Hair follicle stem cell differentiation is inhibited through cross-talk between Wnt/β-catenin and androgen signalling in dermal papilla cells from patients with androgenetic alopecia

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Summary

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Background Hair follicle (HF) regeneration begins when signals from the mesenchyme-derived dermal papilla cells (DPC) reach multipotent epidermal stem cells in the bulge region. Wnt/β-catenin signalling is known to affect mammalian hair growth positively. In androgenetic alopecia (AGA), androgens cause HF miniaturization through a mechanism that remains unclear. Circulating androgens act on DPC and alter paracrine factors that influence hair epithelial cells.

Objectives To elucidate the role of androgens in dermal papilla-induced differentiation of HF stem cells.

Methods HF stem cell differentiation was evaluated in a coculture model with DPC or culturing with media conditioned by DPC after activation of androgen and Wnt/β-catenin signalling pathways. To study the molecular cross-talk between the androgen and Wnt signalling pathway in DPC, we analysed the expression and activation of downstream Wnt signalling molecules in the presence of androgens.

Results In a coculture model with human DPC from patients with AGA and HF stem cells, we observed that androgens abrogate hair differentiation evaluated by hair-specific keratin 6 expression. Wnt signalling activation restored the ability of androgen-treated DPC to induce differentiation. Androgen treatment revealed a significant decrease in the cytoplasmic/total β-catenin protein ratio and upregulation of the activity of glycogen synthase kinase-3β in DPC, indicative of canonical Wnt pathway inhibition.

Conclusions These results suggest that androgens deregulate DPC-secreted factors involved in normal HF stem cell differentiation via the inhibition of the canonical Wnt signalling pathway.

The hair follicle (HF) is a regenerating system, which physiologically undergoes cycles of growth (anagen), regression (catagen) and rest (telogen) numerous times in adult life. The multipotent epithelial stem cells[1–3] of the bulge give rise to a range of differentiated cell types in skin.[4] During adult hair follicle cycling, the signalling between epithelial keratinocytes and underlying specialized mesenchymal dermal papilla cells (DPC) induces stem cell proliferation and initiates the cascade of cell differentiation into the HF cell lineages. Many paracrine factors involved in this cross-talk at different hair cycle stages and some signalling pathways have been implicated.[1,5,6] Hair-specific keratin 6 (K6hf) is a type II keratin, exclusively expressed in the companion layer of the HF[7] and its expression is specific for DPC-induced HF differentiation.[6]

Androgenetic alopecia (AGA)[8] is a common, progressive disorder in which large, terminal scalp hairs are gradually replaced by smaller hairs in precise patterns. Although these changes are driven by androgens, most of the molecular mechanisms are unknown, limiting available treatments. The dermal papilla is the site through which androgens act on follicle cells by altering the regulatory paracrine factors involved in the differentiation and proliferation of HF stem cells. Wnt proteins are known to affect mammalian hair growth positively.[9]

Canonical Wnt signalling causes accumulation of cytosolic β-catenin which then translocates to the nucleus, where it acts as a coactivator of T cell-factor proteins to regulate gene expression. β-Catenin can be phosphorylated at its N-terminal

domain by glycogen synthase kinase-3β (GSK-3β), which leads to its degradation mediated by the stem cell factor/ubiquitin/proteasome pathway.[10] GSK-3β activity is suppressed when this enzyme is phosphorylated by protein kinase B/Akt. Maintenance of Wnt signalling through the β-catenin pathway is required for the hair-inducing activity of dermal papillae and to keep DPC with anagen-phase characteristics.[11]

The present study shows that the action of androgens abrogates the ability of DPC to induce HF stem cell differentiation and inhibits the canonical Wnt signalling in DPC. We provide evidence that the androgen inhibition of the Wnt canonical pathway involves GSK-3β inhibitory activity.

Materials and methods

Cell cultures

Full-depth skin samples were obtained, with written consent, from transitional areas of hair loss on the occipital human scalp of individuals undergoing corrective surgery for the treatment of AGA. The studies were approved by the local institutional review board.

DPC primary cultures were established as previously described.[12] In brief, anagen follicles were dissected and the isolated hair bulbs were incubated in collagenase I (Sigma-Aldrich, Munich, Germany) for 2 h at 37 °C. The papilla explants were grown in culture medium, Dulbecco's modified Eagle's medium (DMEM) (Gibco-Invitrogen, Carlsbad, CA, U.S.A.) containing penicillin (100 U mL^{-1}), streptomycin (100 mg mL^{-1}) and 10% fetal bovine serum (FBS) (GIBCO-Invitrogen).

In order to obtain HF stem cell-enriched primary cultures (HFSC), skin samples were incubated in 1 mg mL^{-1} collagenase/dispase (Sigma-Aldrich) overnight at 4 °C, and telogen follicles were pulled-out and incubated in 0·05% trypsin–ethylenediamine tetra-acetic acid (EDTA) 0·23 mmol mL^{-1} (Gibco-Invitrogen). The isolated cells were resuspended in cFAD medium.[6] The culture was enriched in stem cells by attachment to collagen IV[13] (Sigma-Aldrich) coated plates for 30 min at 37 °C and then cultured with mitomycin C-inactivated 3T3-Swiss cells as a feeder layer (FL), using cFAD medium. After 3 days, the cultures were supplemented with epidermal growth factor (10 ng mL^{-1}). The immortalized human bulge stem cell line Tel-E6E7[14] was kindly provided by Dr Stephen Lyle (University of Massachusetts Medical School, MA, U.S.A.) and cultured under the same conditions.

Transfection of dermal papilla cells with androgen receptor (AR) and AR activity evaluation

DPC cells were electroporated with pSVAR [androgen receptor (AR) gene recombinant plasmid] and pTA-TALuc (luciferase gene, under control of androgen-regulating element, recombinant plasmid) using the BTX ECM 830 electroporator (BTX Harvard Apparatus, Holliston, MA, U.S.A.).

In order to evaluate AR activity, transfected DPC were cultured for 24 h in DPC medium and then cultured for 3 days in DMEM supplemented with 5% charcoalized FBS and with dihydrotestosterone (DHT) or without it (control). The cells were harvested, and luciferase activity was tested using the Luciferase Assay System (Promega, Madison, WI, U.S.A.) using the Berthold FB14 luminometer (Berthold Technologies, Bad Wildbad, Germany).

Cocultures of hair follicle stem cell-enriched primary culture or Tel-E6E7 cell line with dermal papilla cells

HFSC or Tel-E6E7 cells were plated in a 10-cm^2 well and stabilized for 24 h by cocultivation with FL located in 1·0-μm pore-size membrane inserts (Becton Dickinson Labware & Co, Franklin Lakes, NJ, U.S.A.). The FL inserts were removed and replaced with pSVAR-transfected DPC inserts. Cocultures were maintained in cFAD medium with or without 10^{-8} mol L^{-1} DHT. Additional control was performed by coculturing HFSC or Tel-E6E7 cells with FL inserts in cFAD medium without DHT.

Hair follicle stem cell treatment with dermal papilla cell-conditioned media

pSVAR-transfected DPC were cultured for 3 days in DPC medium with 5% charcoalized FBS (control), supplemented with 10^{-8} mol L^{-1} DHT, with 20 mmol L^{-1} LiCl or with 10^{-8} mol L^{-1} DHT and 20 mmol L^{-1} LiCl. The conditioned media were dialysed against water. HFSC or Tel-E6E7 cell line were cultured with cFAD or 1 : 1 DPC-conditioned medium:cFAD medium for 5 days. Cells were lysed for total protein extraction.

Western blot assays and immunoprecipitation

Whole cell extracts were obtained by RIPA lysis buffer [20 mmol L^{-1} Tris, pH 7·5, 1 mmo L^{-1} EDTA, 0·15 mol L^{-1} NaC1, 10 mmol L^{-1} KC1, 1% NP-40, 0·1% deoxycholate, 0·1% sodium dodecyl sulphate (SDS)] with protease inhibitors. Cytoplasmic extracts of DPC were obtained with a lysis buffer containing 10 mmol L^{-1} Tris, pH 7·6, 10 mmol L^{-1} NaCl, 3 mmol L^{-1} MgCl$_2$ and 0·5% Nonidet P-40, supplemented with protease inhibitors. After SDS–polyacrylamide gel electrophoresis and electroblotting, membranes were incubated with the following primary antibodies: K6hf-specific antibody (Progen Biotechnik GmbH, Heidelberg, Germany) for the HF stem cell differentiation assays; and β-catenin (Becton Dickinson Labware & Co), GSK-3β (Cell Signaling Technology Inc., Danvers, MA, U.S.A.) and Ser 9-phosphorylated GSK-3β (Cell Signaling Technology Inc.) specific antibodies for the DPC assays. Anti-β actin antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, U.S.A.) was used as a loading control. Primary antibody application was followed by incubation with horseradish peroxidase-conjugated secondary antibodies (GE Healthcare, Amersham, U.K.). Blots were

developed using an enhanced chemiluminescence detection system (Biolumina; Productos Bio-Logicos, Buenos Aires, Argentina).

For immunoprecipitation analysis, cytoplasmic cell extracts from DPC treated for 4 h with 10^{-8} mol L^{-1} DHT, or not, were subjected to immunoprecipitation with the AR441 antibody (Santa Cruz Biotechnology Inc.). After overnight incubation at 4 °C, protein A or G agarose beads were added and left for an additional 3 h and the immunocomplexes were subjected to immunoblotting analysis using GSK-3β antibody as described above.

Total RNA isolation, cDNA synthesis and real-time polymerase chain reaction

Total RNAs were isolated and cDNAs were obtained by M-MLV reverse transcriptase (Promega). Real-time polymerase chain reaction (PCR) analyses were carried out in the Plus-One Real time PCR machine (Applied Biosystems-Invitrogen, Carlsbad, CA, U.S.A.) using SYBR Green Master Mix (Applied Biosystems-Invitrogen) and the following set of specific primers: K6hf (forward: 5′-CTAGAGCCCCTCTTTGATTCCT-3′, reverse: 5′-GCAGCATCTACGTCCTTTTTCA-3′); β-catenin (forward: 5′-AATACCATTCCATTGTTTGTGCAG-3′, reverse: 5′-AGCTCAACTGAAAGCCGTTT-3′); GSK-3β (forward: 5′-AAGCCGGTGCAGCAGCCTTC-3′, reverse: 5′-ACCCTGCCCAGGAGTTGCCA-3′). A hypoxanthine phosphoribosyltransferase (HPRT)-specific set of primers (forward: 5′-CTCAACTTTAACTGGAAAGAATGTC-3′, reverse: 5′-TCCTTTTTCACCAGCAAGCT-3′) was used as the internal mRNA expression control.
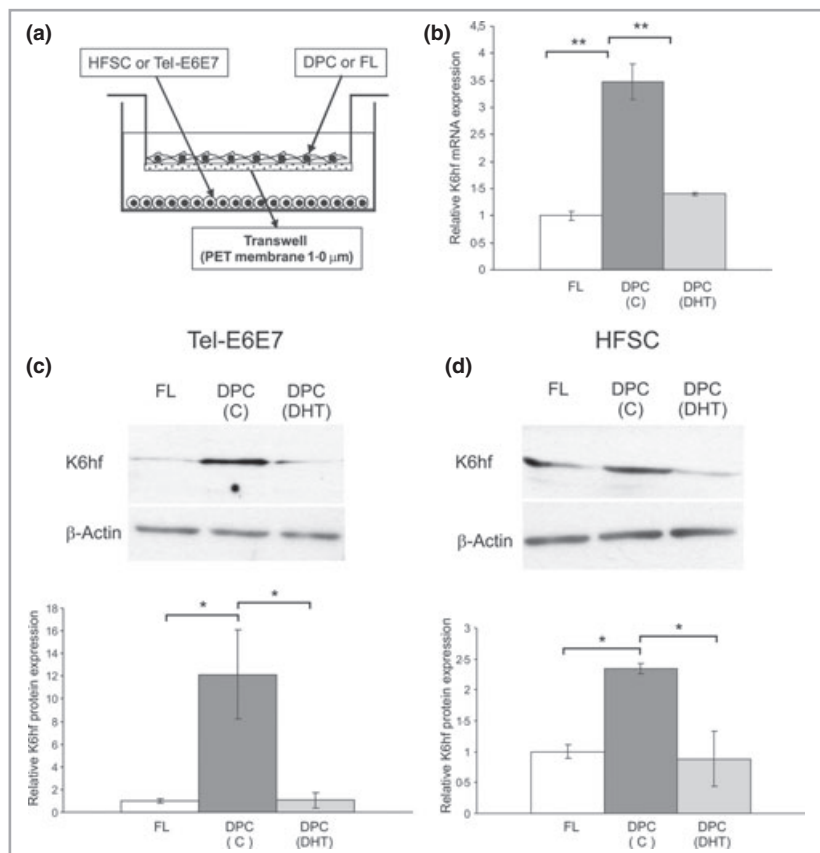
Results

Androgen treatment of dermal papilla cells abrogates hair differentiation

To study the effect of androgens on DPC-induced HF stem cell differentiation during epithelial–mesenchymal interactions, we used a coculture model[6] (Fig. 1a) in which DPC and HF stem cells are cultured in different compartments of a Transwell® system (Fisher Scientific, Whitby, ON, U.S.A.).

It is accepted that the AR expression level is a crucial factor for DPC androgen sensitivity. To enhance androgen sensitivity in DPC, we transfected the AR-expression vector pSVAR and conducted all experiments using DPC transiently transfected with pSVAR.

To see the effect of androgens on DPC-induced HF stem cell differentiation, we analysed the K6hf expression level in HFSC after coculture with DPC in the absence or presence of DHT. An approximate 3·5-fold increase in K6hf mRNA was detected in the immortalized stem cell line Tel-E6E7[14] after 3 days of coculture with DPC (P < 0·01). This induction was not observed when DHT was present in the cocultures (Fig. 1b). Similar results were obtained when K6hf protein expression

Fig 1. Androgen effect on hair stem cell differentiation. (a) Coculture model of hair follicle stem cell-enriched primary cultures (HFSC) or Tel-E6E7 cell line with feeder layer (FL), dermal papilla cells (DPC) alone (DPC-C) or DPC with 10^{-8} mol L^{-1} dihydrotestosterone (DHT) (DPC-DHT). (b) Real-time polymerase chain reaction analysis of hair-specific keratin 6 (K6hf) mRNA expression in Tel-E6E7 cell line from each treatment. Values are means of triplicates ± SD using hypoxanthine phosphoribosyltransferase mRNA as the internal control. (c, d) Representative Western blot of K6hf and β-actin expression in Tel-E6E7 cell line (c) and HFSC (d) from each treatment. Graphs show K6hf protein expression normalized to β-actin expression. Values are means of triplicates ± SD. In all cases, results are expressed relative to FL. Statistical analysis was performed using one-way ANOVA test. *P < 0·05; **P < 0·01.

level was analysed in whole cell lysates from Tel-E6E7 (Fig. 1c) or HFSC (Fig. 1d) cocultured with DPC for 7 days in the absence or in the presence of DHT. These results indicate that the presence of androgens abrogates the capability of DPC to induce HF stem cell differentiation, probably by deregulation of secreted factors.

Androgens are able to inhibit canonical Wnt signalling in dermal papilla cells

As the Wnt/β-catenin pathway positively affects hair growth, we investigated if androgens were able to modulate this pathway. For this purpose, we first analysed the effect of androgens on β-catenin expression in DPC. The analysis of β-catenin by Western blot revealed that the cytoplasmic/total β-catenin ratio was about five times lower in androgen-treated DPC ($P < 0.01$) (Fig. 2). This result suggests that androgens induce inhibition of the canonical Wnt signalling pathway.

LiCl reverses the effect of dihydrotestosterone on the ability of dermal papilla cells to induce hair follicle stem cell differentiation

In the coculture system, we observed that DHT abrogated DPC-induced HF stem cell differentiation. To confirm that this effect is mediated by DPC, we tested the differentiation ability of conditioned media, obtained from DPC grown for 3 days with or without DHT, and into which they would have secreted soluble factors. These media were collected, dialysed and used as growth media for HF stem cells. Medium conditioned by DPC was able to differentiate HF stem cells. A significant increase in K6hf protein level was detected in Tel-E6E7 and HFSC grown in DPC-conditioned medium compared with the control (cFAD medium). This induction was not observed when conditioned medium from DHT-treated DPC was used. This result confirms that DHT acts on DPC and alters paracrine factors involved in the differentiation of HF

stem cells. These factors are not dialysable (Fig. 3, lanes 2 and 3) and are therefore larger than 3·5 kDa.

As we observed that DHT inhibited the canonical Wnt signalling, we further investigated whether the DHT effect on hair differentiation is mediated by a canonical Wnt signalling blockage. For this purpose we collected the conditioned media from DPC cultured in the presence of both LiCl and DHT. Lithium inhibits the activity of GSK-3β, leading to β-catenin stabilization and an increase of the Wnt canonical signalling pathway.[15,16] We observed that conditioned media from DPC treated with LiCl and DHT induced the expression of K6hf both in Tel-E6E7 ($P < 0.001$) (Fig. 3a) and in HFSC ($P < 0.001$) (Fig. 3b), indicating that the activation of Wnt/β-catenin by LiCl in DHT-treated DPC restored its hair differentiation capability.

It is known that HF stem cells differentiate to hair through the Wnt/β-catenin pathway.[6] To avoid a direct effect of LiCl on HF stem cell differentiation, we dialysed all the DPC-conditioned media prior to their use as growth media.

These results confirm that DHT prevents DPC from secreting soluble factors necessary for HF stem cell differentiation. Also, the activation of Wnt signalling in DPC is required for HF stem cell differentiation. Altogether these results indicate that at least some of the paracrine factors involved in DPC-induced HF stem cell differentiation are target genes of the Wnt/β-catenin signalling pathway in DPC.

Androgens activate glycogen synthase kinase-3β in scalp dermal papilla cells

GSK-3β, the protein kinase that targets β-catenin for proteolytic degradation, is inactivated by phosphorylation at Ser-9.[17] Protein complex formation and intracellular localization are other ways to regulate the activity of this kinase.

In order to determine whether the activation of AR signalling involves a complex formation between GSK-3β and AR, DPC extracts were immunoprecipitated with an anti-AR anti-
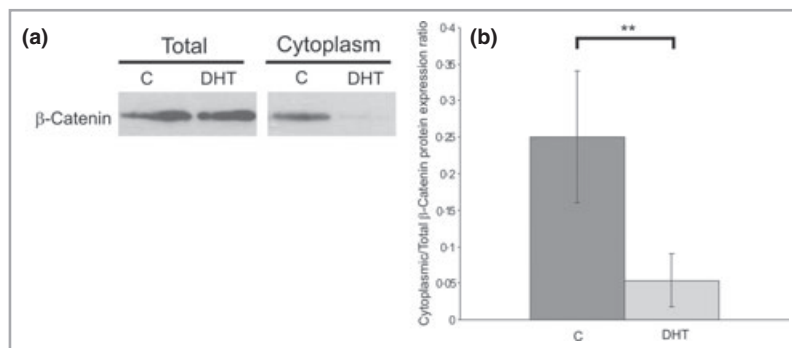

Fig 2. Androgen effect on dermal papilla cell (DPC) β-catenin protein expression. DPC were treated with 10^{-8} mol L^{-1} dihydrotestosterone (DHT), or not treated [control (C)], for 72 h, and total and cytoplasmic protein extracts were obtained from each treatment. (a) Representative Western blot for β-catenin expression in cytoplasmic and total protein extracts. The load of cytoplasmic extract on the blot is from the same amount of cells as the load of total cell extract. (b) Cytoplasmic/total β-catenin protein expression ratio for each treatment. Values are means of triplicates ± SD. Statistical analysis was performed using the unpaired t-test. **$P < 0.01$.
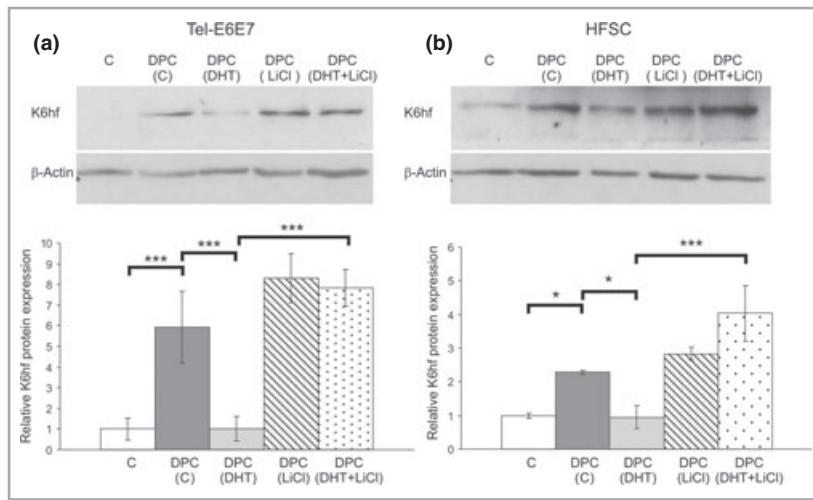
Fig 3. Effect of LiCl on the blocking of hair differentiation by dihydrotestosterone (DHT). (a) Tel-E6E7 cells were cultured for 5 days with cFAD [control (C)] or with dialysed conditioned medium obtained from dermal papilla cells (DPC) (DPC-C), DPC with 10^{-8} mol L^{-1} DHT (DPC DHT), with 20 mmol L^{-1} LiCl (DPC LiCl) or with 10^{-8} mol L^{-1} DHT + 20 mmol L^{-1} LiCl (DPC DHT+LiCl). Representative Western blot of hair-specific keratin 6 (K6hf) expression in Tel-E6E7 cells from each treatment is shown. The graph represents the K6hf protein expression normalized to β-actin. Values are means of triplicates ± SD and results are expressed relative to C. (b) The same experiments as in (a) were performed in hair follicle stem cell-enriched primary cultures (HFSC). Statistical analysis was performed using one-way ANOVA test. *P < 0·05; ***P < 0·001.
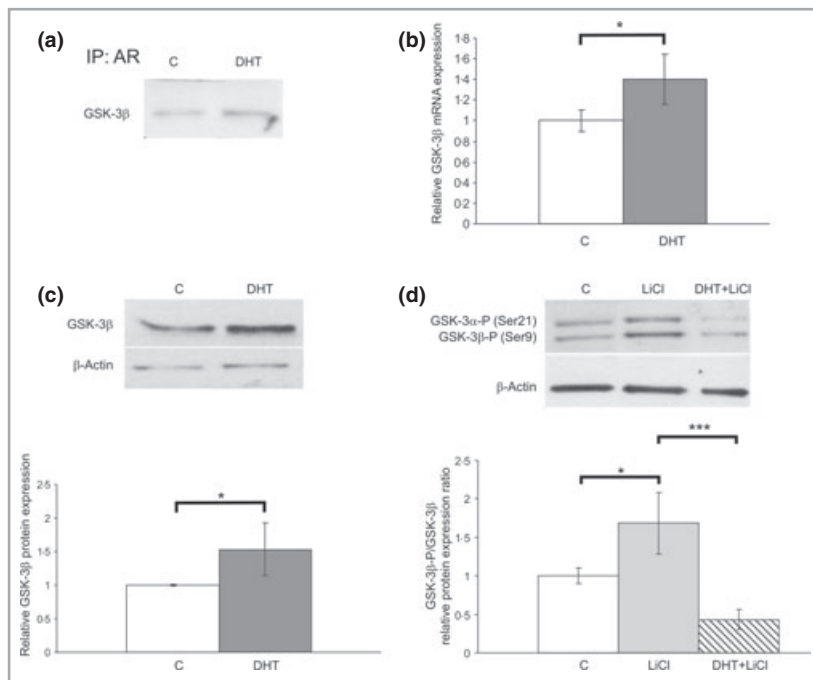
Fig 4. Glycogen synthase kinase (GSK)-3β expression, phosphorylation and androgen receptor (AR) co-immunoprecipitation. (a) AR immunoprecipitated from lysates of dermal papilla cells (DPC) treated for 4 h with 10^{-8} mol L^{-1} dihydrotestosterone (DHT) or not [control (C)] and immunoblotted for GSK-3β. (b, c) GSK-3β expression in DPC after 3 days with 10^{-8} mol L^{-1} DHT: (b) mRNA level by real-time polymerase chain reaction analysis using hypoxanthine phosphoribosyltransferase mRNA as the internal control; (c) immunoblot of GSK-3β normalized to β-actin. (d) Phosphorylation analysis by immunoblot of GSK-3βP in DPC treated with 20 mmol L^{-1} LiCl (LiCl), 10^{-8} mol L^{-1} DHT + 20 mmol L^{-1} LiCl (DHT + LiCl) or not treated (C). Graph shows GSK-3βP/GSK-3β expression ratio and normalized to β-actin. In all cases, values are means of triplicates ± SD. Results are expressed relative to C. For statistical analysis the unpaired t-test (b, c) and one-way ANOVA (d) were used. *P < 0·05; ***P < 0·001.

body in the presence or absence of DHT. As shown in Figure 4a, GSK-3β was detected in anti-AR immune precipitates and the presence of DHT upregulated the amount of

GSK-3β that co-immunoprecipitates with AR, suggesting that the activation of AR signalling promotes the physical interaction between these proteins.

To investigate the expression and the phosphorylation status of GSK-3β in DPC after androgen treatment, total lysates from DPC cultured for 3 days in the presence of 10^{-8} mol L^{-1} DHT were tested for GSK-3β expression. A significant upregulation of GSK-3β expression ($P < 0.05$) was found both at mRNA (1·4 times) (Fig. 4b) and protein levels (1·5 times) (Fig. 4c).

In an attempt to examine the effect of DHT on GSK-3β phosphorylation at Ser-9, total lysates of DPC cultured in the presence of DHT were analysed by Western blotting using an antiphospho-GSK-3 antibody which detects endogenous GSK-3β, only when phosphorylated at Ser9. As mentioned above, LiCl inhibits GSK-3β by Ser9 phosphorylation (GSK-3βP), mimicking a functional activation of the canonical Wnt signalling pathway. We observed an increase in GSK-3βP level in DPC cultures when treated with LiCl. This effect was blocked when these cells were cultured in the presence of both LiCl and DHT ($P < 0.001$) (Fig. 4d). These results suggest that androgens activate GSK-3β by upregulation of its expression and also by inhibition of its phosphorylation at Ser-9.

Discussion

In AGA, androgens are believed to play an important role in the gradual transformation of scalp hair follicles to smaller vellus hair through the mesenchyme-derived dermal papilla.

DPC from HF on a balding scalp contain higher levels of ARs than those from a nonbalding scalp,[18] and this has been proposed[19] as a reason for the regional specificity of the androgen effect and a crucial factor for DPC androgen sensitivity.

It is known that DPC have regulatory effects on HF epithelial cells through the secretion of soluble factors during the hair growth cycle.[20,21] We hypothesized that the miniaturization of the HF seen in AGA may result from deficient HF stem cell differentiation. To our knowledge, androgen action in DPC-induced differentiation of HF stem cells in AGA has not been previously addressed.

The coculture of HF stem cells with DPC recapitulates the interactions occurring at the onset of HF regeneration, which can be assayed by upregulation of K6hf.[22] We observed in our study that androgen action impairs DPC-induced HF stem cell differentiation. A recent study showed that while stem cells were maintained in bald scalp samples, progenitor cells were markedly diminished, supporting the notion that a defect in conversion of HF stem cells to progenitor cells plays a role in the pathogenesis of AGA.[23]

Activation of the Wnt/β-catenin signalling pathway is important for the initiation and maintenance of hair morphogenesis[24] and is critical for the maintenance of DPC inductive properties required for HF regeneration and growth of the hair shaft.[11] In this study, we found that androgen treatment inhibits the canonical Wnt signalling in DPC. Our results are in agreement with Kitagawa *et al.*[25] who observed that in DPC from patients with AGA, the Wnt/β-catenin signalling pathway is negatively influenced by ligand-activated AR.

Activation of the canonical Wnt signalling pathway can be achieved with lithium,[15,16] which has been shown to inhibit GSK-3β. To mimic the effects of Wnt signalling activation, we used lithium together with DHT in our DPC cultures and we found that the ability of the DPC-conditioned media to induce HF stem cell differentiation was restored. Our results indicate that androgens inhibit the secretion by DPC of soluble factors necessary for HF stem cell differentiation and at least some of these paracrine factors involved in DPC-induced HF stem cell differentiation are encoded by target genes of the Wnt/β-catenin signalling pathway in DPC.

A functional cross-talk between the AR and Wnt signalling pathways has been described in target tissues.[26] We found that androgens produce an increase in GSK-3β expression as well as a decrease in its phosphorylation level. These results would involve this kinase in the inhibition of the canonical Wnt signalling pathway, induced by androgen treatment. GSK-3β activity is determined by a combination of several factors, and the phosphorylation of an N-terminal serine residue (Ser-9) is the most extensively studied mechanism that leads to a decrease in its activity. Although, many kinases are involved in phosphorylation of GSK-3β at Ser-9,[27] only PP2A[28] and calcineurin[29] have been reported to dephosphorylate GSK-3β at this residue. Further studies will be necessary to elucidate the mechanism involved in the GSK-3β dephosphorylation mediated by androgens that we have observed in androgen-sensitive DPC. The reduction in GSK-3β phosphorylation levels may be an indirect effect of androgen action due to the inhibition of some of the kinases mentioned above. In accordance with our findings, it has been reported previously that treatment of human DPC with a GSK-3 inhibitor resulted in an increased activity and expression of indicators of hair inductivity.[30]

In addition to its kinase activity, GSK-3β shows specific docking properties that have been extensively investigated in the canonical Wnt signalling pathway.[31] We examined the existence of a GSK-3β/AR complex and found that in the presence of androgens, the physical interaction of AR and GSK-3β is increased.

Even if the putative function of GSK-3β in AR transcriptional activity in DPC was not analysed in this case, our observation may support a model in which the association of GSK-3β with AR leads to elevated AR transcriptional activity as has been reported in prostate cancer cells.[32]

Altogether our findings demonstrate that androgens regulate secreted factors involved in normal HF stem cell differentiation via the inhibition of the canonical Wnt signalling system in androgen-sensitive DPC. We provide evidence that androgen activation of GSK-3β appears to be responsible for the inhibition of Wnt/β-catenin signalling. Target genes of Wnt/β-catenin signalling appear to code for secreted proteins necessary for human hair differentiation.

Shin *et al.*[33] presented for the first time genome-wide transcriptional responses of DPC to the Wnt/β-catenin pathway. Nevertheless, the identity of DPC-secreted proteins regulated

by androgens or Wnt ligands involved in hair stem cell differentiation have not yet been reported.

The identification of the DPC-secreted factors responsible for the differentiation of HF stem cells would contribute to elucidating the regulation of epithelial–mesenchymal interactions that occur at the onset of hair regeneration and will lead to further understanding of the mechanisms involved in AGA development.

What's already known about this topic?

- Wnt/β-catenin signalling maintains the hair-inducing activity of the dermal papilla. In androgenetic alopecia, androgens act on dermal papillae by altering regulatory soluble factors.

What does this study add?

- We report that the action of androgens abrogates the ability of the dermal papilla to induce hair follicle stem cell differentiation. Activation of Wnt signalling restored its differentiating ability.
- We provide evidence that androgens upregulate the activity of glycogen synthase kinase-3β, a Wnt/β-catenin signalling inhibitor.

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