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(Neuro-) endocrinology of epithelial hair follicle stem cells

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Abstract

The hair follicle is a repository of different types of somatic stem cells. However, even though the hair follicle is both a prominent target organ and a potent, non-classical site of production and/or metabolism of numerous polypeptide- and steroid hormones, neuropeptides, neurotransmitters and neurotrophins, the (neuro-) endocrine controls of hair follicle epithelial stem cell (HFeSC) biology remain to be systematically explored. Focussing on HFeSCs, we attempt here to offer a “roadmap through terra incognita” by listing key open questions, by exploring endocrinologically relevant HFeSC gene profiling and mouse genomics data, and by sketching several clinically relevant pathways via which systemic and/or locally generated (neuro-)endocrine signals might impact on HFeSC. Exemplarily, we discuss e.g. the potential roles of glucocorticoid and vitamin D receptors, the hairless gene product, thymic hormones, bone morphogenic proteins (BMPs) and their antagonists, and Skg-3 in HFeSC biology. Furthermore, we elaborate on the potential role of nerve growth factor (NGF) and substance P-dependent neurogenic inflammation in HFeSC damage, and explore how neuroendocrine signals may influence the balance between maintenance and destruction of hair follicle immune privilege, which protects these stem cells and their progeny. These considerations call for a concerted research effort to dissect the (neuro)-endocrinology of HFeSCs much more systematically than before.

Common abbreviations: hair follicle (HF); stem cells (SCs); hair follicle epithelial stem cells (HFeSC); outer root sheath (ORS); transit amplifying cells (TA); connective tissue sheath (CTS); inner root sheath (IRS)

1. The hair follicle as a somatic stem cell repository

The hair follicle (HF) and its special, associated mesenchyme (i.e. the connective tissue sheath, CTS) are repositories of different types of somatic stem cells (epithelial, mesenchymal, neural, mesenchymal) (Cotsarelis, 2006; Kruse et al., 2006; Millar, 2005; Ohyama et al., 2006; Tiede et al. 2007; Waters et al., 2007; Yu et al., 2006). These HF-associated stem cell populations are critical for HF development and function, including HF pigmentation (Nishimura et al., 2002, 2005; Sarin and Artandi, 2007). Fig. 1 shows several important, recognized hair follicle-associated stem cell populations and their best-characterized location(s) in the pilosebaceous unit, though it must be kept in mind that the indicated ones are unlikely to be the only stem cell population associated with mammalian skin appendages (Tiede et al. 2007).

2. Clinical importance of hair follicle stem cells

Hair follicle epithelial stem cells (HFeSCs) are clinically important, since they can be key targets for pathological processes, such as an autoaggressive inflammatory cell attack on HFeSC, e.g. in lupus erythematosus or lichen planopilaris, where the destruction of bulge HFeSCs (see Fig.1) abolishes the hair follicle's capacity to cycle and to rhythmically regenerate itself, leading to scarring alopecia (Cotsarelis, 2006; Hiroi et al., 2006; Mobini et al., 2005; Paus, 2006). Likewise, a gradual loss of HF melanocyte stem cells may exhaust the HF pigmentary unit's capacity to generate itself and to produce melanin and thus likely plays a key role in hair graying (Commo et al., 2004; Nishimura et al., 2005; Sarin and Artandi, 2007).

Moreover, HFeSC also invite multiple uses for regenerative medicine via cell-based therapy with autologous adult SCs, ranging from skin defect repair to neuroregeneration (Chuong et al., 2007; Hoffman, 2005; Kruse et al., 2006; Yu et al., 2006). Finally, HFSC offer excellent targets for both corrective and replacement gene therapy approaches, not only in dermatology (Hoffman, 2005; Jensen, 2007; Sugiyama-Nakagiri et al., 2006). Taken together, this explains why HFeSC have recently attracted so much general interest in many fields of biology and clinical medicine, well beyond the traditional confines of hair research.

3. Hormonal controls of hair follicle stem cells

Since other types of stem cells in the mammalian system are increasingly recognized to underlie hormonal controls (see this special issue, Baik et al., 2005), this raises the question to which extent the various HFSC populations are also subject to (neuro-) endocrine controls, namely during hair follicle development and/or later HF cycling and pigmentation (Alonso and Fuchs, 2006; Paus and Cotsarelis, 1999; Stenn and Paus, 2001; Schmidt-Ullrich and Paus, 2005). As in other areas of stem cell (SC) biology, such hormonal regulation of HFeSC function may offer new targets for therapeutic manipulation, perhaps even in the management of clinically relevant, hormone-dependent disorders of human hair growth or pigmentation (e.g alopecia areata). Moreover, characterization of the (neuro-) endocrine controls of defined HFeSC populations may facilitate their culture and directed differentiation along desired pathways in vitro, and is thus potentially important for regenerative medicine uses of HFeSCs.

Neuroendocrine regulation controls e.g. gonads – and gonadal steroids in turn influence the brain; and so do corticosteroids secreted from the adrenal gland under the influence of ACTH (Adreno Corticotropic Hormone) and regulate the human HF pigmentary unit (Ito et al., 2005; Slominski et al., Tobin and Krauser, 2005; 2007; Watts, 2006). Peptides secreted by hypothalamic neuroendocrine neurons into the blood often appeared to complement with peripheral actions e.g in the HF it is believed to play a role in the pathogenesis of alopecia areata (Kim et al., 2006; Pritchard and White, 2007; Sivilia et al., 2008; Sun et al., 2007). Adrenaline and noradrenaline proved to have properties between endocrine cells and neurons, and demonstrated an outstanding model system for the instance of neuroendocrine systems in the HF (Botchkarev et al., 1999; Peters et al., 1999). The origins and significance of patterning in neuroendocrine secretion are still dominant themes in *neuroendocrinology* today.

Cytokines act like hormones and neurotransmitters but whereas hormones are released from specific organs into the blood and neurotransmitters are produced by neurons, cytokines are released by many types of cells, like the interleukin-6 family member oncostatin M, which is a HF expressed factor with growth inhibitory properties (Yu et al., 2008).

The systemic exploration of hormonal controls of HFeSCs is required since the pilosebaceous unit has become recognized as both, a prominent target organ of multiple endocrine signals and a potent non-classical site of production and/or metabolism of numerous neuro- and steroid hormones, neuropeptides, neurotransmitters and neurotrophins (see below). In the past, almost all interest in the field has focused on the – well-recognized and long-studied, but still insufficiently characterized – impact of these hormones, neuropeptides, neurotransmitters and neurotrophins on the more differentiated progeny of HFeSC, namely on hair matrix keratinocytes and HF outer root sheath (ORS), on the melanocytes of the HF pigmentary unit, and on the specialized, inductive mesenchyme of the HF (connective tissue sheath and follicular dermal papilla) (Alonso and Fuchs, 2006; Gilhar et al., 2007; Slominski et al., 2005; Stenn and Paus, 2001). Now, however, there is growing awareness that HFeSCs themselves may underlie hormonal controls, and that the latter may well impact on hair normal follicle development, growth, cycling, hair shaft formation, and hair pigmentation. Unfortunately, the (neuro-) endocrinology of HFeSCs has not yet been systematic explored and has, thus, remained essentially *terra incognita*.

The current review, therefore, attempts to provide a “roadmap through the unknown” by synthesizing the few known facts in this fascinating, but totally under-investigated area. Specifically this will be done by taking a closer look at published, endocrinologically relevant gene expression profiles of HFeSCs (Tables 1, 2 and 3) and by looking at selected, endocrinologically relevant mouse mutants that display a hair phenotype or a recognized defect of HFeSCs.

We discuss the potential roles of vitamin D receptor and its relation to the hairless gene product and of thymic hormones. In addition, we summarize how or why hormonally regulated key controls of HF biology, like BMPs and their antagonists, SKG-3, may impact on the (neuro-) endocrinology of HFeSCs. Highlighting the HF as a sensitive target tissue for perceived stress that also displays complex, local neuroendocrine stress response-systems, we furthermore elaborate on the potential role of NGF- and substance P-dependent neurogenic inflammation in HFeSC damage. Finally, we explore how neuroendocrine signals such as alpha-melanocyte-stimulating hormone receptor (alpha-MSH), substance P and calcitonin/calcitonin-related polypeptide-alpha (CGRP) may influence the balance between maintenance and collapse of hair follicle immune privilege,

which protects HFeSCs and their progeny from immune destruction. Fig. 2 summarizes salient concepts. The almost completely obscure endocrine controls of HF melanocyte SCs and HF mesenchymal SCs as well as other appendage-associated skin SCs, such as nestin+ cells or SKPs (Tiede et al. 2007) have purposely been excluded from this review.

4. (Neuro-) endocrinology of the hair follicle

When contemplating the hormonal controls of HFeSCs, this must be done on the backdrop of general HF (neuro-) endocrinology, including:

- The pilosebaceous unit (= HF + sebaceous and/or apocrine gland + arrector pili muscle + HF ORS; Fig. 1) is a prominent target tissue for most major endocrine signals, including (but not restricted to) androgens, estrogens, retinoids, glucocorticoids, thyroid hormones, thymic hormones, adrenocorticotrophic hormone (ACTH), corticotrophin-releasing hormone (CRH), melanocortins, parathyroid hormone-related protein, vitamin D3, insulin, prolactin, substance P, melatonin, acetylcholine, endovanilloids, endocannabinoids, catecholamines and prostaglandins. These endocrine signals manipulate all levels of HF biology in mice and man, ranging from HF development, growth, cycling and pigmentation to hair shaft formation, HF immunology and the intrafollicular production of secreted growth factors, cytokines, and hormones.
- The pilosebaceous unit is also a major site of hormone metabolism, e.g. of androgens, estrogens, and prostaglandins.
- The pilosebaceous unit itself also serves as a non-classical, peripheral production site of a surprisingly wide range of neuro- and steroid hormones, neuropeptides, and neurotrophins. These include e.g. CRH, ACTH, alpha-MSH, β -endorphin, cortisol, prolactin, melatonin, erythropoietin, and NGF.
- Both murine and human HFs are highly sensitive target organs of perceived stress and/or classical, neuroendocrine stress response mediators (CRH, prolactin, ACTH, substance P, cortisol). Most of these stress mediators predominantly inhibit hair growth and induce premature HF regression (catagen). CRH, prolactin, ACTH, substance P also manipulate key features of HF immunology e.g. HF immune privilege, connective tissue sheath (CTS) mast cell degranulation.

- Finally, HF (including human scalp HFs) have a fully functional peripheral equivalent of the hypothalamic-pituitary-adrenal axis (HPA), with production of CRH, ACTH and cortisol and classical intrafollicular regulatory feedback loops as they are found in the central HPA.

(see Arck et al., 2005a; Bodó et al., 2005 and 2007; Botchkarev et al., 2004; Colombe et al., 2007; Deplewski and Rosenfield, 2000; Hasse et al., 2007; Kauser et al., 2006; Ohnemus et al., 2006; Paus et al., 2006; Peters et al., 2006b and 2007; Philp et al., 2004; Slominski et al., 2007; Telek et al., 2007; Zouboulis et al., 2007)

While this remains to be conclusively demonstrated, it is likely that HFeSC are fully integrated into this neuroendocrine tapestry of general HF biology. However, all we have to go by currently are some pointers to potentially important elements in the as yet obscure neuroendocrinology of HFeSC.

5. Pointers from HFeSC gene profiling studies

Careful gene expression profiling of epithelial cells from the bulge region of the ORS (Fig. 1) of both murine and human HFs that were classified as “HF epithelial stem cells” (HFeSC) on the basis of GFP (green fluorescent protein)-visualized cytokeratin 15 promoter activity for targeting epithelial SCs in the HF bulge (Liu et al., 2003; Ito et al., 2005). Morris et al., 2004; Ohyama et al., 2006; Tiede et al., 2007 and Tumber et al., 2004, have provided particularly valuable pointers to endocrine controls of this population of HFeSCs (Tables 1 and 2).

Insulin, for example, is a major growth factor for human HF and stimulates IGF-1 expression and secretion (Rudman et al., 1997; Tang et al., 2003; Weger and Schlake, 2005). It is, therefore, interesting to note that HFeSCs in mice show an upregulation of transcription for insulin-like growth factor binding proteins 3, 5, 6, which would be expected to antagonize IGF (insulin growth factor)-1-mediated signalling in this SC compartment. Transgenic Igf-I under the control of involucrin affects follicular proliferation, tissue remodelling, and the hair growth cycle, as well as follicular differentiation. These transgenic effects are partially compensated by ectopic expression of Igfbp3 (Weger and Schlake, 2005). Edmondson et al., 2005, used an Igfbp-3 transgenic mouse to assess the effect of excess epidermal Igfbp-3 on keratinocyte proliferation. The highest levels of

Igfbp-3 mRNA were detected in TA keratinocytes. In the epidermis, Igfbp-3 mRNA is highly expressed by proliferative keratinocytes (TA) and overexpression of Igfbp-3 inhibits keratinocyte proliferation. However, as with most of the following gene profiling data, it remains unclear whether the corresponding protein products are also significantly regulated in HFeSC *in situ*.

Given that the expression of connective tissue growth factor (CTGF) is subject to hormonal regulation e.g. by estrogens (Harlow and Hillier, 2002; Wang et al., 2007) and that estrogens are recognized key hair growth regulatory steroid hormones (Ohnemus et al. 2006), the reported upregulation of *Ctgf* expression by murine HFeSC (**Table 1**) also deserves to be carefully followed up. It is conceivable that increased *Ctgf* release by these SCs may contribute to condition the bulge SC niche (Cotsarelis, 2006), including its mesenchymal component, i.e. the immediately adjacent peri-bulge CTS (**Fig. 1**).

Pregnancy and prolonged lactation in adult female mice can reset complex hair cycle domain patterns. This phenomenon may arise due to the effect of estrogen and/or prolactin levels that are elevated during these physiological states. Both of these hormones are implicated in direct inhibition of anagen induction or anagen initiation in telogen HFs (Plikus and Chuong, 2007) (**Fig. 1**). Resetting occurs following the prolonged telogen associated with pregnancy and lactation. In the post-lactation period, there is simultaneous anagen re-entry of most of the HFs across the skin. Most of the HFs become synchronized, reminiscent to the status in the first post-natal cycle. Subsequently, new complex hair cycle domain patterns can re-form, unless new pregnancies occur. Also, one wonders whether estrogens and prolactin may mediate some of their putative effects on HFeSC (see below) via regulating the CTGF expression of these cells.

Transforming growth factor (TGF β 1 and TGF β 2), whose activity is limited by binding to latent transforming growth factor beta binding proteins (LTBPs), are major modulators of HF development, growth, and cycling (Alonso and Fuchs, 2006; Foitzik et al., 1999 and 2000; Nishyama and Hibino, 2004). In mature human scalp HFs, TGF β 1 and TGF β 2 are now recognized as key hair growth-inhibitory signals, whose up-regulation e.g. by androgens and retinoids (Foitzik et al., 2005; Inui et al., 2002) underlies, at least in part, the hair growth-inhibitory effects of these steroid hormones on susceptible HF

subpopulations. Therefore, it is noteworthy that *LTBP1* transcription is up-regulated in the HFeSC of both mice and man. Again, this would be expected to limit TGF β -mediated signaling in the bulge region, as well as the stimulation of intrafollicular TGF β production by androgens or retinoids. Instead, *LTBP2* is down-regulated in human HFeSC (**Table 2**).

Similarly indirect endocrine controls of HFeSC biology may also be deduced from the finding that the transcription of another *TGF β /BMP* superfamily member, bone morphogenic protein 6 (BMP-6), and of the BMP antagonist, *follicle-stimulating protein*, is upregulated in these cells (Table 1; Ohyama et al., 2006). Since both genes are regulated by hormones and growth factors (Sammons et al., 2005, Bamberger et al., 2005) this could represent another level via which endocrine signals may impact HFeSC functions (Nakamura et al., 2003, Hwang et al., 2005; Thesleff et al., 2007).

Among the additional gene profiling pointers to endocrine controls of HFeSC biology it is of note that HFeSC show a relative overexpression of receptor transcripts for estrogens (ER β), growth hormone, angiopoietins, and endothelins (**Table 1+2**). Also, human HFeSC upregulate β 1-adrenergic receptor (β 1-AR; Ohyama et al., 2006). These expression data may carry particular hair-biological relevance, since beta-adrenergic receptor protein immunoreactivity in murine skin *in vivo* is up-regulated in the bulge region of pelage HF during the early growth phase of the hair cycle (anagen) (Botchkarev et al., 1999) and since catecholamines indeed modulate HF cycling in murine skin organ culture (Peters et al., 1999). Moreover, diffuse hair loss (telogen effluvium) is a fairly frequent, undesired clinical effect of betablocker pharmacotherapy (Shelley et al., 1985). Taken together, this raises the possibility that sustained β 1-AR-mediated signalling is needed for supporting normal HFeSC functions, at least in anagen HFs.

6. Immunohistological patterns of hair follicle located (neuro-) endocrine relevant proteins

Additional phenomenological indicators to potential neuroendocrine controls have arisen from immunohistological studies of relevant protein-immunoreactivity patterns in the bulge region of mouse and human HFs *in situ* (Table 3). For example, vasoactive intestinal peptide receptor (Vip receptor) is expressed in the murine bulge region during anagen V, but disappears in all other phases of the murine hair cycle (Wollina et al. 1995). Though it

is conceivable that this neuropeptide receptor plays a role in hair cycle-associated changes in HFeSC chronobiology (Paus and Foitzik, 2004), the significance of VIP-induced signalling for HFeSC functions has not yet been followed up and thus remains completely obscure.

Comparing bulge and bulb expression patterns of human HFs, Xu et al., 2003, noted that the cell cycle-regulatory protein CYCLIN D1 was increased at transcript and protein level, with the cell cycle regulatory protein localized to the suprabasal cells of the telogen bulge and to ORS. Interestingly, CYCLIN D1 immunoreactivity was not detectable in the rapidly proliferating TA cells of the HF (matrix keratinocytes), despite strong immunoreactivity for Ki-67. This led the authors to hypothesize that CYCLIN D1 may mediate the transition of bulge HFeSC to more differentiated TA cells. Gli-2 induced type 3 iodothyronine deiodinase reduces intracellular active thyroid hormone, thus resulting in increased cyclin D1 and keratinocyte proliferation (Dentice et al., 2007).

In theory, stimulation of any hormone receptor that modulates CYCLIN D1 expression/nuclear accumulation e.g. thyroid hormone, estrogen, or PTH/PTHrP receptors (Datta et al., 2007; Porlan et al., 2007; Zhu and Pollard, 2007), may thus manipulate this crucial transition from the eSC pool of the HF to its direct progeny – provided that HFeSC express such hormone receptors.

In a combination of immunoreactivity and functional assays, Philp et al. 2004, have reported that, thymic hormones (thymosin β 4) can stimulate rodent hair growth and that bulge-derived murine HF keratinocytes express thymosin β 4. Both migration and differentiation of clonogenic keratinocytes from rat vibrissae HFs, at least some of which likely represent HFeSCs, are stimulated by this thymic peptide *in vitro*. Thus, intrafollicularly produced thymosin β 4 may promote HFeSC migration and subsequent differentiation to ORS and hair matrix keratinocytes (Philp et al., 2004).

Besides spatiotemporally restricted β -adrenergic and Vip receptor expression in the bulge during defined segments of the murine hair cycle (Table 1, 3), other key hormone receptors have long been known to be expressed throughout the ORS: this is true e.g. for glucocorticoid, retinoid, estrogen, and vitamin D3 receptors (glucocorticoid receptor (GR), retinoic acid receptor (RAR), estrogen receptor (ER), vitamin D receptor (VDR)); and mutations in VDR and retinoid receptors can cause severe alopecia in mice and man (Chebotaev et al., 2007; Billoni et al., 1994; Ohnemus et al., 2006; Reichrath et al., 1994;

Zouboulis et al., 2007). Even though a marked, immunohistologically prominent up- or down-regulation of these receptors has not been noted, the hair growth effects of appropriate receptor ligands, especially in mice, is impressive. For example, even once weekly topical 17- β -estradiol application suffices to “freeze” murine back skin pelage HFs in the resting stage of the hair cycle (telogen) and induces catagen i.e. apoptosis-driven HF involution (Ohnemus et al., 2006). Topical glucocorticoid administration prevents depilation-induced anagen development and is a very potent catagen-inducer in mice (Paus et al., 1994 a; Stenn et al., 1993). Moreover, murine bulge keratinocytes appear to be much more sensitive to the antiproliferative effect of topically applied glucocorticoids than basal layer epidermal keratinocytes, possibly due to incomplete desensitization by insufficient downregulation of GR in the bulge (Chebotaev et al., 2007). Topical application of VDR agonists (calcitriols) also promotes catagen development in mice (Paus et al., 1996).

These striking, yet mechanistically ill-understood hair growth-modulatory effects of e.g. GR, VDR and ER agonists deserve to be carefully re-evaluated in the light of recent HFeSC gene profiling results e.g., up-regulation of GR and ER β transcripts in HFeSCs (Table 1). There is increasing evidence that HFeSC are important direct targets for steroid hormone signalling *in situ*.

7. Insights on HFeSC endocrinology from mouse genetics

Transgenic overexpression of GR under the control of *cytokeratin-5 (Krt5)* promoter shows a significantly reduced number of HFeSC compared to normal mice. Additionally GR-overexpression reduces the clonogenic potential of HFeSC and modulates the expression of several HFeSC “signature genes”, and makes them less susceptible to experimentally induced skin carcinogenesis (Chebotaev et al., 2007). These findings suggest that GR-mediated signaling is a critical element in both HFeSC biology and in the epithelial tumors that can arise from them. Moreover, they retrospectively offer new explanations for why glucocorticoids are such powerful hair growth inhibitors e.g. potential shut-down of transient amplifying cell production from bulge HFeSCs.

The glucocorticoid-sensitivity of HFeSCs may also reflect another pathway, i.e. interaction of these steroid hormones with serum- and glucocorticoid-responsive kinase 3 (Sgk3). Hair follicles of mice that lack Sgk3 show severe developmental abnormalities and

prematurely enter into catagen, along with reduced proliferation and increased apoptosis of transient amplifying cells in the hair matrix (Alonso et al., 2005). A critical role for Sgk3 in murine hair follicle morphogenesis and cycling has recently been confirmed in other Sgk3 mutants (Okada et al., 2006).

In mice and man, VDR-mediated signaling is essential for normal HF development, growth, and cycling, and VDR mutations result in alopecia. The product of the *hairless* gene – a zinc finger protein like VDR, whose mutation, by itself, produces a very similar hair phenotype in mice and man as VDR mutants - serves as a nuclear co-repressor for VDR (Bergman et al., 2005; Bikle et al., 2006; Miller et al., 2001; Wang et al., 2007).

Despite otherwise complete HF destruction after the first entry into catagen, mutant mice with functionally defective hairless protein seem to retain a residual HFeSC pool (Panteleyev et al., 1999; Panteleyev et al., 2000a; Panteleyev et al., 2000b). Likewise, even in the absence of functional VDR HFs develop, and a HFeSC niche establishes itself. However, subsequently VDR is essential for these SCs to cyclically regenerate the lower hair follicle epithelium during anagen (*in vivo*) and for normal clonogenic potential (*in vitro*). VDR impairs the canonical wingless-type MMTV integration site family (Wnt)-signaling in HFeSCs that ultimately results in alopecia (Cianferotti et al., 2007; Demay et al., 2007; Thompson et al., 2006).

These recent pointers from mouse genomics to HFeSC endocrinology demonstrate a key role of GR, Sgk3 and VDR (and normal VDR-hr interactions) for normal HFeSC function and encourage one to now dissect the relative importance of these signaling elements in human HFeSC biology. For clinical endocrinology, one particularly intriguing question in this context is e.g. whether the stimulation of GR- and/or the suppression of VDR-mediated signaling in the bulge of human HF may be exploited for treating excessive, unwanted hair growth (hirsutism, hypertrichosis) by sharply limiting the HF's SC-based, enormous capacity for self-renewal.

8. Hair follicle stem cells and cutaneous responses to perceived stress

The complexities of general HF neuroendocrinology sketched above render it likely that multiple, indirect neuroendocrinological and neuroimmunological pathways must also be considered when exploring as yet the obscure neuroendocrinology of HFeSCs. The HF's

response to perceived stress and to prototypic neuroendocrine mediators of stress responses adequately illustrate both, the major open questions one faces in this context, and some promising future research avenues.

Many affected patients – as well as some of their physicians - have long suspected that psychoemotional stress can be associated with hair loss, telogen effluvium and alopecia areata. In view of substantial recent progress in dissecting the “brain-skin connection” and cutaneous responses to perceived stress in health and disease (Arck et al., 2006; Paus et al., 2006) this notion has become less speculative. Also, the discovery that rodent and human skin - including human scalp hair follicles - even display fully functional, peripheral equivalents of the central hypothalamic-pituitary-adrenal (HPA) axis stress response system (Ito et al., 2005; Slominski et al., 2007) has added further credence to the concept that psychoemotional stress can negatively affect human hair growth. Yet, until recently, there was no convincing evidence available on whether or not mammalian HFs even respond to perceived stress.

This recently changed with the demonstration that mice which had been subjected to sound stress, already after 24 hours, showed dense inflammatory cell infiltrates around their HFs, in particular around the bulge area, with an increase in the number and activation of perifollicular mast cells as well as MHC class II-positive cell clusters (Arck et al., 2005b; Fig. 3). Interestingly, this was not only associated with reduced baseline proliferative activity throughout the skin epithelium, but also with an increased number of apoptotic cells in the HFeSC region, the bulge (Fig. 3). Subsequent studies in mice revealed that perceived stress challenge shortens the hair cycle growth phase (anagen) and prematurely induces catagen. Furthermore, these studies revealed that the major stress-associated growth factor, NGF, in conjunction with the prototypic stress-associated neuropeptide, substance P, and with skin mast cells (as “central switch-boards of neurogenic inflammation”) could be identified as key mediators in the stress-induced perifollicular neurogenic inflammation (Arck et al., 2003; Joachim et al., 2007; Peters et al., 2004).

Essentially, the key hair-related findings in this sonic stress model were reproduced in a classical foot shock stress model (Katayama et al., 2007). Importantly, when microdissected, organ-cultured human scalp HFs are directly exposed to NGF or substance P, very similar HF responses are seen in this human in vitro system as in

sound-stressed mice: Inhibition of hair matrix keratinocyte proliferation, up-regulation of matrix apoptosis, increased mast cell numbers and degranulation in the CTS, and premature catagen development (Peters et al., 2006 b; Peters et al., 2007). Therefore, at least *in vitro*, the immediate progeny of human HFeSC shows strikingly similar stress responses as one sees in mice *in vivo*.

Interestingly, in the foot shock mouse model, the most proximal element of the central HPA axis, CRH, was seen to be upregulated in the HF epithelium of stressed mice. These also showed higher serum corticosterone levels (Aoki et al., 2003). Thus, both systemic and intracutaneous CRH levels rise in response to perceived stress, which may then induce not only higher adrenal, but also increased intrafollicular glucocorticoid synthesis (Ito et al., 2005). It is conceivable that GR-expressing HFeSC (Table. 1), whose “signature” gene expression profile clearly is subject to glucocorticoid-regulation (Chebotaev et al., 2007), may then generate insufficient transient amplifying cells e.g. hair matrix keratinocytes needed for anagen maintenance so that catagen develops prematurely.

Thus, besides responding to (as yet insufficiently-characterized) growth-inhibitory signals arising from the stress-induced, NGF- and substance P-driven perifollicular inflammatory cell infiltrate (activated mast cells and dendritic cells), HFeSCs may also be directly targeted by both locally synthesized and systemic stress mediators, such as glucocorticoids (Fig. 2). It now needs to be characterized whether human and/or mouse HFeSC in the bulge express constitutively higher or lower levels of receptors for classical secreted stress mediators e.g. CRH, ACTH, NGF, substance P, prolactin and cortisol) – all of which are synthesized in the human HF itself (Peters et al., 2006 a; Slominski et al., 2007). Also, in view of the observation that these cells appear to fail to show appropriate desensitization responses to glucocorticoid stimulation (Chebotaev et al., 2007) one needs to know how HFeSC respond to stimulation with these neuron-endocrine stress mediators.

Our recent finding that substance P stimulation of organ-cultured human scalp HFs down-regulates the expression of cognate receptors (neurokinin 1 receptor; NK1) in the proximal HF epithelium, while it up-regulates immunoreactivity for a key substance P-degrading enzyme (neutral endopeptidase), suggests that negative feedback loops are established (Peters et al., 2007). The fact that substance P down-regulates

immunoreactivity for the hair growth-promoting NGF-receptor TRKA (neurotrophic tyrosine kinase, receptor, type 1), whereas it up-regulated NGF and its apoptosis- and catagen-promoting NGF-receptor associated protein 1 (p75NTR) (Peters et al., 2007) suggests the establishment of intrafollicular cross-signaling between major neuroendocrine stress-mediating signal transduction systems. Unfortunately, the employed organ culture assay (= amputated anagen hair bulbs) contains transient amplifying cells, but no bulge cells. Therefore, it remains to be studied e.g. in a new, serum-free assay for the long-term culture of human scalp skin (Lu et al., 2007), whether the neuroendocrine response patterns of human HFeSC in the bulge *in situ* differ from those of their progeny.

9. Neuroendocrine controls of hair follicle immune privilege and their importance for stem cell survival

We conclude this attempt at developing a roadmap through the *terra incognita* of HFeSC (neuro-) endocrinology with a final digression into HF immunology. It had long been thought that only the anagen hair bulb epithelium is a site of relative immune privilege, where e.g. immunogenic, melanogenesis-associated autoantigens are shielded from potentially deleterious autoimmune responses, and that the collapse of this anagen hair bulb immune privilege is a critical step in the pathogenesis of the most common autoimmune hair growth disorder, alopecia areata (Gilhar et al., 2007; Paus et al., 2005).

We could show that this HF immune privilege underlies striking neuroendocrine controls: The key stress-associated neuropeptide, substance P, induces a collapse of the bulb immune privilege by triggering ectopic MHC class I expression in the proximal HF epithelium (Peters et al., 2007). Interestingly, CGRP, and the immunosuppressive melanocortin, alpha-MSH – both of which are also produced/released in the HF – protect this HF immune privilege from experimentally induced collapse (Ito et al., 2004). Strikingly, upon stress challenge in murine systems, an increased expression of CGRP can be detected in the skin, which may be indicative of a counter-reaction to prevent a severe tissue damage (Joachim et al., 2007). Therefore, even the protection of transient amplifying cells in the HF from undesired autoimmune responses appears to be subject to stringent neuroendocrine controls.

But what about the HFeSCs themselves? Recent human HFeSC gene profiling data have raised the possibility that the bulge may also enjoy a relative immune privilege (Cotsarelis, 2006), since e.g. MHC class I transcripts are down-, and CD200 mRNA are upregulated in K15-GFP+ bulge cells (Morris et al., 2004, Ohyama et al., 2006). Indeed, we have most recently collected protein expression evidence *in situ* that fully supports the concept that the human HF bulge, the seat of HFeSCs, shows many phenotypic features of relative immune privilege, including the down-regulation of MHC class I and class II proteins, along with an up-regulation of alpha-MSH and TGF β 2. This could be important in the context of auto-aggressive inflammatory hair disorders such as lichen planopilaris and lupus erythematosus, where irreversible immune damage to the bulge region abrogates the HFeSC's capacity for self-renewal, thus leading to permanent, scarring alopecia (Cotsarelis, 2006). We are now challenged to elucidate whether the neuroendocrine immune privilege protectants that safeguard the hair bulb's immunological integrity (alpha-MSH, CGRP) do the same for HFeSCs in the bulge.

Whether or not future research into the neuro- and endocrine controls of HFeSC will answer this question in the affirmative, these final considerations only further underscore the importance of dissecting much more systematically than before the role of locally synthesized as well as systemic (neuro-)endocrine signals and their intrafollicularly expressed cognate receptors in HFeSC biology and health and disease.

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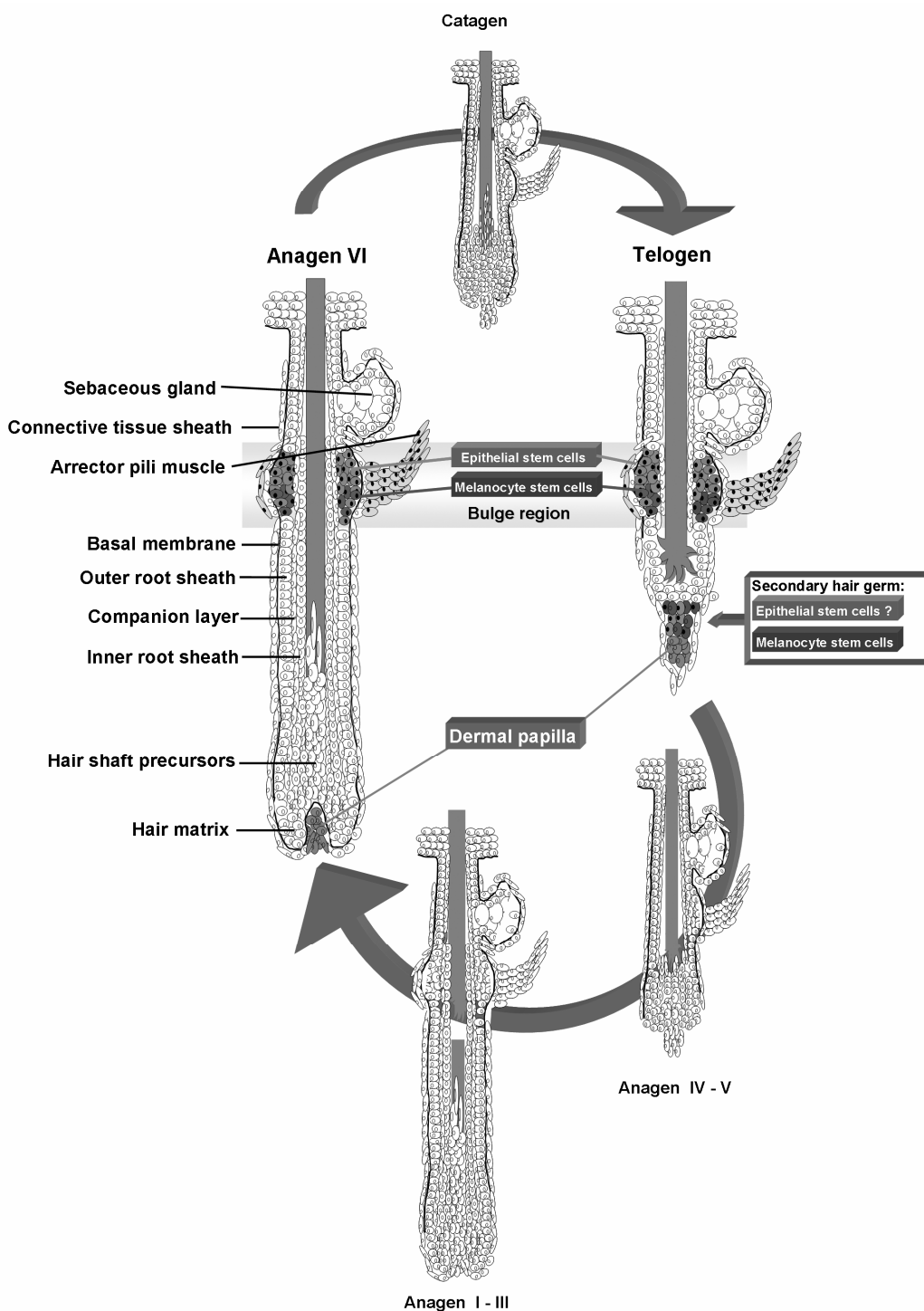


Figure 1. Hair follicle anatomy and localization of different stem cell populations in defined hair follicle compartments. Cyclical changes in hair follicle growth are divided into different stages, referred to as anagen, catagen, and telogen. Hair follicle epithelial and melanocyte SCs localize to the bulge at the site of arrector pili muscle insertion. During anagen, rapidly proliferating progenitor cells in the bulb generate the hair shaft and its surrounding inner root sheath. The onset of catagen is marked by completion of proliferation as well as by apoptosis of the epithelial cells below the bulge. The mesenchymally-derived dermal papilla survives catagen and moves to the lowermost portion of the bulge during telogen, which then forms the secondary hair germ at its base

as a pool for melanocyte SCs, which are moved down from the bulge, and maybe for a second epithelial SC population. As the new hair shaft grows in, the old hair is shed.

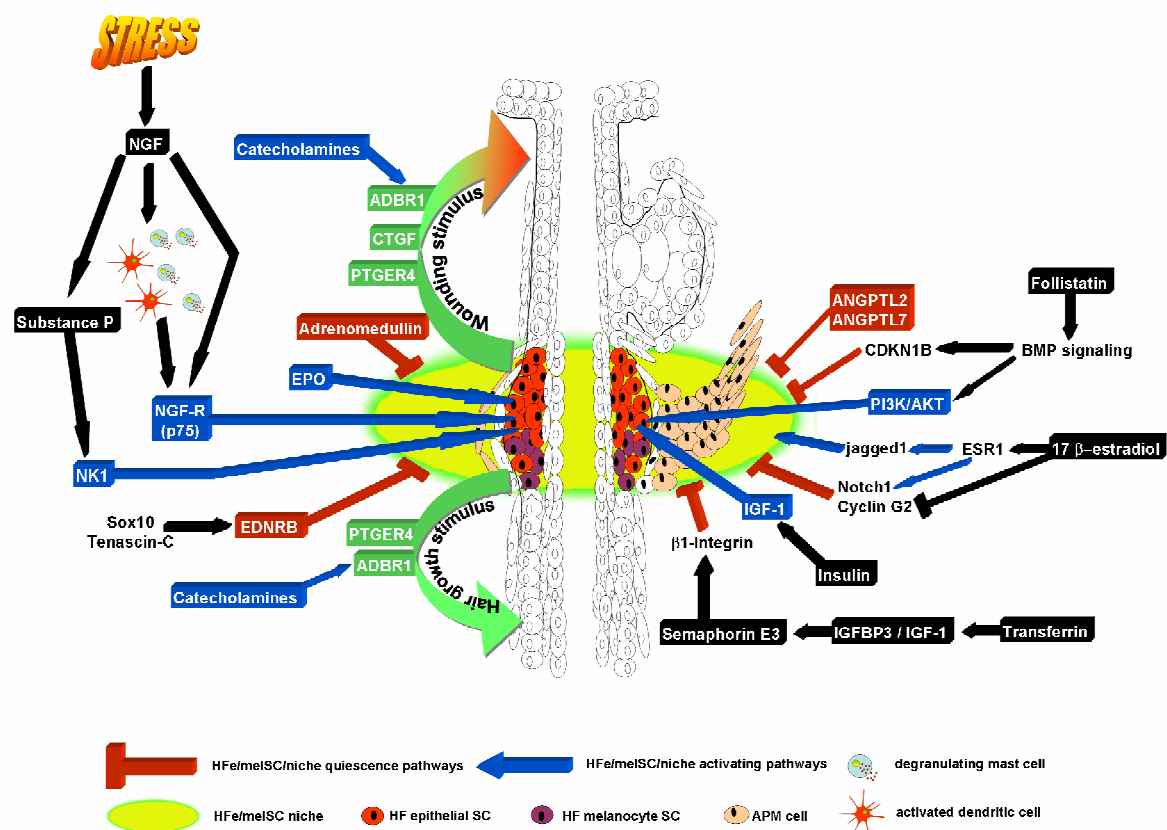


Figure 2. Hypothetical pathways for potential (neuro-) endocrine regulators of epithelial hair follicle stem cells and their niche.

Abbreviations: ADBR1, adrenergic beta1 receptor; ANGPTL2/7, angiopoietin-like protein; APM, arrector pili muscle; BMP, bone morphogenetic protein; CDKN1B, cyclin dependent kinase inhibitor; CTGF, connective tissue growth factor; *EDNRB*, endothelin receptor B; EPO, erythropoietin; ESR1, estrogen receptor beta1; HFe/melSC, hair follicle epithelial and melanocyte stem cells; *IGF1*, insulin like growth factor 1; IGFBP3, IGF binding protein 3; NGF, nerve growth factor; NGF-R, NGF receptor; NK1, neurokinin-1 receptor; PI3/AKT, phosphatidylinositol 3-kinase/ protein kinase B; PTGER4, prostaglandin E receptor; SOX10, (sex determining region Y)-box 10 (for more details, see table 1, 2 and 3)

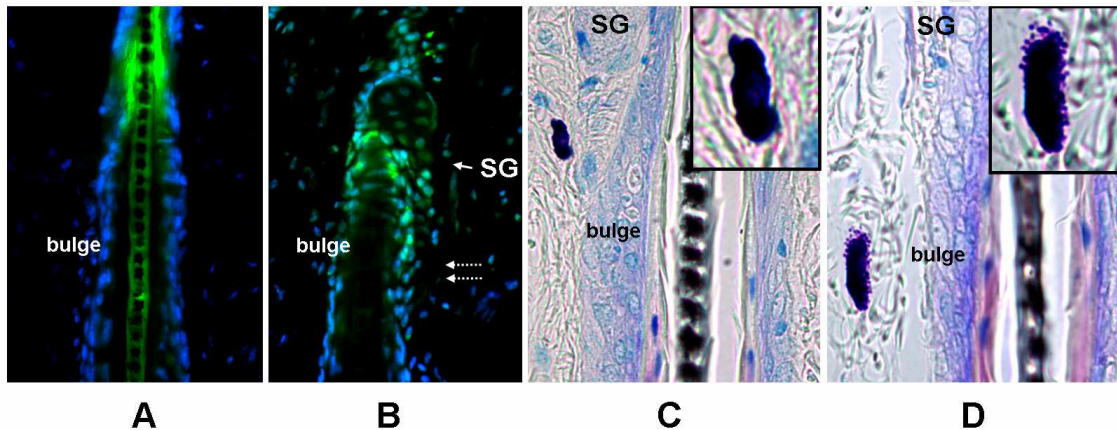


Fig.3. Representative photomicrographs to visualize murine skin reaction to stress challenge. (A, B) The effect of sound stress challenge on hair follicle (HF) keratinocyte apoptosis in the bulge region in dermis of C57BL/6 mice. (A) TUNEL staining in dermis of control mice, very few apoptotic cells can be observed in the bulge region. HF keratinocyte nuclei appear in bright blue, resulting from DAPI counterstaining. (B) Stress exposure 2 days prior to detection of apoptotic signals employing TUNEL staining caused an increase in apoptotic cells in the bulge region of the HF, as depicted by green fluorescence and pointed out in one cases with arrows. SG means sebaceous gland. (C, D) Detection of mast cell activation in dermis upon Giemsa staining. (C) Mostly non activated mast cells are present in the bulge region of non stressed C57BL/6 mice, as confirmed by the lack of extracellular granules in the vicinity of the resting mast cell (dark purple staining, see insert for close up). (D) Stress exposure caused an increase in activated (degranulated) mast cells in dermis, see also insert for better visualization of extra-cellular granules. If more than extracellular 8 granules can be detected, the mast cell respective mast cell can be considered as activated. Magnification in A-D is x 400.

Table 1
GENE PROFILING POINTERS TO ENDOCRINOLOGICALLY RELEVANT ELEMENTS OF HAIR FOLLICLE STEM CELL BIOLOGY (micro-array analysis and qPCR data):
Up-regulated expression (according to expression analysis by Blainpain et al., 2004; Morris et al., 2004 and Ohyama et al., 2006)
 (in HFeSCs compared to basal layer epidermal keratinocytes/non-bulge ORS)

gene/gene product (species)	comments	refs.	potential relevance for endocrinology of HFeSC and/or their progeny	refs.
ADRB1 (adrenergic, beta-1-receptor) (HUMAN) (Mouse)	ADRB1 blockade impairs cutaneous wound healing.	Ohyama et al., 2006	<i>Adrb1</i> expression in murine bulge cells in early (anagen). Murine hair cycle modulation by adrenergic agents.	Botchkarev et al., 1999 Peters et al., 1999 Souza et al., 2006
ANGPTL2 (angiopoietin-like) (HUMAN)	Mesenchymal stem cells enhance wound healing through angiopoietin-like triggered differentiation and angiogenesis.	Ohyama et al., 2006	Angiopoietins have been identified as the major ligands of the endothelial-specific receptor Tie2. Thus, the Tie2/Angiopoietin-like signaling pathway plays a critical role in maintaining HFeSC in the quiescent state in the niche.	Kuroda et al., 2001 Mecklenburg et al., 2000 Oike et al., 2004 Suda et al., 2005 Yano et al., 2001 Yano et al., 2003
BMP6 (bone morphogenetic protein) (HUMAN) (Mouse)	BMPs are a family of secreted signaling molecules that can induce ectopic bone growth. Many BMPs are part of the transforming growth factor-beta (TGF β) superfamily.	Blainpain et al., 2004	BMP signaling inhibits HF anagen induction by restricting HFeSC/TA activation and expansion. 17 β -estradiol activates via BMP signaling PI3K/AKT pathways which augment proliferation of HFeSCs/TAs by loss of the quiescent niche	Kobiela et al., 2007 Murayama et al., 2007 Ohnemus et al., 2005 Ohnemus et al., 2006 Zhang et al., 2006
CALM2 (Calmodulin 2 phosphorylase kinase, delta) (HUMAN)	CALM2 functions include roles in growth and the cell cycle as well as in signal transduction and the synthesis and release of neurotransmitters.	Ohyama et al., 2006	Calmodulin stimulation activates the expression of ADRB1 and activates therefore signaling pathways which modulate epithelial-mesenchymal transformation. Calmodulin binds additionally the erythropoietin receptor which modulates together with TGF β telomerase activity which could play an important role in HFeSC quiescence	Bodo et al., 2007 Lenior et al., 1986 Prade-Houdellier et al., 2007 Siegl-Cachedenier et al., 2007 Sterin-Borda et al., 2007
Cdkn1B (cyclin-dependent kinase inhibitor) (Mouse)	Cyclin-dependent kinase inhibitors, like Cdkn1B, are expressed site- and hair cycle-dependently in rat HFs. Involved in the differentiation of follicular epithelial cells	Blainpain et al., 2004	Activation of Bmp signaling induces growth arrest, Cdkn1B expression and plays an important role in regulating cell by modulating the expression of cell-cycle-associated genes in hair matrix keratinocytes. Somatostatin analogues stimulate Cdkn1B expression	Mitsui et al., 2001 Nanba et al., 2003 Niederhorn, 2003 Sharov et al., 2006

			and inhibit the MAP kinase pathway. Somatostatin itself is an regulator of the HF bulge immunoprivilege (HFeSC niche)	
CtgF (connective tissue growth factor) (Mouse)	Involved in inductive properties of fibroblasts during embryogenesis and in adult wound healing, tissue repair and fibrogenesis.	Blainpain et al., 2004	Relevant for HFeSC/TA communication with the CTS e.g. with CTS component of the bulge SC niche ?	Friedrichsen et al., 2005 Kennedy et al., 2007
EDNRB (endothelin receptor B) (HUMAN)	EDNRB appears essential for the continued survival of migrating melanocytes	Ohyama et al., 2006	SOX10 interacts with EDNRB which regulates melanocyte SCs. Binding of Tenascin-C to EDNRB may regulate HFeSC niche properties.	Boissy and Nordlund, 1997 Lange et al., 2007 Slominski et al., 2005 Yoshida et al., 1996
ESR1 (estrogen receptor beta) (HUMAN)	The estrogen receptor (ESR) is a ligand-activated transcription factor composed of α and β domain.. These receptors display different immunohistochemical localizations in human scalp skin, where ESR β is predominantly expressed, suggesting their different functional roles in cutaneous biology.	Ohyama et al., 2006	17 β -estradiol activates via the ESR jagged1- and notch1 expression. Jagged1 regulates HF differentiation (HFeSC/TA activation), whereas notch signalling is required for the maintenance of melanocyte SCs in the HF	Ambler and Watt, 2007 Kwon et al., 2004 Nishikawa and Osawa, 2007 Soares et al., 2004 Thornton et al., 2003 Yoo et al., 2007
FS (Follistatin) (HUMAN)	FS is a gonadal protein that inhibits follicle-stimulating hormone release.	Ohyama et al., 2006	FS antagonizes the functions of BMPs and Activin. Both mediate interactions between the mesenchymal SC and HFeSC within the niche and form an integrated network	Hwang et al., 2005 Nakamura et al., 2003 Thesleff et al., 2007
GHR (growth hormone receptor) (HUMAN)	Binding of growth hormone (GH) to the receptor leads to receptor dimerization and the activation of an intra- and intercellular signal transduction pathway leading to growth. In transgenic rats in which the GH expression is suppressed the hair enters a long-lasting telogen phase.	Ohyama et al., 2006	HF bulge cells which express GHR also express prolactin receptors. These two factors play an important role for the immunocompetent bulge region (HFeSC niche).	Dardenne et al., 1998 Umeda-Ikawa et al., 2006
Nr3c1 (GR; glucocorticoid receptor) (Mouse)	Keratinocytes from both the HF bulge and interfollicular epidermis of mice express Nr3c1.	Ohyama et al., 2006	GR signaling has an important role in the homeostasis of HFeSC, and they are more sensitive to the antiproliferative effect of glucocorticoids implicating that the reduction in their number may underlie the tumor suppressor effect of Nr3c1 in the skin.	Chebotov et al., 2007 Morris, 2007
Igfbp-3, -5, -6 (insulin-like growth factor binding proteins 3, 5, 6) (Mouse)	Igfbp-3 inhibits hair matrix keratinocyte proliferation, possibly involved in catagen induction. Igfbp-5 involved in hair shaft formation. Igfbp-6 promotes beta-	Blainpain et al., 2004 Morris et al., 2004	Igf-1 production stimulated by major hair growth-regulatory hormones e.g. insulin, prolactin; main effect likely on TA and their more differentiated progeny. Key role of	Alonso and Fuchs, 2006 Aminik et al., 2007 Paus and Foitzik, 2004 Paus, 2005 Schlake, 2005a Schlake, 2005b Stenn and Paus, 2001 Weger and Schlake,

	catenin transcriptional activity		beta-catenin in HFeSC commitment to HF-type differentiation.	2005
LTBP1 (latent transforming growth factor beta binding protein 1) (HUMAN) (Mouse)	LTBP1 targets latent complexes of TGF β to the extracellular matrix, where the latent cytokine is subsequently activated by several different mechanisms. LTBP1 interacts with fibrillin and is a microfibril-associated protein.	Blainpain et al., 2004 Ohyama et al., 2006	TGF β 1/2 are catagen-inducing growth factors. TGF β 2 is an inducer of HF morphogenesis. Epidermal growth factor stimulated TGF β 1 expression in stromal cells, whereas keratinocyte growth factor stimulated TGF β 2 in epithelial cells (mobilization and inactivation of HFeSC)	Foitzik et al., 1999 Foitzik et al., 2000 Isogai et al., 2003 Itoh et al., 1998 Paus and Foitzik, 2004
Mmp-2 (matrix metalloproteinase) (Mouse)	Mmp's control extracellular matrix remodelling, including during HF cycling.	Blainpain et al., 2004	Expression of Mmp-2 is increased by thymosin β 4. Thymosin β 4 accelerates hair growth due to its effect on the active phase of the HF cycle. Activation of EGF receptor by the release of Gprc-ligands through proteolytic action of matrix Mmp's promotes proliferation, survival, differentiation and migration of HFeSC/TA due to remodelling the basement membrane of the HFeSC-niche.	Karelina et al., 2000 Paus R., et al., 1994 Philp et al., 2004 Shah and Catt, 2004
Sema3E (Semaphorin H 3E) (Mouse)	Signaling by Sema3E and its receptor plexin-D1 controls endothelial cell positioning and the patterning of the developing vasculature in the mouse	Morris et al., 2004	Sema3E binds to α - and β -integrins which are regulators of the HFeSC niche. Transferrin modulates Sema3E -expression through an Igfbp3/Igf-1-dependent pathway by regulation of adhesion proteins (HFeSC-niche remodeling)	Cotsarelis et al., 1999 Herman et al., 2007 Tanentzapf et al., 2007
VDR (Vitamin D receptor) (Mouse)	The major physiological role of the VDR is the maintenance of mineral ion homeostasis.	Morris et al., 2004	Mutation of the VDR, in humans and mice, results in alopecia. The absence of VDR impairs canonical Wnt signaling in keratinocytes and leads to the development of alopecia due to a defect in keratinocyte stem cells.	Cianferotti et al., 2007 Pálmer et al., 2008

Legend

HF = hair follicle

HFeSC = hair follicle epithelial stem cells (located predominantly in the bulge region of the outer root sheath [ORS])

TA = transit amplifying cells (=rapidly proliferating hair follicle keratinocytes derived from HFeSC)

CTS = connective tissue sheath of the hair follicle

IRS = inner root sheath of the hair follicle

Table 2:

GENE PROFILING POINTERS TO ENDOCRINOLOGICALLY RELEVANT ELEMENTS OF HAIR FOLLICLE STEM CELL BIOLOGY micro-array analysis and qPCR data):
Down-regulated expression (according to expression analysis by Blainpain et al., 2004; Morris et al., 2004 and Ohyama et al., 2006)

(in HFeSCs compared to basal layer epidermal keratinocytes/non-bulge-ORS)

gene/gene product (species)	comments	refs.	potential relevance for endocrinology of HFeSC and/or their progeny	refs.
ANGPTL7 (angiopoietin-like 7) (HUMAN)	ANGPTL7 is expressed at high levels in the avascular corneal stromal layer suggested that the protein, similar to certain angiopoietins, acts as a negative regulator of angiogenesis.	Ohyama et al., 2006	Angiopoietins have been identified as the major ligands of the endothelial-specific receptor Tie2. Thus, the Tie2/Angiopoietin-like signaling pathway plays a critical role in maintaining HFeSC in the quiescent state in the niche.	Kuroda et al., 2001 Mecklenburg et al., 2000 Peek et al., 2002 Suda et al., 2005 Yano et al., 2001 Yano et al., 2003
Cyclin G2 (Mouse)	Estradiol represses expression of the <i>cyclin G2</i> gene, which encodes a negative regulator of the cell cycle	Morris et al., 2004		Stossi et al., 2006
Ir1 (Interleukin 1 receptor) (Mouse)	The IL-1 system plays a key role in skin physiology and pathology	Morris et al., 2004	Ir1 plays a role in initiation and maintenance of inflammatory reactions of the immune privileged HFeSC niche.	McGirt and Beck, 2006 Palmer et al., 2007 Schiller et al., 2006
LTBP2 (latent transforming growth factor beta binding protein) (HUMAN)	It has thus been suggested that LTBP2 may have multiple functions: as a member of the TGF-beta latent complex, as a structural component of microfibrils, and a role in cell adhesion.	Ohyama et al., 2006	LTBP2 has a regulatory mechanism of elastic fiber assembly in which LTBP-2 regulates targeting of DANCE on microfibrils to form elastic fibers and this could be a prerequisite for the HFeSC niche.	Hirai et al., 2007
Ntf3 (Neurotrophin-3) (Mouse)	Ntf3 and its receptor tyrosine kinase C are essential for nervous system development. Both are expressed in human scalp skin and HF and exert many growth-regulatory functions beyond the nervous system.	Morris et al., 2004	Neurotrophins, including NGF, BDNF, NT-3, and NT-4, and their cognate receptors, tyrosine kinase A-C and p75 neurotrophin receptor exhibit significant, hair cycle-dependent fluctuations on the gene and protein level. Related with a loss of quiescence in the HFeSC-niche by modulating the surrounding neural network.	Adly et al., 2005 Botchkarev et al., 2004
PTGER4 (prostaglandin E receptor) (HUMAN)	Knockout studies in mice suggest that PTGER4 may be involved in the neonatal adaptation of circulatory system, osteoporosis, as well as initiation of skin	Ohyama et al., 2006	Prostanoid pathway in HF may regulate hair regrowth. Local production of prostaglandin in stem cell compartments controls injury-induced HFeSC proliferation.	Colombe et al., 2007a Colombe et al., 2007b Kabashima et al., 2003 Rakoff-Nahoum and Medzhitov, 2007

	immune responses.			
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Legend

HF = hair follicle

HFeSC = hair follicle epithelial stem cells (located predominantly in the bulge region of the outer root sheath [ORS])

TA = transit amplifying cells (=rapidly proliferating hair follicle keratinocytes derived from HFeSC)

CTS = connective tissue sheath of the hair follicle

IRS = inner root sheath of the hair follicle

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Table 3:

OTHER ENDOCRINOLOGICALLY RELEVANT OBSERVATIONS IN THE BULGE REGION

gene/gene product (species)	comments	refs.	potential relevance for endocrinology of HFeSC and/or their progeny	refs.
ADM (Adrenomedullin) (HUMAN)	ADM is a potent vasodilator, but also exerts other functions, such as regulating cell growth and antimicrobial defence.	Müller et al., 2003	Expression of <i>ADM</i> especially in proximity to the bulge region displaying immune privilege (quiescent HFeSC niche conditions)	Müller et al., 2003
Cald1 (Caldesmon-1) (Mouse)	Cald1 was present during the early anagen phases within the bulge and is involved in cyto skeleton remodeling processes which could inhibit HF growth	Wollina et al., 1995	Cald1 modulates the actin cytoskeleton in the bulge region and could be therefore a regulator of the HFeSC-niche.	Greenberg et al., 2007 Hayashi et al., 2002 Wollina et al., 1995
CCND1 (Cyclin D1) (HUMAN) (Mouse)	CCND1 forms a complex with and functions as a regulatory subunit of the cyclin-dependent kinases-4 or -6, whose activity is required for cell cycle G1/S transition.	Xu et al., 2003 Blainpain et al., 2004	Localized to suprabasal cells of telogen bulge and anagen ORS. Mediate proliferation HFeSCto differentiated TA cells in the suprabasal ORS. NF-kappaB activation is essential for induction of <i>cyclin D1</i> and <i>SHH</i> expression and hair placode down growth.	Schmidt-Ullrich et al., 2006 Xu et al., 2003
EPO (Erythropoietin) (HUMAN)	HFs express <i>EPO</i> and transcripts for <i>EPO</i> -receptor and the <i>EPO</i> -stimulatory transcriptional cofactor hypoxia-inducible factor-1alpha.	Bodo et al., 2007	TGFβ blocks <i>EPO</i> signaling downstream of c-myc induction through a SMAD3-dependent mechanism.	Bodo et al., 2007
HSD17B4 (hydroxysteroid (17-beta) dehydrogenase 4; 17 β-estradiol) (HUMAN)	It has long been known that estrogens also profoundly alter HF growth and cycling by binding to locally expressed high-affinity estrogen receptors (ESRs).	Ohnemus et al., 2006	Estrogen arrests murine pelage HFs in telogen and demonstrate that it is a potent inducer of premature catagen development. 17 β-estradiol activates via the ESR <i>JAGGED1</i> - and <i>NOTCH1</i> expression. <i>JAGGED1</i> regulates HF differentiation (HFeSC/TA activation).	Ambler and Watt, 2007 Nishikawa and Osawa, 2007 Ohnemus et al., 2005 Yoo et al., 2007
NGF-R (Nerve Growth Factor Receptor) (HUMAN)	Expression of <i>NGF-R</i> in the bulge region of plucked human HFs	Moll, 1996	NGF-R regulates the immune privilege of the HF which is linked to a the HFeSC niche.	Peters et al, 2006b Peters et al., 2007
Substance P (HUMAN) (Mouse)			Substance P down-regulates immunoreactivity for the growth-promoting NGF receptor, whereas it up-regulated NGF and its apoptosis- and catagen-promoting receptor (p75NTR).	Peters et al., 2007

TMSB4 (Thymosin β 4) (HUMAN) (Mouse)		Philp et al., 2004	TMSB4 upregulates MMP2 and promotes the migration of HFeSCs/TAs to the base of the HF.	Karelina et al., 2000 Paus et al., 1994 Philp et al., 2004
Vipr1 (vasoactive intestinal peptide (Vip) receptor 1) (Mouse)	It was demonstrated that <i>Vipr1</i> is expressed sequentially in the bulge area and the IRS as well as in the sebaceous gland epithelium.	Wollina et al., 1995	<i>Vipr1</i> is expressed in the bulge region during anagen V, but is absent during cycle phases. This suggests a physiological function of <i>Vipr1</i> in terminating the HF growth during anagen I to IV. Additionally <i>Vip</i> could be a regulator for the immunoprivileged bulge (HFeSC-niche).	Luger, 2002 Wollina et al., 1995

Legend

HF = hair follicle

HFeSC = hair follicle epithelial stem cells (located predominantly in the bulge region of the outer root sheath [ORS])

TA = transit amplifying cells (=rapidly proliferating hair follicle keratinocytes derived from HFeSC)

CTS = connective tissue sheath of the hair follicle

IRS = inner root sheath of the hair follicle