Trends in Endocrinology & Metabolism

CellPress
REVIEWS

Review

The Role of Immature and Mature Adipocytes in Hair Cycling

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Hair follicles (HFs) strongly interact with adipocytes within the dermal white adipose tissue (dWAT), suggesting a strong physiological dependence on the content of immature and mature adipocytes in this layer. This content is regulated by the proliferation and differentiation of adipocyte precursors, as well as by dedifferentiation of mature existing adipocytes. Spatially, long-range interactions between HFs and dWAT involve the exchange of extracellular vesicles which are differentially released by precursors, preadipocytes, and mature adipocytes. Different exogenous factors, including light irradiation, are likely to modify the release of adipocyte-derived exosomes in dWAT, which can lead to aberrations of the HF cycle. Consequently, dWAT should be considered as a potential target for the modulation of hair growth.

Introduction

A rapidly growing body of data suggests that HF cycling strongly correlates with spatiotemporal behavior of dWAT located in rodents above the panniculus carnosus and in humans in the superficial layer of the subcutaneous adipose tissue adjacent to the dermis [1–5].

Different correlations between HFs and dWAT have been observed in various murine knockout models. Myelin protein zero-like 3 (*Mpzl3*$^{-/-}$) knockout mice [6], which develop alopecia soon after birth, demonstrate no significant changes in the HF cycle, but a striking reduction in dWAT thickness as well as in the average size of adipocytes. Loss of *Mpzl3* function may cause defects in the HF structures formed during anagen (the different phases of HF cycling are summarized in Box 1). Knockout mice for early B cell factor 1 *(Ebf1*$^{-/-}$*)* are characterized by reduced dWAT and lack of adipocyte progenitors in the postnatal skin. HFs in these animals fail to re-enter anagen [1,7], which leads to the abortion of the HF cycle. It should, however, be mentioned that *Ebf1* is also highly expressed in dermal papilla fibroblasts, and its deficiency can directly perturb HF cycling.

Mice lacking platelet-derived growth factor (PDGFA) demonstrate phenotypic similarities to *Ebf1*$^{-/-}$ mice and display a block in anagen [1]. At the same time, intradermal preadipocytes have a high expression level of PDGFA, and local injections of PDGFA were able to induce anagen in murine HFs [1,8]. Transgenic mice overexpressing human apolipoprotein CI in the skin, fatty acid transport protein (FATP)-4-deficient mice, and *Dgat1*$^{-/-}$ and *Dgat2*$^{-/-}$ mice also have reduced dWAT layers and display hair loss [2].

Cyclosporine A (CsA) is a well-known HF stimulator that causes prolongation of anagen both *in vitro* and *in vivo* [9]. CsA downregulates the expression of secreted Frizzled-related protein 1 (SFRP1) in human HFs *ex vivo* [10]. On the one hand, SFRP1 is an antagonist of Wnt/β-catenin signaling, while on the other it is a known adipokine [11]. Suppression of SFRP1 with subsequent activation of Wnt/β-catenin signaling in preadipocytes inhibits their differentiation,

Highlights

HFs strongly interact with adipocytes within dWAT, suggesting a strong physiological dependence on the content of immature and mature adipocytes in this layer.

This content is regulated by the proliferation and differentiation of adipose precursors and preadipocytes, as well as by dedifferentiation of mature existing adipocytes leading to the production of adipocyte-derived preadipocytes.

Long-range spatial interactions between HFs and dermal adipocytes involve the exchange of extracellular vesicles which are differentially released by precursors, preadipocytes, and mature adipocytes, and are likely to carry various proteomic fingerprints during different phases of the HF cycle, effectively modulating the transcriptome profile of the recipient HFs.

Different exogenous factors, including light irradiation and some chemical agents, can affect immature and immature adipocytes, and are likely to modify the release of adipocyte-derived exosomes in dWAT, which can lead to aberrations of the HF cycle.

Dermal adipocytes should be considered as a potential target both for the modulation of hair growth and for the removal of unwanted hair.

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Box 1. HF Development

HFs are skin appendages that demonstrate a remarkable level of cyclic development. HF cycling is traditionally subdivided into three phases: anagen (growth), catagen (regression), and telogen (rest). Morphologic and physiologic features of HFs significantly vary not only between the phases but also within a specific phase. As a result, anagen is further subdivided into six subphases, the first two of which ('early anagen') are connected with modifications of HF structure, subphases III–V ('mid-anagen') with downgrowth and full HF development, and subphase VI ('late anagen') with development of a hair shaft.

Morphologic and physiologic variations of HFs demonstrate a strong spatiotemporal correlation with behavior of adjacent superficial adipose tissue known as dWAT. This suggests a long-range interaction between HFs and dermal adipocytes. Such correlations are observed not only as a function of varying thickness of the dermal adipose layer, but also at the level of modulation of the relative ratios of immature and mature dermal adipocytes during HF cycling.

whereas both intra- and extracellular activation of SFRP1 and inactivation of the Wnt/β-catenin pathway induce adipogenesis [12]. The latter observation indicates that SFRP1 modulating HF development is not intracellular because SFRP1 is transported from differentiating preadipocytes located in dWAT to HFs. A more recent report indicates that CsA inhibits adipogenic differentiation in adipocyte precursors [13]. Generally, interactions between dWAT and HFs must be reciprocal, and the influence of epidermal Wnt/β-catenin signaling on adipocyte differentiation was investigated in [14].

Whereas most of the studies referenced above do not describe direct cellular pathways and interactions, periodical cycling of dWAT synchronized with the HF cycle [3,5] together with above results suggest that modification of dWAT content can influence HF cycling. We discuss here possible mechanisms for these interactions and how their modification can lead to aberration of HF cycles.

Immature and Mature Adipocytes in HF Cycling

Adipocytes are derived from mesenchymal stem cells which can differentiate into various cell lineages. These stem cells (also known as adipose-derived stem cells, ADSCs) mainly reside in the vascular stroma of adipose tissue and can undergo a multistep commitment process to produce preadipocytes (immature adipocytes) which bear some typical adipocyte markers, among them PPARγ. Transformation of precursors to the adipocyte lineage is initiated by the activation of several factors, including bone morphogenetic proteins (BMPs), as well as by components of the Wnt and hedgehog signaling pathways [15]. These preadipocytes can further differentiate into mature, lipid-laden adipocytes (Figure 1).

ADSCs, preadipocytes, and mature adipocytes (Table 1) are differentially involved in HF cycling: while the appearance of mature adipocytes inhibits HF progression, there are different indications that stimulation of immature adipocytes in dWAT can induce the telogen-to-anagen transition [2,5,16]. Non-committed ADSCs have stimulatory effects on dermal papilla cells and are able to promote HF cycling [17,18]. At the same time, defects in the generation of immature adipocytes block follicle stem cell activation, and immature adipocytes thought to be not only necessary, but also sufficient, for proper HF cycling [1].

ADSCs and preadipocytes play distinct roles in HF cycling. Although almost normal HF cycling is observed in A-ZIP/F1 mice, which contain both ADSCs and preadipocytes, but lack mature white adipocytes system-wide, depletion of immature adipocytes (e.g., with PPARγ antagonists or genetically in *Ebf1*$^{-/-}$ mice) caused complete HF cycle disruption. It should be noted that the application of PPARγ antagonists, such as BADGE or GW9662, is postulated to deplete preadipocytes, but not ADSCs, which suggests a unique role for preadipocytes in HF

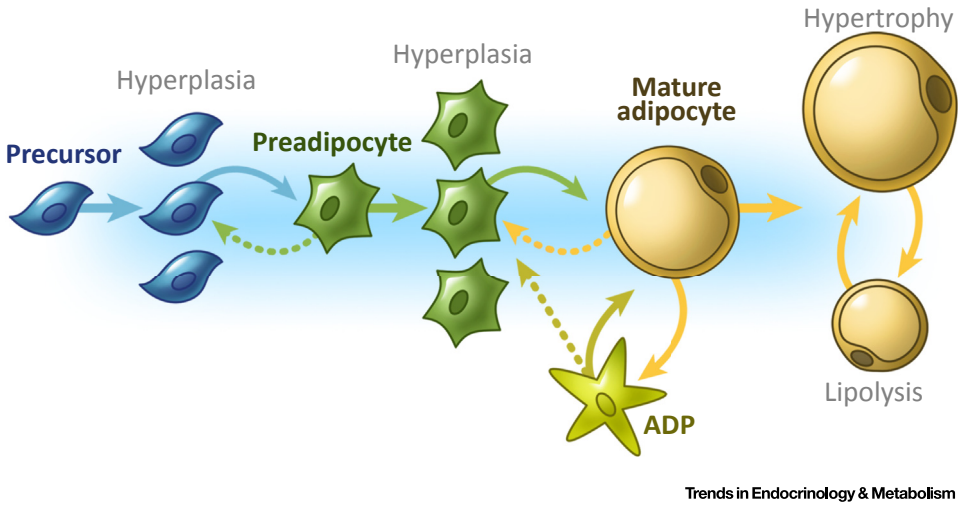

Figure 1. Precursors, Preadipocytes, and Mature Adipocytes Can Undergo Different Conversions. Mature adipocytes can dedifferentiate, producing adipocyte-derived preadipocytes (ADPs). This mechanism can contribute to modification of dermal white adipose tissue volume during the hair follicle cycle.

cycling [1,5]. On the other hand, transplantation of ADSCs into the skin was able to induce anagen in both wild-type and *Ebf1*$^{-/-}$ mice [1]. This clearly demonstrates that immature adipocytes in dWAT can serve as a target for modulation of the HF cycle.

Mature adipocytes can also influence the HF cycle through expression of BMPs. Adipocyte-derived BMP2 reaches HF stem cells while they are in the quiescent state [5,19] and thus suppresses hair growth. BMP expression levels increase during anagen, along with the increased concentration of mature adipocytes around HFs. On the other hand, BMPs can significantly influence the appearance and behavior of immature adipocytes: BMP4 not only promotes adipogenic commitment and subsequent differentiation of adipose precursor cells but it is also induced in precursor cells during their differentiation, as well as promoting the differentiation of preadipocytes and the activation of PPARγ in these cells [20]. Deletion of *Bmpr1* in myofibroblasts results in a lack of new adipocytes, which demonstrated that BMP signaling in myofibroblasts is necessary for adipocyte regeneration in wound healing [21]. In addition, mature adipocytes can regulate some differentiation processes in HFs during late anagen [19,22].

The local volume of dWAT adjacent to HFs increases in anagen and significantly decreases in catagen and telogen [3,5]. Such remodeling of dWAT not only includes hyperplasia and hypertrophy but it is also highly likely that some dedifferentiation and perhaps transdifferentiation events are part of the process.

Table 1. Differences among Skin Adipose-Derived Stem Cells, Skin Preadipocytes, and Mature Dermal Adipocytes

	Skin adipocyte-derived stem cells (ADSCs)	Skin preadipocytes	Mature dermal adipocytes
Location	Lower dermis	Lower dermis	Rodents: under the reticular dermis and above the panniculus carnosus Human: under the reticular dermis, superficial to subcutaneous adipose tissue
Morphology	Fibroblast-like	Fibroblast-like	Unilocular with large lipid droplets
Markers	Lin$^-$, Sca1$^+$, CD34$^+$, CD29$^+$, CD24$^+$, Pdgfra$^+$	Lin$^-$, Sca1$^+$, CD34$^+$, CD29$^+$, Pdgfra$^+$, CD24$^-$, CEBPa$^+$	Common adipocyte markers

Transitions between Immature and Mature Adipocytes as a Hallmark of Proper HF Cycling

Modification of the dWAT volume and structure during HF cycling demonstrates two unique features. First, 20–40% of mature adipocytes in fully expanded dWAT (mid-anagen) are thought to be newly formed cells derived from differentiated progenitors, and the remaining cells are thought to be pre-existing adipocytes [23,24]. This means that proper HF cycling can take place only if a sufficient number of immature adipocytes in dWAT have the ability to proliferate and differentiate. Consequently, depletion of immature adipocytes in dWAT should lead to aberration of the HF cycle. However, the field is awaiting more detailed lineage-tracing studies to establish whether this is actually true.

Second, as HFs enter telogen, dWAT abruptly decreases to approximately half of its maximum local volume. Such rapid transformation of the dWAT layer is not caused by apoptotic cell death, and it was assumed that this effect is facilitated by the stimulation of lipolysis [5]. However, as it was recently stated in [25], the relative contributions of hypertrophy/lipolysis and hyperplasia in modulating dWAT volume during HF cycling are different in spontaneous and induced HF cycling. Whereas all these processes are evidently important for dWAT modulation during the HF cycle, another phenomenon may also significantly contribute to this process – namely the dedifferentiation of adipocytes.

Mature adipocytes can indeed undergo trans- and dedifferentiation [26]. In the latter case, they produce a population of adipocyte-derived preadipocytes (ADPs), which have an intermediate fibroblast–adipocyte phenotype and bear markers for both cell types, even though most ADPs exhibit a fibroblast-like morphology and are very similar to adipose tissue-derived stem/stromal cells. They do express some specific markers *in vitro*, and can differentiate into adipogenic/osteogenic/chondrogenic lineages in culture. Among others, the expression of transforming growth factor β (TGF-β1) and of different collagen genes is significantly higher in ADP cells compared to normal adipocytes; moreover, it was shown that stimulation of TGF-β1 expression enhances adipogenic dedifferentiation [27]. Such dedifferentiation was reliably reproduced in different experiments *in vitro*, and dedifferentiated cells can either differentiate back into mature adipocytes or into other different lineages in tissue culture [28]. This awaits validation *in vivo*.

Another transformation of adipocytes was observed *in vivo* after subcutaneous injections of bleomycin that are known to induce systemic sclerosis in murine models. Bleomycin strongly suppresses the adipogenic commitment of adipocyte precursors and simultaneously increases the expression of TGF-β1 in injected areas [29]. Such TGF-β1 induction is thought to lead to an adipocyte–myofibroblast transition, which involves formerly adiponectin-positive intradermal progenitors from dWAT [30]. Already 24 h after stimulation with TGF-β1, dermal adipocytes demonstrate an intermediate phenotype bearing both perilipin (typical of adipocytes) and α smooth muscle actin (typical of myofibroblasts) markers. Such an intermediate state is characteristic of adipocytes from the interfacial adipose layer adjacent to the skin. Similar effects were observed after chronic UV irradiation of murine skin (discussed in [31]) and was described in cancer progression, where these cells were named cancer-associated adipocytes [32]. Recently, it was also argued that adipocytes with intermediate phenotypes may be involved in the pathophysiology of androgenetic alopecia [33] and melanoma [34].

Dedifferentiation of adipocytes and induction of a quasi-fibroblast phenotype in dedifferentiated cells in a murine model was postulated to be triggered by expression of resistin-like molecule α (RELMα/FIZZ1) [35,36]. Moreover, *Fizz1* knockout mice treated with bleomycin exhibit

significantly impaired pulmonary fibrosis [37], which may be linked to the suppression of adipocyte dedifferentiation. The main sources of FIZZ1 in rodent skin are adipocytes, macrophages, and HF epithelial cells. FIZZ1 is much more highly expressed in mature adipocytes than in preadipocytes, and is able to inhibit the differentiation of preadipocytes [35]. It is likely that the abrupt reduction of dWAT volume in murine model when HFs enter telogen is connected with local activation of RELMα/FIZZ1 expression that may lead to dedifferentiation of existing mature adipocytes. Whereas the key trigger leading to adipocyte dedifferentiation in humans is unknown, it is possible that RELMβ/FIZZ2 is also involved.

Two other phenotypic changes – epithelial–mesenchymal and endothelial–mesenchymal transitions – were earlier reported to be reversible both *in vitro* and *in vivo* [38,39]. It was even postulated that the cells involved must have very high flexibility in their phenotype, allowing them to undergo multiple rounds of conversions [38]. By analogy, one could envisage that, under proper conditions, not only hyperplasia, hypertrophy, and lipolysis, but also multiple cycles of transitions between immature and mature adipocytes in dWAT could take place during HF cycling *in vivo* (Figure 1). This important postulate remains to be formally demonstrated.

The ratio of immature-to-mature adipocytes in dWAT correlates with the HF cycle and reaches its maximum during the mid-anagen phase, associated with downward growth of HFs [1,5]. This ratio in a given adipose depot is, however, not constant and can be affected by different exogenous and endogenous factors.

Mechanisms for Long-Range Spatial Interactions between HFs and dWAT

Selective modulation of adipogenic differentiation is likely to solicit a reciprocal reaction in adjacent HFs by inducing their cycling or bringing this process to a halt. For this to occur, the induction of HF-modulating factors must be spatially linked between dWAT and HFs. Normally, proliferating stem cells divide asymmetrically, producing one stem cell and one adipogenic progenitor committed to differentiation. However, in some tissues stem cells produce fast-cycling 'transit-amplifying cells' (TACs), which undergo differentiation only after several rounds of mitosis [40]. TACs have a very high capacity to amplify the number of differentiated cells and are typical in HFs. These cells are known as an early intermediate in tissue regeneration [41], and their appearance generally means that the regeneration processes initiated by stem cells have reached the 'point of no return'. These cells first appear in the HF matrix during anagen II, and their content significantly increases during anagen progression [41]. HF-TACs are strongly involved in the downgrowth of HFs and actively express *Sonic hedgehog* (Shh) in an HF cycle-dependent manner. Moreover, these cells may be responsible for morphological and physiological coupling between HFs and dWAT through the regulation of proliferation of adipose precursors [23].

Hedgehog signaling is known to progressively decrease during adipocyte differentiation in humans [42]. Activation of this pathway (similarly to Wnt signaling) impairs adipogenesis and lipid accumulation, whereas suppression of Hedgehog signaling was reported to be a necessary condition for adipocyte differentiation [42]. However, activation of the Hedgehog pathway does not inhibit the early steps of adipogenesis associated with the commitment and proliferation of precursors [23,42]. Nevertheless, it impairs the differentiation of preadipocytes [15].

Interactions between dWAT and HFs can be provided by different mechanisms, among them extracellular exchange of microRNA (miR) and some signaling proteins. One of the most important miRs involved in HF cycling is miR-214 that has an inhibitory effect on β-catenin and thus modulates Wnt/β-catenin signaling [43]. This miR also suppresses the osteogenic

differentiation of bone marrow-derived mesenchymal stem cells, thereby effectively promoting adipogenic differentiation in these cells [44]. In addition, miR-214 plays a dominant role in exosome-mediated signaling between endothelial cells [45].

Transport of miRs in extracellular vesicles between the source and recipient cells permits spatially long-range intercellular communication and can effectively alter the transcriptome of the recipient cells [46]. Extracellular vesicles can carry different factors, effectively modulating the physiological state of HFs and dWAT. Among these factors transported in extracellular vesicles are SFRP1 [47], a well-known antagonist of Wnt/β-catenin signaling [10], and caveolin [48]. Primary vertebrate cells secrete Shh in distinct vesicular forms [49] which can significantly increase the spatial correlation of Shh signaling versus simple diffusion. Adipose tissue-derived exosomes carry miRs to regulate TGF-β and Wnt/β-catenin signaling [50,51], and thus can provide reciprocal regulation of HFs and dWAT.

Extracellular vesicles released by immature and mature adipocytes have different proteomic profiles *in vitro* [52] (Figure 2). Moreover, the concentration of extracellular vesicles released by preadipocytes may be higher than for mature adipocytes [53], even though this may depend on the nutritional state of the system [54]. Adipocyte precursors also produce high concentrations of extracellular vesicles which, however, demonstrate very high interindividual variations [55] and which are regulated by PDGF [56].

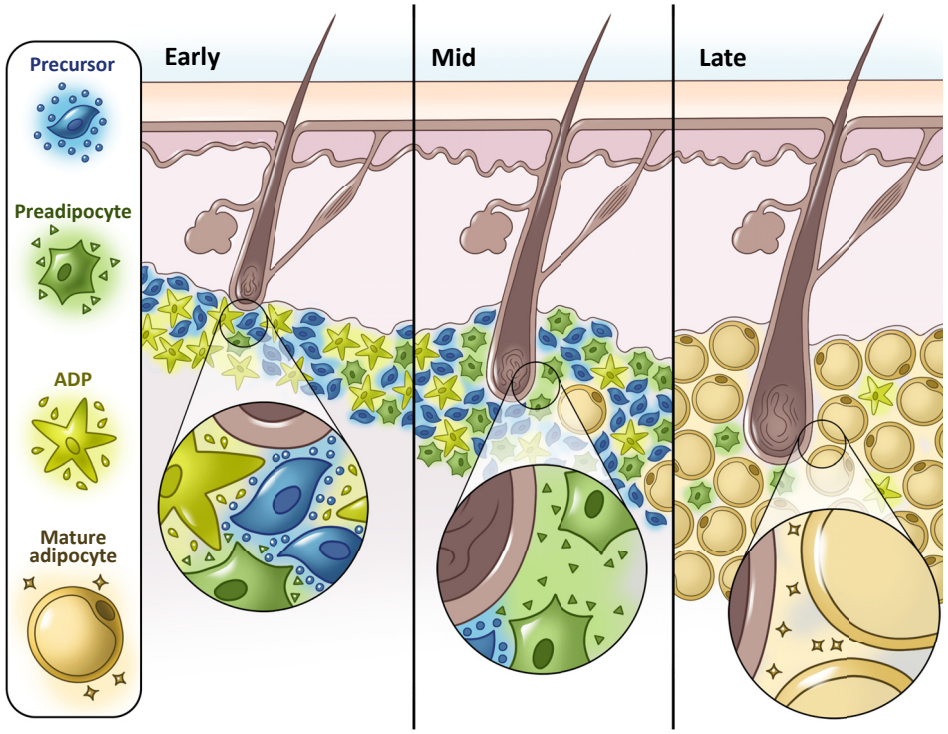

Figure 2. Local Content of Dermal White Adipose Tissue Results in a Specific 'Fingerprint' of Exosomes with Specific Content in This Area. Precursors and preadipocytes may produce higher levels of extracellular vesicles than mature adipocytes. The concentration and loading of exosomes vary strongly according to the phase of hair follicle cycle. Abbreviation: ADP, adipocyte-derived preadipocyte.

It must be mentioned that very recently, effective vesicular exchange of caveolin regulated by systemic metabolic state, was demonstrated between endothelial and adipose cells [54]. Such exchange was strong enough to replace the protein levels of caveolin-1 in genetically caveolin-1-deficient adipocytes.

Adipose tissue content-specific profiling of adipocyte-derived vesicles may allow distinct interactions between HFs and dWAT during different stages of the HF cycle; however, this awaits further elucidation in *in vivo* models.

Selective Modification of Immature and Mature Adipocytes by Light Irradiation

The absorption coefficients of light waves in human subcutaneous adipose tissue (sWAT) demonstrate strong dispersion and were reported to be 2.3 cm^{-1} at a wavelength of 400 nm and 1.0–1.2 cm^{-1} at a wavelength of 600–1200 nm [57]; much higher values of 16.0 cm^{-1} at 400 nm were measured in [58]. Absorption coefficients determined with the same method were higher in adipose tissue than in the adjacent skin [59]. In addition, extinction coefficients of porcine sWAT are significantly higher in the superficial than in the deep adipose tissue layers [60]. All of this suggests that dWAT depots should have much higher light absorption than classical deep sWAT.

Immature and mature adipocytes have substantially different light absorption properties [61]. Absorption spectra obtained with Fourier transform infrared (FTIR) spectrometry in 3T3-L1 preadipocytes and corresponding mature adipocytes revealed that light absorption in some wavelength intervals was much higher in preadipocytes than in the corresponding mature adipocytes. We can infer that this effect may lead to preferential reaction to light by immature cells compared to mature adipocytes, at least in some intervals of the light spectrum.

Generally, the response of adipocyte precursors to light irradiation is biphasic: whereas low light intensity demonstrates stimulatory effects on the proliferation of these cells [62], high intensity normally produces inhibitory effects. Such inhibition was observed, for example, at fluences of 10–15 J/cm^2 in [63] and over 20 J/cm^2 in [64]. Visible light can also effectively interact with adipocytes: irradiation of mature 3T3-L1 adipocytes demonstrated that light with a wavelength of 500–600 nm was able to significantly reduce lipid droplets and increase the efflux of glycerol from these cells, thus effectively reducing their volumes [65].

Taken together, immature and mature adipocytes differentially react to the same light irradiation, and selective absorption of light energy by these cells should be able to modify their content in dWAT and thus regulate HF cycling.

The Relationship between HF Pigmentation and Content of Immature and Mature Adipocytes in dWAT

Hair pigmentation is strongly dependent on the number and activity of melanocytes present in HFs. Black hair (containing eumelanin) demonstrates the highest light sensitivity, followed by blond, red (containing more pheomelanin), and grey hairs (containing less melanin). In accordance with the theory of selective photothermolysis, this effect was linked to the reduced concentration of light-sensitive eumelanin in red and grey HFs. At the same time, if the light sensitivity of HFs depends on the content and physiological state of adjacent dWAT, then distinctly pigmented HFs should have different dWAT structures: specifically, different ratios of immature-to-mature adipocytes.

The biosynthetic pathways for eumelanin and pheomelanin are mainly determined by the signaling activity of melanocortin receptor 1 (MC1R) and Wnt/β-catenin signaling [66]. Two different MC1R ligands demonstrate opposing effects on this receptor: α-melanocyte stimulating hormone (α-MSH) is an agonist, while agouti signaling protein (ASIP in human) is an antagonist. Activation of MC1R stimulates the production of eumelanin, whereas inactivation of this receptor leads to a reduction of eumelanin synthesis and to a corresponding increase of pheomelanin content in HFs [67]. On the other hand, the MC1R/α-MSH signaling pathway was found to be strongly activated in cutaneous wound repair as well as in hypertrophic scarring [68], demonstrating that this pathway has other functions in the skin in addition to pigment production.

As discussed earlier, both cutaneous wound repair and the formation of hypertrophic scars are connected with activity of dWAT [4]. From this point of view, it is not surprising that a genetic link involving α-MSH/ASIP signaling was recently found between adipose tissue and pigmentation [34]. Human adipose tissue indeed demonstrates high expression levels of ASIP [69] and MC1R [70]. Expression of MC1R is much higher in mature adipocytes than in preadipocytes [70], and stimulation of MC1R in adipose precursors inhibits their proliferation [71]. In addition, precursors can directly affect the behavior of melanocytes, inhibiting their proliferation, differentiation, and even melanogenesis. This effect was demonstrated in an engineered skin-equivalent model [72], and it was linked to high expression of TGF-β1 which maintained melanocytes in an immature state and thus prohibited melanin synthesis.

On the other hand, TGF-β signaling is tightly linked to plasma membrane structures known as caveolae, which are characteristic Ω-shaped plasma membrane invaginations. Caveolae contain different types of caveolins, one of which, Cav-1, is highly expressed in mechanically stressed cells, including mature adipocytes. Cav-1 physically interacts with TGF-β membrane receptors [73] and participates in their internalization [74]. Internalized TGF-β receptors undergo rapid degradation, thereby leading to an effective reduction of TGF-β signaling. At the same time, Cav-1 expression was reported to be very low in adipose precursors, gradually increasing in preadipocytes, and reaching high values in mature adipocytes [75,76]. This explains how the relative content of adipocyte precursors, preadipocytes, and mature adipocytes can influence TGF-β expression, and thus modulate the local activity of melanocytes. In this context, it should be once more noted that both Wnt/β-catenin signaling and TGF-β can be spatially regulated through exosomal exchange [50].

MC1R expression in HFs and dWAT should demonstrate a long-range spatiotemporal correlation which is highly likely regulated by extracellular vesicles. We can infer that a long-lasting reduction of MC1R activity in HFs leading to modulation of their pigmentation must correspond to modified activity of this receptor and/or of its ligands in adjacent dWAT. Taking together, HF pigmentation correlates with the structure of adjacent dWAT, especially with its content of immature and mature adipocytes. Variation of cellular content in dWAT adjacent to diversely pigmented HFs should cause a distinct response of these dWAT structures to the same light irradiation.

Possible Applications for the Reduction of Unwanted Hair Growth

Removal of unwanted hair remains one of the most important fields in esthetic medicine and dermatology. Photoepilation, currently the leading method in hair removal, is based on the widely accepted theory of selective photothermolysis that was originally formulated for light absorption by microvessels and melanosomes [77,78]. Extension of this theory to light absorption by HFs restricted the effect of the light to its absorption by melanin. Melanin content is variable in

different stages of HF cycling, being generally higher in anagen and correspondingly lower in the catagen and telogen phases. According to the theory of selective photothermolysis, light sensitivity of a given HF should be higher in anagen than in other phases of HF cycle.

These observations were widely integrated into clinical applications of photoepilation, but led to some contradictions between the theory and experimental/clinical results, as discussed in detail in [79,80]. The most serious is the well-known reduced photosensitivity of HFs in hirsutism. Hirsutism is generally connected with an elongation of the late anagen. Thus, according to the theory of selective photothermolysis, the hirsute HFs should theoretically demonstrate higher photosensitivity than normal HFs.

As discussed in [79], this contradiction can be resolved if the HFs are much less photosensitive in the subphase anagen VI than in early- and mid-anagen subphases. During anagen VI, only hair shaft growth takes place, and not downgrowth of HFs. This is typically found not only in rodents [81] but also in humans. A direct comparison between the stages of hair growth is shown in Table 2. In addition, the processes of mesenchymal remodeling at the distal end of the HF are known to be strongly reduced or even completely suppressed in anagen VI. This leads to the conclusion that modulation of mesenchymal remodeling at the distal ends of down-growing HFs, and not photothermolysis in HFs *per se*, is mainly responsible for the aberration or even the abortion of the HF cycle after sufficiently intensive light exposure [79,80]. In line with this, focusing microwave energy to the interface of the dermis/subcutis can produce an axillary hair reduction of up to 75% 1 year after irradiation. This effect is independent of hair color [82].

Interactions between HFs and dWAT depend on the content of immature and mature adipocytes. The selective character of light absorption in these cells allows effective modulation of the ratio of immature-to-mature adipocytes in dWAT. Therefore, we can assume that interference or even the complete abortion of the HF cycle, which can often be observed after light irradiation, is at least partly linked to absorption of the light energy not in HFs, but in dWAT (Figure 3). At the same time, interference in the HF cycle after light irradiation is known to be strongly dependent on hair pigmentation. Because dWAT is involved in this process, the relative contents of immature and mature adipocytes in dWAT adjacent to differently pigmented hairs should also be different.

Concluding Remarks

HFs and dWAT strongly interact with each other, demonstrating a highly coordinated behavior. This interaction is substantially dependent on the content of immature and mature adipocytes in

Outstanding Questions

Are there phenotypic differences between ADPs and 'virgin' preadipocytes that had not previously reached the adipocyte stage?

What is the difference between ADPs in dermal adipose tissue versus other fat pads?

Do ADPs play a role in obesity, weight loss, and diabetes?

Is the number of 'back and forth' transitions between immature and mature adipocytes in dWAT limited?

How does the pigmentation of hair follicles correlate with the structure of adjacent dermal adipose tissue?

Is there any difference in light sensitivity between cycling adipocytes and 'virgin' adipocytes?

What is the difference between the normal skin fibroblasts and dermal adipocyte precursor cells?

What is the natural turnover rate of dermal adipocytes in adult mice?

Are mature dermal adipocytes necessary for hair cycling?

Table 2. Comparison of Hair Growth Stages between Rodents and Humans

	Mouse	Human
Main cycle stage	Anagen, catagen, telogen	Anagen, catagen, telogen
Length of anagen	1–3 weeks	Can last for several years
Length of catagen	About 2–4 days	Around 2–3 weeks
Length of telogen	First cycle, 1–2 days From second cycle, more than 2 weeks	A few months
Synchronization	Highly synchronize for the first two cycles Only small patches of synchronous hair growth after the second cycle	Synchronized fetal hair follicle cycling becomes asynchronous soon after birth Mosaic cycling pattern

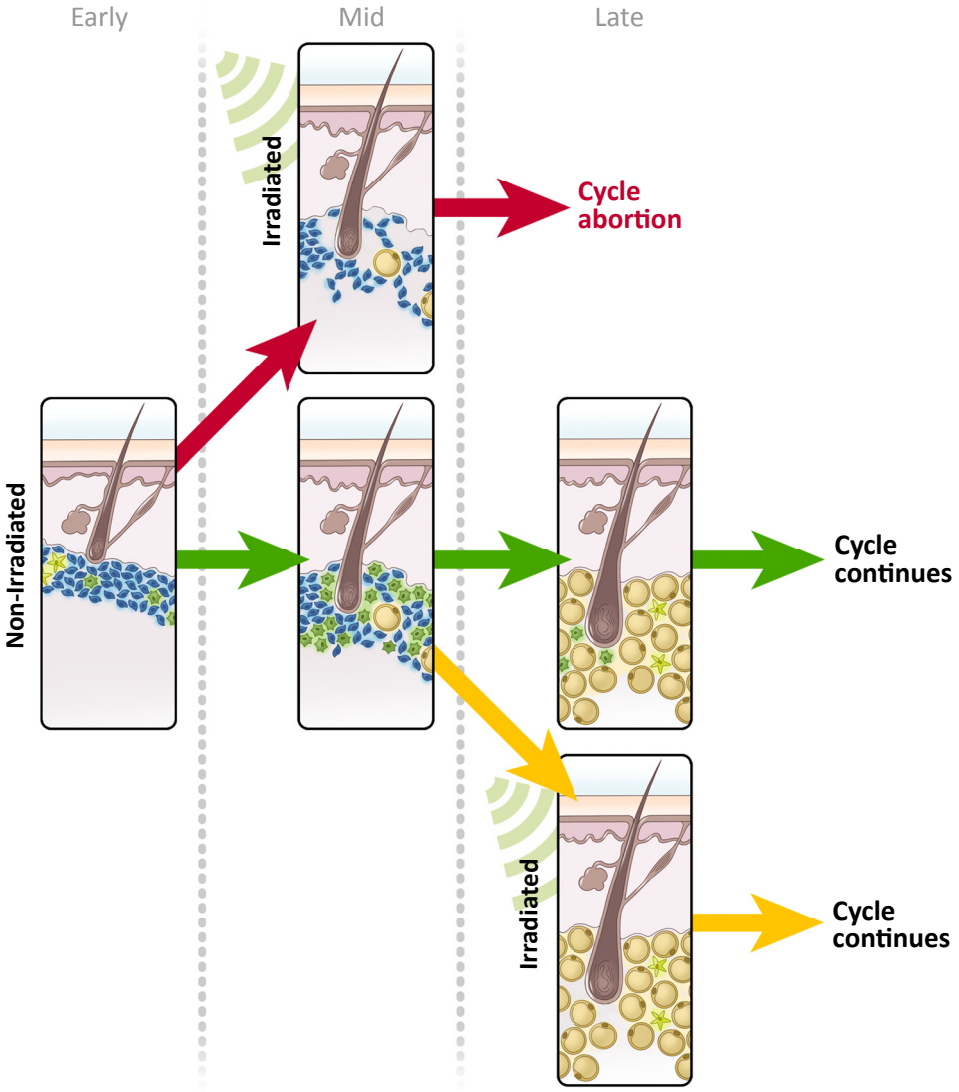

Figure 3. Different Contents of Precursors, Preadipocytes, and Mature Adipocytes in Dermal White Adipose Tissue (dWAT) in Distinct Phases of the Hair Follicle (HF) Cycle, as well as the Differential Sensitivity of These Cells to the Same Light Irradiation, Is At Least Partly Responsible for Differential Light Sensitivity of HFs in the Corresponding Phases of the HF Cycle.

dWAT adjacent to HFs. Such content may be regulated not only by the proliferation and differentiation of adipose precursors and preadipocytes but also by the dedifferentiation of mature adipocytes. Thus, this involves mechanisms and factors that promote such dedifferentiation, including TGF-β signaling, among others.

Interactions between HFs and dWAT can be provided through exchange of extracellular vesicles. Because vesicles released by precursors, preadipocytes, and mature adipocytes probably have different proteomic profiles and are released in different concentrations, such mechanism can provide fine regulation of the HF–dWAT interaction. However, this is mostly

based on *in vitro* observations and awaits *in vivo* validation. dWAT exposure to different exogenic factors could lead to rapid direct modification of exosomal release in this tissue or modify this release on a long-lasting scale through modulation of the ratio of immature to mature adipocytes. Both types of modification can lead to consequent changes in the HF cycle.

One such factor demonstrating selective effects on immature and mature adipocytes is light irradiation in some specific wavelength intervals. Adipose precursors, preadipocytes, and mature adipocytes react differently to the same light irradiation, demonstrating significantly higher light absorption in immature adipocytes. Because the tissue content of precursors, preadipocytes, and mature adipocytes significantly varies over the course of the HF cycle (and even during different subphases of anagen), this effect leads to variable photosensitivity of HFs in different phases of the HF cycle. Together, this argues that more attention should be paid to dWAT as a potential target in modulating hair growth.

Acknowledgments

I.L.K. is a managing partner of Wellcomet GmbH. No methods or devices of Wellcomet were used in this publication. I.L.K. received no financial support from Wellcomet GmbH. I.L.K. did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. P.E.S. was supported by US National Institutes of Health (NIH) grants R01-DK55758, R01-DK099110, P01-DK088761, and P01-AG051459 (P.E.S.; Project 3) as well as by an unrestricted grant from the Novo Nordisk Research Foundation.

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