



Growth Hormone Operates as a Neuroendocrine Regulator of Human Hair Growth Ex Vivo

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TO THE EDITOR

The human hair follicle (HF) is a mini-organ that undergoes cyclical tissue remodeling, tightly coupled to profound hair cycle-dependent changes in follicular pigmentation (Oh et al., 2016; Paus and Cotsarelis, 1999). To regulate these complex biological processes, among many other signals, the HF also recruits locally and/or systemically generated neurohormones and neuropeptides (Paus et al., 2014). Indeed, a thorough characterization of the neuroendocrine regulation of human follicular biology, and its role in diseases affecting the HF, promises to help identify important novel neuroendocrine targets for therapeutic intervention. Although the role of prolactin in HF biology is increasingly appreciated (Fitzzik et al., 2006; Langan, 2018; Ramot et al., 2010), the role of several other pituitary-derived hormones in human HF biology, notably that of growth hormone (GH), remains to be fully characterized and explored.

Human GH (also called somatotropin) is a 191-amino acid polypeptide secreted by the somatotrophs of the anterior pituitary gland (Bartke and Darcy, 2017). Human skin is an important target for GH, with both GH excess and deficiency being associated with phenotypic changes, namely, hypertrichosis/hirsutism and thin, brittle hair, respectively (Kanaka-Gantenbein et al., 2016). GH receptor (GHR) expression has been reported in dermal fibroblasts, epidermal keratinocytes, sebocytes, melanocytes, outer root sheath (ORS), and matrix keratinocytes of human HFs (Póvoa and Diniz, 2011). Despite these well-known clinical associations and the recognized intrafollicular GHR expression, the function of GH in human HFs remains to be systematically investigated.

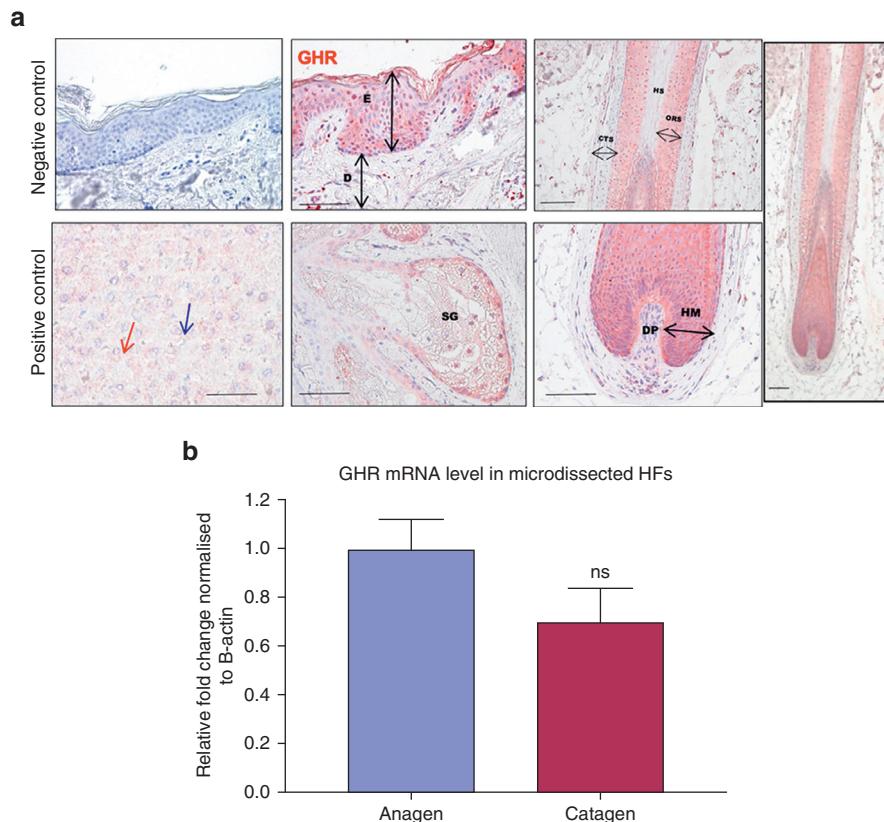


Figure 1. Growth hormone receptor is expressed by human scalp HFs. (a) GHR expression was seen in the epidermis and the pilosebaceous unit in human scalp skin. It was prominently expressed in the HF epithelium, in particular in the ORS, with a nuclear and cytoplasmic expression pattern. Liver tissue was used as a positive control for GHR; cytoplasmic expression of GHR was seen in hepatocytes (red arrow) but not in the liver sinusoids (blue arrow). The staining was performed using avidin-biotin complex–horseradish peroxidase and aminoethyl carbazole as substrate. (b) Quantitative real-time reverse transcriptase–PCR analysis showed a decrease in the expression of GHR mRNA in catagen HFs compared with anagen VI HFs. n = 5–6 HFs per group, mean ± standard error of the mean. Student *t* test, ns = not significant, *P* > 0.05. Data pooled from three female donors. Scale bar = 100 μm. CTS, connective tissue sheath; D, dermis; DP, dermal papilla; E, epidermis; GHR, growth hormone receptor; HF, hair follicle; HM, hair matrix; HS, hair shaft; ORS, outer root sheath; SG, sebaceous gland.

Therefore, after having confirmed and further characterized GHR expression in female human HFs (see below), we hypothesized in this pilot study that pituitary GH may be an undiscovered hair growth regulator in human scalp skin via GHR-mediated signaling. To probe this

hypothesis, we asked whether microdissected, organ-cultured human scalp HFs (Langan et al., 2015) directly respond to GH stimulation in the absence of systemic endocrine stimuli by assessing changes in human HF cycling, growth, and pigmentation and the expression of the key hair growth regulators IGF-1 and transforming growth factor-β2 (Chéret et al., 2018; Kloepfer et al., 2010). To exclude potential sex- or location-dependent GH effects, only occipital HFs from female patients undergoing

Abbreviations: GH, growth hormone; GHR, growth hormone receptor; HF, hair follicle; ORS, outer root sheath; TGF, transforming growth factor

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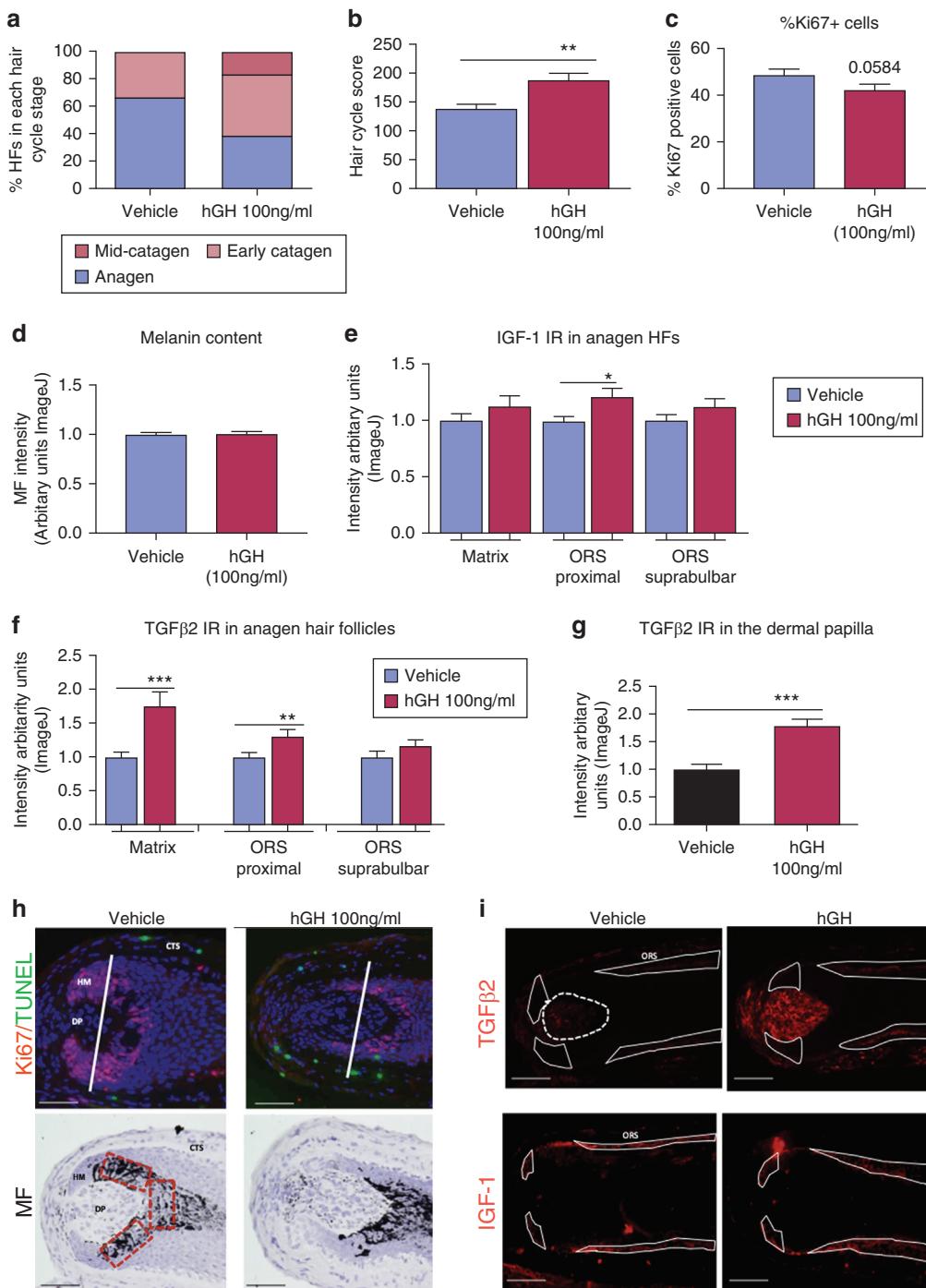


Figure 2. Recombinant growth hormone induces premature catagen in organ-cultured microdissected HFs. (a) Hair cycle staging based on established morphological criteria and hair cycle score (b) indicates that premature catagen is induced in female occipital HFs treated with recombinant GH. (c) Quantitative immunohistomorphometry of Ki67 and (d) Masson Fontana. GH also increases protein immunoreactivity of IGF-1 (e, i) and TGF β 2 (f, g, i) in the hair matrix and the outer root sheath (ORS) in the proximal and suprabulbar region and dermal papilla (TGF β 2 only). (h) Representative images of Ki67/TUNEL (white lines demarcate the Auber's line) and Masson-Fontana staining (red boxes indicate areas of analysis) and (i) TGF β 2 and IGF-1 in vehicle and treated groups. N = 39-44 HFs analyzed, mean \pm standard error of mean, Mann-Whitney test, **P < 0.02, ***P < 0.01, data pooled from three female donors. CTS, connective tissue sheath; DP, dermal papilla; HM, hair matrix; IGF, insulin growth factor 1; MF, Masson Fontana; ORS, outer root sheath; TGF, transforming growth factor.

routine facelift surgery were used (see Supplementary Table 1 online). Written informed patient consent and an institutional ethics license (University of Münster 2014-041-b-N and 2015-602-f-S) were obtained.

Immunohistochemistry confirmed GHR protein expression in healthy female human scalp skin, which was particularly prominent in the HF epithelium, in line with previous studies (Ginarte et al., 2000; Lobie

et al., 1990), with both cytoplasmic and nuclear GHR protein expression seen in ORS keratinocytes (Figure 1a). Quantitative reverse transcriptase-PCR of mRNA extracts from HFs not only confirmed substantial intrafollicular

GHR transcription in human anagen HFs but also showed a decline in GHR mRNA expression in female catagen versus anagen VI HF (Figure 1b, and see Supplementary Figure S1 online), suggesting that human anagen VI HF are more receptive to GH stimulation than regressing catagen HF.

Next, we explored whether these intrafollicular GHRs are functional and whether their stimulation by exogenous GH alters human hair growth and/or pigmentation ex vivo. Female occipital scalp HF were stimulated over 6 days with recombinant human GH (Sigma-Aldrich, St. Louis, MO) at a concentration of 100 ng/ml (the level seen in patients suffering from acromegaly) (Espinosa de los Monteros et al., 2018), and quantitative hair cycle histomorphometry was performed as described previously (Hawkshaw et al., 2018; Kloepper et al., 2010). As shown in Figure 2a, b, and h, 100 ng/ml recombinant growth hormone induced premature catagen development. A significant acceleration of catagen development in recombinant growth hormone–treated HF ex vivo was independently confirmed by calculating the hair cycle score ($P < 0.01$) (Samuelov et al., 2012) (Figure 2b), and a reduced percentage of proliferating hair matrix keratinocytes below Auber's line was determined ($P < 0.0538$) (Figure 2c) by quantitative Ki-67 immunohistomorphometry in GH-stimulated HF when all HF (i.e., anagen and catagen) were assessed, as well as when only anagen VI HF were assessed (see Supplementary Figure S2 online). No significant difference was found in the histomorphometrically quantified melanin content of test and control HF when only anagen VI HF were compared with each other (Figure 2d), suggesting that GH does not inhibit follicular melanogenesis in a hair cycle-dependent manner. Thus, GH induces complex HF regression changes that promote the catagen transformation of this mini-organ, at least ex vivo, but does not appear to exert hair cycle-independent effects on human HF melanocytes in situ, in striking contrast to thyrotropin-releasing hormone (Gáspár et al., 2011), another recognized key neuroendocrine regulator of human hair biology (Gáspár et al., 2010; Paus et al., 2014; Ramot et al., 2013; Vidali et al., 2014).

Finally, we showed that HF stimulation with GH ex vivo significantly up-regulated the intrafollicular protein expression of IGF-1 in the ORS (Figure 2e and i), that is, the key peripheral tissue mediator of GH bioactivity (Cooke et al., 2016) and a known key growth factor that promotes anagen maintenance (Chéret et al., 2018; Schneider et al., 2009). However, this was coupled with a significant increase in protein expression of the key catagen-promoting growth factor, TGF- β 2 (Ramot et al., 2013; Vidali et al., 2014), not only in the HF epithelium but, most prominently, also in the dermal papilla (Figure 2g and i), where TGF- β 2 protein is notably expressed during catagen induction (Fischer et al., 2014; Hibino et al., 2004). This suggests that GH-induced premature catagen development in female human HF likely results from a functionally dominant increase in TGF- β 2 expression, namely, in the "mesenchymal command center" of the HF (i.e., the follicular dermal papilla), which cannot be overridden by the up-regulation of IGF-1 upon GH stimulation.

In summary, our pilot study shows not only that GHR-expressing human scalp HF in anagen VI are highly receptive to GH stimulation but also that GH operates as a neuroendocrine inhibitor of female human HF ex vivo, despite the well-documented overall growth-promoting properties of GH in many other tissues (Kaiser and Ho, 2016), possibly via up-regulating intrafollicular TGF- β 2 production. We also show that human HF organ culture provides an excellent research tool for interrogating the as yet insufficiently explored peripheral effects of GH/GHR-dependent signaling in adult human tissues (Ocaranza et al., 2016; Wang et al., 2017). Future studies now will have to clarify whether human HF also express GH itself, just as they express prolactin, thyrotropin-releasing hormone, corticotropin-releasing hormone, proopiomelanocortin-derived hormones (Paus et al., 2014), and/or additional players of the GH signaling axis, namely GH-releasing hormone. Moreover, future studies should clarify whether there are sex- and/or location-dependent differences in the response of human HF to GH stimulation.

Given the clinical skin manifestations associated with abnormalities in GH serum levels and the currently available treatments (see Supplementary Materials), further elucidating the role of GH/GHR-dependent signaling could pave the way for the development of future therapeutics.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

MA performed the experiments and analyzed data with substantial help from DAB. MA, EAL, and RP wrote the manuscript. MA and RP conceived, designed, supervised the study, and interpreted the data. All authors contributed to data analysis and writing of the manuscript.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <https://doi.org/10.1016/j.jid.2018.12.022>.

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Desmoglein 1 Deficiency Causes Lethal Skin Blistering

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TO THE EDITOR

Pemphigus is an autoimmune bullous disorder affecting both mucous membranes and the epidermis (Kasperkiewicz et al., 2017). It is widely accepted that pemphigus is caused by autoantibodies primarily targeting desmosomal cadherins desmoglein (Dsg) 1 and 3, which are crucial for intercellular cohesion of keratinocytes. The relevance of autoantibodies against other antigens detectable in pemphigus patients is

unclear yet (Spindler et al., 2018). There are two main forms of pemphigus, which differ with respect to their clinical phenotype: in pemphigus vulgaris, skin blistering affects the deep epidermis when, in addition to antibodies against Dsg3, autoantibodies targeting Dsg1 are detectable (Spindler and Waschke, 2018). In pemphigus foliaceus, epidermal blistering is also associated with autoantibodies against Dsg1, but is confined to the superficial epidermis.

By passive transfer, it has been shown in mouse models that autoantibodies against Dsg1 and Dsg3 induce pemphigus phenotypes similar to humans (Mahoney et al., 1999). More recently, it was proposed that different signaling mechanisms in pemphigus correlate with different autoantibody titers against Dsg1 and Dsg3 and define, at least in part, the different clinical phenotypes in pemphigus (Walter et al., 2017). However, antibodies against Dsg1 in contrast to those against Dsg3 where not directly interfering with Dsg binding (Heupel et al., 2008; Waschke et al., 2005). Deletion of Dsg3 without loss of Dsg1 function causes a mild pemphigus vulgaris-like phenotype in

Abbreviation: Dsg, desmoglein

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