AGE-RELATED HAIR PIGMENT LOSS

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Abbreviations: ATM: Ataxia-telangiectasia mutated; cAMP: Cyclic adenosine monophosphate; Dopa: 3,4-dihydroxy phenylalanine; DCT: Dopachrome tautomerase; HF: Hair follicle; MITF: Micro-phthalmia-associated transcription factor; ROS: Reactive oxygen species; UVR: Ultra violet radiation

Abstract:

Humans are social animals that communicate disproportionately via potent genetic signals imbued in skin and hair, including racial, ethnic, health, gender and age status. For the vast majority of us age-related hair pigment loss becomes the inescapable signal of our disappearing youth. The hair follicle pigmentary unit is a wonderful tissue to study mechanisms regulating to general aging, and often before this becomes evidence elsewhere in the body. Given that follicular melanocytes (unlike those in the epidermis) are regulated by the hair growth cycle, this cycle is likely to impact on the process of aging in the hair follicle pigmentary. The formal identification of melanocyte stem cells in the mouse skin has spurred a flurry of reports on the potential involvement of melanocyte stem cell depletion in hair graying (i.e., canities). Caution is recommended however, against simple extrapolation of murine data to humans. Regardless, hair graying in both species is likely to involve an age-related imbalance in the tissue's oxidative stress handling that will impact not only melanogenesis but melanocyte homeostasis and survival. There is some emerging evidence that the hair follicle pigmentary unit may have some regenerative potential, even after it has begun to produce white hair fibers. It may be feasible to develop strategies to modulate some aging-associated changes and so maintain melanin production for longer.

Background: Mixed indole-rich compounds (melanins) significantly contribution to the color of skin and hair color, and appear to be one of nature's favorite molecules, a view supported by its ancient origins and frequent re-use throughout numerous different phyla, not just in mammals. In mammals melanin is synthesized via a complex biochemical pathway [1-SlominskiPhys Rev] that given its cytotoxic potential occurs within unique lysosome-related organelles called *melanosomes* in a biologically-mysterious neural cell type called the melanocyte. It was initially thought that melanocytes were present only in a limited number of body sites, principally the skin, hair follicle, eyes and ears but recent evidence suggests these cells may be found in several other non-cutaneous sites including sites where they would not be expected to transfer their melanin e.g., the heart [2-Extracut Mel].

Our rather simplistic, pre-epigenetics age, understanding of mechanisms employed by evolutionary selection pressures on our phenotypic traits has essentially confused up rather the 'value' of canities in humans when one considers that humans would have been long dead and definitely well past reproductive peak age for pretty much all of human evolutionary time on planet earth to give hair graying some selective survival advantage [2 2 Allende MF].

That said, much of the human species' success appears to flow from its powerful social strategies, so changing hair color contributes significantly in modern human social communications. One of the striking differentiating features of young adult humans and other primates is the extra-long and commonly deeply pigmented scalp hair fibers, evidence of the enormous melanogenic activity in growing pigmented scalp hair follicles. Some have suggested this trait derived from melanin (especially 'wildtype' black-brown eumelanin) being an important sink for noxious materials (metals, toxins etc), thereby sparing the living tissue of a highly vascularized scalp. While nature favors a human population with dark brown/black scalp hair (and skin) given the climatic imperatives associated with our origins in Africa and general UVR exposure risk [3 9- Rees JL], ≈5% of humans concentrated in northern Europe exhibit a remarkably diverse palette of hair colors e.g, white blonde, yellow blonde, red, auburn and all shades in between.

The hair follicle pigmentation unit: Pigment loss from skin epidermis, and more dramatically from hair follicles (HF), approaches the end stage in the life history of the cutaneous melanocyte [4, Tobin 2013 PCMR review]. Beginning with its pigment cell lineage commitment in the neural crest during embryogenesis the HF melanocyte embarks on an eventful and protracted course that includes highly variable activity states from quiescent melanoblasts (stem cell), to proliferating, differentiating, senescencing terminally-differentiated melanocytes that are more sensitive to cell death by apoptosis. Casual recognition of white hair on the scalp of elderly African subjects points to the variable fate of skin versus HF melanocytes. It appears that microphthalmia-associated transcription factor (MITF), SOX10, Pax3, KIT, fibroblast growth factor-2, endothelin 3 etc., are all involved in committing the early neural cell to the melanocyte lineage [5, 6, 15,16] and in several aspects of their subsequent differentiation pathways. However, even late adult skin contains immature melanocytes that appear to retain some plasticity [6, 16].

Several follicular melanocyte subpopulations exist in the adult human scalp (Fig. 1). These can be distinguished most easily on the basis of their (variable) dopa oxidase activity of tyrosinase [19,20], and this level of cell differentiation appears to be regulation in the context of their microenvironmental cues within different components of the hair follicle. While melanogenically-active melanocytes can be found distributed in the HF at the infundibulum and basal layer of the sebaceous gland, it is the upper hair bulb matrix were fully-differentiated follicular melanocyte synthesize and transfer the melanin into the developing hair fiber. An immature subpopulation of non-dendritic, dopa oxidase-negative follicular melanoblasts/ melanocytes can also be detected in the outer root sheath and most proximal bulb, and may represent a pool of "transient" melanocytes that migrate from precursor melanocyte stores in the hair follicle bulge to other areas of the outer root sheath [23].

Proposed mechanisms for loss of pigment production in aging human scalp hair follicles: Given the tight coupling of the follicular pigmentary unit with the hair growth cycle [refs], does continual HF cycling have implications for the aging of the hair pigmentary unit? Key to this question is the apparent instability of the hair pigmentary unit compared to the continuously-active melanocytes in the epidermis, which is an inevitable consequence of the hair cycle, be it either smooth or stochastically bistable [Bernard 2012]. This instability is an outcome of cycles of melanocyte activation and proliferation during hair growth (anagen), selective apoptosis of the hair bulb's complement of follicular melanocytes during HF regression (catagen), and their quiescent status in telogen [ref]. It is not clear whether the extended duration of anagen in scalp HFs (3-5 years), and therefore the corresponding time bulbar melanocytes will engage in melanogenesis and melanin transfer, has direct implications for follicular melanocyte homeostasis and survival. One possible clue may come from the apparent robustness of the eyebrow follicular pigmentary unit with its ultra-short (1 month) anagen duration. These hairs are commonly more pigmented that scalp hair at an age when canities may already be extensive in the latter. There is also evidence from rare 'blocked-in-anagen' case reports [7], where extremely long hair fibers representing 3 decades of continuous anagen and can be similarly pigmented along their entire length. These cases suggest anagen duration may not be a limiting factor for the maintenance of an optimal pigmentary environment. This view however, is based on the unproven assumption that no replenishment of bulbar melanocytes i=occurs during a single anagen phase.

The transition between full anagen and the start of catagen-associated regression of the hair follicle during the adult hair cycle may hold some clues about the stability of the follicular pigmentary unit. For example, tyrosinase expression and activity decreases rapidly in late catagen [28,29] causing a physiologic decrease in follicular melanogenesis that is likely to result from the general withdrawal of growth and differentiation-supporting morphogens and mitogens in the regressing HF. However, the follicular melanocyte appears to be exquisitely sensitive to the changing tissue microenvironment in catagen HF, such that melanocyte changes [34] significantly predate in time by at least a few days the cessation of keratinocyte proliferation (explaining the depigmented proximal end of telogen hair fibers). We now know that many of the terminally-differentiated melanocytes that were actively pigmented the hair fiber during anagen as selectively deleted (by apoptosis) from the regressing HF [34,36, 37] and are replenished during the following telogen-anagen transition from the melanoblast reservoir located in the upper HF [34,23].

Aging of melanocytes of the follicle pigmentary unit: The intrinsic instability of the follicular pigmentary unit is likely to be a feature of life-long continuous de- and reconstructive HF cycling, as well as relative 'frailty' of neural crest-

derived cells and the unique cytotoxic potential of redox biochemistry in melanogenesis. These features and others probably make this unit uniquely susceptible to age-related change. We know that the overlying epidermal melanin unit is very stable – there is extremely low turnover of melanocytes in the epidermis, despite continuous constitutive activity and additional facultative activity due to repetitive UVR exposure. However, even here there is a 10-20% reduction in number (whether in sun-exposed or unexposed skin) for every decade after 30 years of age [41,42]. The intrinsic resistance to apoptosis in epidermis melanocytes is thought to be in part due to their relatively high expression of Bcl2 equipping these cells to survive both endogenous (via melanogenesis) and exogenous (i.e. UVR) sources of oxidative stress in the form of reactive oxygen species (ROS). Most of the available data on human skin melanocyte aging is limited to the relatively stable epidermis melanocyte population and then from i*n vitro* studies characterized by enforced melanocyte proliferative and replicative senescence states. Caution is needed here, as these are unnatural states for an essentially post-mitotic adult cell type.

Biology of melanocyte aging: Melanocytes are lost with age from the skin epidermis and hair follicle and from nevi and eye [43,44,45,21], suggesting the possibility of a common molecular clock controlling aging. However, all melanocyte subpopulations do not appear to be similarly affected; differentiated dopa-positive (i.e. tyrosinase-positive) melanocytes appear to go first, followed by amelanotic undifferentiated melanocytes survive longer (especially in the outer root sheath of the adult HF), and lastly by stem cell melanoblasts located in an undamaged niche. There also appears to be some sufficient variance in the rates of melanocyte loss in different body site that may reflect both their starting positions during embryogenic stem cell seeding patterns [46] as well as their experience during their time distributed in variously stable (e.g, epidermis), unstable or bi-stable (e.g., HF) tissue microenvironments during adult life. It will be important to determine whether an aging melanocyte is compelled to be deleted from the tissue, eg., via apoptosis, or whether these cells can remain in a senescence state for a prolonged period, even if sub-functional. There will, inevitably, be an over-arching genetic element (probably also an epigenetic one, Botchkareva et al 2013) governing the fate of the aging melanocyte. A polygenic heredity (autosomal dominant) appears to be a dominant factor in the expression of canities and this would explain how entire kinships can experience both early and extensive graying or conversely be protected via unusually late graying.

We know only very little about the mechanisms underlying melanocyte loss from the adult human HF with age, despite several high-profile papers in the world's leading journals. Perhaps some of the reason for this limited progress has been the mouse-centric approach to much of 'human' pigmentation research. In this way pigment cell researchers may have overly focused on the follicular melanocytes of the nocturnal and UVR-shy mouse as a proxy for

human epidermal melanocytes (much less human HF melanocytes). Indeed, there continues to be a steady stream of data suggesting that human and mouse pigment cell biology differs in several other important ways ([Tobin PCMR 2013] [47]. That said, the formal identification of melanocyte stem cells in the upper mouse hair follicle [23, 48] has shed some light on the fate of their progeny in the epidermal and follicular melanin units during mammalian adult life.

One of the proposed drivers of melanocyte aging in general and for canities in particular relates to an increasing unbalanced redox with age, and its impact on both melanocyte stem cells and differentiated melanocytes in the anagen hair bulb. Such reducing redox support is thought to derive from age-related reduction in anti-oxidant enzyme expression and support [Kauser et al JID 2010], reduction in anti-apoptosis proteins like Bcl2 family proteins [48], failure of maintenance of stemness in the melanocyte reservoir [Nishimura] that could in part be due to deficiency in the melanocyte master transcriptional regulator Mitf and Ataxia-telangiectasia mutated (ATM) [Sikkink] as well as aberrant pro-differentiation and MAP kinase signalling [Nishimura, 50,52,53]. These observations dovetail well with the dominant "free radical" theory of aging [54, 55], where accumulation of oxidative damage determines the rate of aging. While aging melanogenic melanocyte could be expected to exhibit 'free-radical'-associated anomalies, just how these are degenerative is not yet clear.

ROS can damage all categories of biomolecules in the cell, but their effects on DNA (both nuclear and mitochondrial) can drive an accumulation of mutations. While aging impairs the melanocyte's ability to mount a robust antioxidant response, the fate of the latter be worse in differentiated melanocytes than that for other skin cell type, given that much of the biochemistry of these cells involves melanogenesis - replete with oxidative stress generation (e.g., quinone and semi-quinone production) [56, 25]. However, the prolonged melanogenesis characteristic of hair bulb melanocytes during anagen is likely to generate large amounts of ROS via the oxidation of tyrosine and dopa to melanin [5,59]. Failure to remove ROS may cause the resultant oxidative stress to damage the melanocyte itself. It is noteworthy that differentiated HF melanocytes have not been associated with melanoma, suggesting that assuming a post-mitotic (pre)senescent status may be the safest outcome, even if increasing the likelihood of canities.

Recent studies have reported increased melanocyte death by apoptosis and oxidative stress in the human follicular–melanin unit of graying HF [57, TObin]. In one study the "common" deletion in mitochondrial DNA (a marker of oxidative stress) was reported to occur more prominently in graying HF than in matched normally-pigmented HF when stressed with a pro-oxidant [57]. A reasonable interpretation here may be that melanocytes in canities-affected HFs are less capable of handling exogenous oxidative stress due to impaired antioxidant systems, and this concurs with findings we have recently reported [Kauser et al JID], especially in terms of a depleting expression and activity of

catalase [Kauser et al JID]. This redox imbalance appears to be preferentially affect the melanocyte population, as hair growth in gray/white HF can exceed that seen in matched pigmented HF [57, 30,58,50]. This unexpected finding suggests that hair bulb keratinocyte proliferation is enhanced in the absence of either melanocytes or melanin and that at very least keratinocytes are more capable of handling age-related increases in oxidative stress than melanocytes. Indeed, white or gray hair fibers may accumulate hydrogen peroxide [33] when produced from HFs that express little catalyse and methionine sulfoxide reductase A and B protein expression. Unfortunately, the cause or effect of this pro-oxidant environment in canities, even if it damages tyrosinase function, is not known.

Hair follicle changes in canities: A hallmark of aging HFs their markedly reduced numbers of differentiated and functioning melanocytes located in the hair bulb melanocytes. Indeed, canities-affected HFs is also known to produce 'senile white' hair. While those engaged in canities research in mice tend to focus on the status of the melanocyte reservoir, researchers of human hair graying focus principally on the anagen hair bulb, and (upper) outer root sheath, where some (mostly immature) pigment cells can survive for some considerable time after graying begins elsewhere in the HF. Thus, it is not in my view currently possible to know exactly when white HFs contain absolutely no more melanocytes of any kind, despite some statements in the current literature [60, 2,3,4]. The description 'gray' HF can be taken to mean that at least some differentiation melanocytes are still present in the anagen hair bulb and are attempting to transfer some melanin to the recipient pre-cortical keratinocytes, even if apparently rather haphazardly (Fig. 2). Similarly, these gray hair bulbs exhibit reduced but still detectable dopa-oxidase reaction, indicating that melanocytes remain with at least some tyrosinase activity. Indeed, some pre-cortical keratinocytes close to apparently degenerating pigmented melanocytes fail to accept melanin granules despite the presence of considerable levels of melanin [2,3,4]. In this way, canities can also be defined as a disruption in the normal relationship of melanocyte to keratinocyte in the 'follicular melanin unit'. This also indicates that at least some significant elements of the canities mechanism must occur during the same anagen VI phase of the hair cycle, rather than requiring events specific to the transition point between previous telogen and subsequent anagen in order to grow-out the first gray/white hair fiber.

Observations from graying scalp hair fibers suggest that canities-affected bulb melanocytes may exist in a dystrophic hypertrophic state for some time. Cytoplasmic organelles and machinery in targeted melanocytes show evidence of enhanced auto-phagolysosomes, some containing defected melanosomes, suggesting that the melanocytes may be attempting to remove aberrant melanosomes. Abnormal melanosomes may become toxic in the cell if they become leaky in terms of ROS and other pro-oxidants [Ref Des]. If the melanocyte is ultimately unable to efficiently detoxify and re-cycle damaged cellular products it may become so compromised it will undergo apoptosis

and die [61]. Indeed, histological sections of canities scalp commonly show significant evidence of melanocyte death, not least as evidenced by deposits of melanin debris in the follicular dermal papilla and connective tissue sheath. Further support for the involvement of ROS in the histopathology of canities is suggested by the observation that melanocytes in graying and white hair bulbs may be vacuolated, a common cellular response to increased oxidative stress [ref Westerhoof}].

Does melanocyte loss from aging hair follicles affect their growth behavior? All normal HFs are populated with melanocytes [ref-Des), and these cells are also present even in dermatological disorders feature little or no melanin (e.g, albinism) [Eva and Des book]. However, melanocytes are clearly the minor population of both epidermis and hair bulb cells – not surprising as both epidermis and HF are essentially epithelial keratinous structures. Also, the limits of human longevity are clearly associated with retained ability to make a skin epidermal barrier, showing of much regenerative plasticity there is imbued in the skin keratinocyte population. Similarly, though clearly not essentially, scalp hair growth can continue for well over 100 years, even it is invariable gray by then. As can be seen from very old people with dark skin however, that cutaneous melanocyte survival is also possible for well over 100 years of life. Still, there appears to be significant regional variations is progression of melanocyte loss throughout the body, and it is not likely accidental that this is most striking in the scalp where the greatest call on follicular melanogenesis was made during life [check 65, 66, 58].The hair follicle pigmentary unit functioning most optimally during post-adolescence and early adulthood life, when terminal hair growth is also optimal and the follicular melanin unit has arrived at its most stable tonal variant and responding to our post-puberty hormonal status. By that point the scalp follicular melanin units will have experienced only a few hair growth cycles involving only a few stem cell re-seedings following by differentiation. If you consider an average scalp hair growth cycle length of 3.5 years and depending on the pattern of melanocyte stem cell distribution to individual scalp HF during embryogenesis, some individual scalp HFs will experience approximately 10 rounds of melanocyte re-seeding in the typical “gray-free” Caucasians aged 30-40 years [67, also new Loreal 50:50].

While Caucasians are most prone to early and extensive hair graying, the onset and progression of canities, this phenotype still correlates very closely with chronological aging (but not with photo-aging) regardless of race or ethnicity. Age of canities onset is genetically controlled and heritable. Om average Caucasians start to gray by their mid-30s; Asians about 5 years later, and those of African ancestry latest in their mid-40s. Despite the cosmetic concerns of may your people, premature hair graying is really only accepted if it occurs in the mid-late teen for Whites, before 25yr in Asians, and before 30yr in Africans. A recent reassessment of the rule of thumb that by 50yr,

50% of people have 50% gray hair has concluded that globally this applied to less than 23% of humans and then differs significantly by ethnic or geographical origin as well as natural hair colour [Panhard S, Lozano I, Loussouarn G. Greying of the human hair: a worldwide survey, revisiting the '50' rule of thumb. Br J Dermatol. 2012 Oct;167(4):865-73.]. However, even the term “gray” can be controversial; some consider this color to be derived from an admixture of fully-white and fully-pigmented hair only rather than via pigment dilution within single hairs. This author has observed canities to affect individual HFs during a single anagen VI growth phase, with resultant gradual loss of pigment along the same hair shaft. Furthermore, the extent to which hair is perceived as gray/white may not reflect absolute its melanin content, as this can be influenced by hair fiber morphology/geometry that contribute to fiber curvature, shine, luster. Also, important is the background contextual color of the non canities-affected scalp hair - thus, early graying is first noticeable in dark-haired individuals. Paradoxically canities appears to be more extensive in blonde-haired individuals, who more quickly appear blanched before a similar fraction of total hair turns white. The rate of graying is also highly variable, not only on different areas of the scalp but also across the body surface - a phenotype that reflects perhaps variations in original seedings during melanoblast migrations in embryogenesis. Alternatively, there may be site-specific variations in the quality or ‘stemness’ of melanocyte stem cell niches across the body. This may be reflected in the observations of scalp hair graying first at the temples, and then spreading to the vertex and thereafter to the remainder of the scalp, affecting the occiput last. Beard and body hair is usually only affected later. Most of us will also have noticed that striking autonomy of individual HFs, and this is also apparent in scalps sporting so-called ‘silver hair’, characterized by an admixture of white/gray and deeply pigmented hair. While, the optical effect here may be silver rather than white, this phenotype shows that there is little systemic control of contiguous HFs and that pigmentation status in the HF is controlled from within individual follicles. The juxta-positioning of HFs with variably intact or depleted HF pigmentary units within the same precise region of the scalp suggests that their shared skin dermis and presumably vasculature contribute little to determine the ultimate fate of the respective pigmentatary units. It is also difficult to see how these hugely variable outcomes result from significantly different initial seedings of their respective stem cell niches during embryogenesis.

Hair bulb melanocytes need to interact closely with pre-cortical keratinocytes in order to distribute melanin to the hair fiber [13, 21, 24]. There has long been intense research interest in the nature of melanocyte-keratinocyte crosstalk in the epidermis [ref, boissy-Boots grant], and with the advent of in vitro and ex vivo culture models for HFs attention have been turning also to the nature of the cellular partnership in HFs [Des, Ralf etc]. Evidence from mixed skin-phototype origin melanocytes and keratinocytes suggests that it is the keratinocyte partner that directs telanocyte

behavior to produce the type of melanin matching the keratinocyte donor's skin phototype. Similarly, melanin transfer to the keratinocyte appears to reduce its proliferative potential and so may stimulate their terminal differentiation. Thus, the absence of melanocytes and more important melanin from the HF is likely to influence the life-cycle of hair bulb matrix keratinocytes in a number of ways. For example, white HFs (e.g., most easily observed in beard hair) appear to grow more rapidly than co-located pigmented hair *in vivo* [59]. This superior growth rate of white HFs over pigmented HFs can also be seen in *ex vivo* culture where HFs lack a patent vasculature or direct neural regulation, again suggesting very much the involvement of an autonomous system [57]. Why should hair growth be checked by its pigmentary status? Some clues may be found in the unique properties of the indole-rick biopolymer that is melanin, and off their originating cytoplasmic lysosome-related organelle, the melanosome. The later have indeed a be viewed as regulatory packages [68], especially on account of how their ability to buffer calcium. Calcium is a critical second messenger not only for cell signaling in melanogenesis and melanosome transfer, but also for keratinocyte differentiation. Saturation binding of transition metals like iron and copper to melanin will also impact on the antioxidant defenses of the keratinocyte interacting directly with its partner melanocyte and its complement of transferring melanin granules [3,4,5]. Melanocytes are also likely to influence keratinocytes is other more generic (i.e., non pigmentary) ways. As a very statement and long-living sentinel dendritic cell of the skin, melanocytes produce a wide range of regulatory cytokines, growth factors, eicosanoids, adhesion molecules, and extracellular matrix molecules, and these will influence the behavior of neighboring keratinocytes [69, 70]. The reduction to absence of these bio-response modifiers in the canities-affected HF is therefore most likely to have implications for the highly active matrix keratinocytes in this HF compartment. Just only example of this is in the altered nature of the hair fiber in early graying - which are commonly characterized by increased hair fiber caliber (a result for higher keratinocyte output in the hair bulb), altered more 'unmanageable' physico-mechanical characteristics (e.g., commonly is coarser, wirier etc) [30]. These are not trivial changes, and have significant implications for how the personal care industry will approach addressing age-related changes in hair phenotype. We have noticed a particular canities-related change in the relationship between how the hair cortex and the (facultative) medulla are produced. One interpretation is that an early response to the diminishing melanin-rich environment of pre-cortical keratinocytes in graying HF is the reprogramming of matrix keratinocytes to increase the production of medullary, rather than pre-cortical, keratinocytes. There is however, some evidence that with increasing time the non-pigmented HF alters again its mode of keratinocyte differentiation to return to a largely medulla-free scallop hair fiber norm.

Conclusion:

Despite the recent flurry of mouse-related data on the status of the HF pigmentary unit during induced hair graying as a model for canities, progress in the study of the regulation of human hair pigmentation and associated changes with aging has been slow. There is also some indication that over-zealous extrapolation of murine data to human canities has been distracting us from the fundamentals of the human aging HF given its mosaic pattern of growth, its super-long anagen phase on the scalp, and striking level of individual HF autonomy. Obne should not focus attention on the human HF pigmentary unit, especially on the function of follicular dermal papilla and amelanotic melanocyte distributed in the outer root sheath of human scalp HF. Do the latter really represent melanocyte stem cells or at least their early progeny and if so, whether do they retain some stem cell characteristics. Selective recruitment of these immature outer root sheath melanocytes for the re-pigmentation of the HF (or even the overlying epidermis especially after wounding, vitiligo etc. [70]) may offer significant clinical and therapeutic advances. There is some rationale for this approach, not least from observations of reversal of canities after some types of therapy e.g., radiation/drug. The latter scenarios likely involve a cytokine-induced activation of these outer root sheath melanocytes. The future therefore looks bright, and potentially colorful, for human hair pigmentation research.

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Figure Legends

Fig. 1 Fully-pigmented human anagen scalp hair follicles showing intense melanization (brown and red) of the hair bulb and hair shaft. Whole-mount (i.e. entire hair follicle) bright-field light microscopy.

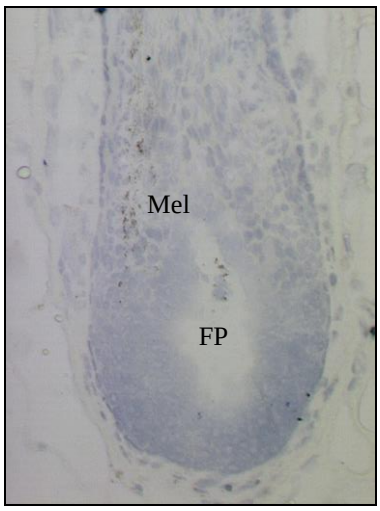

Fig. 2 Loss of melanocytes from the hair bulb of aging human anagen scalp hair follicles.

FP; follicular papilla, Mel; melanin. B**right-field light microscopy with toluidine blue staining.**

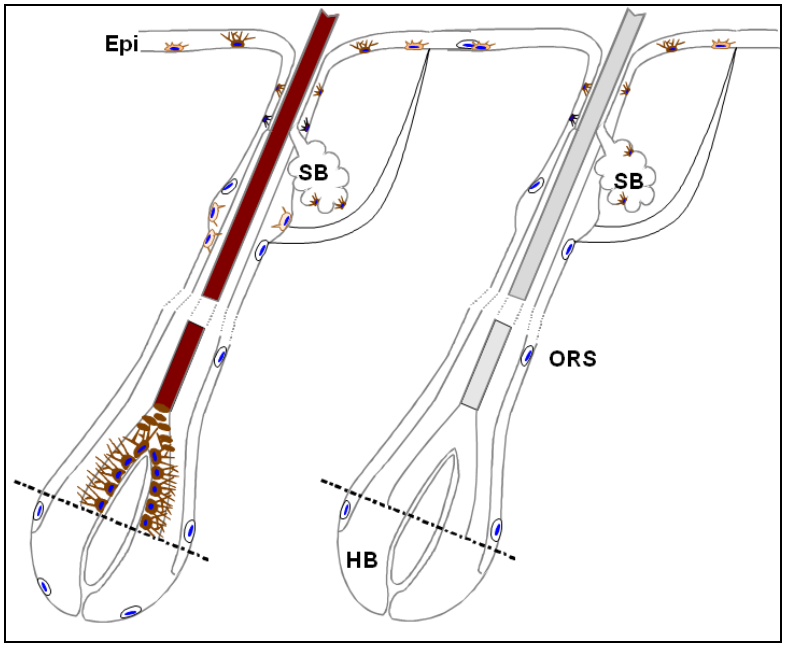

Fig. 3 Cartoon of pigmented and canities-affected human anagen scalp hair follicle, showing loss of melanization in the hair bulb and hair shaft with graying. Some amelanotic melanocytes can be seen in the outer root sheath (ORS) and in the most proximal and peripheral hair bulb (HB). SB, sebaceous gland; Epi, epidermis.