

Melanocytes and melanogenesis

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ABSTRACT

Pigmentation is heritable and is regulated by genetic, environmental and endocrine factors. During embryogenesis, specific cells (melanoblasts) migrate from the neural crest into the basal epithelium of the epidermis, hair bulbs of the skin and specific areas of the eye, ear and brain. There are 3 types, viz. eumelanosome, pheomelanin, neuromelanin. They have special staining methods: antigens as well as antibodies.

Key words: Eumelanosome; pheomelanin; neuromelanin

INTRODUCTION

Pigmentation is heritable and is regulated by genetic, environmental and endocrine factors [1]. Skin colour results from [1-3]:

1. Concentration and admixture of the types of melanins in melanocyte (most important),
2. carotenoid pigments,
3. the number of blood vessels in the cutis, the colour of blood in them.

EMBRYOLOGY

During embryogenesis, specific cells (melanoblasts) migrate from the neural crest into the basal epithelium of the epidermis, hair bulbs of the skin and specific areas of the eye, ear and brain [2].

Slac2-a simultaneously interacts with Rab27A on the melanosome and with an actin-based motor myosin Va, the resultant tripartite protein complex (Rab27A-Slac2-a-myosin Va) mediates actin-based melanosome transport. After actin-dependent melanosome transport, the second Rab27A effector Slp2-a promotes the anchoring of melanosomes to the plasma membrane of melanocytes through direct interaction of the C2A domain with phosphatidylserine. Melanosomes are attached to a framework of microtubules and are transported up

the dendrites on ladders of actin. The little "feet" that walk up the actin fibers are called myosin 5. Melanin is transferred through dendritic processes from melanocytes to basal keratinocytes, where it is first stored and later degraded. The greater number of melanin is present in Basal keratinocytes than in the melanocytes and often basal cells at the tips of Rete ridges are preferentially more melanized. 10% of cells in basal layer are melanocytes. Each melanocyte supplies 36 keratinocytes with melanin, forming Epidermal-Melanin unit [2-4].

ANATOMY

They are located in the Basal layer of epidermis, Hair bulb and Outer root sheath of hair follicles [2-7].

Melanocytes in epidermis

Epidermal have round to oval, dark stained nuclei, smaller than those of basal keratinocytes. A clear halo of surrounding cytoplasm.

Melanocytes in hair

Melanocytes are differentiated into

1. Differentiated melanocytes, located in the hair matrix region
2. Melanocyte stem cells, located at the lower portion of hair follicle.

How to cite this article: Zonunsanga. Melanocytes and melanogenesis. Our Dermatol Online. 2015;6(3):350-355.

Submission: 25.10.2014; **Acceptance:** 26.02.2015

DOI: 10.7241/ourd.20153.94

Differentiated melanocytes

They express

1. Sox 10 (Sry-related HMG box/sex determining region box 10),
2. kit,
3. mitf (microphthalmia associated transcription factors),
4. Pax 3 (paired bx 3),
5. Dct (Dopachrome tautomerase)

Melanocyte stem cells

They express only Pax 3 and Dct.

Ear

Melanins are found in Striae vascularis of Inner ear.

Eye

Melanocytes are also present in the Iris stroma in the front of Iris, Iris pigment epithelium on the back of Iris, Retinal pigment epithelium.

Adrenal glands

They are also seen in Medula and Zona reticularis.

Others

Melanin is also found in heart, liver, muscles and intestine.

TYPES OF MELANIN [4-7]

1. Eumelanin (dark brown)
2. Pheomelanin (pale red or yellow)
3. Neuromelanin (produced in dopaminergic neurons of the human substantia nigra).

Phaeomelanosomes

- They spherical in shape
- they lack TRP1, TRP2 and p-protein (TRP = tyrosine related proteins)
- they have 1/3rd the level of tyrosinase as eumelanosomes.

Eumelanosomes

- They are oval-shaped melanosomes,
- they have TRP1, TRP2, p-protein,
- they have 3 times the tyrosinase as compared to phaeomelanosomes.

Neuromelanin

Sites

1. dopaminergic neurons of Substantia nigra,
2. locus ceruleus,
3. dorsal motor nuclei of vagus nerve,
4. median raphe nucleus of Pons.

They are located in the lysosomes and are made from oxy-radical metabolites of mono-amines neurotransmitters such as Dopamine and Norepinephrine.

LABORATORY METHODS FOR STAINING OF MELANIN

1. Fontano-masson method

Melanin granules reduce ammoniacal silver nitrate to metallic silver [6-14].

2. Schmorl's method

Melanin has ability to reduce Ferricyanide to Ferrocyanide which in presence of Ferrous ion forms Prussian blue [6-14].

3. Dopa-oxidase method [6-14]

This is the Most specific test, based on action of Dopa-oxidase upon Dopa.

4. Bleaching technique

Using strong oxidising agents like KMnO₄ or Hydrogen peroxide [6-14].

5. Formaldehyde-induced fluorescence

Formalin fixation imparts a strong yellow fluorescence to unstained tissues with biogenic amines [6-14].

6. PAS (Periodic acid schiff)

Pseudomelanin (melanosis coli) is PAS +ve while true melanin is PAS -ve [6-14].

ANTIGENS AND THEIR RESPECTIVE ANTIBODIES

Antigens are [7-13]:

- a. S-100 proteins
- b. gp 100

- c. Melan-A/MART-1
- d. Tyrosinase
- e. PNL 2 Antigen
- f. MITF

Antibodies are [7-13]:

- a. Anti-S-100
- b. HMB 45
- c. A 103/M2-7C10
- d. T 311
- e. PNL 2 Antibodies
- f. D 5

Melanogenesis

TYR Gene (located in the long arm of chromosome 11 i.e chr 11 q 14-q 21) is involved in melanogenesis [13-17].

Genes involved in development of melanoblast

The genes/transcription factors involved in melanogenesis are Pax3, Sox10, Mitf, Edn3 (endothelin 3), Ednrb (endothelin receptor B), Kit (c-Kit tyrosine kinase receptor), Kitl (Kit ligand/asSCF/steel factor) and Snai2 (also called as Slug) [13-17].

Sites of melanogenesis

Melanosomes are synthesised in [13-18]:

1. mammalian skin melanocytes,
2. choroidal melanocytes,
3. retinal pigment epithelial (RPE) cells in the eye.

Steps of melanin synthesis

The survival and migration of neural crest-derived cells [13-19]

It is dependent on interactions between specific receptors on the cell surface and their extracellular ligands. For example, FGF, steel factor, formerly known as mast cell growth factor, KIT ligand or stem cell factor (SCF), binds the KIT receptor on melanocytes and melanoblasts. Mutations in the KIT gene decrease the ability of the KIT receptor to be activated by the steel factor.

Differentiation of melanoblasts into melanocytes [13-19]

When melanoblasts reached epidermal-dermal junction, it is differentiated into melanocytes at around sixth month of fetal life.

Survival and proliferation of melanocytes [13-19]

Melanocytes have been identified within fetal epidermis as early as 50 days of gestation. Dermal melanocytes decrease in number during gestation and virtually disappear by birth. Epidermal melanocytes established at the epidermal-dermal junction continue to proliferate and start to produce melanin.

Formation of melanosomes and production of melanins [13-19]

Melanocytes start producing melanosomes, highly organized elliptic membrane-bound organelles in which melanin synthesis takes place.

BIOCHEMICAL EVENTS

The Tyrosine, under the influence of Tyrosinase and Cu^{2+} forms DOPA and then Dopakinone. This Dopakinone with the help of Cystathione or Cysteine, forms Cysteine-L-Dopa which further forms pheomelanin. When Cysteine is depleted, the Dopakinone forms Leucodopachrome which ultimately forms eumelanin. The ratio of these two types of Melanin determines visible pigmentation. The variation in skin color among various races is determined mainly by the number, melanin content and distribution of melanosomes produced and transferred by each melanocyte to a cluster of keratinocytes surrounding it [17-21].

STAGES OF MELANOSOMES

Stage 1

Melanosomes are spherical endosomal vacuoles lacking tyrosinase (TYR) activity (the main enzyme involved in melanogenesis) and have no internal structural components. No melanin is present yet. They contain the melanosomal protein Pmel17, which is sorted into intraluminal vesicles (ILVs) within the organelle. A partial clathrin coat is seen which is involved in sorting proteins into ILVs of vacuolar endosomes. The presence of Pmel17 gives rise to the structurally important intraluminal fibrils that characterise stage II melanosomes [13-20].

Stage 2

Melanosomes are ellipsoidal, around 0.5 micro-mm diameter. At this point, the presence and correct processing of Pmel17, an important melanosomal structural protein, determine the transformation

of stage I melanosomes to elongated, fibrillar organelles known as stage II melanosomes. They contain MELANOSOMAL enzymes tyrosinase TYRP1. They exhibit minimal deposition of melanin. Melanin is deposited within cross-linked longitudinal filaments. Enzymes activity is localised in surrounding membrane. Tyrosinase and TYRP1 are trafficking to melanosomes from early endosomes. They are present in tubular endosomal domain. Tyrosinase- and TYRP1-positive endosomal membranes have buds that are coated with the adaptor proteins AP1 or AP3, which play roles in sorting tyrosinase and TYRP1 to melanosomes [13-20].

BLOC1 and BLOC2 (protein complexes) plays important role in the regulation of endosome-to-melanosome transport.

- a. BLOC1 – They are located in the tubular regions on early endosomes and control the exit of cargo from early endosomes.
- b. BLOC2 – They regulate subsequent step i.e. the direction of cargo to maturing melanosomes.

Stage 3

Melanosomes are ellipsoidal. Melanin deposition increases by enzymatic activity and non-enzymatic polymerization. The pigment is uniformly deposited on the internal fibrils [13-20].

Stage 4

Melanosomes are ellipsoidal. Melanosome is fully developed and is filled with electron-opaque organelles. Melanin production is through polymerization.

Key protein involved in melanosome assembly is NCKX5, encoded by the gene SLC4A5 [13-20].

FACTORS INFLUENCING MELANOGENESIS

1. Genetics and endogenous factors

Migration, proliferation, differentiation and survival of melanocytes is influenced by tyrosine kinase receptor KIT and its ligand stem cell factors [7-9,11-16].

Microphthalmia transcription factor (MITF) is a nuclear protein involved in:

1. embryonic development of melanocytes,

2. regulation of transcription of genes involved in melanin synthesis e.g ‘mi’ gene located at chromosome 3p

The two Rab27A effectors are Slac2-a (also called melanophilin) and Slp2-a; they are abundantly expressed on melanosomes and sequentially regulate melanosome transport in melanocytes. TGF-beta suppress melanogenesis by inhibiting activation of Pax3. P 53 promotes melanogenesis by suppressing TGF- beta, upregulation of alpha-MSH and KITL. E-cadherin (mediating cell-to-cell interaction) also regulates melanocytes. Dermal fibroblast is also involved in regulation of melanocytes. Notch signalling in melanocytes, an essential cell-to-cell interaction mechanism, regulates processes such as cell proliferation, cell fate decision, differentiation or stem cell maintenance [7-9,11-16].

2. Environmental or exogenous factors

(A) UV Rays

UVA Cause immediate dark pigmentation within minutes and persists for several hours. Followed by persistent pigment darkening, which occurs within several hours and lasts for several days. UV exposure leads to increase expression of MITF (master transcriptional regulator of pigmentation) and its downstream melanogenic proteins, including Pmel 17, MART-1, TYR, TRP-1, Dct. Thereby, leading to increase in melanin content. It also increases levels of PAR 2 (Protease activated receptor) in keratinocytes which increases uptake and distribution of melanosomes by keratinocytes in the epidermis. It also increases Alpha-MSH, endothelin and ACTH which upregulate MC-1R, thereby enhancing melanocytes response to melanocortins. It also increases IL-1 secretion by keratinocytes, stimulating secretion of ACTH, Alpha-MSH, SCF, NGF Endothelin-1 and hFGF by keratinocytes. UV-R also causes peroxidation of lipids in cellular membranes, leading to generation of ROS, which may stimulate melanocytes to produce excess melanin [7-9,11-17].

(B) Retinoic acid

It upregulates differentiation and proliferation of melanocytes through melanocortins receptors.

(C) Other exogenous factors

- vitamin D metabolites - retinoids
- forskolin (extract from Indian coleus plant)
- cholera toxin

- isobutyl-methyl-xanthines
- di-acylglycerol and its analogues

3. Endocrine factors

The endocrinal products and substances responsible for melanogenesis are CRH (Corticotropin-releasing hormones), UROCORTINS (CRH-like neuro-peptide), POMC (Pro-opio-melanocortin), ACTH, alpha-MSH And beta-ENDORPHIN [7-9,11-16].

Mechanism of action of melanocytes

Melanocyte acts on Melanocyte-receptors thereby activating signaling pathways [7,9,11-16,19-21].

1. Melanocyte-receptors are:
 - MC1R,
 - Melatonin receptor,
 - G-protein-coupled receptors – Frizzled 5
 - Receptor Tyrosine kinase- c-Kit,
 - Hepatocyte Growth factor (HGF).
2. Signalling pathways involved in actions of melanocytes
 - RAS/RAF/MEK/ERK pathway,
 - PI3K/AKT pathway,
 - Notch signalling pathway.

Role of melanocytes

Melanocytes or melanin [4,7,8,10,12-16,21,22].

- Gives skin colour,
- Protects skin from UV-induced skin damaged and skin cancer,
- Development of striae vascularis of cochlea and production of Endocochlear potential thereby helping hearing function,
- Absorb toxic substances in the inner ear,
- Maintain proper hair colour,
- Role in eye color,
- In CNS, different stressors, like chemicals, oxidative damage and high temperature are also suppressed by melanin,
- Melanocortins have effects on appetite and sexual activities.

Deleterious effects

In vitro studies, melanin reacts with DNA and is known to act as photosensitiser. In contrast to eumelanin, pheomelanin is prone to photodegradation and contribute to damaging effects of UVR because it can

generate Hydrogen peroxide and superoxide anions and may cause mutations in melanocytes. Pheomelanin is associated with higher rates of apoptosis after UVR. Pheomelanin increases release of Histamine, thereby contributing to sun-induced erythema and oedema. Pheomelanin is UVA sensitiser that cause cell death [4,7,8,10,12-16,21,22].

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Source of Support: Nil, **Conflict of Interest:** None declared.