MELANOGENESIS

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Melanogenesis by definition is the production of melanin pigments. Melanocytes, the cells in which melanogenesis exclusively takes place, have a wide array of functions. The structure and function of melanocytes, the process of melanogenesis, and variations and diseases resulting in altered melanogenesis and pigmentation will be covered.

STRUCTURE OF MELANOCYTES

Melanoblasts, the precursor cells of melanocytes, are unpigmented cells that originate from embryonic neural crest cells. During embryogenesis, melanoblasts migrate to various regions of the body and develop into melanocytes as well as cells of the peripheral nervous system, bone and cartilage of the head, and the choroid of the eye. Melanoblast migration and differentiation into melanocytes is influenced by a number of signaling molecules produced by neighboring cells. These include Wnt, endothelin-3 (ET3), bone morphogenetic proteins (BMPs), steel factor (stem cell factor), and hepatocyte growth factor.

Melanocytes located in the skin are primarily located in two distinct locations that distinguishes them both structurally and functionally; epidermal melanocytes and follicular melanocytes. In the epidermis melanocytes are primarily found in the basal layer. Follicular melanocytes are located in the outer root sheath and the hair matrix of hair follicles. Melanocytes predominantly express the adhesion molecules E and P-cadherins and it is thought that E-cadherin is the prime mediator of adhesion between melanocytes and keratinocytes. Other locations of melanocytes include the ducts of sebaceous and sweat glands, the superficial dermis, the uvea of the eye, the cardiovascular system, the cochlea (stria vascularis), the central nervous system, and adipose tissue.

Melanocytes have long dendrites (cytoplasmic processes) that weave intercellularly between keratinocytes. The function of the dendrites is to transfer melanosomes to keratinocytes. Melanosomes are subcellular lysosome-like organelles in which melanin pigments are synthesized and stored. One melanocyte is typically associated with 10 to 20 keratinocytes in the basal layer of canine skin and approximately 36 keratinocytes in the basal layer of human skin. The melanocyte and associated keratinocytes are called an *epidermal melanin unit*. Once melanosomes are transferred to keratinocytes they often cluster as “caps,” dorsal to nuclei. This orientation is thought to play a photo-protective role to decrease damage to DNA in the nuclei. Melanocytes do not stain readily with hematoxylin and eosin staining and will appear clear on *normal* histopathology slides. To visualize melanocytes, special stains (DOPA oxidase reaction, Fontana-Masson stain, Schmorl’s method) are needed. Immunocytochemistry can also be used to identify melanocytes as they are positive for vimentin and S-100 protein. In humans, it has been shown that melanocyte stem cells reside in the hair follicle bulge where they can leave, migrate, and differentiate into the epidermis or hair follicles.

Coat and hair color in mammals and feather color in birds is determined by melanins produced by melanocytes. However, other species, including amphibians, fish, reptiles, crustaceans, and cephalopods have specialized cells called chromatophores that contain pigment and reflect light in different ways that determine coloration. Chromatophores are sub-classified by their appearance under white light: xanthophores (yellow), erythrophores (red), iridophores (reflective/iridescent), leukophores (white), melanophores (black/brown), and cyanophores (blue).

FUNCTION OF MELANOCYTES

Melanocytes have a variety of functions, most of which are related to their unique ability to produce melanin. However, they also participate in inflammatory mediated reactions. A list of their functions include: (1) cosmetic appearance, allowing for camouflage, mimicry, social communication, and sexual attraction, (2) protection against ionizing radiation; can absorb a wide spectrum of ultraviolet and visible light wavelengths, (3) activing as a scavenger of cytotoxic free radicals and intermediates – this function provides probably the most protection against UV light damage. Other, non-pigment related functions, include the secretion of IL-8, IL-1α, and TNFα in response to inflammation to aid in inflammatory responses, inhibition of keratinocyte proliferation, and calcium homeostasis.

Pigment from melanin is the main determinant of skin and hair coloration. Other influences on skin color include: yellow carotenoids found in epidermal cells, red from oxygenated hemoglobin, and blue from unoxygenated hemoglobin in the dermal venules. Melanin can influence pigmentation in both a constitutive and facultative fashion. Constitutive pigment is defined as pigmentation that is genetically determined in the absence of external influences while facultative pigment is pigmentation that occurs in response to stimuli (such as UV light).

The synthesis and distribution of melanin in the epidermis involves several key steps, including: transcription of proteins required for melanogenesis, melanosome biogenesis, transport of the melanosome to the tips of melanocyte dendrites, and transfer of the melanosome to keratinocytes. All melanins arise from a common pathway in which dopaquinone is the key intermediate. While melanins have a wide range of color variation, they are classically divided in to two main melanin types, eumelanin (black-brown) and pheomelanin (red-yellow). Major differences are listed in Table 1. The major differentiating factor is the high levels of sulfur found in pheomelanins. Oxymelanins are yellow-red/brown non-dark pigments devoid of sulfur. These have been identified in humans, Argentinean goats, Addis sheep, and wild boar.

Eumelanin	Pheomelanin
Black-brown Low in cysteine Alkali-Insoluble Elliptical melanosomes Lamella/ highly structured melanosomes Produced w/ high levels of tyrosinase	Red-yellow High in glutathione or cysteine Alkali-soluble Spherical melanosomes Unorganized/microvessicle melanosome Produced w/ lower levels of tyrosinase

Table 1: Major differences of eumelanin and pheomelanin

Melanin synthesis

Eumelanin and pheomelanin are synthesized within melanosomes of melanocytes by a series of reactions that are catalyzed by specific enzymes (Figure 1). The conversion of Tyrosine to L-DOPA by the copper containing enzyme tyrosinase is the rate limiting step. Copper is considered a critical co-factor for tyrosinase.

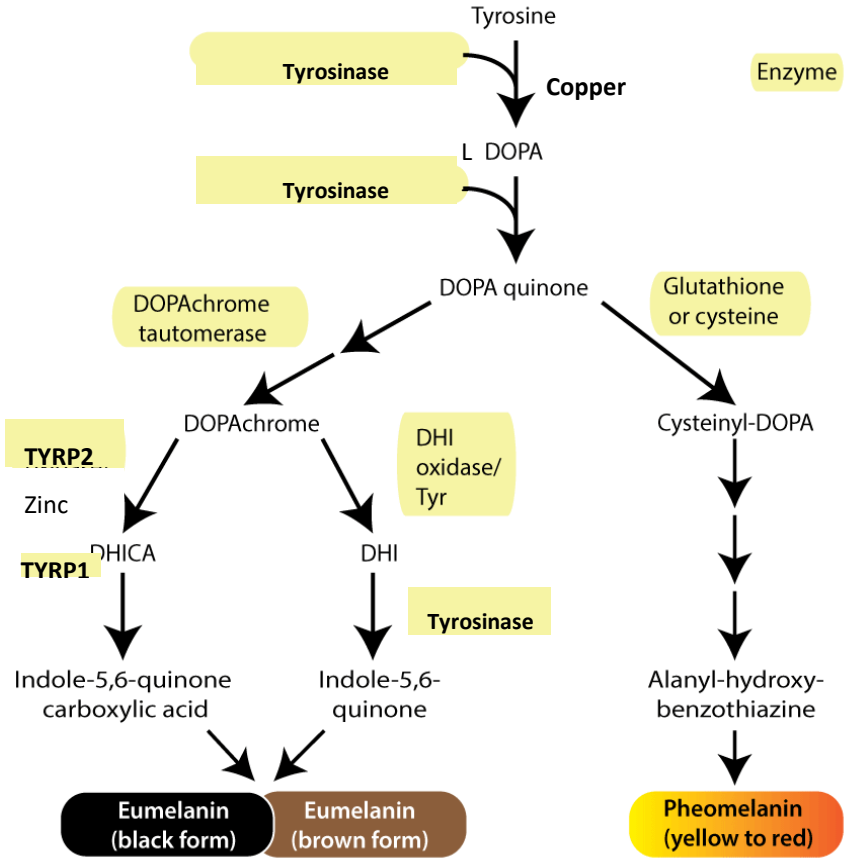

Figure 1 (edited version of original from biologystackexchange.com): The synthesis of melanin takes place in the melanosome in a series of enzymatic conversions. Melanin biosynthesis begins with the conversion of Tyrosine to L-DOPA (3,4 dihydroxyphenylalanine) by tyrosinase (Tyr) in the rate limiting step. L-DOPA is subsequently converted to DOPAquinone by the same enzyme. DHI (5,6-dihy-droxyindole) and DHICA (5,6-dihydroxyindole-2-carboxylic acid) are then formed to produce either black or brown eumelanin. Alternatively, through incorporation of glutathione or cysteine, DOPAquinone can form pheomelanin. TYRP = Tyrosinase related protein (1 and 2).

Regulation of Melanogenesis

Regulation of melanogenesis occurs primarily through the transcription factor MITF (microphthalmia-associated transcription factor). MITF induces transcription of Tyrosinae, TyRP1, DOPA and TyRP2 allowing melanogenesis to occur. MITf is upregulated by numerous processes that include the following ligand-receptor interactions: binding of c-kit with steel factor (stem cell factor) leading to activation of the cAMP pathway and the binding of the MC1-R (melanocortin 1 receptor) by α-MSH and ACTH (eumelanin) and ASP (agonist stimulating protein) (pheomelanin). Wnt (Wingless-related integration site) glycoproteins also play a role in melanogenesis. Wnt3a acts on melanoblasts to maintain MITF expression and promote melanoblast differentiation into melanocytes. Wnt signals also play a critical role in the development of melanocytes from neural crest cells and melanoblast differentiation into melanocytes.

α-MSH is one of the main drivers of melanogenesis. It is cleaved from a precursor protein called pro-opiomelanocortin (POMC) which is produced by the pituitary gland and epidermal keratinocytes. Ultraviolet radiation (UVR) acts as a stimulatory factor on POMC gene expression, and it is thought that UVR-triggered oxidative stress leads to POMC peptide production. POMC is the precursor molecule of ACTH as well. α-MSH can then activate the process of melanogensis through binding on the MC1-R receptor. UVR also upregulates the levels of α-MSH, ACTH and MC1R which in turn increase cAMP production which increases MITF expression. This is how UVR can stimulate melanogenesis and produce increased pigmentation.

Stimulators of melanogenesis include α-MSH, ACTH, Endothelin1, steel (stem cell) factor, leukotrienes (LTB4, LTC4), prostaglandins (PGE2, PGF2α),UV radiation, nitric oxide, histamine and neurotrophins. Inhibitors of melanogenesis include TNF-α,IL-1, IL-6. BMP-4 (bone morphogenetic protein -4) has been shown to inhibit the differentiation of neural crest cells in to melanocytes.

Another important regulator of melanogenesis is the endothelin (ET) family of peptides. ET's are involved in the late steps of melanoblast migration from the dermis to the basal layer of the epidermis. ET3 which is produced by ectodermal cells, interact with endothelin receptor B (ednrB) on melanoblasts. Proper interaction of ET3 with its receptor is required for survival, proliferation, and migration of melanoblasts as well as normal formation of enteric nerves. Defects in ET3 or EdnrB result in prominent melanocytes loss. A type (Type IV) of Waardenburg Syndrome, which is a hereditary syndrome characterized by varying degrees of deafness and facial bone defects and by variation in pigmentation of the skin, hair, or eyes, is caused by mutations of the gene encoding EdnrB. Lethal white foal syndrome, an autosomal recessive disorder resulting from the breeding of two Overo spotted paint horses, is also caused by a mutation of EdnrB. Affected foals, when born, are white in color and appear normal, but typically die 1-5 days after birth due to inability to defecate. This inability is due to a lack of the formation of myenteric plexuses resulting in colonic atresia.

Transfer of melanosomes to keratinocytes

Melanosomes are designated Stage I-IV by their stage of maturation. Stage I melanosomes

contain no melanin. As melanin is produced, melanosomes become more electron dense, move to the periphery of the melanocyte dendrite and await transfer to keratinocytes. The transfer of melanosomes to keratinocytes is critical for proper pigmentation to occur. Melanosomes are transported along the melanocyte dendrites attached to two microtubule proteins: dyneins (retrograde motion along dendrites) and kinesins (anterograde). At the dendrite tip, myosin-Va plays an important role in actin binding and melanosome transfer. Melanosomes are transferred to keratinocytes through multiple different processes including exocytosis, cytophagocytosis, fusion of plasma membranes, and transfer by membrane vesicle.

Hair Follicle Melanocyte Specifics

There are some important differences between epidermal melanocytes and follicular melanocytes. The follicular melanin unit undergoes cyclic modifications in coordination with the hair cycle. Follicular melanocytes provide melanin for hair shaft pigmentation and the result is the variety of hair and coat colorations seen. Follicular melanocytes are larger and more dendritic than epidermal melanocytes and produce larger melanosomes. Follicular melanocytes undergo maturation during anagen phase and their proliferation and maturation is dependent on c-Kit expression by melanocytes and steel factor synthesis by follicular keratinocytes. Melanogenesis occurs and melanosomes are transferred to developing hair shafts only during anagen phase. Melanocytes are located in the proximal hair bulb during anagen; during catagen they undergo apoptosis.

DISORDERS OF PIGMENTATION

Due to the complexity of the process of melanogenesis, numerous genetic defects and acquired diseases can occur that can result in varying changes in pigmentation, hearing, vision, and sometimes death. Many of these have been fully characterized in humans while some have been characterized in animals.

Genetic Pigmentary Disorders

Waardenburg Syndrome

Many genetic pigmentation diseases are well characterized in humans. Many of these have animal counterparts and some do not. For example, as described above, Waardenburg syndrome (type IV) is similar to lethal white foal syndrome. Other types of Waardenburg syndrome, which encompass a variety of genetic defects, including mutated MITF, PAX3, and SOX10, to name a few, all result in similar facial bone deformities in people with varying degrees of deafness. The deformities are the result of the failure of migration and development of melanoblasts. Many pet ferrets are affected by Waardenburg syndrome. Affected ferrets are deaf and exhibit a slightly flatter skull than normal ferrets and may have unusually wide-set eyes. Affected ferrets have a “blaze” coat color pattern, exhibiting a small white stripe on the top of the head. Similarly, affected humans often have a white forelock, again due to abnormal migration and differentiation of melanoblasts.

Chediak-Higashi Syndrome

Chediak-Higashi Syndrome is another genetic disorder seen in both humans and animals. It is an autosomal recessive disorder resulting from a genetic mutation in the LYST gene that results in defective lysosome and vesicular transport. This results in in a failure of phagolysosome formation and increased susceptibility to disease. Large lysosomes can be seen in neutrophils and macrophages on blood smears. Melanosomes are affected as well, and trichograms reveal macromelanosomes. Affected individuals exhibit albinism and photophobia. The most commonly affected animal are Persian cats with blue-smoke hair color and yellow eyes. Fundic exams reveal red light reflection instead of the normal yellow/green. Other reported affected species, include: Hereford cattle, Australian blue rats, mice, mink, foxes, and an albino orca. There is no cure and affected animals should not bred.

Pigment and Hearing

Melanocytes reside in the cochlea, where they exist as intermediate cells of the stria vascularis. These cells play an important role in maintaining the endocochlear potential in endolymph which allows hearing to take place. Mitf has been shown to be essential for the development and maturation of the cochlea due to its important role in melanocyte regulation.

Congenital Hereditary Sensorineural Deafness

Hereditary deafness is usually seen in dog and cat breeds with white pigmentation. In can be seen in dogs carrying the piebald gene. Deafness is the result of strial degeneration, the cause of which is unknown. Histologic studies have demonstrated an absence of strial melanocytes in these individuals. In the Dalmatian, postnatal auditory development has been shown to proceed normally up until 3 weeks of age, at which point the strial degeneration produces rapid loss of hair cell function.

There are numerous forms of oculocutaneous albinism. One of the more common forms, or oculocutaneous albinism type 1 has a genetically defective gene for tyrosinase, resulting in less of function, thereby causing an inability to produce melanin in otherwise normal melanocytes. Interestingly, in these animals, hearing is preserved. Therefore, melanin production within otherwise viable melanocytes is not essential for hearing. However, albinos are more susceptible than the normal population to hearing loss from noise and/or exposure to toxic agents. Therefore, melanin does provides some protective effect. Other types of oculocutaneous albinism are the result of defective TYRP1 or MATP genes.

Piebaldism

This is an autosomal dominant disorder that results from mutations of c-Kit or Steel factor. This mutation leads to the failure of melanoblasts to migrate appropriately to the skin and/or survive. Affected humans have broad depigmented areas, most commonly observed on the central forehead and trunk. Piebaldism rarely affects the melanocytes in the eyes or inner ear, so hearing usually remains intact. Piebaldism has been described in mice and affected individuals exhibit complete absence of melanocytes as well as abnormalities of the reproductive and hematopoietic

systems with homozygous loss of SF or c-Kit. Generally, in animals, the only abnormality seen is a coat appearing to have pigmented spots on a white background of hair, feathers or scales. The location of the pigmented spots is where melanoblasts were able to migrate during development. In this sense, piebaldism is generally not recognized as a problem in most domestic animals, as white spot patterns are considered a normal variant.

Lethal lavender foal syndrome (coat color dilution lethal)

This is an autosomal recessive disease that exclusively affects Arabian horses of Egyptian descent. Affected individuals are born a pale lavender, silver or pale-pinkish gray color. They are unable to stand and typically have an arched neck or back. They die shortly after being born. It has recently been shown that this is caused by a genetic mutation in the myosin-Va (MYO5A) gene. Myosin-Va plays an important role in melanosome transfer from melanocytes to keratinocytes.

Color Dilution Alopecia

This is a form of alopecia seen in "dilute" color dogs; those dogs that are blue or fawn in color, which are dilutions of black and brown respectively. Not all blue and fawn dogs are affected. The disorder is thought to be due to a a mutation(s) with coat color genes at the D locus. Under the influence of these genes, especially a mutation near the MLPH gene, dilute hairs gain significantly enlarged melanosomes (macromelanosomes). These hairs contain more melanin than their non-dilute counterparts, even though they appear lighter in color due to the clumping and abnormal melanosomes. The melanocytes of affected dogs also have abnormal dendrites and therefore have abnormal melanosome transfer to keratinocytes. Different breed variations exist in both phenotype (and possible genotype). It is most commonly seen in blue or fawn Doberman pinschers. Clinical signs include bacterial folliculitis which is often recurrent and hypotrichosis and/or complete alopecia with scale. Only dilute hairs are affected. The macromelanosomes cause the hair shafts to be to fracture. There is no cure and therapy is often aimed at limiting hair loss (by avoiding harsh shampoos like those containing benzoyl peroxide) and preventing recurrent infections. Some reports claim success with oral retinoic acids. The author has had success preventing infection and decreasing scale and overall skin health in affected dogs with phytosphingosine and essential fatty acid spot-on products.

Silver Labrador retrievers are a new "designer breed" of dog with some individuals exhibiting what may be a variant of color dilution alopecia. It is not known whether these dogs have been crossbred with Weimeraners or have a spontaneous mutation causing the dilute silver color. Some "silver" dogs exhibit similar clinical signs to color dilution alopecia.

Acquired Pigmentary Disorders

Vitiligo

This is an acquired disease that is caused by melanocyte destruction resulting in leukoderma and less commonly leukotrichia. It has been reported in dogs, Siamese cats, humans, mice, horses, pigs, and chickens. The pathogenesis is not well characterized and it is considered a

multifactorial disease involving both autoimmune and genetic mechanisms. In humans, numerous trigger factors such as trauma, sunburn, stress and systemic illness have been reported. In some cases the destruction of melanocytes may be directly mediated by autoreactive cytotoxic T-cells. In humans, it is thought that genetics play a large role in the disease by causing melanocytes to be more susceptible to damage from the immune system as well as oxidative damage from free radicals. In some dogs, there may be a genetic component, as a possible hereditary vitiligo has been described in Belgian Tervuren dogs, rottweilers, and Old English sheepdogs. Anti-melanocyte antibodies have been demonstrated in Belgian Tervuren dogs with vitiligo. Diagnosis is made through clinical signs and biopsy if needed. Histopathology will reveal a complete lack of melanocytes and inflammatory infiltrate. However, in early lesions some melanocytes and inflammatory infiltrate may still be present. In animals there is no successful therapy, but some cases may resolve without treatment.

Uveodermatologic Syndrome (Vogt-Koyanagi-Harada Syndrome – like syndrome)

In humans Vogt-Koyanagi-Harada (VKH) Syndrome is a rare, systemic, T cell-mediated autoimmune disease that targets melanin containing tissue. This results in leukoderma, uveitis, aseptic meningitis, dyascusis (hearing impairment), tinnitus (ringing in the ears), poliosis, and alopecia. Three phases have been described: (1) *Meningoencephalic* phase during which patients experience headaches, seizures, muscle weakness/paralysis and can vomit. (2) *Acute Ophthalmic* phase during which patients exhibit photophobia, eye pain and altered visual acuity. During this phase some patients exhibit uveitis, iridocyclitis, choroiditis, and retinal detachment. (3) *Dermatologic* phase during which vitiligo, poliosis and alopecia appear.

A similar condition has been described in dogs called Uveodermatologic Syndrome, or VKH-like syndrome. Affected dogs experience granulomatous uveitis and depigmenting dermatitis. While this is an autoimmune disease, there is a genetic component as well as Akitas are predisposed. Similar to VKH, affected dogs typically experience acute uveitis as the first clinical sign. Following the "ophthalmologic phase," depigmentation of the hair, usually on the face and the skin of the nose, lips, eyelids and occasionally the footpads, scrotum, anus, and hard palate occur and can be striking.

Uveitis can result in blindness due to secondary glaucoma as well as other ocular abnormalities and aggressive therapy to control the uveitis is warranted. Immunosuppressive medications such as glucocorticoids, azathioprine, and cyclosporine can be helpful at controlling the dermatologic signs. Therapy is typically life-long.

Other Pigmentary Changes

Graying

The process of graying hairs is seen when there is a gradual decline in melanogenically active melanocytes in the hair bulb (determined by age and genetics). A component of graying also involves ineffective melanosome transfer.

Post-inflammatory hyperpigmentation

This the most common cause of acquired hyperpigmentation seen in dogs and cats. The exact mechanism of hyperpigmentation is unknown, but it is thought to be due to keratinocytes releasing melanocyte-stimulating factors. Both diffuse hyperpigmentation in response to chronic inflammatory disease) can be seen and typically appears in a reticulated or lattice-like pattern. This is commonly seen in atopic patients in the inguinal or axillary regions. Focal hyperpigmentation can also be seen in focal inflammatory diseases like folliculitides or superficial pyoderma. Melanotrichia can also occur after deep inflammatory diseases.

Feline Acromelanism

Considered a genetic form of oculocutaneous albinism and seen in Siamese, Himalayan, Balinese, Birman, Burmese, and Singapura cats. This mutation has been genetically selected for and results in tyrosinase that is temperature sensitive and is inactivated in hair bulbs at temperatures above 35°C. Kittens are born white and develop points as adults due to the influence of external temperature. In these cats high temperatures produce light hairs while low temperatures produce dark hairs. If cats are shaved or exhibited self-induced alopecia, environmental temperature drops due to decreased hair, and new hair will grow in dark. The change in coat color is reset with the next hair cycle.

Leukoderma in horses

Leukoderma is a common change seen in horses from a variety of inciting factors including onchocerciasis, dourine, herpes coital exanthema, lupus erythematosus, pressure sores, ear papillomas, ventral midline dermatitis, regressing viral papillomatosis, or freezing and burns (chemical, thermal, radiation). Additionally, it has been reported in horses following contact with phenols and rubber (many rubbers contain monobenzyl ether of hydroquinone which inhibits melanogenesis). Leukoderma can be localized or multifocal and may be permanent or temporary depending on the underlying cause.

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