Opinion

Lichen Planopilaris and Frontal Fibrosing Alopecia as Model Epithelial Stem Cell Diseases

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Inflammation-associated, irreversible damage to epithelial stem cells (eSCs) of the hair follicle in their immunologically privileged niche lies at the heart of scarring alopecia, which causes permanent difficult-to-treat hair loss. We propose that the two most common and closely related forms, lichen planopilaris (LPP) and frontal fibrosing alopecia (FFA), provide excellent model diseases for studying the biology and pathology of adult human eSCs in an easily accessible human mini-organ. Emphasising the critical roles for interferon (IFN)-γ and peroxisome proliferator-activated receptor (PPAR)-γ-mediated signalling in immune privilege (IP) collapse and epithelial–mesenchymal transition (EMT) of these eSCs respectively, we argue that these pathways deserve therapeutic targeting in the future management of LPP/FFA and other eSC diseases associated with IP collapse and EMT.

Scalp Hair Follicles as a Model to Study the Biology and Pathology of human eSCs

eSCs (see Glossary) are increasingly being appreciated to be critically affected in several human diseases, such as lung disorders, inflammatory bowel disease, and liver cirrhosis [1–3]. While animal models have provided important insights, these models remain limited in their instructiveness and relevance for the comparable human pathology, and SCs are often difficult to access in the corresponding human organs. Instead human scalp hair follicles (HFs), which become frequently available during facelift and hair transplantation surgery and can be kept for several days in organ culture [4], have long been appreciated as optimally accessible human mini-organs for characterizing and interrogating the biology and pathology of human eSCs and their progeny within their natural tissue habitat [5,6].

Therefore, we argue that investigators interested in the biology, pathology, and therapeutic targeting of human eSCs are well advised to turn their attention to primary cicatricial alopecias (PCAs), an important, but underinvestigated group of rare inflammatory scalp disorders that result in progressive permanent hair loss; namely the two most common PCA variants in European populations [7], LPP and FFA [8] (Figure 1). PCAs are typically disfiguring and difficult to treat, and are associated with distressing scalp symptoms and significant secondary morbidity, marked psychological impact, and significant loss of quality of life [8–15] (Box 1). This unmet clinical need alone already justifies why these hair loss disorders deserve to attract more interest from the SC research community.

Here, however, we focus on portraying LPP/FFA as prototypic model stem cell diseases in which one can exemplarily study how human eSCs are physiologically protected from inflammation-induced
Figure 1. Clinical Observations and Histopathological Features of LPP and FFA. (Ai) LPP is an irregular patch of permanent (scarring) alopecia on the central scalp. (Aii) When viewed with a trichoscope (10x magnification) loss of follicular ostia, perifollicular scale and perifollicular erythema (visible signs of inflammation) are evident at the margins of the patch. (Aiii) LPP histology shows a dense perifollicular lymphocytic immune infiltrate (white arrow) and partial destruction of the outer root sheath (red arrow). (Bi) FFA is characterised clinically by a symmetrical band-like recession of the frontal and temporal hairline with loss of sideburns (white line shows the original hairline). Loss of eyebrows is a prominent and early feature in FFA. (Bii) FFA – close up view of frontal hairline showing loss of follicular ostia and perifollicular inflammation. Lone hairs (arrows) remain as the hairline recedes. (Biii) Histologically (horizontal section), perifollicular fibrosis can be seen (grey arrows) with squamatisation of the basal layer suggesting epithelial-mesenchymal transition (green arrows). The immunopathology of both diseases shows a loss in the stem cell marker K15 (Cii) when compared to healthy skin (Ci). Upregulation of MHC class 1 suggesting immune privilege collapse (Di, ii) and a decline in the expression of the epithelial marker E-cadherin (Ei, ii). Ci, ii and Di, ii reproduced, with permission, from [28]; Ei, ii reproduced, with permission, from [59]. Abbreviations: APM, arrector pili muscle; FPP, frontal fibrosing alopecia; LPP, lichen planopilaris.
damage, which eSC pathology and clinical consequences arise from a failure of these protective mechanisms and what strategies for therapeutic intervention promise to be most clinically useful [16]. Simultaneously scrutinizing LPP/FFA also permits new general insights into the biology and pathology of human HFs and their specific SC populations [5,16,17].

LPP and FFA – Two Branches of the Same Pathobiology Tree?

While LPP and FFA are clinically distinct entities, their histopathology is deceptively similar [11,18,19] (Figure 1 and Box 1). This has encouraged the – still controversial – concept that FFA is a variant of LPP [11,16,20,21]. However, some investigators feel that FFA is a distinct entity from LPP and it remains conceptually unclear how (i) both HF-targeting dermatoses are related to each other and (ii) what mechanisms generate such different clinical phenotypes [22]. We propose that LPP and FFA represent two phenotypically distinct branches of the same pathobiology tree (Figure 2), thus expanding upon the recent concept that both entities share clinical and histological characteristics of a lichenoid folliculitis [20] (i.e., a group of entities that clinically display scarring alopecia with follicular papules and histological features of follicular interface dermatitis).

While the prevalence of LPP is unknown, it is classified as an orphan disease (ORPHA: 525), a rare often chronic disease affecting less than two in 2000, and its numbers appear to have remained stable over recent years; whereas cases of FFA have substantially increased over the past two decades [7,14,21,23,24]; despite it being only a relatively recently described entity [23]. This raises the question to what extent environmental and/or epigenetic components impact on FFA pathobiology. Intriguingly, this also suggests that FFA may provide a model disease for studying pathogenic environmental influences on adult human eSC [25–27].

The central pathobiological process in both LPP and FFA is inflammation-induced permanent loss of eSCs in the HF bulge region that are vital for HF cycling and regeneration [16,28–30]. In

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**Box 1. Clinician’s Corner**

The human HF immune system exhibits an array of characteristic properties that enable ongoing hair growth and cycling, while protecting the follicle from microbial pathogens.

LPP and FFA are characterised clinically by inflamed HFs surrounding an area of permanent scarring alopecia. Histologically, both conditions display a distal perifollicular lymphocytic infiltrate, squamatization of the basal epithelium, and progressive perifollicular fibrosis. Eventually, these affected HFs are completely replaced with fibrous tissue.

In addition to distressing symptoms (e.g., itch and pain) that can accompany these disorders, it is important to recognize the significant psychological impact of such permanent hair loss. Associated autoimmune conditions (particularly thyroid dysfunction) are also seen more frequently.

Current treatments for LPP/FFA use nonspecific suppression of the immune system (e.g., with glucocorticosteroids or other immunosuppressants) to control symptoms and reduce inflammation to prevent further hair loss. Hair regrowth is not possible with medical treatment and hair transplantation is ineffective since most transplanted HF grafts are destroyed by the inflammatory infiltrate.

Researchers might use our growing understanding of disease pathogenesis to identify novel targets for treatment in LPP/FFA. Potential targets for treatment include using PPAR-γ agonists to prevent EMT, and IP-restorative therapies, such as FK506/tacrolimus, to protect eHFSCs from further immune assault.

Human HFs, both in health and disease (e.g., LPP/FFA), are easily accessible human tissue that allows researchers to better understand the normal physiological processes in a functional mini-organ while observing different pathological processes associated with inflammatory disease. Thus, these model diseases could provide insights into stem cell functioning, autoimmunity and fibrotic processes relevant to other systems away from the skin.

**Glossary**

**Alopecia areata:** autoimmune condition characterised by reversible patchy to complete hair loss.

**Epithelial hair follicle stem cells (eHFSCs):** stem cell subpopulation located at the bulge of the HF that are positive for K15 and CD200, but negative for connexin 43.

**Epithelial stem cells:** slow-cycling undifferentiated cells with the potential to differentiate and replenish damaged cells within the tissue compartment.

**Epithelial–mesenchymal transition (EMT):** process in which epithelial structures take on a mesenchymal/fibroblast phenotype. EMT is characterised by loss of the epithelial marker E-cadherin and increased expression of the mesenchymal marker, vimentin.

**Frontal fibrosing alopecia (FFA):** recently described scarring alopecia characterised by frontal hairline recession and loss of eyebrows, predominantly seen in post menopausal women.

**Hair cycle:** series of stages in which the HF produces and then sheds a hair shaft. The three main stages of the hair cycle are anagen, catagen, and telogen.

**Hair follicle bulb:** the most proximal part of the HF that contains rapidly dividing cells that generated the hair fibre.

**Hair follicle bulge:** area of hair follicle epithelium at the insertion of the arrector pilis muscle where epithelial HF stem cells reside.

**Immune privilege (IP):** series of mechanisms in a specific tissue that restricts antigen presentation to normal immune surveillance. One theory is that this mechanism protects vulnerable tissue from the damaging effects of an unrestricted immune response (e.g., inflammation in the eye or brain).

**Köbner phenomenon:** appearance of skin lesions at the site of trauma.

**Lichenoid folliculitis:** new term coined to describe a group of similar entities characterised by scarring alopecia and follicular papules with histological findings of follicular interface changes.

**Lichen planopilaris (LPP):** type of PCA characterised by patchy scarring alopecia and a lymphocytic
contrast to the often significant inflammation seen in the most common autoimmune hair disease **alopecia areata**, where massive inflammation can attack the proximal hair **bulb** (yet without destroying the HF) [31], even fairly discrete infiltrates around the bulge can suffice to damage the HF irreversibly. The mechanisms that normally protect these eSCs from immune attack in a healthy HF, namely by the creation of an area of relative **immune privilege** (IP) in the bulge under physiological conditions, and how these eSCs respond when these protective mechanisms fail [28,32] (Figures 2 and 3), constitute key problems in general human stem cell biology. In fact, the niche that surrounds eSCs seems to dictate stem cell behaviour [33] and thus PCAs constitute a superb model to dissect the importance of preservation of individual constituents of the local microenvironment for stem cell function.
In order to understand the pathobiology of LPP and FFA, a few key features of human HF physiology need to be called to mind.

**The HF Immune System**

Immunohistologically, the human bulge displays high protein coexpression of keratin 15 (K15) and CD200 on epithelial hair follicle stem cells (eHFSCs) [5,34], which are also negative for...

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*Figure 3. LPP and FFA Pathogenesis Timeline and Pathological Features.* Summary diagram of key processes in LPP and FFA pathogenesis. We display a proposed progression from healthy follicle (left image) to permanent scarring alopecia (right images). Factors predisposing the healthy hair follicle to LPP/FFA are displayed. Inflammation and immune privilege collapse develops due to unknown triggering factors, leading to cell death and EMT of HF bulge cells, eventually resulting in complete replacement of the HF with fibrous tissue. The HF is now completely lost with no potential to regenerate, which manifests clinically as a patch of permanent scarring alopecia. White arrows denote inflammatory infiltrate, black arrows highlight apoptosis cells, grey arrows show early stage perifollicular fibrosis, thin black lines demarcate fibrosis tissue and thick black lines highlight a wedge shape loss in elastin expression. Abbreviations: EMT, epithelial–mesenchymal transition; FPP, frontal fibrosing alopecia; HF, hair follicle; LPP, lichen planopilaris; PPAR-γ, peroxisome proliferator-activated receptor-γ.
connexin 43 and quiescent in terms of cell cycle activity [6,35]. Healthy human HFs recruit several mechanisms to protect these eSCs from potentially damaging immune responses [36,37]. Central to this is the downregulation of MHC class Ia and II molecule protein expression not only in the proximal anagen HF epithelium but also in the bulge, thus restricting (auto-) antigen presentation [28,32]. Furthermore, the bulge shows reduced resident immune cell populations [38] and constitutes a special tissue niche characterised by the expression of potent, locally generated immunosuppressants, including α-melanocyte-stimulating hormone (MSH) and transforming growth factor (TGF)β2, as well as prominent expression of the immunoinhibitory cell surface ‘no-danger’ signal, CD200. Combined, these mechanisms restrict immune responses against the bulge and bestow a relative IP on this eSC niche, from which the melanocyte SCs that reside here also profit [28,32].

**General Considerations on Scarring Hair Loss (PCA)**

In the skin of healthy mice, inflammatory attacks are regularly launched on the bulge region of isolated HFs; possibly as a mechanism by which damaged HFs are selectively eliminated without causing widespread tissue damage (programmed organ deletion) [39]. Therefore, one wonders whether PCAs represent a pathologically exaggerated and ill-controlled variant of an otherwise physiological phenomenon of selective HF elimination [16]. What remains unclear is how such T lymphocyte- and macrophage-dominated inflammatory infiltrates, which are prominently accentuated in mice under conditions of perceived stress [40,41], are attracted to the human bulge in the first place and what distinguishes HFs affected in this manner from their unaffected neighbours.

In mice, different regions of the HF epithelium secrete a distinct spectrum of chemokines, which guide the intracutaneous trafficking of defined immunocyte populations and selectively attract them to distinct locations within the HF epithelium [42], which may explain (in part) the major fluctuations in the number and location of intracutaneous immunocytes that are hair cycle dependent [36,37]. It is plausible that these principles also apply to the human HFs. Therefore, changes in chemokine secretion from the bulge region in PCAs may well attract a lymphocytic infiltrate to the stem cell niche of the HFs, similar to what has been shown in alopecia areata (AA), in which excessive chemokine CXC ligand (CXCL)10 secretion from the anagen HF bulb epithelium attracts a Th 1-type infiltrate [43,44]. Since the location of an inflammatory attack on the HF ultimately determines the clinical phenotype and outcomes [16], in PCA one needs to take a closer look at the bulge region.

**Bulge Region**

In lesional LPP/FFA HFs the bulge undergoes major pathological changes such as reduced protein expression of the key bulge stem cell markers K15 and CD200 [16,28–30] (Figures 2 and 3). Moreover, microarray analysis of mRNA extracted from laser-capture microdissected bulge epithelium from lesional human LPP HFs shows loss in the expression of eHFSC signature genes, and K15+ cells in the bulge of lesional LPP and FFA HFs undergo increased apoptosis *in situ* [28]. Indeed, mutant mice with targeted deletion of eHFSCs, using a K15 promoter-driven suicide gene approach, develop permanent HF loss, yet without substantial inflammation or fibrosis [45]. Therefore, while the loss of eHFSCs explains the disappearance of HFs and the resulting permanent alopecia seen in LPP/FFA, it does not explain the other characteristic clinical manifestations, such as fibrosis, epidermal atrophy, or follicular plugging seen in active disease [16,45,46].

Notably, when healthy human scalp HFs are cultured *ex vivo* with the proinflammatory cytokine IFN-γ, expression of K15 and CD200 mRNA and protein is significantly reduced and MHC class I is upregulated, demonstrating experimentally induced IP collapse and reduction of the eHFSC...
compartment [28,32]. CD200 may be particularly critical for the maintenance of bulge IP and downregulation of CD200 may promote its collapse, since mutant mice whose K15+ cells fail to express CD200 develop a severe scarring alopecia phenotype as a result of a major lymphocytic HF inflammation that attacks the bulge [47]. Therefore, IFN-γ-induced collapse of the physiological bulge IP likely lies at the heart of LPP/FFA pathobiology [28].

**Common Pathobiology Features in Both LPP and FFA – the Trunk**

Th-1-Biased Inflammation and IP collapse – Lessons from AA

Let us first look at common pathobiology features that are likely to be shared between LPP and FFA. Distal HF inflammation is a key histological feature of both LPP and FFA, characterised by an activated Th1-biased CXC receptor (CXCR)3+ cytotoxic T cell infiltrate, along with increased numbers of IFN-γ-secreting (CD123+) plasmacytoid dendritic cells [28]. Since IFN-inducible chemokines (CXCL9/10/11) are upregulated in the bulge epithelium, this is one plausible mechanism for recruitment and propagation of the inflammatory response [28,48]. Furthermore, IFN-γ-driven increased expression of IP collapse indicators (MHC class I and class II; β2-microglobulin) on the transcript and protein level and down-regulation of locally generated key immunosuppressants (e.g., CD200 and TGF-β) induce a collapse of the physiological HF IP, thus exposing usually hidden HF (auto-)antigens to immune surveillance (Figure 3) [28]. However, it is unknown what initiates these inflammatory processes (e.g., HF stress or dysbiosis of HF microbiota); whether IP collapse is the primary event preceding the inflammatory response or a secondary phenomenon occurring after inflammation remains to be established.

Notably, bulge IP collapse in LPP/FFS shows striking similarities with the bulb IP collapse seen in AA [31,36]. In AA, the innate immune system, namely natural killer cells and possibly also γδ T cells, induce an IFN-γ-dependent collapse of HF bulb IP via the NKG2D receptor expressed on these (and CD8+) T cells, for which the proximal HF epithelium can express high levels of NKG2D ligands like MHC class I polypeptide-related sequence A (MICA) and UL16 binding protein (ULBP)3 under conditions of tissue stress [50,51]. Presumably, activation of their NKG2D receptors by MICA overexpression on HF keratinocytes stimulates the secretion of IFN-γ [36,50] from the innate immunocytes, inducing IP collapse [36,50]. Moreover, perifollicular mast cells switch from their physiological immunoinhibitory phenotype to a proinflammatory one and show increased contact with CD8+ T cells [52]. It is conceivable, but remains to be formally demonstrated, that a similar pathobiology scenario underlies the bulge IP collapse seen in LPP/FFA [28]. However, despite these similarities in immunopathobiology, the clinical phenotype between LPP/FFA and AA is very different (i.e., permanent vs. reversible alopecia, epidermal atrophy and fibrosis vs. normal epidermis and absence of scarring) reflecting the location of the inflammatory attack on key HF structures (LPP/FFA = bulge eHFSC; AA = HF bulb).

Current therapies in LPP and FFA are directed at reducing inflammation by nonselective local or systemic immunosuppression [13]. However, as in AA [36], the targeted reestablishment of bulge IP, with candidates therapies such as FK506 (tacrolimus) or αMSH analogues, may successfully re-establish bulge IP. This concept can bepreclinically probed in full-length human HF organ culture [4] to screen for new candidate compounds already established in AA [e.g., Janus kinase (JAK) inhibitors [53]].

**EMT**

In LPP/FFA, affected HF display progressive perifollicular fibrosis with eventual replacement of the entire HF with fibrous tissue. One explanation for the fibrosis seen is EMT [54,55]; a process by which epithelial cells lose polarity and cell-to-cell contact and acquire a mesenchymal phenotype as it is, for example, seen in wound healing, cancer development, and various
fibrotic diseases [56–58]. Notably, the dermis of FFA lesions shows cells positive for the EMT marker Snail1 [54], raising the possibility that EMT is somehow involved in the pathogenesis of FFA.

EMT does indeed occur within the bulge epithelium of lesional human LPP HF s, which shows increased gene and protein expression of mesenchymal markers (e.g., vimentin, fibronectin, and CDH2) and downregulation of the epithelial marker E-cadherin (CDH1) (Figure 3). Furthermore, the transcription factors SNAI1 (Snail), SNAI2 (Slug), and TWIST1, which can be induced by TGF-β, epidermal growth factor (EGF), and Wnt/β catenin signalling, are also prominently expressed in lesional HF bulge epithelial samples [59]. Moreover, colocalisation of K15 with vimentin in affected human LPP bulge tissue, and transmission electron microscopy confirming collagen fibre production by affected eSCs within the bulge, support this claim [59].

K15+ cells in the bulge of healthy, human scalp HF s ex vivo can be experimentally induced to undergo molecular changes consistent with EMT by exposing them to a defined cocktail of four well-known EMT-promoting agents (i.e., IFN-γ, TGF-β1, EGF, and the E-cadherin-inhibiting peptide SWELYYPLANL) [59]. This shows that normal adult human eHFSCs can easily switch on an EMT programme within their native stem cell niche when given appropriate signals; at least under conditions of tissue stress associated with HF culture [4]. Taken together with the K15/vimentin coexpression evidence mentioned above, this suggests that human eHFSCs undergo EMT in LPP [59], thus further depleting the pool of eHFSCs that have survived apoptosis induction by cytotoxic CD8+ T cells and excessive IFN-γ signalling and are no longer protected by bulge IP [28]. Thus, EMT of bulge eSCs may explain at least in part the prominent scarring and fibrosis of advanced LPP, and perhaps eSC EMT-derived progenitors may be more profibrotic than if the transition occurred in a non-stem cell area of the perifollicular mesenchyme, where instructive local microenvironment signals may not be present.

Importantly, experimentally induced EMT in human eSCs ex vivo can be used to dissect the key mechanisms that underlie fibrosis development and to test pharmacological agents to prevent or reverse these effects. In fact, PPAR-γ agonists partially prevent induced EMT in this model [59]. This is translationally relevant since PPAR-γ agonists can be clinically beneficial in some patients with LPP and FFA (see below) [60]. Notably, PPAR-γ and its ligands are important in inhibiting fibrosis in other systems [61] with reduction in TGF-β1 signalling central to suppressing fibrogenesis. This model could also help to investigate other (nondermatological) fibrotic conditions where eSC EMT is prominent.

PPAR Signalling
Karnik and colleagues [62] pioneered the concept that the inflammatory pattern seen in active LPP may develop from an inhibition of fatty acid metabolism and cholesterol and peroxisome biogenesis, thus predisposing affected individuals to proinflammatory lipid accumulation and immune cell infiltration. Pathway analysis identified PPAR-γ as the key regulator of these processes with significantly reduced PPAR-γ levels identified in both affected and unaffected LPP tissues compared to healthy controls [62]. Furthermore, deletion of PPAR-γ in K15+ eSCs in mice (PPARγ−/−) results in an LPP-like phenotype with progressive hair loss, perifollicular inflammation, and scarring alopecia [62]. This work has led to the therapeutic use of the PPAR-γ agonist, pioglitazone, with reported response rates of up to 50% [60]. Moreover, PPAR-γ signalling downmodulates inflammatory responses [63], and upregulates K15 protein expression in the bulge of normal human scalp HF s ex vivo [63]. Therefore, PPAR-γ stimulation may not only suppress and perhaps even partially reverse bulge EMT, but PPAR-γ-mediated signalling may exert functionally relevant eHFSC-protective and immunoinhibitory effects.
Less clear is why some groups of HF develop a cicatricial alopecia phenotype while others within the same scalp region remain clinically unaffected. This cannot be explained by PPAR-γ-mediated effects alone, since there are no significant differences in PPAR-γ transcription between lesional and nonlesional HF bulge regions from the same LPP-affected individual [28]. Thus, other intrafollicular parameters, such as the level of relative bulge IP, the secretory profile of each individual HF (e.g., for selected chemokines) and interfollicular differences in the HF microbiome may determine which HF develop LPP/FFA and which ones do not. Alternatively, one might invoke the existence of regional differences or spatiotemporal domains within the human scalp that might affect disease spreading via quorum sensing, similar to HF cycling and regeneration in mouse skin [64,65].

**Disrupted Cholesterol Biosynthesis**

Gene expression profiling reveals that the cholesterol biosynthesis pathway is perturbed early in PCA development [66]. Genes encoding the post-squalene cholesterol biosynthesis enzymes 7-dehydrocholesterol reductase (DHCR7) and emopamil-binding protein (EBP) were significantly decreased in all PCA samples. Furthermore, using the cholesterol biosynthesis inhibitor BM15766 (that targets DHCR7 and EBP), in both human cell culture and in vivo (mice), accumulation of the cholesterol biosynthesis intermediates lathosterol and 7-dehydrocholesterol (7-DHC) could be demonstrated. This accumulation of intermediate sterols resulted in the production of proinflammatory cytokines and induction of eHFSC apoptosis; two critical processes in PCA development [28,49,66].

**Differences between LPP and FFA – The Branches**

We suggest that the pathobiology principles delineated above represent a shared pathogenesis core or (to remain within botanical imagery) the common pathogenesis trunk of both LPP and FFA (Figure 2). Case reports documenting the typical LPP and FFA clinical features side by side in the same patient indeed underscore shared pathobiology elements [14,15]. However, the two distinct clinical manifestations branch out, guided by as yet unknown local, systemic and/or environmental regulatory factors that determine whether the LPP or the FFA phenotype predominates.

One of the most important open questions in this field of research, therefore, is what these regulatory factors might be: candidates are discussed below. Although this remains to be formally demonstrated, this discussion might also be of relevance for eSC pathology in other tissues, since dissection of some of the regulatory factors mentioned might also impact on human eSC niches elsewhere.

**Environmental Factors in FFA**

Environmental factors may well drive the distinct FFA phenotype. Predominantly, but not exclusively, affecting individuals those of higher socio-economic status [15,67,68]. FFA has seen a rapid rise in incidence (as opposed to the stably low incidence of LPP); this, along with the distinctive, symmetrical and predominantly frontal, band-like hair loss pattern and the clustering of FFA cases in postmenopausal women, have all encouraged research into potential environmental exposures in patients affected by FFA. For example, significantly increased regular use of dedicated sunscreens in people with FFA compared to age-matched controls is reported [69,70], whereas this association has not been identified in LPP. Dissecting the environmental regulatory factors that have an impact on development of the FFA versus LPP phenotype could also serve as a human model system for exploration of the role of environmental stimuli in human eSC pathology, namely in promoting EMT and fibrosis.
Sex Steroids
In contrast to LPP, sex steroids may also play a functionally important role in FFA: (i) although FFA rarely occurs in men [15,70,71], postmenopausal women are by far the most frequently affected population [15]; (ii) coexisting androgenetic alopecia is frequently seen in FFA [14,15]; (iii) there is a higher incidence of FFA in women with early menopause/hysterectomy/oophorectomy [15,22]; and (iv) FFA reportedly responds to antiandrogen therapy (i.e., 5α-reductase inhibitors), at least in some patients [13]. Thus, androgen changes, age-related decline in dehydroepiandrosterone (DHEA) activity, or oestrogen decline after menopause might predispose to FFA development [22,72]. However, normal or deficient blood sex hormones levels [21,67,73] and the involvement of non-androgen-dependent HFs (e.g., posterior hairline or eyebrows) may argue against a key role of androgens in FFA pathobiology [67]. Clarifying the role of sex steroids in FFA could also shed further light on the role of eSCs in other, much less accessible tissues, for example, in hepatic fibrosis.

Notably, DHEA (and DHEA sulfate) are essential for nuclear PPAR-γ activation, fat metabolism, and mitochondrial activity, and its dysregulation can be associated with inflammatory and autoimmune changes [72,74,75]. Furthermore, DHEA levels are reduced in other fibrotic conditions and can prevent induced EMT/fibrosis in cultured cells [76]; although, a DHEA-induced mouse model of ovarian fibrosis mediated by TGF-β signalling suggests a more complex interplay [77]. Finally, sex steroids and their receptors (e.g., androgen receptor, oestrogen receptor-α) have long been implicated in the regulation of EMT in diverse extracutaneous human pathologies [78,79].

Role of Genetics?
A UK-based genome-wide association study in FFA is currently underway that may help to clarify the role of genetics in this disorder [22,80]. Reports of familial cases [80–85,90] and data from case series suggest that 5–8% of reported FFA cases display a positive family history [15,82,83], with links to specific HLA alleles being identified in some [86,87] but not all [88] cases. Arguments to explain the (albeit rare) family associations as well as the late age of FFA onset include autosomal dominant inheritance with incomplete penetrance or a germline predisposition in these women [22]. Epigenetic factors and miRNA signalling may also play a role [22,89]. Again, once the role of genetic and epigenetic factors in the shared and distinct elements of eSC pathology that underlies FFA versus LPP has been clarified, this invites scrutiny of other human eSC pathologies for abnormalities in the expression/activity of the same genes and pathways.

Mitochondrial Dysfunction and Oxidative Stress in FFA
Transmission electron microscopy and global metabolomic profiling data have identified defects in mitochondrial β oxidation of fatty acids leading to accumulation of medium- and long-chain fatty acids, along with decreased levels of (antioxidant) glutathione and elevated levels of oxidised glutathione (i.e., a marker of oxidative stress) in both lesional and nonlesional FFA scalp samples [91]. Thus, accumulation of damaged and oxidised proteins may trigger inflammatory responses in FFA and raises the possibility of mitochondrial dysfunction as an early process in disease pathogenesis. We are currently investigating whether LPP shows similar indications of eSC mitochondrial dysfunction (as yet unknown). Given recent insights into the role of mitochondrial energy metabolism and reactive oxygen species production in human HF biology [92], this line of enquiry, which remains to be rigorously applied to eHFSCs, deserves to be followed up. That mouse eHFSCs are exquisitely susceptible to mitochondrial damage [93], and that the transfer of mitochondria from mesenchymal SCs to lung or cornea epithelial cells can protect these from oxidative damage [94], supports the importance of
mitochondrial function for eHFSC health. Once this has been dissected in these accessible eSC populations, corresponding working hypothesis can then be pursued in a well-targeted manner in other human eSC pathologies.

Localized Trauma
As members of the lichen planus family of dermatoses that characteristically show an appearance of skin lesions at sites of skin injury (Köbner phenomenon), it is not surprising that an association between skin injury and development of LPP has been reported [95]. Moreover, cases of LPP following hair transplantation and FFA after face-lift surgery have been observed [96,97]. Localized skin trauma resulting in a nonspecific inflammatory response triggering cytokine and chemokine production, upregulation of stress proteins and adhesion molecules promoting collapse of HF IP, or downregulation of PPAR-γ expression, are all potential factors in PCA development. It is interesting to ask whether similar nonspecific inflammatory cascades may promote eSC pathology in other human tissues, even if an initiating, localized physical tissue trauma cannot be evoked.

Neurogenic Skin Inflammation
Psychoemotional stress has long been suspected as a triggering or aggravating factor for hair loss [9]. Mouse studies clearly show that perceived stress can induce major perifollicular neurogenic inflammation, causing increased mast cell degranulation and production of hair-growth-inhibitory stress mediators such as corticotropin-releasing hormone and substance P that inhibit hair growth [98,99]. As substance P is a known growth factor for fibroblasts, and substance P+ nerve fibres are particularly dense around the murine and human bulge, these mechanisms might contribute not only to bulge inflammation, but also to fibrosis and the prominent bulge apoptosis seen in LPP/FFA [16,100]. However, whether this actually happens in patients with LPP or FFA remains to be conclusively shown. Yet, given that extracutaneous eSC niches, for example, in the lungs and gastrointestinal tract, are also supplied with dense sensory innervation, it is conceivable that similar neurogenic inflammation principles that impact on LPP and FFA could also apply to eSC pathology beyond the human HF.

Concluding Remarks and Future Perspectives
If one views LPP and FFA as model eSC diseases, more systematic investigation of the branching factors that determine into which clinical manifestation the underlying shared stem cell pathobiology trunk develops is not only of interest to PCA experts but also promises to reveal general principles of how local, systemic, epigenetic, and/or environmental factors affect the function and dysfunction of human eSCs within their specific tissue niche.

Here, we present LPP and FFA as disease entities that share the phenomenon of eHFSC depletion and pathological stem cell EMT on the basis of a compromised or collapsed bulge IP, which invites a cytotoxic inflammatory attack on the bulge. This common pathobiology core explains why it has been so difficult to distinguish LPP and FFA microscopically. However, what induces the above pathobiology scenario, how inflammatory cells are attracted to the bulge (e.g., chemokine secretion, and excessive expression of NKG2D-activating danger signals like MICA), and whether it is primarily damaged/stressed HFs (e.g., as a consequence of abnormalities in the HF microbiome) that are singled out for immune elimination in these diseases remains to be determined. Given that the HF microbiome profoundly affects the murine HF and skin immune system [101,102], we need to understand whether and how dysbiosis of the complex human HF microbiome [103] may predispose to eSC damage (see Outstanding Questions).
Most importantly, we have delineated here how LPP and FFA can serve to generate important, clinically relevant insights, for example, into how inflammation-induced eSC damage occurs; which immune tolerance mechanisms are normally in place; why the IP of the eSC niche collapses and how it can be therapeutically restored; and what drives EMT of these stem cells and how this can be halted or even reversed. This knowledge will not only help to develop more effective, pathobiology tailored strategies for the management of PCA, but likely also for related human eSC diseases. In particular, current evidence suggests that it is particularly promising to pharmacologically target IFN-γ and PPAR-γ-mediated signalling by inhibiting the former and stimulating the latter, thus preventing and potentially restoring both IP collapse and pathological EMT in the bulge.

One limitation of our proposal that the eSC pathology seen in LPP and FFA can teach important lessons on how human eSCs in other tissue niches become dysfunctional, undergo EMT, and/or apoptosis, is that little is known about the pathobiology of human eSC niches in extracutaneous tissues, because they are more difficult to access and manipulate experimentally. However, given the many parallels that have already surfaced, for example, between gut, corneal limbal, and skin eSCs [104,105], we believe that investigators specifically interested in the pathobiology of human eSCs within their natural tissue habitat will find it most instructive to turn their attention to interrogating LPP and FFA as model eSC diseases.

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Disclaimer Statement
R.P. is founder and owner of a CRO (Monasterium Laboratory, Münster/Germany) that also performs contract preclinical research on LPP/FFA. None of the authors’ views expressed here are influenced by this.

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