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# Melanogenesis, Its Regulatory Process, and Insights on Biomedical, Biotechnological, and Pharmacological Potentials of Melanin as Antiviral Biochemical

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**Abstract:** Melanin is s most widely distributed pigment and is found in bacteria, fungi, plants, and animals. Melanogenesis is under complex regulatory control by multiple agents interacting through pathways activated by hormonal and receptor-dependent and -independent mechanisms. There are about 20 genes that are involved in the biochemical pathway of melanogenesis and its regulation, which include: tyrosinase, microphthalmia-associated transcription factor, melanocortin1 receptor, adenylate cyclase, protein kinase A. Human melanogenesis regulatory proteins such as MAPK1, CREB3, and CREBP, have binary interaction with the protein of herpesvirus, hepatitis C virus, Human immunodeficiency virus type 1, Simian virus 40, and Human adenovirus A and C. Melanin is a double-edged sword in host-pathogen interaction (e.g., human-bacteria and/or fungi interaction). The inducers of upregulation of melanogenesis include fluvoxamine, famotidine, terbutaline, heliotrine, sirolimus, dicoumarol, Prestwick-860, carbimazole, (-)-MK-801, rilmenidine, hydrastine hydrochloride, haloperidol, scopolamine N-oxide, raubasine, and dihydroergocristine. In melanogenesis, GSK3B, CSNK2A, MAPK1, MAPK3, MAPK14, ERK1, and HIPK2 were the major kinases, while RUNX1, GATA1, and REST, SUN12, and RCOR1 were the major transcription factors. This study has reviewed the melanogenesis pathway, its regulations as well as applications to viral infection. The antiviral

activity of melanin and its complex in the presence of antibacterial and antifungal compounds should be investigated to further provide insight for biomedical, biotechnological, and pharmacological applications.

**Keywords:** melanin; melanogenesis; regulation; biomedical; biotechnological; pharmaceutical; antiviral; melatonin.

## 1. Introduction

Melanins, the end-products of L-tyrosine complex multistep transformations, are polymorphous and multifunctional biopolymers [1]. Melanin is a widely distributed pigment and is found in bacteria, fungi, plants, and animals. In animals, it is secreted by melanocyte cells distributed in the basal layer of the dermis [2]. Melanins are majorly insoluble and naturally consist of four types; allomelanin, eumelanin, neuromelanin, and pheomelanin [3]. It is a heterogeneous, polyphenol-like biopolymer with a complex structure and color varying from yellow to black, namely allomelanin in plants and fungi, neuromelanin, pheomelanin, and eumelanin in human [4]. A study has determined that eumelanin is more resistant than pheomelanin towards UV-Vis radiation [5]. Melanin is a radical-free polymer presenting a highly-conjugated structure that enables the transformation of UV-Vis radiation into heat [6]. Basophils, mast cells, and eosinophils are important in the viral microenvironment, contributing majorly to viral growth, as well as tumor and cancer proliferation [7]. Eumelanin inhibits the proliferation of mast cells in disease involving immune cells [8]. Melanins have multifunctional biological properties such as antioxidant, anti-inflammatory, radioprotective, antimicrobial, metal chelation, radical scavenger, chemoprotective, and antiviral property [9-12].

## 2. Melanogenesis and it's Regulation

Melanogenesis is under complex regulatory control by multiple agents interacting through pathways activated by receptor-dependent and -independent mechanisms, in hormonal, auto-, para-, or intracrine manner [1]. The control of melanogenesis is an important strategy in treating abnormal skin pigmentation for cosmetic purposes [13]. Melanin synthesis (as shown in Figure 1) is mainly controlled by tyrosinase, a copper-containing enzyme that catalyzes two distinct reactions in melanin synthesis, *viz* the hydroxylation of tyrosine by monophenolase action and the oxidation of 3,4-dihydroxy-L-phenylalanine (L-DOPA) to *o*-dopaquinone by diphenolase action [14]. *o*-Dopaquinone is unstable in an aqueous solution and rapidly suffers a non-enzymatic cyclization to leukodopachrome, which is further oxidized non-enzymatically by another molecule of *o*-dopaquinone to yield dopachrome and one molecule of regenerated L-DOPA [15]. Although the Raper-Mason pathway and its neuronal or insect variants are the routes for the synthesis of animal melanins, the biosynthesis of allomelanins in lower organisms occur from precursor different from *L*-tyrosine which are catechols in plants, and 1,8-dihydroxynaphthalene in fungi [16]. Under physiological conditions, melanin synthesis in melanocytes is restricted to melanosomes. Melanosomes contain a proton pump that allows regulation of intra-melanosomal pH. It also internalizes cell surface melanocyte-stimulating hormone (MSH) receptors via the endocytic pathway [17]. Unlike animal melanocytes, where melanin is located almost exclusively within melanosomes

(as shown in Figure 2), fungal and bacterial melanin can be formed in intracellular and/or extracellular spaces in insects, plants, and many bacterial species, where the enzyme and/or the precursors are secreted to give place to melanogenesis for defensive purposes, as for hardening exocuticle, cell walls, and even neutralizing host defenses in pathogenic species [16].
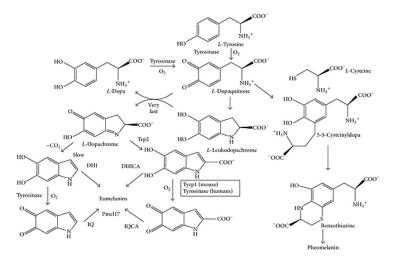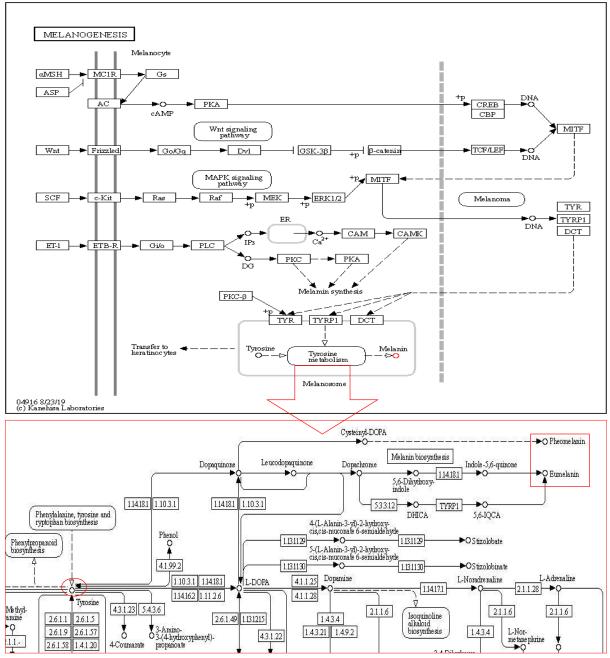


**Figure 1.** Raper-Mason pathway for eu- and pheomelanin formation. Adapted from Solano [16].

Melanogenesis is a complex process that is regulated by many genes [18]. According to Pahari *et al*. [19], the genes that are involved in the biochemical pathway of melanogenesis and its regulation include: Microphthalmia Associated Transcription Factor (MITF), Paired box gene 3 (PAX3), Melanocortin1 receptor (MC1R), Adenylate Cyclase (AC), Protein Kinase A (PKA), Beta-catenin (β-Catenin), Tyrosine kinase receptor (C-kit), Ras GTPase protein (RAS), RAF kinase protein (RAF), Mitogen-activated protein kinase (MEK), Extracellular signal-Regulated Kinase (ERK), Tyrosinase (TYR), Tyrosinase Related Protein 1 and 2 (TYRP1 and TYRP2). Other possible regulators of melanogenesis that remain to be characterized and cloned include dihydroxyindole (DHI) inhibitory factor, which decreases the rate of DHI transformation to melanin, and stablin, which prevents autoxidation of DHI carboxylic acid (DHICA) to melanin [1].

D'Mello *et al*. [20] have explained that eumelanin and pheomelanin are synthesized within melanosomes of melanocytes by a series of reactions that are catalyzed by specific melanogenic enzymes whose production is driven by the MITF. The activity of MITF drives the expression of a number of genes (including SOX10 and PAX3) and is regulated by a number of signaling pathways,including Protein kinase C (PKC); cyclic AMP (cAMP); MAPK/ERK Kinase (MEK); Wingless-related integration site (WNT). These signaling pathways are activated upstream by receptors such as tyrosine kinase receptor KIT (ligand: Stem Cell Factor (SCF)) and Melanocyte-specific melanocortin-1 receptor (MC1R) (ligands: αmelanocyte-stimulating hormone (α-MSH); adrenocorticotropic hormone (ACTH); agonist stimulating protein (ASP)). According to Guyton and Hall [22], a preprohormone called proopiomelanocortin (POMC) is the precursor of adrenocorticotropic hormone (ACTH) as well as several other peptides, including melanocyte-stimulating hormone *(MSH),* β-*lipotropin,* β-*endorphin*, and a few others. α-Melanocyte stimulating hormone (α-MSH) is a 13-residue peptide that stimulates the release of melanin by skin melanocytes.α-MSH also binds to the melanocortin 4 receptor (MC4R), modulating food intake and energy utilization [23]. It has been reported that transcription factors such as lymphoid-enhancing factor-1 are involved in the expression of tyrosinase-related proteins such as TRP-1 and TRP-2 [24]. Moreover, another

transcription factor called the microphthalmia-associated transcription factor (MITF), played a key role in melanocyte survival, development, and differentiation [25].



**Figure 2.** Melanogenesis pathway, showing regulatory elements and tyrosine metabolism leading to melanin [21]. Reprinted with permission.

However, tyrosinase plays a critical regulatory role in melanin biosynthesis. The activation of MITF, a transcription factor that regulates tyrosinase gene expression, is a critical event during melanogenesis [26,27]. During the melanogenesis in melanocytes, the ERK cascade depresses MITF protein stability in the nucleus [26-30]. Moreover, ascorbic acid plays an important role (significantly enhanced) in the ERK pathway in α-MSH-induced B16 melanoma cells [31]. The mechanism of cAMP regulation of melanogenesis involves the activation of protein kinase A (PKA), which then phosphorylates enzymes, ion channels, and several regulatory proteins [1]. The transcriptional control of melanogenesis by cAMP is coordinated predominantly by MITF. Besides, activation of ras oncogene inhibits melanogenesis in normal and malignant melanocytes [32,33]. Another signal transduction pathway important in regulating melanogenesis is represented by protein kinase C (PKC) [34-

36]. Moreover, it has been reported that angiotensin II stimulates melanogenesis via the protein kinase C pathway [37].

## 3. Melanin and Melanogenesis Interactions with Virus Proteins and Melatonin

### 3.1. Melanogenesis regulatory proteins interact and modulate specific virus proteins.

Based on curated data on UniProt database (ref.: www.uniprot.org), catenin beta-1 (CTNNB1) interacts with herpes virus 8 protein vPK; and this interaction inhibits the Wnt signaling pathway. Mitogen-activated protein kinase 1 (MAPK1) interacts with HIV-1 Nef through its SH3 domain. CREB-binding protein (CREBBP) interacts with HTLV-1 Tax, p30II, and HBZ; human herpesvirus 8/HHV-8 protein vIRF-1; and HIV-1 Tat. Cyclic AMP-responsive element-binding protein 3 (CREB3) plays a role in virus protein expression in human immunodeficiency virus type 1 (HIV-1). It also plays a role in herpes simplex virus-1 (HSV-1) latent infection and reactivation from latency; where it represses the VP16-mediated transactivation of immediate early genes of the HSV-1 virus by sequestering host cell factor-1 (HCFC1) in the endoplasmic reticulum (ER) membrane of sensory neurons, thereby preventing the initiation of the replicative cascade leading to latent infection. It activates the transcription of genes required for reactivation of the latent HSV-1 virus. CREB3 may play a role as a cellular tumor suppressor that is targeted by the hepatitis C virus (HCV) core protein, and CREBZF inhibits its transcriptional activity in an HCFC1-dependent manner by the viral transactivator HCV core protein.

Moreover, Human MAPK1 shows binary interaction with a protein (Uniport ID: Q69559) from Human herpesvirus 6A (strain Uganda-1102). Human CREB3 shows binary interaction with a protein (Uniport ID: P29846) from Hepatitis C virus genotype 1b (isolate Taiwan). Human CREBBP show binary interactions with several proteins, which include protein (Uniport ID: P04608) from Human immunodeficiency virus type 1 group M subtype B (isolate HXB2); protein (Uniport ID: P03070) from Simian virus 40 (SV40); protein (Uniport ID: P03255) from Human adenovirus (HAV) C serotype 5; and protein (Uniport ID: P03259) from Human adenovirus A serotype 12. A study has shown that L-DOPA is active against a Simian immunodeficiency virus, exhibiting selective and restricted antiviral activity [12].

### 3.2. Melatonin Interaction with melanogenesis.

Melatonin (N-acetyl-5-methoxytryptamine) controls the changes in pigmentation by aggregating melanin into the melanocytes within the skin. Sunlight suppresses the secretion of melatonin, and increases the secretion of melanocyte-stimulating hormone (MSH), which suppresses $T_H1$ cell activity [38], and stimulates the release of melanin by the skin melanocytes. α-Melanocyte-stimulating hormone (α-MSH) decreases feeding (anorexigenic), whereas melanin-concentrating hormone (MCH) increase feeding (orexigenic) in the hypothalamus [22]. According to Slominski and Pruski [39], melatonin has antagonistic activity against melanogenesis inducers (L-tyrosine or MSH). Melatonin and MSH are potential inhibitors of tyrosine-protein kinase receptor and dopamine/histamine $H_2$ receptor, respectively. These receptors could be essential for coronavirus (SARS-CoV-2) virulence by indirectly interact with several SARS-CoV-2 cellular targets such as ACE2, BCL2L1, JUN, and IKBKB [40, 41].

Unlike melanin, melatonin synthesis occurs in pinealocytes from tryptophan at night-time (absence of sunlight) [42]. The protective effects of melatonin against viral infection such

as encephalomyocarditis virus, Semliki Forest virus, West Nile virus ad respiratory syncytial virus have been studied [43]. T-lymphocytes, natural killer (NK) cells, eosinophils, and mast cells possess melatonin receptors [44]. Eumelanin inhibits the proliferation of mast cells in disease involving immune cells [8]. Microscopically, there was no definite evidence that melatonin caused the melanin granules within the melanocytes to change their position. The length and complexity of the melanocytes' dendritic processes remained unaltered in most of the animals [44]. Melatonin administration increases the proliferative response of rat lymphocytes, increases the number of NK cells, stimulates the release of pro-inflammatory cytokines interleukin (IL)-1 and tumor necrosis factor (TNF)-α, enhances phagocytosis and modulates apoptosis [43]. In contrast, melanin showed inhibitory effects on these immunological biomarkers [45, 46]. Thus, it could be hypothesized that melatonin responds against virus infection that downregulates immunological biomarkers, while melanin responds against virus infection that upregulates immunological biomarkers. This understanding will help in clinical diagnosis in biomedicine and pave the way for antiviral agents' biopharmaceutics development. Melatonin synthesis depends on the induction of adrenergic signal transduction. It is a cross-section with melanin synthesis via the cAMP-PKA-CREB-CRE pathway (see Figure 2) [42].

## 4. Antiviral Applications of Melanin and Melanogenesis

The study of melanin and melanogenesis have found applications in pharmaceutical, biomedical, and biotechnological fields. Inhibition of melanin synthesis is sometimes a desirable process for several purposes, mainly for cosmetical reasons in human skin, for anti-browning treatments in fruit technology, and for diminishing some pathogenic fungi' virulence and bacteria [16]. Melanin is a double-edged sword in host-pathogen interaction (e.g., human-bacteria and/or fungi interaction), as these organisms (host and pathogen) synthesize melanin with antagonist purposes; host melanin is a defense against the pathogen infection, and microbial melanin is a defense against the oxidative attack and release of reactive oxygen species from the host [16].

### 4.1. Biomedical application.

Biomedically, melanin may play a role in regulating the activity of immunological cytokines. A study has shown that melanocytes produce numerous immunological biomarkers, including interleukin 1 (IL-1) and IL-6 [45]. Also, a study has shown that synthetically derived melanin at non-cytotoxic concentrations reversibly suppressed the production of tumor necrosis factor (TNF), inhibited the production of interleukins IL-1b, IL-6, and IL-10 by lipopolysaccharide (LPS) stimulated monocytes [46], inhibited macrophage migration inhibitory factor (MIF) which possesses oxidoreductase (tyrosinase-like) activity necessary for the formation of neuromelanin precursors [47]. The interferons (IFN) consist of a large family of antiviral peptides. IFN-β mRNA is rarely expressed in melanoma cells and suppresses the proliferation of melanoma cells [48].

Polyinosinic-polycytidylic acid (poly(I:C)), is a synthetic analog of double-stranded RNA synthesized by various types of viruses. γδ T-cell activation by immunostimulatory double-stranded RNA, such as poly(I:C), is indirectly mediated *via* type I interferons (IFN-α, IFN-β) and may contribute to effective antiviral responses in human [49]. Poly(I:C) induces IFN-β mRNA, and it is an agonist of toll-like receptor (TLR) 3 and retinoic acid-inducible gene

I (RIG-I)-like receptors (RLRs), including RIG-I and melanoma differentiation-associated gene 5 (MDA5). The activation of TLR3 and RLR signaling by poly(I:C) can directly trigger apoptosis in some cancer cells [50]. According to Blalock and Harp [51], adrenocorticotropic hormone (ACTH) and human but not mouse interferon caused induction of melanin synthesis and antiviral activity in human melanoma cells. Thus, interferon has species-specific hormonal activity, and ACTH has cell-specific antiviral activity. The natural function of interferon is hormonal, and that of hormones includes the protection of tissues against viruses.

The enzyme cascade, which leads to melanin, the prophenoloxidase-activating system, forms the central antimicrobial defense system in many animals. Most melanins exist in an insoluble form, but synthetic soluble melanins have been shown to inhibit the replication of human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) in two human lymphoblastoid cell lines (MT-2 and H9) and phytohemagglutinin-stimulated human T cells, as well as blocked the HIV-1 envelope surface glycoprotein, and T cell-specific monoclonal antibody leu-3a (CD4+), with no effect on HIV-1 reverse transcriptase activity in viral lysates [12,52].

It has been reported that skin darkness due to melanins and melanocytes have a vital role in defending against infectious disease [53]. The skin darkness was negatively associated with rates of HIV infection in sub-Saharan Africa. This relationship was attributed to possible lower infection rates of other parasites, especially bacteria and fungi, that lead to tissue damage in the genital tract and hence increased the chance for contracting HIV [54]. Melanin production directly favors bacterial and fungal infections but not a viral infection. A bacterial and fungal infection could serve as a template for viral infection but not vice versa. The production process of melanin may protect bacteria and fungi such as *Bacteroides melaninogenicus* (recently renamed as *Prevotella melaninogenica*) *Sporothrix schenckii,* and Cryptococci from the oxidative injury of phagocytes. The melanin pigments are produced in *Cryptococcus neoformans*, where it played a role in its virulence [55,56]. Melanisation often becomes a virulence factor in some pathogenic bacteria because melanin protects bacterial cells from a defense mechanism in the infected host [57].

In *Streptomyces*, melanin formation is a protective response to adverse environmental conditions [58]. *Bacillus thuringiensis* synthesizes melanin that protects against pesticides [59]. Melanin is inherent in the tropical region, especially in populations of Africans, Asians, Hispanics, etc. Mosquito is an agent that transmit many human viruses such as Chikungunya virus (CV), Dengue virus (DV), Japanese encephalitis virus (JEV), and West Nile virus (WNV), and its antiviral mechanism has been linked to the gene Prophenoloxidase (PPO), encoding a prototype of phenoloxidase, which is predominantly expressed in mosquito hemocytes [60]. Melanization in *Lepidoptera* hemolymph mediates antiviral activity during infection with *Microplitis demolitor* bracovirus [61]. A study has shown that melanin produced by a *Pseudomonas balearica* strain possessed antimicrobial activity against phytopathogenic strains, which include *Erwina carotovora* and *Erwina chrysanthemi*, as well as against *Candida albicans, Escherichia coli,* and *Staphylococcus aureus* [62].

The pigmentary effects of small oligonucleotides, which involved amplifying the melanogenic effect of α-MSH [35,63], could follow a pathway functionally similar to the SOS response system of bacteria [63]. The SOS response involves the induction of several proteins that serve to enhance the integrity of DNA through complex regulation. It includes error-prone factors that allow for improved survival and continuous replication in the presence of extensive DNA damage but at the cost of elevated mutagenesis [64]. Melanogenesis and its intermediates

can switch cell metabolism from aerobic to anaerobic glycolysis, stimulate the pentose phosphate pathway, and/or inhibit glycoprotein phosphorylation [1]. Melanin and melanin-like nanoparticles have found application in both biomedical and biotechnological fields [65,66].

### 4.2. Biotechnological application.

In biotechnology, microbial infection such as viral, bacterial, and fungal challenge can induce PPO activity. According to Escobar *et al*. [67], protective effects of polyphenol oxidase (PPO) have generally been attributed to the generation of reactive quinones, which may 1) possess direct bacteriocidal/insecticidal properties; 2) generate toxic reactive oxygen species through secondary oxidation reactions; 3) reduce protein palatability and digestibility by oxidizing nucleophilic amino acids; and 4) form an impermeable melanin barrier, preventing the spread of pathogen infection. In-plant infection, fungal melanin has a different particular function as the pigment is essential for cell wall penetration in appressorial processes [68,69]. PPO-overexpressing tomatoes display enhanced resistance to the bacterial pathogen *Pseudomonas syringae pv.* tomato [70]. Thang *et al*. [71] have reported that melanin-containing diets may be applied in aquaculture to protect shrimp against white spot syndrome virus infection.

### 4.3. Pharmacological application.

In pharmacology, microbial tyrosinase is used to produce synthetic melanin, which protects against radiation and is used as cation exchangers, drug carriers, antioxidants, antiviral agents, or immunogens [72]. Significant antiviral activity has been observed in the synthesis of lipophilic catechols by tyrosinase, suggesting a new inhibition mechanism based on both redox and lipophilic properties [73]. L-DOPA inhibits *in vitro* phosphorylation of melanoma glycoproteins [74]. Diacylglycerol (an endogenous activator of PKC) can stimulate melanin synthesis both in cell culture and *in vivo* [75,76]. At the same time, melanogenesis could be blocked by PKC inhibitors or cellular depletion of PKC [35,36]. It has been reported that an aqueous solution of melanin and melanin-glucan complex derived from Chaga fungus *Inonotus obliquus* have antiviral activity against HSV-2 and HIV-1 [77].

Ellagic acid interferes with the melanin biogenesis pathway [78]. Ellagic acid (DB08846) is an inhibitor of human PRKCA (UniProt ID: P17252) and PRKCB proteins (UniProt ID: P05771). Ito and Wakamatsu [79] reported that ellagic acid could act as an alternative tyrosinase substrate to be oxidized to form o-quinones. Semiquinones may then react with nucleophilic compounds. High doses or long exposure of melanin-containing cells such as eyes and skin to chloroquine can cause toxicity of the skin, blood, and eyes. It becomes concentrated in melanin-containing structures, which can lead to corneal deposits and blindness [80]. However, ellagic acid has been reported for antiviral activity against a spectrum of viruses, including coronavirus [40]. A study has shown that Liquiritin (LQ) and liquiritigenin (LQG), which are the major flavonoids in licorice root (*Glycyrrhiza* spp.), induced melanogenesis (the expression of tyrosinase, TRP-1, and TRP-2; MITF, and CREB protein phosphorylation), through enhancement of p38 and PKA signaling pathway [81]. Also, inhibition of C-terminal Src kinase induced melanogenesis by phosphorylation of p38 mitogen-activated protein kinase (MAPK) and CREB pathways in human G361 cells [82].

Melanogenesis regulatory proteins and their associated pathologies due to mutation are shown in Table 1. Another study has provided a review of these pathologies [18]. Based on

Figure 2, twenty (20) genes involved in the regulation of melanogenesis, as shown in Table 1, were obtained from the UniProt database. The transcription factors and kinases associated with the 20 genes were analyzed and visualized on the eXpression2Kinases webserver at default setting [83] as shown in Figure 3. The top 15 drugs that can induce and reverse the up-regulation and down-regulation of these melanogenesis regulatory proteins was predicted on Expression2Kinases software, at a default setting using human as an organism of interest [84] as shown in Table 2. In melanogenesis, GSK3B, CSNK2A, MAPK1, MAPK3, MAPK14, ERK1, and HIPK2 were the major kinases, while RUNX1, GATA1, and REST, SUN12, and RCOR1 were the major transcription factors.

**Table 1.** Melanogenesis regulatory proteins and their associated pathologies due to mutation.

| S.N. | Regulatory Protein | Gene | UniProt ID | Pathology Due To Mutation (https://www.orpha.net/) |
|---|---|---|---|---|
| 1 | Adenylate cyclase type 1 | ADCY1 | Q08828 | 90636, Autosomal recessive non-syndromic sensorineural deafness type DFNB |
| 2 | Calcium/calmodulin-dependent protein kinase type II subunit alpha | CAMK2A | Q9UQM7 | 178469, Autosomal dominant non-syndromic intellectual disability |
| 3 | Calcium/calmodulin-dependent protein kinase type II subunit gamma | CAMK2G | Q13555 | - |
| 4 | Catenin beta-1 | CTNNB1 | P35222 | 210159, Adult hepatocellular carcinoma; 54595, Craniopharyngioma; 873, Desmoid tumor; 891, Familial exudative vitreoretinopathy; 85142, Aldosterone-producing adenoma; 33402, Pediatric hepatocellular carcinoma; 91414, Pilomatrixoma; 404473, Severe intellectual disability-progressive spastic diplegia syndrome |
| 5 | L-dopachrome tautomerase | DCT | P40126 | - |
| 6 | Endothelin receptor type B | EDNRB | P24530 | 388, Hirschsprung disease; 895, Waardenburg syndrome type 2; 897, Waardenburg-Shah syndrome |
| 7 | Frizzled-4 | FZD4 | Q9ULV1 | 891, Familial exudative vitreoretinopathy; 91495, Persistent hyperplastic primary vitreous; 90050, Retinopathy of prematurity |
| 8 | Mast/stem cell growth factor receptor Kit | KIT | P10721 | 98834, Acute myeloblastic leukemia with maturation; 98829, Acute myeloid leukemia with abnormal bone marrow eosinophils inv(16)(p13q22) or t(16;16)(p13;q22); 102724, Acute myeloid leukemia with t(8;21)(q22;q22) translocation; 280785, Bullous diffuse cutaneous mastocytosis; 79455, Cutaneous mastocytoma; 44890, Gastrointestinal stromal tumor; 158778, Isolated bone marrow mastocytosis; 158772, Nodular urticaria pigmentosa; 2884, Piebaldism; 158769, Plaque-form urticaria pigmentosa; 280794, Pseudoxanthomatous diffuse cutaneous mastocytosis; 544260, Selection of therapeutic option in melanoma; 158775, Smoldering systemic mastocytosis; 98849, Systemic mastocytosis with associated hematologic neoplasm; 90389, Telangiectasia macularis eruptiva perstans; 842, Testicular seminomatous germ cell tumor; 158766, Typical urticaria pigmentosa |
| 9 | Melanocyte-stimulating hormone receptor | MC1R | Q01726 | 618, Familial melanoma; 626, Large congenital melanocytic nevus; 79432, Oculocutaneous albinism type 2 |
| 10 | Microphthalmia-associated transcription factor | MITF | O75030 | 404511, Clear cell papillary renal cell carcinoma; 618, Familial melanoma; |

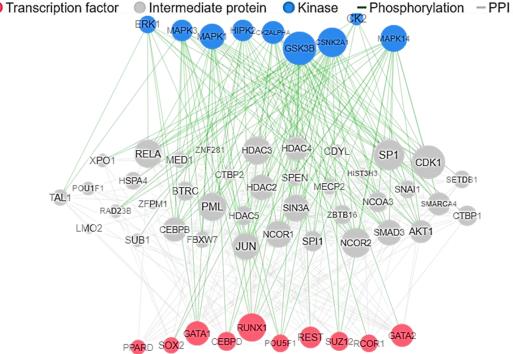| S.N. | Regulatory Protein | Gene | UniProt ID | Pathology Due To Mutation (https://www.orpha.net/) |
|------|--------------------|----|-----------|--------------------------------------------------|
| | | | | 293822, MITF-related melanoma and renal cell carcinoma predisposition syndrome; 352740, Ocular albinism with congenital sensorineural deafness; 319298, Papillary renal cell carcinoma; 42665, Tietz syndrome; 895, Waardenburg syndrome type 2; 897, Waardenburg-Shah syndrome |
| 11 | cAMP-dependent protein kinase catalytic subunit alpha | PRKACA | P17612 | 401920, Fibrolamellar hepatocellular carcinoma; 189439, Primary pigmented nodular adrenocortical disease |
| 12 | Protein kinase C alpha type | PRKCA | P17252 | - |
| 13 | Protein kinase C beta type | PRKCB | P05771 | - |
| 14 | Tyrosinase | TYR | P14679 | 352734, Minimal pigment oculocutaneous albinism type 1; 352740, Ocular albinism with congenital sensorineural deafness; 79431, Oculocutaneous albinism type 1A; 79434, Oculocutaneous albinism type 1B; 352737, Temperature-sensitive oculocutaneous albinism type 1 |
| 15 | 5,6-dihydroxyindole-2-carboxylic acid oxidase | TYRP1 | P17643 | 79433, Oculocutaneous albinism type 3 |
| 16 | 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-1 | PLCB1 | Q9NQ66 | 293181, Malignant migrating focal seizures of infancy; 3451, West syndrome |
| 17 | Mitogen-activated protein kinase 1 | MAPK1 | P28482 | 261330, Distal 22q11.2 microdeletion syndrome |
| 18 | Transcription factor 7 | TCF7 | P36402 | - |
| 19 | CREB-binding protein | CREBBP | Q92793 | 370026, Acute myeloid leukemia with t(8;16)(p11;p13) translocation; 353281, Rubinstein-Taybi syndrome due to 16p13.3 microdeletion; 353277, Rubinstein-Taybi syndrome due to CREBBP mutations |
| 20 | Cyclic AMP-responsive element-binding protein 3 | CREB3 | O43889 | - |



**Figure 3**. Network expression of transcription factors and kinases associated with 20 regulatory genes of melanogenesis.

**Table 2**. Top 15 drugs that can induce and reverse the up-regulation and down-regulation of melanogenesis regulatory proteins.

| S.N. | Up-Regulation | | Down-Regulation | |
|------|---------------|---|-----------------|---|
| | **Inducer** | **Reverser** | **Inducer** | **Reverser** |
| 1 | Fluvoxamine | Acetylsalicylic acid | Acetylsalicylic acid | Fluvoxamine |
| 2 | Famotidine | Prednisone | Prednisone | Famotidine |
| 3 | Terbutaline | Propoxycaine | Propoxycaine | Terbutaline |
| 4 | Heliotrine | CP-320650-01 | CP-320650-01 | Heliotrine |
| 5 | Sirolimus | Emetine | Emetine | Sirolimus |
| 6 | Dicoumarol | Dicycloverine | Dicycloverine | Dicoumarol |
| 7 | Prestwick-860 | Dopamine | Dopamine | Prestwick-860 |
| 8 | Carbimazole | Cinchocaine | Cinchocaine | Carbimazole |
| 9 | (-)-MK-801 | Pancuronium bromide | Pancuronium bromide | (-)-MK-801 |
| 10 | Rilmenidine | Isoetarine | Isoetarine | Rilmenidine |
| 11 | Hydrastine hydrochloride | Nitrendipine | Nitrendipine | Hydrastine hydrochloride |
| 12 | Haloperidol | Indapamide | Indapamide | Haloperidol |
| 13 | Scopolamine N-oxide | Ethoxyquin | Ethoxyquin | Scopolamine N-oxide |
| 14 | Raubasine | Amrinone | Amrinone | Raubasine |
| 15 | Dihydroergocristine | Folic acid | Folic acid | Dihydroergocristine |

Because of antiviral activity of melanin and melanogenesis based on antioxidant and anti-inflammatory activities, some of the recently reported inducers include (i) 7,8-Dimethoxycoumarin, which stimulated the expression of tyrosinase, TRP-1, TRP-2, and MITF, thereby activating melanin production and Akt phosphorylation was increased in the Akt signaling pathway but interfered with the phosphorylation of ERK in the MAPKs pathway [85], (ii) Cistanche deserticola polysaccharide, which activated MAPK signal pathway, then upregulated the expression of MITF, and downstream genes TYR, TRP1, TRP2, and RAB27A [86], (iii) Argania Spinosa fruit shell extract, which upregulated the expression of the melanogenic enzymes through the cAMP-MITF signaling pathway [87], (iv) Fosfomycin disodium salt, which upregulated the phosphorylation of c-Jun N-terminal kinases (JNK) and p38 pathways [88], and (v) Dasatinib, which induced melanogenesis via ERK-CREB-MITF-tyrosinase signaling in normal human melanocytes [89]. Inducers of melanogenesis find biomedical, biotechnological, and pharmaceutical applications to treat depigmentation disease and anti-gray hair product development.

## 5. Conclusions

There is controversy on the interaction of melatonin with melanogenesis. Some researchers have reported that melatonin, and its precursor (N-acetylserotonin), inhibited melanogenesis in human melanotic melanoma cells in human epidermis, human MNT-1, and rodent [39,90,91]. In contrast, others have reported that melatonin induced melanogenesis in human melanoma SK-MEL-1 cells [92,93]. On the roles of inflammation factors in melanogenesis, Fu *et al*. [94] have reported that IL-18, IL-33, interferon-γ, granulocyte-macrophage colony-stimulating factor, prostaglandin E2 have the effect of promoting melanogenesis, while IL-1, IL-4, interleukin-6, IL-17, and TNF can inhibit melanogenesis.

This study has reviewed melanogenesis regulation and applications of melanin as an antiviral chemical. Insights from the bacteria and fungi but not the virus showed negative implications on human melanogenesis. The proposition is that melanin is an antiviral agent. It downregulates dysfunctional immunological biomarker in an infectious state by specific viruses such as HIV-1, HIV-2, HSV-1, HCV, SV40, HAV, CV, DV, JEV, WNV, and SARS-CoV-2. Since melanosomes could modify the cellular energy-yielding metabolism by switching oxidative catabolism to anaerobic glycolysis, altering the intracellular NAD/NADH

and NADP/NADPH ratios and/or stimulating the pentose phosphate pathway [1], defects in the regulation which lead to overproduction of melanin will favor cancer, while underproduction of melanin will favor viral infection. The antiviral activity of melanin and its complex in the presence of antibacterial and antifungal compounds should be investigated to further provide insight into the biomedical and pharmacological applications of melanin and melanogenic genes.

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## Conflicts of Interest

The authors declare no conflict of interest.

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