BIOLOGY AND DISORDERS OF MELANOCYTES

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1. INTRODUCTION

The biology of each significant step along the pathway from embryonic melanocyte development to skin pigmentation will be reviewed as a means to inform the missteps that lead to pigmentary disorders in domestic animals. The best and most up-to-date source of information on the genetic mutations affected pigmentation is the OMIA website (https://omia.org/key_articles/). Melanocytic neoplasia will not be addressed.

2. MELANOCYTE ORIGIN AND MIGRATION TO THE SKIN

In development, transient neural crest cells (NCCs) delaminate from the neural tube and form migratory, bipotent, melanoblast-schwann cell precursors that express SOX10. Down regulation of FOXD3 followed by expression of PAX3 and ultimately MITF specifies melanoblasts. Successful migration via dorsal and ventral embryonic routes to skin requires survival, expansion and differentiation of melanoblasts into melanocytes that is dependent on these and other factors, such as KIT, EDNRB, FGF, WNT, P-cadherin, and E-cadherin. Melanoblasts generate melanocytes in the skin, oral mucosa, eye, leptomeninges, and ear. NCCs also contribute to facial connective tissues, heart, enteric nervous system, etc., which explains the syndromic components of several pigmentary neurocrestopathies – the disorders of neurocrest.

2.1. Disorders of melanoblast migration

In humans, mutations in genes affecting embryonic melanoblast migration, proliferation, and survival from the neural crest result in **piebaldism** (KIT, SCF, SLUG), **Waardenburg syndrome** (PAX3, SOX10, MITF, SNAI2, EDNRB, EDN3), and **Tietz syndrome** (MITF). Thus, all three conditions are considered neurocrestopathies and, in each, hypopigmentation results from an absence of melanocytes in the skin due to failed migration and survival. Syndromic effects in Waardenburg syndromes result from concurrent deficiencies of NCC contributions to other organs, (eye, ear, intestine, facial bones, muscle, etc).

White coat spotting, or patches, in several domestic animals is phenotypically similar to piebaldism in humans, even occurring in similar midline areas such as the forehead, chest, abdomen and/or limbs. Hypopigmented irides and deafness are not features. KIT mutations cause nearly all cases in humans with piebaldism. Many domestic animals have KIT mutations and develop a similar white spotting pattern, for example in dogs, Birman cats (white gloving), cats (white spotting), horses, cattle, and pigs without congenital deafness, and are similar to peibaldism in humans, varying in extent of white areas or in position of white spots. In animals, other white spotted or white belted phenotypes occur in restricted skin areas and are similar to piebaldism but affect other genes, such as MITF in Brown Swiss cattle,Holstein and Simmental cattle, and TWIST2 in different breeds of cattle with the white belting pattern. Some conditions, such as nearly all white Northern Finn cattle and Swedish Mountain cattle with a mutation in KIT and segmental hypoplasia of the reproductive tract are not easily classified into a human condition but are syndromic conditions. Mutations in the KIT-ligand (KITLG; stem cell factor (SCF)) cause the roan coat color in short horned cattle and Belgian blue cattle. Mutations in KITLG contribute with other genes, likely MC1R and others, to increased incidence of digital squamous cell carcinomas in large-breed black dogs, like the Standard poodle and Giant schnauzers. Coat color

intensity also appears to be partly controlled by copy number variants in of the KITLG gene in dogs.

Conditions with white coat color in animals occurring with congenital sensorineural deafness (CSD) and/or hypopigmented irides are similar to human Waardenberg syndrome type-2 and mostly have the same genes affected as humans. Gene mutations are described in dogs (MITF, SILV), cats (KIT), cattle (MITF), pigs (MITF) and horses (ENDRB, PAX3, and/or MITF). Diseases expression in neurocrestopathies is known to be highly variable and not all animals with mutations in these genes have the ear and/or eye defects. Involvement of MITF also occurs in Tietz syndrome in humans, which overlaps with Waardenberg syndrome type-2, and occurs with generalized hypopigmentation of skin, hair and eyes as well as congenital deafness. One case, report in a nearly all white calf with congenital deafness and microphthalmia, was reported with a mutation in MITF and this is similar Tietz syndrome. Microphthalmia is only very recently reported in Teitz syndrome in humans and it is not a usual feature or a fully accepted clinical feature. Waardenberg syndromes type-4 has features of type-2 plus aganglionic megacolon in affected people and this presentation is reported in lethal white foal syndrome and lethal white (lamb) syndrome, both with mutations in EDNRB. Compared to humans, the genotype phenotype relationships in animals for coat color are more varied, often more pronounced, due to intense selective pressure by humans that value the unique coat colors.

3. MELANOCYTE STEM CELLS

Melanocyte stem cells of hair follicles are better characterized than those of the epidermis. Evidence also supports melanocyte stem cells in the dermis and eccrine sweat glands in some species. Dermal neural crest stem cell-like cells and Schwann cell precursors (stem cells) along nerves are considered possible sources of melanocytes. Cyclical hair follicle growth and arrest requires a constant source of transiently amplifying melanocytes from stem cells, which share their niche with hair follicle keratinocyte stem cells in the bulge/isthmus region. Localization and maintenance of melanocyte stem cells to these epithelial niches is thought to be partially dependent on KIT/KIT-ligand interactions with keratinocytes as well as, cadherins, integrins, and matrix interactions, such as Collagen-XVII. Additional factors important for melanocyte stem cell interactions in the hair follicle include WNT, B-RAF C-RAF, NOTCH, TGF-B, EDN, and NFIB. KIT/KIT-ligand interactions between melanocytes (KIT) and keratinocytes (KIT-ligand) control migration of activated melanocytes from the stem cell pool to the anagen hair bulb to pigment each new hair shaft via establishment of the follicular melanin unit (see below). Interestingly, ancient horses had a dilute hair coat because of asymmetrical positioning of melanocytes in the hair bulb that appears to be under transcription factor control of TBX3. Asymmetric melanocytes in the bulb leads to asymmetric pigmentation of the hair shaft only, thus less pigment in hair, and a dilute appearance. Modern horses have inactivating mutations in TBX3 and more uniform melanocytes in hair bulbs and thus pigment in hair shafts. TBX3 suppresses KIT-ligand on keratinocytes and thus melanocytes cannot be fully recruited to the bulb. Melanocyte stem cells can be lured out of their follicle niche to the epidermis in response to wounding or UVB irradiation and this is dependent on MC1R signaling.

3.1. Disorders of melanocyte stem cells

Hair graying occurs with aging, irradiation and chemotherapy in humans and animals. Inadequate melanin transfer to the growing hair shaft leads to this graying and is correlated with loss of melanocyte stem cells in hair follicles, possibly from oxidative injury. Melanocyte stem cells activated during follicle cycling, or with injury, fail to repress differentiation, do not return stem cells to a quiescent state and thus deplete stem cell mass. Keratinocyte stem cells support melanocyte stem cells in their shared niche and can be injured along with melanocytes to affect graying, for example with irradiation. Reversible hair graying occurs with use of receptor tyrosine kinase KIT inhibitors and anecdotal

evidence indicates that this phenomenon occurs dogs. In the **gray horse phenotype**, premature graying is autosomal dominant and is due to a mutation in STX17. Nearly complete loss of hair coat pigmentation occurs by 6 to 8 years of age and cutaneous melanomas occur in 70 to 80% of horses that reach 15 years of age. Progression of graying suggests a defect in hair follicle melanocyte stem cell survival. This mutation is largely responsible for the gray coat phenotype, melanoma formation, vitiligo-like depigmentation, and coat speckling but is modified by other genes, including ASIP.

4. MELANOSOME FORMATION

Melanin production and storage are the key characteristic features of melanosomes. Melanosomes are formed only by melanocytes, and by iris and retinal pigment epithelial cells of the eye. Importantly, melanosomes are transferred to keratinocytes of the epidermis, mucosae, and hair follicles. Melanosomes belong to the family of lysosome-related organelles (LRO), which include dense granules of platelets, type II pneumocyte lamellar bodies, etc., because they share with lysosomes an acidic pH, certain proteins, and a common pathway of organelle biogenesis. This shared biogenesis of different LROs explains the pleiotropy of clinical effects observed in several related pigmentary disorders. Eumelanosomes hold the brown-black eumelanin pigment and are distinct organelles from pheomelanosomes that hold yellow-red pheomelanin pigment. Much more is known about eumelanosomes; melanosome formation involves four distinct morphological stages. Stages-I and -II are melanin free. Stage-I establishes the organelle vesicular structure and Stage-II adds internal protein fibrils (premelanosome protein 17 (PMEL), also called Silver (SILV)) that hold melanin and give the ellipsoid melanosome shape. MART-1 and GPNMB also contribute to structure. Acquisition of melanin pigment biosynthetic enzyme machinery starts in Stage-II melanosomes and is dependent on organelle, cargo sorting, protein complexes that shuttle proteins from the golgi complex. In Stage III melanosomes, melanin deposits along, and thickens, luminal fibrils, which are still visible ultrastructurally. In Stage IV melanosomes, melanin deposition obscures fibrils completely.

4.1. Disorders of melanosome formation and cargo sorting

Hermansky-Pudlak syndrome (HPS) is a heterogeneous group of autosomal-recessive disorders in humans that cause cutaneous and ocular hypopigmentation along with variable extra-pigmentary clinical signs. Nine human genes identified involve one of four protein complexes: BLOC-1, BLOC2, BLOC-3 or Adaptor Protein Complex-3 (AP-3), which transport cargo proteins to the developing lysosomes and LROs, including melanosomes. In the nine HPS types, the combination of clinical signs depends on the type of LROs concurrently affected in different cell types, such as platelets, neutrophils, T-cells, and type-2 pneumocytes. For example, bleeding occurs in nearly all HPS patients due to defects in platelet dense granules. **Gray Collie syndrome** (GCS; also called canine cyclical hematopoiesis) presents with dilute skin and hair coat, cyclical neutropenia, thrombocytopenia, anemia and recurrent infections. A gray or pale-tan planum nasale is a typical feature. Dilution is due to decreased melaninization of melanosomes due to failure of complete delivery of melanin synthesis enzymes to the melanosome. GCS resembles HPS type-2 in humans and, in both, the causes is a mutation in AP3B1, which encodes the β-subunit of the AP-3 complex that delivers the enzymes.[38] **HPS type-3 of French bulldogs** occurs with a cocoa coat phenotype and has mutation in BLOC gene similar to humans. Affected French bulldogs have significantly decreased platelet dense granules but do not suffer any recognized bleeding tendency. **HPS type-5 of Donskoy cats** is associated with light-brown skin, yellow irises and a red-eye effect. Affected cats have a candidate mutation in the gene for the subunit-2 of the BLOC-2 complex. Similarly, **HPS type-5 in horses** is recognized in association with some variants of white marking, mutation occurs in the same gene, but there are no known deleterious effects.

Chediak-Higashi syndrome (CHS) is an autosomal recessive disorder of humans characterized by extensive hypopigmentation of hair, skin and eyes as well as a silvery sheen to the hair. CHS has been linked to mutations in CHS1 (also called lysosomal trafficking regulator (LYST)), which codes for the CHS-1 protein, thought to affect vesicle membrane fission/fusion events; this affects multiple lysosomes and LROs, in addition to melanosomes in different cell types in the body, thereby explaining the variable clinical signs. Distorted lysosomes and LROs form giant intracytoplasmic organelles that are visible as large granules, for example in the cytoplasm of neutrophils (pathognomonic). Affected patients suffer severe immunodeficiency with recurrent infections, neurologic signs, pulmonary fibrosis and bleeding abnormalities variably, among other features. Chediak-Higashi syndrome in animals has been reported in Herford, Brangus and Japanese Black cattle, Persian cats, beige mice, beige rats, Aleutian mink, blue and silver foxes, and a killer whale. Identified mutations in CHS1 are reported for Japanese Black cattle, beige mice and beige rats. Animals also develop hypopigmentation of the skin, hair coat and eyes and show silver colored hair. Bleeding abnormalities are attributed to platelet dense granule defects and immunodeficiency to multiple effects on leukocyte function, especially cytotoxic granule release.

Mutations in SILV cause the **merle coat phenotype** in dogs associated with CSD and blue irides (see above), **equine multiple congenital ocular anomalies** in silver and silver dapple horses and contributes to coat color dilution and hypotrichosis in calves **("rat-tail syndrome")** along with PMEL17 and one other unidentified locus. Leopard spotting complex in many horse breeds is associated with **congenital stationary night blindness** in horses and is attributed to a mutation in TRPM1 that is a channel protein thought to affect melanosome microenvironment, melanocyte survival, and retinal cell function. Leopard spotting patterns vary greatly and are affected by other genetic factors, most notably RFWD3 at the PATN1 locus that extends areas of white in the pattern. RFWD3 encodes for an E3 Ubiquitin ligase involved in P53 stabilization and DNA repair. A common theme emerges in multigenic disorders with variable hypopigmentation severity. Several pigment disorders appear to induce cytotoxicity that lead to hypopigmentation, and sometimes other defects of NCC origin, through loss of melanocytes. If other genes that protect the cells from this cytotoxicity are also affected, then pigment changes, and often the associated deleterious non-pigment changes, tend to increase in severity due the lack of cellular protection or compensation. Thus, as in **Harlequin** locus due to PSMB7 in Great Danes and the RFWD3 locus in horses with the leopard complex, non-pigment genes are altered that extend the phenotype, which are involved in the damaged protein or DNA responses and cell survival. Possibly the missing gene in "rat tail" syndrome in cattle will have a similar relationship. If the cytotoxicity is related to pigment production, then white spotted areas that lack melanocytes, tend to be protected, which might explain this observation in the rat tails syndrome. Obviously, when the additional defect is alopecia, then genes that alter hair cycle control and follicle stem cell maintenance also could be involved, making it not quite so simple.

5. MELANIN SYNTHESIS

In mammalian skin, melanin is a non-protein polymer of modified tyrosine and is present as two main types, brown-black eumelanin and yellow-red pheomelanin. Eumelanin is composed of oligomers of two related indoles, 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA), while pheomelanin is built from benzothiazine units. Melanosomes with pheomelanin production are unique from those with eumelanin. Initially, both pigments share a portion of their biosynthetic pathway. Tyrosinase (TYR), located in the melanosome membrane, converts L-tyrosine to L-Dopa, along with tyrosine hydroxylase isoform I (THI), and subsequently converts L-Dopa to dopaquinone. Copper is an important tyrosinase cofactor. Subsequently, the melanogenesis pathways split into those for eumelanin and pheomelanin. For eumelanin, reactive dopaquinone converts to dopachrome spontaneously.

Subsequently, dopachrome is converted to 5,6-dihydroxyindole (DHI) spontaneously and to 5,6-dihydroxyindole-2-carboxylic acid (DHICA) by dopachrome tautomerase (DCT, also called tyrosinase-related protein 2 or TYRP2). DHI, spontaneously, and DHICA, with the help of tyrosine-related protein 1 (TYRP1), combine to form oligomers of eumelanin. If sufficient cysteine is available, dopaquinone is converted to cysteinyl-L-Dopa and additional steps yield pheomelanins. Enzymes specific to pheomelanin production are not well described.

5.1. Disorders of melanin synthesis

Oculocutaneous albinism (OCA) presents with hypomelanosis of tissues, including the skin, hair and eyes due to a decrease or absence of melanin synthesis in melanosomes. Ocular albinism alone is not reviewed here. Melanocytes are still present in tissues and thus congenital deafness is not a feature. OCA can be accompanied by, but not limited to, delayed visual development, nystagmus, decreased visual acuity and photophobia. Neuronal retinal projections to the visual cortex are altered due altered functions of melanocytes in the choroid/PPE of the eye and nystagmus is a hallmark of nearly all forms of OCA in humans, which in animals, has only been addressed in Siamese cats.

OCA in humans is sub-classified into seven types depending on clinical signs and the gene mutation altering melanogenesis. Comparable OCA types-1 through 4 are reported in animals. OCA types-5, -6 and -7 are very rare in humans and are not yet associated skin hypopigmentation in animals. Note OCA type-5, due to a mutation in SLC24A5, is associated with “tiger eye” in Paso fino horses but these horses do not present with changes in skin pigmentation. In cats, mutations are reported in TYR that cause complete albinism similar to OCA type-1 and temperature sensitive seal-point variants similar to OCA type-1B and in TYRP1 that cause a brown/chocolate coat consistent with OCA type-3 in humans. There is considerable variation in the alleles associated with TYR and OCA type-1 in cats, for example a new TYR allele allows for some pigment production, for example with the Mocha phenotype in Burmese cats. In dogs, mutations are reported in the OCA2 gene (encoding the OCA2 melanosomal transmembrane protein) similar to OCA2 in humans, TYRP1 that causes a brown coat similar to OCA type-3, and SLC45A2 causing near white, cream coat similar to OCA type-4. Interesting, the latter has a higher risk of eye and skin melanomas in Dobermans (“white Dobermans”, which are actually cream). In dogs, a mutation in MFSD12, coding for major facilitator superfamily domain containing 12, appears to cause dilution of pheomelanin but not eumelanin and leads to cream and white coat colors in dogs that have eumelanin production blocked in hair. A mutation in MFSD12 gene is reported in humans and this may be a new type of OCA, but it is not yet classified as such. Interestingly, MFSD12 appears to be expressed in lysosomes but not eumelanosomes. In sheep and cattle, albinism is reported in several breeds but often without genetic investigation. The condition in the Braunveih calves is due to TYR mutation and is similar to OCA type-1 in humans. A mutation in TYRP1 that was strongly associated with the dun (brown) coat color in Dexter Cattle, similar to OCA type-3. Cattle are also described with a mutation in SCL45A2 that is seen in OCA type-4. In horses, the cream, pearl, and sunshine coat colors are very pale to nearly white in homozygous animals, some occur with blue eyes, and are attributed to a mutation in SLC45A2 similar to OCA type-4 in humans. SLC45A2 codes for a protein called solute carrier family 45, member A2 - proteins in the SLC family function as channels and transporters. Horses with the very light coated champagne phenotype are reported to have a mutation in SLC36A1 for which a comparable OCA type in humans is not reported. Similar to dogs, a mutation in MFSD12 is associated pheomelanin dilution in Shetland ponies. In pigs, polymorphisms in the OCA2 gene in a red strain of Iberian pigs possibly contributes to coat color variation, similar to OCA type-2 in humans and mutations in TRYP1 are reported akin to OCA type-3.

Dietary amino acid deficiency can cause phenylalanine (Phe; essential) and tyrosine (Tyr) deficiency and both can lead to decreased melanin synthesis and changes in coat color. In the best example, black

cats fed Tyr and/or Phe restricted diets develop red-brown hair coats, with decreased melanin in hair shafts, and gave birth to kittens with red discoloration of normally black hair coats. **Dietary copper deficiency** leads to graying of the hair coat because copper is an essential cofactor for tyrosinase, the rate-limiting enzyme in melanin synthesis. Graying of the periocular hair coat in black areas gives rise to the common name of "spectacle disease" or "ghost eye". Depletion of copper in puppy dogs caused hair coat graying.

6. MELANOSOME TRANSFER TO KERATINOCYTES

Melanosome transfer to keratinocytes at the tips melanocyte dendrites is referred to as the *pigmentary synapse* and different mechanisms for transfer are proposed. One epidermal melanocyte is estimated to transfer melanosomes to 36 keratinocytes, which form an *epidermal melanin unit*, and one follicular melanocyte transfers melanosomes to 5 keratinocytes, the *follicular melanin unit.* At the melanocyte dendrite tip, three proteins (Rab27a, melanophilin, myosin-5a) facilitate transfer of melanosomes to keratinocytes.Basal keratinocytes accumulate more melanosomes than suprabasal keratinocytes, where most melanosomes are degraded by autophagy prior to cornification. Smaller melanosomes distribute to the perinuclear cap (microparasol), often in membrane bound vesicles, while larger melanosomes distribute widely in the cytoplasm. After endocytosis, smaller melanosomes are bound by dynactin to dynein motors, for transport along microtubules towards the nucleus, and to microtubules in the perinuclear cap, after arrival.

6.1. Disorders of melanosome transfer

Griscelli syndrome (GS) in humans comprises three disorders related to mutations in three genes (MLPH, MYO5A, RAB27a) that are important for melanosome transfer to keratinocytes. Melanin dispersal to keratinocytes is limited, which leads to color dilution of skin and hair. Silver-gray hairs have pigment clumping and large melanosomes accumulate in the cell bodies of melanocytes. **Lavender foal syndrome** (LFS, also called coat color dilution lethal) in Arabian horses, characterized by dilute coat color and multiple neurological signs in new born foals, is due to a mutation in MYO5A and is similar to GS type-1 in humans. Similarly, and mutation in MYO5A is described in a **Miniature Dachshund with GS type-1**, which included coat color dilution and neurological signs similar to humans.. **Diluted coat color** associated with mutations in MLPH is reported in several species and examples are in cats, dogs and cattle similar to GS type-3 in humans. **Color dilution alopecia** (color mutant alopecia), possibly related to Black hair follicle dysplasia, is a hereditary condition associated with coat color dilution in over 15 breeds of dogs and cross breeds. In dogs, mutations in MLPH contribute to CDA but additional modifying factors appear to be required.

7. ACQUIRED PIGMENT ABNORMALITIES

Once the skin and hair are pigmented, acquired pigmentary abnormalities occur from disruption of the epidermal melanin and/or follicular melanin units. Hyopigmentation occurs in diseases that either specifically or non-specifically disrupt melanocytes, keratinocytes, or both cell types. For these reasons, acquired depigmentation is very common in veterinary medicine. Simple, generic physiochemical injuries, such as wounds, burns, etc, disrupt the epidermal and follicular melanin units and lead to depigmentation. More specific cell targeting of melanocytes is seen with vitiligo, uveoderatologic syndrome, and alopecia areata. In the latter two disorders, keratinocyte targeting can be a feature of the epidermis and hair bulb respectively. Cell targeting of keratinocyte occurs in the epidermal cytotoxic disorders such as keratinocyte viral infections, cutaneous lupus variants, erythema multiforme, Stevens-Johnson syndrome/toxic epidermal necrolysis, and others. Less specific cell targeting leading to depigmentation is seen with vascular disease and ischemia, epitheliotropic lymphoma, autoimmune

subepidermal blistering diseases, and others. Hyperpigmentation is common and not particularly specific to one disorder and is easily trigger in the skin from many different stimuli. Solar and inflammatory stimuli lead to acquired hyperpigmentation and the latter is very common in veterinary medicine and is non-specific. Pharmacological interventions can lead to depigmentation or hyperpigmentation and these effects are recognized in human medicine but are not well described in veterinary medicine.