid, a potential inhibitor of NF- $\kappa$ B activation in transfectant human HaCaT and SCC-13 cells. *Arch Pharm Res* 24: 307-311.
93. Gupta AK, Gover MD, Nouri K, Taylor S (2006) The treatment of melasma: a review of clinical trials. *J Am Acad Dermatol* 55: 1048-1065.
94. Kanwar AJ, Dhar S, Kaur S (1994) Treatment of melasma with potent topical corticosteroids. *Dermatology* 188: 170.
95. Neering H (1975) Treatment of melasma (chloasma) by local application of a steroid cream. *Dermatologica* 151: 349-353.
96. Usuki A, Ohashi A, Sato H, Ochiai Y, Ichihashi M, et al. (2003) The inhibitory effect of glycolic acid and lactic acid on melanin synthesis in melanoma cells. *Exp Dermatol* 12 Suppl 2: 43-50.
97. Ros JR, Rodríguez-López JN, García-Cánovas F (1993) Effect of L-ascorbic acid on the monophenolase activity of tyrosinase. *Biochem J* 295: 309-312.
98. Chedekel MR, Smith SK, Post PW, Pokora A, Vessell DL (1978) Photodestruction of pheomelanin: role of oxygen. *Proc Natl Acad Sci U S A* 75: 5395-5399.
99. Picardo M, Carrera M (2007) New and experimental treatments of chloasma and other hypermelanoses. *Dermatol Clin* 25: 353-362, ix.
100. Kobayashi S, Takehana M, Itoh S, Ogata E (1996) Protective effect of magnesium-L-ascorbyl-2 phosphate against skin damage induced by UVB irradiation. *Photochem Photobiol* 64: 224-228.
101. Yokota T, Nishio H, Kubota Y, Mizoguchi M (1998) The inhibitory effect of glabridin from licorice extracts on melanogenesis and inflammation. *Pigment Cell Res* 11: 355-361.
102. Amer M, Metwalli M (2000) Topical liquiritin improves melasma. *Int J Dermatol* 39: 299-301.
103. Seiberg M, Paine C, Sharlow E, Andrade-Gordon P, Costanzo M, et al. (2000) Inhibition of melanosome transfer results in skin lightening. *J Invest Dermatol* 115: 162-167.
104. Seiberg M, Paine C, Sharlow E, Andrade-Gordon P, Costanzo M, et al. (2000) The protease-activated receptor 2 regulates pigmentation via keratinocyte-melanocyte interactions. *Exp Cell Res* 254: 25-32.
105. Greatens A, Hakozaiki T, Koshoffer A, Epstein H, Schwemberger S, et al. (2005) Effective inhibition of melanosome transfer to keratinocytes by lectins and niacinamide is reversible. *Exp Dermatol* 14: 498-508.
106. Hakozaiki T, Minwalla L, Zhuang J, Chhoa M, Matsubara A, et al. (2002) The effect of niacinamide on reducing cutaneous pigmentation and suppression of melanosome transfer. *Br J Dermatol* 147: 20-31.
107. Bissett DL, Farmer T, McPhail S, Reichling T, Tiesman JP, et al. (2007) Genomic expression changes induced by topical N-acetyl glucosamine in skin equivalent cultures in vitro. *J Cosmet Dermatol* 6: 232-238.
108. Draelos ZD (2014) A split-face evaluation of a novel pigment-lightening agent compared with no treatment and hydroquinone. *J Am Acad Dermatol*.
109. Dooley TP, Gadwood RC, Kilgore K, Thomasco LM (1994) Development of an in vitro primary screen for skin depigmentation and antimelanoma agents. *Skin Pharmacol* 7: 188-200.
110. Kim DS, Kim SY, Park SH, Choi YG, Kwon SB, et al. (2005) Inhibitory effects of 4-n-butylresorcinol on tyrosinase activity and melanin synthesis. *Biol Pharm Bull* 28: 2216-2219.
111. Park SH, Kim DS, Park SH, Shin JW, Youn SW, et al. (2008) Inhibitory effect of p-coumaric acid by *Rhodiola sachalinensis* on melanin synthesis in B16F10 cells. *Pharmazie* 63: 290-295.
112. Chawla S, deLong MA, Visscher MO, Wickett RR, Manga P, et al. (2008) Mechanism of tyrosinase inhibition by deoxyarbutin and its second-generation derivatives. *Br J Dermatol* 159: 1267-1274.
113. Bang SH, Han SJ, Kim DH (2008) Hydrolysis of arbutin to hydroquinone by human skin bacteria and its effect on antioxidant activity. *J Cosmet Dermatol* 7: 189-193.
114. Boissy RE, Visscher M, DeLong MA (2005) Deoxyarbutin: a novel reversible tyrosinase inhibitor with effective in vivo skin lightening potency. *Exp Dermatol* 14: 601-608.
115. Hamed SH, Sriwiriyanont P, deLong MA, Visscher MO, Wickett RR, et al. (2006) Comparative efficacy and safety of deoxyarbutin, a new tyrosinase-inhibiting agent. *J Cosmet Sci* 57: 291-308.
116. Kim DS, Hwang ES, Lee JE, Kim SY, Kwon SB, et al. (2003) Sphingosine-1-phosphate decreases melanin synthesis via sustained ERK activation and subsequent MITF degradation. *J Cell Sci* 116: 1699-1706.
117. Kim DS, Park SH, Park KC (2004) Transforming growth factor-beta1 decreases melanin synthesis via delayed extracellular signal-regulated kinase activation. *Int J Biochem Cell Biol* 36: 1482-1491.
118. Kim DS, Park SH, Kwon SB, Youn SW, Park KC (2004) Effects of lysophosphatidic acid on melanogenesis. *Chem Phys Lipids* 127: 199-206.
119. Kim DS, Kim SY, Moon SJ, Chung JH, Kim KH, et al. (2001) Ceramide inhibits cell proliferation through Akt/PKB inactivation and decreases melanin synthesis in Mel-Ab cells. *Pigment Cell Res* 14: 110-115.
120. Kim DS, Kim SY, Chung JH, Kim KH, Eun HC, et al. (2002) Delayed ERK activation by ceramide reduces melanin synthesis in human melanocytes. *Cell Signal* 14: 779-785.
121. Kim DS, Park SH, Kwon SB, Park ES, Huh CH, et al. (2006) Sphingosylphosphorylcholine-induced ERK activation inhibits melanin synthesis in human melanocytes. *Pigment Cell Res* 19: 146-153.
122. Ando H, Ryu A, Hashimoto A, Oka M, Ichihashi M (1998) Linoleic acid and alpha-linolenic acid lightens ultraviolet-induced hyperpigmentation of the skin. *Arch Dermatol Res* 290: 375-381.
123. Ando H, Funasaka Y, Oka M, Ohashi A, Furumura M, et al. (1999) Possible involvement of proteolytic degradation of tyrosinase in the regulatory effect of fatty acids on melanogenesis. *J Lipid Res* 40: 1312-1316.
124. Ando H, Wen ZM, Kim HY, Valencia JC, Costin GE, et al. (2006) Intracellular composition of fatty acid affects the processing and function of tyrosinase through the ubiquitin-proteasome pathway. *Biochem J* 394: 43-50.

125. Ando H, Watabe H, Valencia JC, Yasumoto K, Furumura M, et al. (2004) Fatty acids regulate pigmentation via proteasomal degradation of tyrosinase: a new aspect of ubiquitin-proteasome function. *J Biol Chem* 279: 15427-15433.
126. Mishima Y, Imokawa G (1983) Selective aberration and pigment loss in melanosomes of malignant melanoma cells in vitro by glycosylation inhibitors: premelanosomes as glycoprotein. *J Invest Dermatol* 81: 106-114.
127. Franchi J, Coutadeur MC, Marteau C, Mersel M, Kupferberg A (2000) Depigmenting effects of calcium D-pantetheine-S-sulfonate on human melanocytes. *Pigment Cell Res* 13: 165-171.
128. Park SH, Kim DS, Kim WG, Ryoo IJ, Lee DH, et al. (2004) Terrein: a new melanogenesis inhibitor and its mechanism. *Cell Mol Life Sci* 6: 2878-2885.
129. Park SH, Kim DS, Lee HK, Kwon SB, Lee S, et al. (2009) Long-term suppression of tyrosinase by terrein via tyrosinase degradation and its decreased expression. *Exp Dermatol* 18: 562-566.
130. Schallreuter KU, Moore J, Tobin DJ, Gibbons NJ, Marshall HS, et al. (1999) alpha-MSH can control the essential cofactor 6-tetrahydrobiopterin in melanogenesis. *Ann N Y Acad Sci* 885: 329-341.
131. Schallreuter KU (1995) Beta-adrenergic blocking drugs may exacerbate vitiligo. *Br J Dermatol* 132: 168-169.
132. Pullar CE, Grahn JC, Liu W, Isseroff RR (2006) Beta2-adrenergic receptor activation delays wound healing. *FASEB J* 20: 76-86.
133. Wood JM, Chavan B, Hafeez I, Schallreuter KU (2004) Regulation of tyrosinase by tetrahydropteridines and H2O2. *Biochem Biophys Res Commun* 325: 1412-1417.
134. Garcia-Molina F, Munoz-Munoz JL, Acosta JR, Garcia-Ruiz PA, Tudela J, et al. (2009) Melanogenesis inhibition by tetrahydropterines. *Biochim Biophys Acta* 1794: 1766-1774.
135. Martínez-Esparza M, Ferrer C, Castells MT, García-Borrón JC, Zuasti A. (2001) Transforming growth factor beta1 mediates hypopigmentation of B16 mouse melanoma cells by inhibition of melanin formation and melanosome maturation. *Int J Biochem Cell Biol* 33: 971-983.
136. d'Ischia M, Napolitano A, Prota G (1991) Peroxidase as an alternative to tyrosinase in the oxidative polymerization of 5,6-dihydroxyindoles to melanin(s). *Biochim Biophys Acta* 1073: 423-430.
137. Kasraee B (2002) Peroxidase-mediated mechanisms are involved in the melanocytotoxic and melanogenesis-inhibiting effects of chemical agents. *Dermatology* 205: 329-339.
138. Kasraee B (2002) Depigmentation of brown Guinea pig skin by topical application of methimazole. *J Invest Dermatol* 118: 205-207.
139. Ito Y, Kanamaru A, Tada A (2006) Centaureidin promotes dendrite retraction of melanocytes by activating Rho. *Biochim Biophys Acta* 1760: 487-494.
140. Ichihashi M, Funasaka Y, Ohashi A, Chacraborty A, Ahmed NU, et al. (1999) The inhibitory effect of DL-alpha-tocopheryl ferulate in lecithin on melanogenesis. *Anticancer Res* 19: 3769-3774.
141. Nishiyama T, Ohnishi J, Hashiguchi Y (2001) Fused heterocyclic antioxidants: antioxidative activities of hydrocoumarins in a homogeneous solution. *Biosci Biotechnol Biochem* 65: 1127-1133.
142. Yamamura T, Onishi J, Nishiyama T (2002) Antimelanogenic activity of hydrocoumarins in cultured normal human melanocytes by stimulating intracellular glutathione synthesis. *Arch Dermatol Res* 294: 349-354.
143. Saliou C, Kitazawa M, McLaughlin L, Yang JP, Lodge JK, et al. (1999) Antioxidants modulate acute solar ultraviolet radiation-induced NF-kappa-B activation in a human keratinocyte cell line. *Free Radic Biol Med* 26: 174-183.
144. Funasaka Y, Komoto M, Ichihashi M (2000) Depigmenting effect of alpha-tocopheryl ferulate on normal human melanocytes. *Pigment Cell Res* 13 Suppl 8: 170-174.
145. Su TR, Lin JJ, Tsai CC, Huang TK, Yang ZY, et al. (2013) Inhibition of melanogenesis by gallic acid: possible involvement of the PI3K/Akt, MEK/ERK and Wnt/ $\beta$ -catenin signaling pathways in B16F10 cells. *Int J Mol Sci* 14: 20443-20458.
146. Solano F, Briganti S, Picardo M, Ghanem G (2006) Hypopigmenting agents: an updated review on biological, chemical and clinical aspects. *Pigment Cell Res* 19: 550-571.
147. Kim YJ, Uyama H (2005) Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future. *Cell Mol Life Sci* 62: 1707-1723.
148. Jones K, Hughes J, Hong M, Jia Q, Orndorff S (2002) Modulation of melanogenesis by aloesin: a competitive inhibitor of tyrosinase. *Pigment Cell Res* 15: 335-340.
149. Lin CB, Babiarz L, Liebel F, Roydon Price E, Kizoulis M, et al. (2002) Modulation of microphthalmia-associated transcription factor gene expression alters skin pigmentation. *J Invest Dermatol* 119: 1330-1340.
150. Ohguchi K, Akao Y, Nozawa Y (2006) Stimulation of melanogenesis by the citrus flavonoid naringenin in mouse B16 melanoma cells. *Biosci Biotechnol Biochem* 70: 1499-1501.
151. Takeyama R, Takekoshi S, Nagata H, Osamura RY, Kawana S (2004) Quercetin-induced melanogenesis in a reconstituted three-dimensional human epidermal model. *J Mol Histol* 35: 157-165.
152. An SM, Kim HJ, Kim JE, Boo YC (2008) Flavonoids, taxifolin and luteolin attenuate cellular melanogenesis despite increasing tyrosinase protein levels. *Phytother Res* 22: 1200-1207.
153. Imokawa G, Kobayashi T, Miyagishi M, Higashi K, Yada Y (1997) The role of endothelin-1 in epidermal hyperpigmentation and signaling mechanisms of mitogenesis and melanogenesis. *Pigment Cell Res* 10: 218-228.
154. Amer M, Metwalli M (1998) Topical hydroquinone in the treatment of some hyperpigmentary disorders. *Int J Dermatol* 37: 449-450.
155. Slominski A, Plonka PM, Pisarchik A, Smart JL, Tolle V, et al. (2005) Preservation of eumelanin hair pigmentation in proopiomelanocortin-deficient mice on a nonagouti (a/a) genetic background. *Endocrinology* 146: 1245-1253.
156. Scarparo AC, Visconti MA, de Oliveira AR, Castrucci AM (2000) Adrenoceptors in normal and malignant human melanocytes. *Arch Dermatol Res* 292: 265-267.
157. Sivamani RK, Porter SM, Isseroff RR (2009) An epinephrine-dependent mechanism for the control of UV-induced pigmentation. *J Invest Dermatol* 129: 784-787.
158. Zouboulis CC, Chen WC, Thornton MJ, Qin K, Rosenfield R (2007) Sexual hormones in human skin. *Horm Metab Res* 39: 85-95.
159. Bischitz PG, Snell RS (1958) Effect of ovariectomy, oestrogen and progesterone on the activity of the melanocytes in the skin. *Nature* 181: 1413.
160. Diaz LC, Das Gupta TK, Beattie CW (1986) Effects of gonadal steroids on melanocytes in developing hamsters. *Pediatr Dermatol* 3: 247-256.
161. Resnik S (1967) Melasma induced by oral contraceptive drugs. *JAMA* 199: 601-605.
162. Spark RF (2002) Dehydroepiandrosterone: a springboard hormone for female sexuality. *Fertil Steril* 77 Suppl 4: S19-25.
163. Yamaguchi Y, Itami S, Watabe H, Yasumoto K, Abdel-Malek ZA et al. (2004) Mesenchymal-epithelial interactions in interactions in the skin: increased expression of dickkopf1 by palmoplantar fibroblasts inhibits melanocyte growth and differentiation. *J Cell Biol* 165: 275-285.
164. Ohguchi K, Akao Y, Nozawa Y (2005) Involvement of calpain in melanogenesis of mouse B16 melanoma cells. *Mol Cell Biochem* 275: 103-107.
165. Hoogduijn MJ, Hitchcock IS, Smit NP, Gillbro JM, Schallreuter KU, et al. (2006) Glutamate receptors on human melanocytes regulate the expression of MITF. *Pigment Cell Res* 19: 58-67.
166. Pollock PM, Cohen-Solal K, Sood R, Namkoong J, Martino JJ, et al. (2003) Melanoma mouse model implicates metabotropic glutamate signaling in melanocytic neoplasia. *Nat Genet* 34: 108-112.

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