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Short Communication Open Access

Effects of Trichosol™ on Increasing the Anagen Phase of the Capillary Cycle of Volunteers

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Abstract

Trichotech™ technology had its mechanism of action published in 2017. Trichosol™ is a vehicle presentation (with Trichotech™ technology) with other hair growth formulations such as Minoxidil, an important drug in the treatment of hair loss. hair and other hair disorders. Through dermoscopy, the percentage of anagen phase was quantified between groups of volunteers who used Minoxidil (3%) associated or not with the Trichosol™ vehicle, which contains Trichotech™ technology. This study aimed to investigate the potential of Trichosol™, associated with Minixodil (3%), in the promotion of hair growth through the analysis of the increase of the capillary anagen phase (about 20%) compared to the telogen phase of the volunteers. The results also indicated that after treatment for 90 days with the combination of Minoxidil (3%) and Trichosol™, both sexes presented increase in the percentage of anagen phase. Further investigation concerning Trichosol™ could be useful in the development of new therapeutic associations for the treatment of hair loss.

Keywords: Trichosol; Minoxidil; Dermoscopic analysis

Introduction

Hair loss or alopecia is a problem in modern society, which is usually related to hair loss on the scalp [1]. The most common forms of non-cicatricial hair loss are androgenetic alopecia, alopecia areata and telogen effluvium [2]. Telogen effluvium was first described by Kligman in 1961 and is one of the most common causes of diffuse hair loss. A variety of potential triggers have been associated with the pathogenesis of telogen effluvium. Diffuse telogenic hair loss are seen after 3-4 months of triggering event [3]. Among the possible therapeutic targets, we have the inhibitors of the catagen phase and the inducers of the anagen phase [4]. Thus, in this sense, topical minoxidil is approved, a drug for prolonging the anagen period [5,6]. The Trichotech™, is a technology for hair growth, a phytocomplex that had its mechanism of action published in 2017 by Amaral et al. [7]. Trichosol™ is a base presentation for the use of vehicles (with Trichotech™ technology) with other hair growth formulation as an example, Minoxidil, an important drug in the treatment of hair loss and other capillary disorders. This study investigated the potential of Trichosol™, associated with Minixodil (3%), in the promotion of hair growth through the analysis of the increase of the capillary anagen phase compared to the telogen phase of the volunteers.

Materials and Methods

Volunteers grouping and treatment

Twenty volunteers were selected and then diagnosed with telogen effluvium, ranging in age (25-50 years of age) and gender. Four groups of volunteers were trained and treated as follows:

- Group I - without treatment
- Group II - with minoxidil (3%) conventional vehicle (alcohol)
- Group III - with minoxidil (3%) in vehicle Trichosol™
- Group IV - treated only with Trichosol™ vehicle

The groups were then treated for 90 days after the first diagnosis. The protocols were approved by the local Ethics Committee and written consent was obtained from each subject.

Solutions

Minoxidil and Trichosol™ were obtained from Fagron Group Brazil Ltda. Other inactive ingredients were procured locally.

Dermoscopic analysis (Trichoscan)

The FotoFinder Trichoscale softwear was used to evaluate the parameters of the capillary cycle phases, such as the anagen and telogen phases. All patients were assessed and subjected to photographic records with a 10x magnification dermatoscope and a digital camera with 20x and 40x magnification on the small area of the shaved headscalp. The dermoscopy findings were evaluated by two dermatologists at the time of the exam and later revised in photographs on the computer.

Statistical analysis

Results were given as mean ± SEM (standard error of the mean). The results obtained were statistically analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's test. Analyses were performed using GraphPad Prism version 5.0 (GraphPad Software Inc., CA, USA).

Results and Discussion

All groups of patients were treated for 90 days, in addition to the initial verification performed in the trichoscan the verification of 90 days. Equipment reports were collected, and data analyzed. Figure 1 shows the enlarged image taken by the trichoscan in the patient at the time of diagnosis, prior to the initiation of any treatment. This is an important parameter because besides being measurable it is qualitative, influencing the patient's direct perception that the treatment has been effective because there is significant visual change.

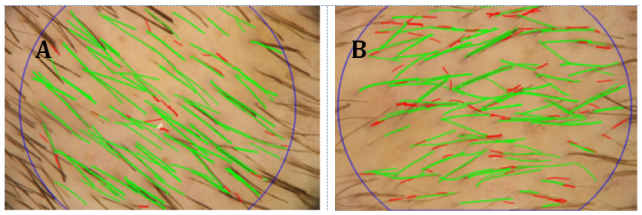

Figure 1: Trichoscan scalp image of a patient at the time of diagnosis (A), indicating in red a small number of patients with wires in the anagen phase and 90 days (B) after treatment with Trichosol™ associated with minoxidil (3%), evidencing the increase of wires in the anagen phase (red).

After data collection, the variation of the anagen phase was verified. Figure 2 shows the data analysis, comparing the variation of the percentage of anagen phase between the studied groups, with an increase of more than 20% in the group treated with Minoxidil (3%) on Trichosol™ in the anagen phase, when compared with untreated volunteers. Note that, although not statistically significant, the group treated with minoxidil (3%) and Trhicosol™ was the one that most reduced the percentage of telogen phase. There are few published information on the efficacy of minoxidil, in addition to several studies reporting subjective data, rather than quantitative data [8,9]. Minoxidil is a medication not related to androgenic mechanisms, which assists in hair loss, at least in part, by causing premature termination of the telogen phase and probably prolonging the anagen phase [10]. Thus, our results suggest that the Trichosol™ vehicle acts in synergism with the minoxidil (3%) through the mechanism of action of Trichotech™ technology (present in Trichosol), activating proliferation pathways such as ERK, in addition to increasing mRNA levels of FGF-7 and FGF-10, corroborating the the hypothesis cited by [7], that Trichotech™ would act in the neogen phase, a transition phase is active between the anagen and telogen stages as a phase of neomorphogenesis, the neogenic phase, where it is believed that the oscillating controllers of mesenchymal and epithelial cells autonomously control stochastic switching in these two stationary states [11].

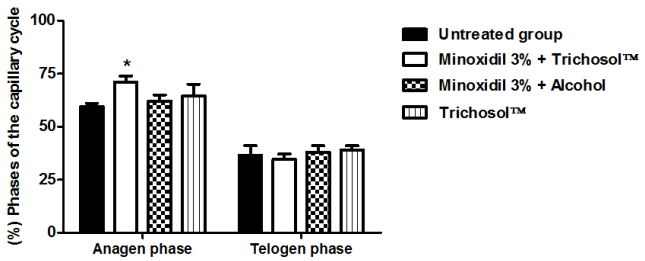

Figure 2: Data comparing treatment evolution and variations of the anagen and telogen phase in the volunteers treated with minoxidil (3%) on Trichosol™; minoxidil (3%) on Alcohol; Trichosol™ and the untreated volunteers. There was a significant increase about 20% in the anagen phase when compared with untreated volunteers. *p<0.05, ANOVA and Tukey's comparison.

The increase in hair in the anagen phase of the capillary cycle was verified in both sexes, in both women and men, always comparing them with the group without treatment (control). Figure 3 shows the results obtained in both men and women. The results obtained in this study are superior to the significant results in relation to placebos, especially those reported in men, with increases in hair growth of approximately 20% on average [12], against an increase of approximately 38% with the group that was treated with minoxidil (3%) associated with the Trichosol™ vehicle. The same was true for the results observed for women where mean increases in counts of approximately 23% [13] were observed, compared to approximately 36% in this study.

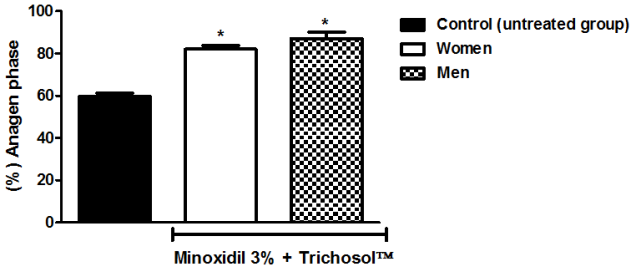

Figure 3: Control group without treatment versus group of women and men treated with Minoxidil (3%) on Trichosol™ after 90 days. *p<0.05. ANOVA and Tukey's comparison.

Conclusions

The analysis performed after obtaining data from the trichoscan equipment allows the conclusion that the treatment of volunteers with Minoxidil™ 3% based on Trichosol™ was able to increase the percentage of anagen phase in these volunteers. In addition, the sex of the volunteers did not interfere with the response of treatment with Minoxidil (3%) on Trichosol™ base.

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In Vitro Effects of the Phytocomplex TrichoTech™ on Human Fibroblasts: Proliferative Potential and Effects on Gene Expression of FGF-7 and FGF-10

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Abstract

The human hair follicle, a mini-organ formed with neuroectodermal-mesodermal interaction, is a complex structure, in the active steady state (anagen) the dermal papilla can be considered as a ball of extracellular matrix, surrounding specialized fibroblasts. The cross-talk of dermal papilla with neighbouring matrix cells results in the maintenance of hair fibre production. This study aimed to investigate the proliferative potential of the compound Trichotech™, a phytocomplex obtained from a mixture of essential oils, on cultured human fibroblasts and its ability to modulate the gene expression of FGF-7 and FGF-10. Trichotech™ was shown to enhance fibroblasts proliferation in concentrations of 0.5% to 2.0%, and also increase the percentage of cells in the S/G2/M phases of the cell cycle. Trichotech™ at both 1.0% and 2.0% induced a statistically significant effect on wound healing assay compared to the untreated control. We examined the interaction between cell survival (PI3K/Akt) and mitogenic (Ras/MAPK) signal transduction pathways after Trichotech™ treatment (1.0% and 2.0%) on the fibroblast cell line. Trichotech™ caused phosphorylation of ERK1/2, as well as greater phosphorylation of MEK in comparison with both the untreated control and ERK1/2. PI3K and AKT, however, were not shown to be significantly more phosphorylated following Trichotech™ exposure. To verify the relative expression of

mRNA for FGF-7 and FGF-10 genes, a real-time polymerase chain reaction (qPCR) protocol was used. Results show the increase in mRNA expression by fibroblasts after treatment with Trichotech™. In both concentrations tested, Trichotech™ was found to increase the expression of FGF-7 and FGF-10. Sirius red staining allows for rapid assessment of collagen content, it showed a significant increase in collagen content in treated fibroblasts. Further investigation concerning Trichotech™ could be helpful towards the development of new bioactive phytocomplexes for dermatological and trichological use.

Keywords

Trichotech™, Fibroblasts, Proliferation, FGF-7, FGF-10

1. Introduction

The human hair follicle, a mini-organ formed with neuroectodermal-mesodermal interaction [1], is a complex structure consisting of an outer root sheath, an inner root sheath, the hair shaft, the bulge and the sebaceous gland [2].

The follicle undergoes successive steps of fibre production, regression and rest, which in humans last for an average of 3 years, 3 weeks and a few months, respectively. An additional phase involving the active release of the club fibre has also been described, and is thought to be independent from the rest of the hair cycle [3], while bearing no direct consequence on fibre production initiation [4].

Human hair follicle dynamics are regulated through a bi-stable equilibrium state, including an active steady state (the anagen stage) and a resting steady state (the telogen stage); the transition between these two steady states involves either a degradation phase (the catagen phase) or a neo-morphogenesis phase (the neogen phase). It is now believed that mesenchymal and epithelial oscillators control the stochastic autonomous switching between these two steady states [5].

In the active steady state (anagen), the dermal papilla can be considered as a ball of extracellular matrix, surrounding specialized fibroblasts. The cross-talk of dermal papilla with neighbouring matrix cells results in the maintenance of hair fibre production [6].

The dermal papilla maintains bulge stem cells and secondary hair germ cells quiescent during telogen through production of bone morphogenetic protein 4 (BMP4) and fibroblast growth factor 18 (FGF-18). Cell proliferation during anagen is triggered via production of BMP inhibitors (e.g. Sosrdc1 and Bmbi), as well as secretion of FGF-7 and FGF-10 [7] [8]. Thus, a combination of factors secreted by dermal papilla fibroblasts generates a signaling environment that dictates whether hair follicles will remain dormant or enter the anagen stage.

In a recent study, [9] found that topical application of FGF-10 was able to induce significant hair growth in C57BL/6 mice, linked to induction of anagen phase and increase in the number of hair follicles due, at least in part, to upre-

gulation of β-catenin and Shh signaling.

The use of certain phytocompounds as stimulants of hair growth has been considered an effective secondary measure for the treatment of hair loss, especially when common first-line treatments such as minoxidil application or finasteride administration yield poor results or cause adverse reactions. Many plant extracts and fractions thereof have been shown to elicit hair growth in mice [10] [11] [12], thus the prospecting of plant extracts as a source of hair growth-promoting compounds is a promising strategy.

The present study aimed to investigate the proliferative potential of the compound Trichotech™, a phytocomplex obtained from a mixture of essential oils, on cultured human fibroblasts and its ability to modulate the gene expression of FGF-7 and FGF-10, as well as to propose further applications on hair growth.

2. Materials and Methods

2.1. Chemicals

Propidium iodide, Direct Red 80 (Sirius Red) and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The AnnexinV/FITC Apoptosis Detection Kit was obtained from BD Pharmigen (CA, USA). Iscove's Modified Dulbecco's Medium (IMDM) and all cell culture reagents were purchased from Life Technologies (Thermo Fisher Scientific, USA). MEK, ERK, PI3K and AKT primary antibodies and Alexa Fluor 488-conjugate monoclonal antibodies were acquired from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

2.2. Cell Culture

CCD-1072Sk (ATCC® CRL2088™) fibroblasts were cultured in ISCOVE'S medium with 10% fetal bovine serum, 0.292 g/l L-glutamine, 1.0 g/l D-glucose, 2.2 g/l $NaHCO_3$, 10.000 UI penicillin, and 0.060 g/l streptomycin. Cells were kept in 25 cm^2 flasks (1 × 105 cells/ml) in a humidified incubator at 37˚C with an atmosphere of 5% CO_2 for a maximum of 30 population doublings. In all experiments, the fibroblast cultures were subjected to cell viability assays using Trypan blue dye, and readings were performed in a hemocytometric chamber under a light microscope. All experiments described were performed when cell viability was equal or above 95%.

2.3. MTT Reduction Cell Viability Assay

The MTT reduction assay ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide]) is employed with great success for estimating the number of viable cells in initial drug screenings. Its interpretation serves as an indicative of cellular metabolic activity, and the redox reactions occur in both the mitochondria and the cytosol. The reduction of the MTT salt to formazan happens mainly because of the succinate dehydrogenase enzyme, and results in purple insoluble formazan crystals. The intensity of the color is used to measure mitochondrial activity and, therefore, cell viability [13]. Cells were seeded at a density of 5×10^4

cells/well and treated with different concentrations of Trichotech™ (0.5, 1.0, 1.5 and 2.0%) for 24 hours. Next, 10 µl of a 5 mg/ml MTT solution (Sigma-Aldrich) were added to each well. After 4 hours the samples was reincubated with 100 µl of Sodium dodecyl sulfate (SDS) solution [10%] for 12 hours, and then optical density was measured in a FlexStation® 3 multimode Benchtop Reader (Molecular Devices, CA, USA) at 540 nm.

2.4. Propidium Iodide (PI) Incorporation Assay

Propidium iodide incorporation assays were performed using flow cytometry to assess the cellular fraction in the S/G2/M phase of the cell cycle (*i.e.* proliferating cells). To summarize, cells were seeded in 24-well plates at an initial density of 2×10^5 cells/well, to which was added a hypotonic fluorochrome solution (HFS—0.1% w/v sodium citrate, 0.5% w/v Triton X-100 and 50 µg/ml propidium iodide). After an incubation period of 4 h at 4˚C and shielded from light, the cells and supernatant were collected and analyzed. A FACS can flow cytometer and the CellQuest software were employed, and the data obtained were analyzed with WinMDI 2.8, considering 20,000 events per analysis for each assay.

2.5. Wound Healing

For this assay, fibroblasts were seeded in 6-well microplates and cultured as described above until observation of a confluent monolayer. The cell monolayers were carefully "scratched" with a sterile pipette tip, and washed with saline and PBS to remove loose cells and debris. Next, the cells were incubated at 37˚C with culture medium without fetal bovine serum (nutrient deprivation) and with 0.2% low molecular weight HA. Reference points near the "wound" were demarcated to ensure the same area of image acquisition. Images were obtained at different times using a digital camera attached to the microscope, and the percentage of wound closure was calculated using IMAGEJ (NIH, USA).

2.6. Measurement of MEK/ERK and PI3K/AKT Signaling Activity in Fibroblasts

Protein phosphorylation is a dynamic process controlled by the enzymatic activities of kinases and phosphatases. In order to inhibit these processes rapidly, fixation was done by adding BD FACS™ Lysing Solution. Following fixation, the cells were pelleted by centrifugation, and cell permeabilization was carried out either by use of a saponin solution, for analysis of cytoplasmatic proteins, or Triton X-100 solution for nuclear proteins. Following fixation and permeabilization, the samples were washed once with PBS and processed for antibody labeling. Antibody dilutions were prepared using PBS with BSA and sodium azide, with primary antibody concentrations according to the manufacturer's instructions. Samples were incubated for 2 hours at room temperature for labeling. Then the samples were washed and incubated with the secondary antibody for 45 minutes also at room temperature. After labeling with the primary and secondary antibodies, samples were read at 100.000 events in a BD Accuri™ C6 flow cyto-

meter.

2.7. Real-Time PCR (qPCR)

Total RNA extracted from fibroblast samples was converted to cDNA using a SuperScript® III RT kit (Invitrogen, Carlsbad, CA). A qPCR analysis was performed in 10 µL reactions with the SYBR GREEN PCR Master Mix and analyzed on a StepOnePlus™ Real-Time PCR instrument (Invitrogen, Carlsbad, CA). Relative standard curves were generated by serial dilutions and all samples were run in triplicates. Primers used are: FGF-7 forward (5'-ATCAGGACAGTGGCAGT TGGA-3'); FGF-7 reverse (5'-AACATTTCCCCTCCGTTGTGT-3') and FGF-10 forward (5'-CACATTGTGCCTCAGCCTTTC-3'); FGF-10 reverse (5'-AGGTGA TTGTAGCTCCGCACA-3'). The PCR reaction was performed under the following conditions: 50˚C (2 min), 95˚C (10 min), and 40 cycles of 95˚C (15 s) and 55˚C (1 min). GAPDH was used as a control gene.

2.8. Sirius Red Collagen Quantification

After cells were cultured, the medium was removed and the wells were washed three times with 0.1 M PBS. Next, 100 µl of Bouin's solution (picric acid 0.9%, formaldehyde 9.0% and glacial acetic acid 5.0%) were added for fixation for 1 h. Samples were washed with PBS, then the Sirius Red dye was added. After 1 h, the maximum possible amount of dye was removed, followed by washing with 150 µl of a 0.01 M hydrochloric acid solution for 30 seconds to remove the dye that did not bind to collagen. Next, the dye was removed from cell layers by the addition of 0.1 M NaOH for 30 min. 100 µl aliquots of the solution contained in the wells were transferred to a new plate. Absorbance was measured with an Elx-800-UV (Bio-Tek Instruments, USA) microplate reader at 570 nm.

2.9. Statistical Analysis

Results were given as mean ± SEM (standard error of the mean). The results obtained were statistically analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's test a posteriori. Semi-quantifications were analyzed using Student's t-test. P-values < 0.05 were considered significantly different. Analyses were performed using GraphPad Prism version 5.0 (GraphPad Software Inc., CA, USA).

3. Results and Discussion

3.1. MTT to Formazan Reduction Assay

The MTT colorimetric assay is an established method of determining viable cell number in proliferation and cytotoxicity studies. This colorimetric assay provides accurate and reliable quantification of viable cell number and is based on the cleavage of the yellow tetrazolium salt, MTT, to form a soluble blue formazan product by mitochondrial enzymes, and the amount of formazan produced is directly proportional to the number of living, not dead cells, present during

MTT exposure [14]. Trichotech™ was shown to enhance fibroblasts proliferation (Figure 1). This effect of Trichotech™ in concentrations of 0.5% to 2.0% was visible by MTT assay after 24 hours of treatment ($P < 0.05$). Trichotech™ at concentrations of 1.5% and 2.0% significantly enhanced the proliferation of fibroblasts compared to the untreated group. Notice a dose-dependent increase in fibroblast proliferation.

3.2. Propidium Iodide (PI) Incorporation Assay

Progression through the cell cycle is one of the most fundamental features of cells and can be measured by staining cells with propidium iodide (PI). The level of PI fluorescence in a cell is, directly proportional to the DNA content of that cell, the quantification of which indicates the percentage of cells in each phase of the cell cycle in a sample [15]. As seen in Figure 2, the percentage of cells in the S/G2/M phases of the cell cycle increased in a dose-dependent fashion upon Trichotech™ treatment. This shows that Trichotech™ acts in accordance with several lines of evidence which support a molecular mechanism in the response to stimulation by natural compounds, increasing G2/M phase in fibroblasts [16] [17].

3.3. Wound Healing

Cell migration and proliferation coupled with controlled cell cycle are beneficial for the repair of sagged and wrinkled skin, dermal, and gastrointestinal wound healing. The *in vitro* scratch assay is a well-developed method to measure cell migration and its steps involve creating a "scratch" in a cell monolayer, capturing the images at the beginning and at regular intervals during cell migration to close the scratch, and comparing the images to quantify the migration rate of the

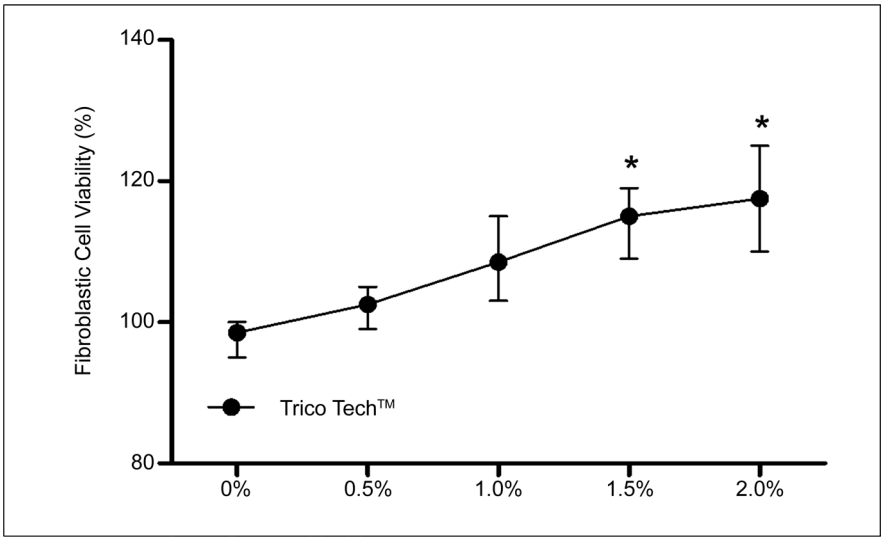

Figure 1. Results of cell proliferation from MTT reduction assay after 24-hour exposure to different concentrations of Tricotech™. Before starting the tests, cells were deprived of fetal bovine serum. (*) $P < 0.05$—significant in relation to control. ANOVA, Tukey. Assays performed in triplicate. GraphPad Prism v5.0.

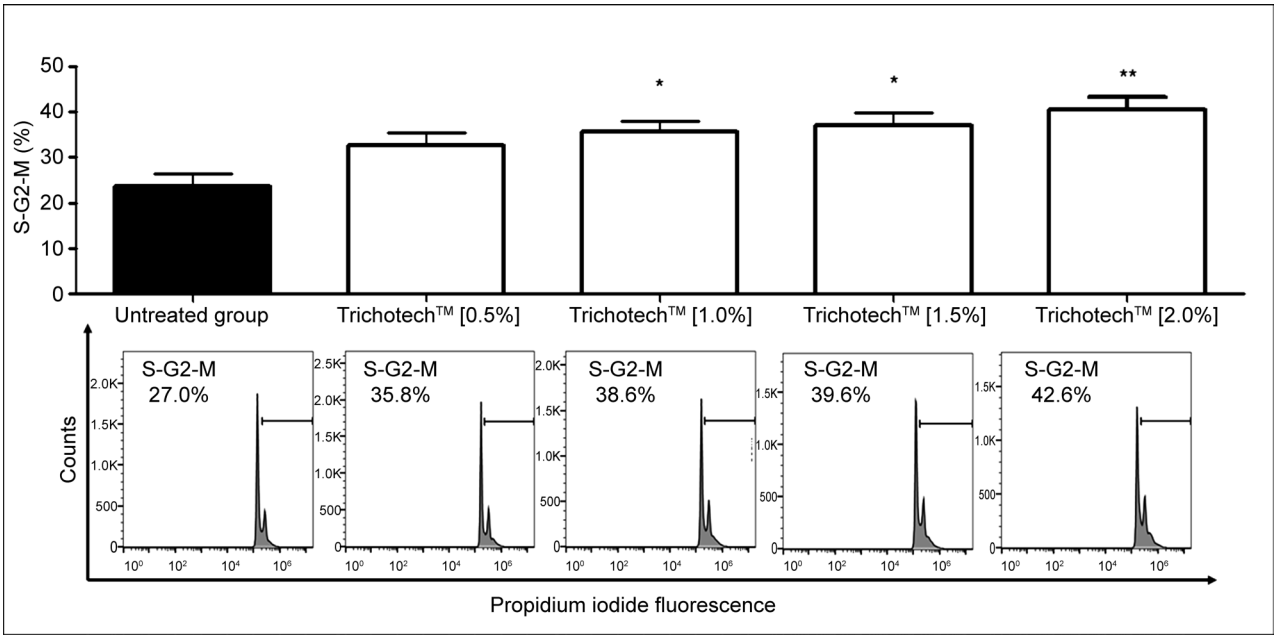

Figure 2. (a) Percentage of cells in S/G2/M phase obtained after 24-hour exposure of CCD-1072Sk cells to different concentrations of Tricotech™. Before starting the tests, cells were deprived of fetal bovine serum. (*) $P < 0.05$—significant in relation to control. ANOVA, Tukey. GraphPad Prism v5.0. (b) Histogram representing statistically significant concentration (10%). FlowJo v10.0.

cells [18]. We evaluated Trichotech™ wound healing stimulating activity on fibroblast cells using the scratch assay. Scratches were made on confluent fibroblast monolayers, which were then exposed to Trichotech™ for 24 h at two concentrations (1.0% and 2.0%). Trichotech™ at both 1.0% and 2.0% induced a statistically significant effect on wound closure compared to the untreated control (**Figure 3(b)**). In **Figure 3(a)** and **Figure 3(b)**, respectively, we can see images obtained at different times and the graphic representation of the distance between the edges of the scratch.

3.4. Measurement of MEK/ERK and PI3K/AKT Signaling Activity in Fibroblasts

An understanding of the mechanisms that regulate the cell migration and proliferation of dermal fibroblast cells by a natural compound could be beneficial in devising novel therapies to regulate fibrosis and wound contraction to ultimately improve the wound healing process [19]. The most highly studied intracellular signaling cascades in the context of cancer are the mitogen activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/AKT pathways [20]. We examined the interaction between cell survival (PI3K/Akt) and mitogenic (Ras/MAPK) signal transduction pathways after Trichotech™ treatment on the fibroblast cell line. The cells were stimulated with 1.0% and 2.0% Trichotech™. The treatment with Trichotech™ caused phosphorylation of ERK1/2, as well as greater phosphorylation of MEK in comparison with both the untreated control and ERK1/2 (**Figure 4**). PI3K and AKT, however, were not shown to be significantly more phosphorylated following Trichotech™ exposure. Similar results

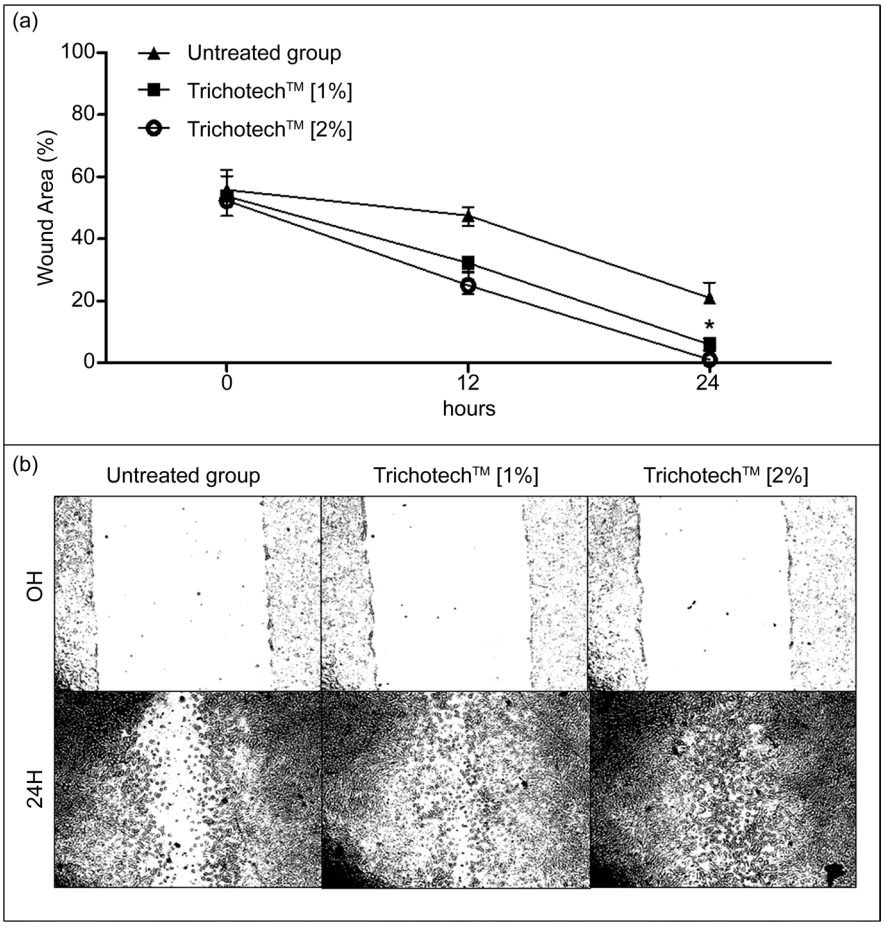

Figure 3. (a) Photographic representation of *in vitro* samples subjected to a simulated wound and exposed for 24 hours to different concentrations of Trichotech™ (1% and 2%). (b) Representative graphic of the percentage of wounded area at 0 h and 24 h after the same treatment. Before starting the tests, cells were deprived of fetal bovine serum. (*) $P < 0.05$—significant in relation to group 0 h treated with Trichotech™, Student's t-test, GraphPad Prism v5.0.

were obtained [21], whereby the proliferative effects of camphor were shown to be mediated by the PI3K/AKT/mTOR and MAP kinase pathways—the key signaling pathways involved in the control of cell proliferation. In this same study, camphor-induced phosphorylation of ERK, but not PI3K and AKT, was also reported. Taken together, this evidence indicates that Trichotech™ induced fibroblast proliferation possibly through upregulation of MAP kinase signaling pathways.

3.5. Real-Time PCR (qPCR)

Several growth factors (e.g., FGF-1, FGF-2, FGF-7 and FGF-10) can promote cell cycle and proliferation and have the potential to rescue hair loss and facilitate hair cell regeneration *in vivo* and *in vitro* [9]. Among these FGF genes, FGF-7 was found to be expressed in the hair follicle. FGF-7 RNA is localized to the dermal papilla during anagen, but expression is down-regulated by the late-anagen VI stage [22]. Besides, no FGF-7 RNA was detected in follicles during

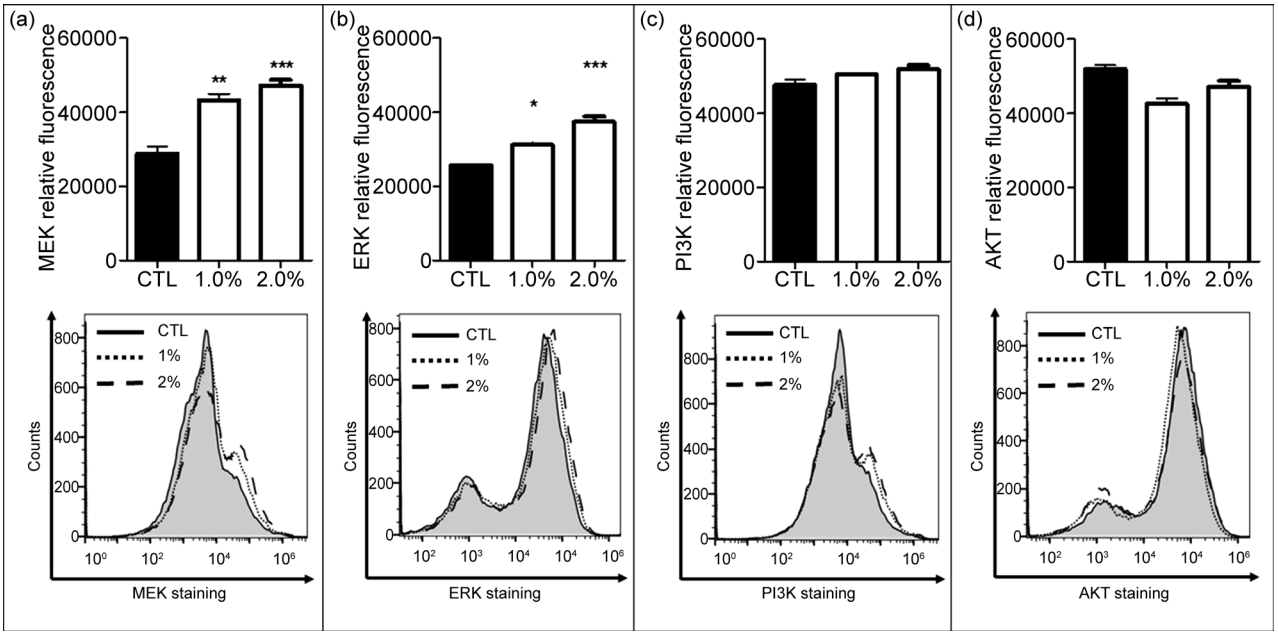

Figure 4. Graphic and corresponding representative histogram of MEK (a), ERK (b), PI3K (c) and AKT (d) phosphorylated proteins signaling after exposure to Trichotech™ [1%] and [2%] for 1 h. Results are expressed by the MFI (median fluorescence intensity) and compared with the untreated group (CTL). (*) $P < 0.05$—significant in relation to CTL group. ANOVA, Tukey, GraphPad Prism v5.0.

catagen or telogen [23]. FGF-10 is found in the dermal papilla fibroblasts and its receptor FGFR2IIIb is found in the neighboring outer root sheath of the keratinocytes [24], suggesting that FGF-10 is a mesenchymally derived stimulator of hair follicle cells, which contribute to the hair-promoting activity. To verify the relative expression of mRNA for fibroblast growth factor-7 (FGF-7) and fibroblast growth factor-10 (FGF-10) genes, a real-time polymerase chain reaction (qPCR) protocol was used. **Figure 5** shows the increase in mRNA expression by fibroblasts after treatment with Trichotech™. In both concentrations tested, Trichotech™ was found to increase the expression of FGF-7 and FGF-10 by several fold, thereby constituting a dermal papilla signal instructing hair germ cells to proliferate and initiate a new hair cycle.

3.6. Sirius Red Collagen Quantification

Sirius red staining of collagen has been used for many years. The present colorimetric plate assay allows for rapid assessment of collagen content [25]. The sirius red assay showed a significant increase in collagen content in treated fibroblasts (**Figure 6**). The magnitude of the increase in collagen between control and treated samples was markedly increased following treatment with 2.0% Trichotech™. Previous reports have demonstrated similar increases in collagen production following exposure to natural compounds, akin to the data obtained in the present work. Jung [26] showed that Camellia japonica oil was capable of inducing type I collagen synthesis in fibroblasts. Studies also observed increased type I collagen content in human dermal fibroblasts following exposure to ginseng and cinnamon preparations, respectively [27] [28].

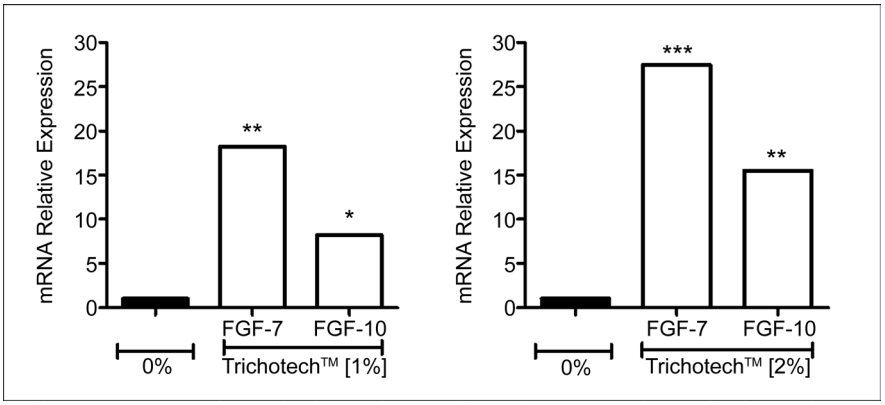

Figure 5. Relative expression levels of mRNA for FGF-7 and FGF-10 in human fibroblasts assessed by quantitative RT-PCR. Bars represent the range of relative expression. *P < 0.05, **P < 0.01, and ***P < 0.0001 compared to internal control GAPDH. Graphs were plotted with GraphPad Prism v5.0.

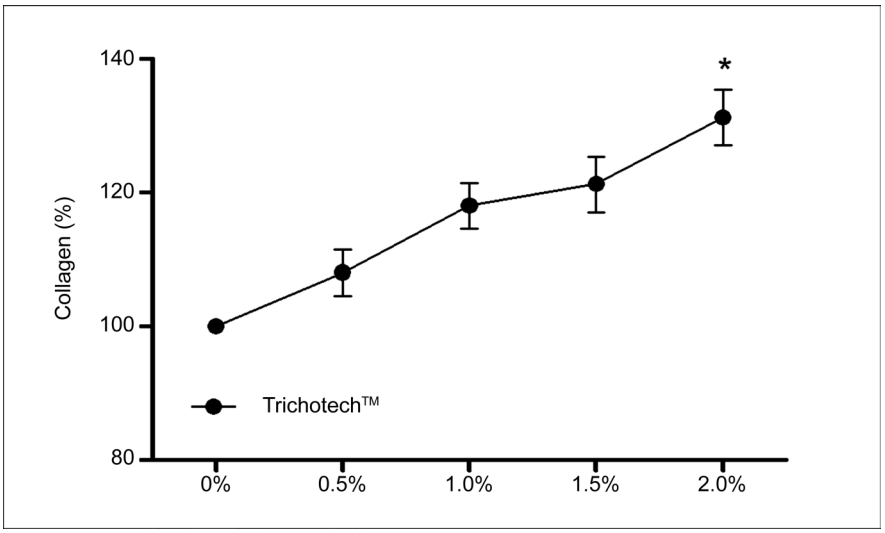

Figure 6. Total collagen content in fibroblasts as measured by incorporation of Sirius Red dye after 24-hour exposure to different concentrations of Trichotech™. Before starting the tests, cells were deprived of fetal bovine serum. The exposure did not cause a significant increase in relation to the control group (untreated cells). (*) P < 0.05—significant in relation to control. Student's t-test. GraphPad Prism v5.0.

4. Conclusion

Fibroblasts are found in the dermal papilla of hair follicles, and as such are heavily implicated in hair growth regulation. Taken together, our data show a stimulating effect of Trichotech™ on cultured fibroblasts. Indeed, a pronounced increase in cell growth was observed after exposure to Trichotech™ at concentrations of 0.5% - 2.0%. Our results suggest that Trichotech™ induced fibroblast proliferation by activating ERK signaling pathways. In addition, FGF-7 and FGF-10 mRNA levels were shown to be increased compared with untreated controls. Further investigation concerning Trichotech™ could be helpful towards the development of new bioactive phytocomplexes for dermatological and trichological use.

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Viewpoint

The human hair follicle, a bistable organ?

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Abstract: The hair cycle and its control remain today an object of debate. A number of factors, which can modulate this process, have been identified but its choreography remains elusive. For years, the hunt for the conductor has been on, but nobody ever caught him. Intuitively, the process being considered as cyclic, an automaton controlling this cycle should be looked for, by analogy with a clock. However, the putative hair follicle oscillator that would control hair cycle failed to be identified and characterized. In fact, we have revealed that human hair follicle has an autonomous behaviour and that the transitions from one phase to the next occur independently for each follicle, after time intervals given stochastically by a lognormal distribution characterized by a mean and a variance. From this analysis, one can conclude that instead of a cyclical behaviour with an intrinsic automaton, a bistable steady state controls human hair follicle behaviour, which under a stochastic way jumps from the dormant to the active steady state and vice versa.

Key words: bistable steady state – hair cycle – hair follicle – neogen

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Introduction

For decades, the hair cycle and its control have been an object of debate. Although several systems, like endometrium, cycle in the mammalian body, the hair follicle is clearly one of the only organs in mammals, together with the mammary gland for example (1), which 'cyclically' degenerates and regenerates from stem cells (2). The understanding of such a unique behaviour would certainly give clues to tissue homoeostasis and regeneration. Interestingly, a number of factors that can modulate, trigger, stimulate or repress this process have been identified (3). Furthermore, the stem cells have been identified, localized and even molecularly characterized (4–6), although recent data suggest an impressive diversity in hair follicle stem cell populations (7). Although the list of actors keeps steadily increasing, the choreography remains elusive. For years, the hunt for the chief of orchestra has been on, but nobody ever caught him. Intuitively, the process being considered as cyclic, an oscillator controlling this cycle should be looked for, by analogy with a clock (8). Even though circadian clock genes were recently identified as possible contributors to the regulation of hair follicle cycling (9), a famous paper evidenced the failure in finding the regulators of the hair cycle (3) and the question, Why is it so difficult to identify and characterize this oscillator? remains. My answer is simple: it simply does not exist.

Neogen – a new phase taking into consideration the morphogenesis process

In fact, by carrying out monthly phototrichograms during 14 years on a group of ten male, alopecic and non-alopecic volunteers (10), we studied the behaviour of 930 individual follicles and recorded about 9000 hair cycles. We then discovered that the duration of each phase of the so-called hair cycle was highly variable, from a few weeks to several years, generating an apparently chaotic behaviour shared by all follicles, whatever the alopecia grade. We had indeed revealed that each follicle had an autonomous stochastic behaviour, the probability of duration of each phase fitting with a lognormal equation (11,12). Of note, even though a deterministic model would predict the average durations of anagen, telogen and kenogen phases around which fluctuations are observed, it would not be capable of accounting for these fluctuations of phase durations (12). Considering this peculiar dynamics, characterized by an absence of synchronized oscillations, one should reconsider the entire process of degeneration–regeneration of the hair follicle. Classically, the follicle undergoes successive steps of fibre production (anagen), regression (catagen) and rest (telogen), which in humans last for an average of 3 years, 3 weeks and a few months, respectively. A side phase, termed 'exogen', has been described, independent from the rest of the hair cycle, during which the club fibre is actively released (13) without direct consequence on anagen initiation (14). After hair loss, a latency period is observed in 80% of hair cycles (10), between elimination of a hair in exogen (14) and the appearance of the replacement hair in anagen. The duration of this period, called kenogen (15), varies from 2 to 5 months on average (10). Interestingly enough, if catagen designates the shift from anagen to telogen, no name characterizes the shift from telogen to anagen, only anagen stages being given (16). Indeed, to date, anagen phase includes a very quick and active morphogenetic process followed by a long-lasting steady fibre production state. It is nevertheless striking that the hair follicle undergoes steady periods (telogen and anagen) that are interrupted by short and intensively active periods of remodelling, regression and regeneration. If regression phase is termed catagen, I propose to call the regeneration phase 'neogen' in order to highlight, in a symmetric way to catagen, the dynamic and short-lasting character of this crucial process. The entire process of resting, regeneration, fibre production and regression would thus include four main successive phases, namely telogen, neogen, anagen and catagen. Two of those are very short, neogen and catagen, and two are quite long, telogen and anagen.

The hair follicle, a bistable organ

Instead of a cycle, the human hair follicle behaviour would rather be described as a stochastic process operating on a bistability. The hair follicle would exist in two steady states, active and dormant. From time to time, under a stochastic way, the follicle would

jump from one state to another one. Thus, the follicle would not have a cyclic behaviour, but would undergo a succession of steady states (Fig. 1). Considering that many of the individual cell-fate decisions which control organism and organ development are binary in nature (life or death, proliferation or quiescence), that stochasticity of gene expression could lead to bimodal output (17) and bistable gene expression (18) and that binary choices are typically made by bistable switches (19,20), one might define the hair follicle – at a higher order – as a bistable organ.

Under this model, the bistable steady state is controlled by a combination of numerous factors with stochastic incremental variations, and the jump from one steady state to the other one would be triggered when given thresholds are reached. To capture this behaviour, an integrative multiparametric equation remains to be elaborated, which would include as variables all the factors so far identified in hair growth control, like growth factors, hormones, nuclear receptors, transcription factors and circadian molecular clock genes (21). Owing to the existence of thresholds, one prediction of this model is that both steady states would be endowed with refractory and competent phases, as recently shown for telogen (22–24). Indeed, refractory telogen is characterized by high bone morphogenetic proteins (BMPs) while competent telogen is characterized by low BMPs (22), a condition required for neogen to take place. Depending on the follicle considered, neogen would thus start after a variable time in the competent telogen phase (22) and last until full anagen development. Similarly, one could predict that anagen would be characterized by competent and refractory phases; the former involving IGF-1, HGF, GDNF and VEGF signalling, and the latter involving FGF5, TGFβ and BDNF in the onset of catagen (3). By analogy with the bistable calcium-/calmodulin-dependent protein kinase II switch that could control long-term memory upon stable persistent activation (25), a second prediction is that follicle could be blocked on either steady states that is an active state or a dormant state. In fact, several examples of unlimited hair growth have been recently reported in China, with anagen duration over 25 years (26). On the contrary, eyelashes are an example of follicles mainly blocked in dormant state, since 70–90% of them are in telogen (27). Chronic telogen efflu-vium and androgenetic alopecia would in fact translate subtle changes in the general multiparametric equation controlling the stochasticity of hair follicle behaviour (28) and more specifically the transition thresholds of the bistable steady states.

The dermal papilla, a key controller

Finally, if it is conceivable that catagen translates the jump from active to dormant state and stops when the follicle regression is completed, how can we explain that the neogen phase also stops, when it reaches the active steady state? Probably, this is partially controlled by the balance between extracellular matrix (ECM) and morphogens production by the dermal papilla (DP), linked to its dynamics. Indeed, DP is voluminous with the cells far apart in anagen, and flattened with the cells compacted in telogen (29). In anagen phase, the DP can be considered as a ball of ECM, surrounding specialized fibroblasts. The cross-talk of DP with neighbour matrix cells results in the maintenance of hair fibre production. An alteration in this cross-talk, induced by modifications of some variables of the multiparametric equation controlling the steady states, such as the transient FGF5 expression (30), would result in the onset of catagen and initiate the jump to the dormant steady state. During this phase, DP is left behind the regressing follicle, while its ECM starts degrading. When the DP ultimately reaches the telogen follicle, it is a simple cell aggregate, with no ECM. A new cross-talk can take place, and morphogenetic signals can be exchanged which, after having reached a given threshold, trigger the neogen phase, that is the jump from dormant to active steady state. Simultaneously, the synthesis of DP ECM is reinitiated. This ECM is rich in components like GAGs

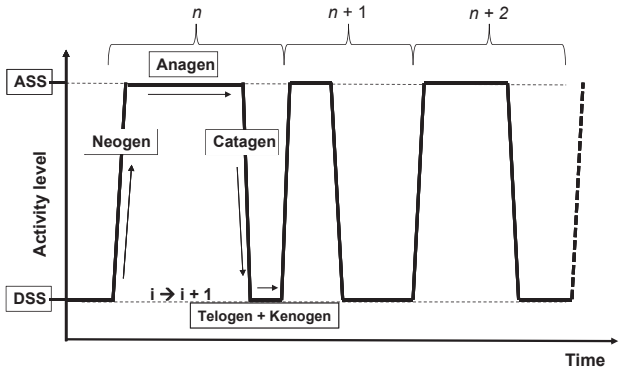

Figure 1. A new representation of the hair follicle behaviour, with an active steady state (ASS) of fibre production (anagen) and a dormant steady state (DSS) (telogen/kenogen), interspaced by short-lasting phases of neomorphogenesis (neogen) and regression (catagen). Three successive periods (n, $n + 1$, $n + 2$) are represented.

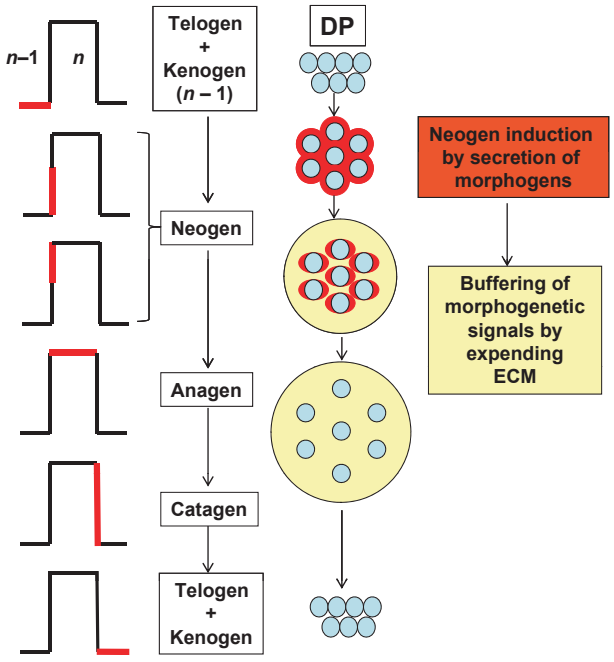

Figure 2. Dynamics of dermal papilla (DP) and consequences. On the left column are represented the successive phases of human hair follicle behaviour (see Fig 1). (n – 1) and (n) correspond to two successive periods. The red fragment denotes the phase corresponding to associated DP drawings (on the right column). In this model, the competition between morphogens and extracellular matrix (ECM) secretion is postulated to regulate the duration and extent of neogen phase. Morphogens secretion corresponds to the red area surrounding DP cells (depicted in blue) while DP ECM is depicted in pale yellow.

(31), which can progressively trap morphogenetic factors and buffer matrix cells activating factors (Fig. 2). A new steady state is established, the neogen phase is finished, and the anagen phase starts.

Conclusion

Although seasonal changes and periodicity in the growth and shedding of human hair have been reported (32,33), large-amplitude oscillations resulting from follicular synchronization are not observed in human scalp, likely because each follicle behaves independently of each other and hair regeneration/degeneration only depends on intrinsic activation/inhibition mechanisms. Of note, desynchronized hair follicle behaviour looks specific for human being, as a different type of dynamic behaviour with periodic moulting is observed in a number of mammalian species (34–37). We have previously demonstrated that moultings would correspond to oscillations of follicular cycles resulting from synchronization by periodic external and/or hormonal signal(s) (34–37), which would trigger the transition from anagen to telogen phase (12). Moreover, regenerative wave patterns in adult mouse hair follicle populations have been linked to macroenvironmental regulation of stem cell activity instead of – or superimposed to – hair follicle intrinsic microenvironment (22,23).

To summarize, the human hair follicle appears as a prototypic systems biology model (38) and as such, the first example of an organ under the control of bistable steady state, which under a stochastic way jumps from dormant to active state and vice versa. This implies that minute variations of some key variables might trigger these jumps to neomorphogenesis or degeneration. If this concept holds true, it might be of value to consider tissue homoeostasis as the result of a bistable steady state and to identify key variables involved in the control of normal and pathological epithelial–mesenchymal interactions, the hair follicle being a true paradigm of this type of interactions.

Conflict of interests

Bruno A. Bernard is an employee of L'Oréal Company.

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Review

Mesenchymal–epithelial interactions during hair follicle morphogenesis and cycling

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ABSTRACT

Embryonic hair follicle induction and formation are regulated by mesenchymal–epithelial interactions between specialized dermal cells and epidermal stem cells that switch to a hair fate. Similarly, during postnatal hair growth, communication between mesenchymal dermal papilla cells and surrounding epithelial matrix cells coordinates hair shaft production. Adult hair follicle regeneration in the hair cycle again is thought to be controlled by activating signals originating from the mesenchymal compartment and acting on hair follicle stem cells. Although many signaling pathways are implicated in hair follicle formation and growth, the precise nature, timing, and intersection of these inductive and regulatory signals remains elusive. The goal of this review is to summarize our current understanding and to discuss recent new insights into mesenchymal–epithelial interactions during hair follicle morphogenesis and cycling.

Contents

1. Introduction

A hair follicle is the primary unit that produces a single outgrowing visible hair shaft. In mice, multiple hairs are induced all over the body and patterned to form rows of eyelashes, discrete whiskers, or densely clustered pelage hairs. All fulfill a wide range

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of functions, including control of body temperature, providing physical protection, relaying sensory and tactile input, and serving decorative purposes for social interactions. At least eight different major hair types can be distinguished in mice [1], and the hair coat alone contains four separate hair subtypes [2].

All hair follicles have the same basic arrangement, with epithelial progenitor cells at the base giving rise to multiple intermediary cell lineages that form the hair shaft and its guiding channel. Epithelial progenitors themselves surround a core cluster of mesenchymal cells, the dermal papilla (DP), which is thought to provide signals to coordinate hair growth [3]. The exchange of molecular cues between epithelial and mesenchymal compartments begins during embryogenesis, when hair follicles are first formed [4]. Remarkably, many of the fundamental signaling programs required for hair morphogenesis are evolutionarily conserved across species with different types of skin appendages, such as feathers and scales [5]. Furthermore, parallels exist between the mechanisms driving hair, tooth and mammary gland formation, all of which require mesenchymal–epithelial interactions [6]. After initial hair follicle formation and a prolonged period of growth, follicles undergo cycles of destruction and regeneration throughout life [7]. For new hair re-growth, signal exchange between DP cells and stem/progenitor cells is thought to occur in a process that is reminiscent of embryonic hair follicle formation [8]. Many diverse developmental programs require coordinated mesenchymal–epithelial interactions for completion, and studies of hair growth provide an exquisite system in which to study the complexities of this universally important process.

Numerous methods have been used to characterize the interplay of signals exchanged between the mesenchymal and epithelial components during embryonic follicle initiation, postnatal growth and adult regeneration. An early approach involved tissue recombination experiments, which determined that dermal signals initiate follicle formation [9]. Subsequent microdissection and transplantation experiments revealed the inductive and nurturing role of specialized DP cells [10] and localized multipotent epithelial stem cells to the follicle bulge [11]. The identification of putative ligands and receptors involved in mesenchymal–epithelial interactions came from tissue stainings performed since the 1990s, and more recently from studies systematically assessing gene expression with the help of genetic fluorescent reporter tools [12–15]. The functional relevance of many ligands has been explored by bead implantation experiments, complete gene knockout mice and spontaneous mouse mutants [16]. Most recently, compartment-specific gene ablation [17] and transgenic overexpression in the epidermis [18] and bulge stem cells [19] of candidate ligands and receptors yielded many insights into the requirement and timing of several signaling pathways for hair morphogenesis. In this review, we will highlight the basic concepts of hair follicle development, discuss our current understanding of the signal exchange during this process, and review recent new insights into the mesenchymal–epithelial interactions driving follicle induction, growth and regeneration.

2. Overview of hair follicle development, growth and regeneration

2.1. Hair follicle formation

Classically, the initiation of hair follicle morphogenesis is described in terms of an ordered series of mesenchymal–epithelial interactions: a “first signal” emanating from the dermis acts on an unspecified epidermis, and the formation of morphologically recognizable hair placodes follows next [4,8]. Several studies have proposed that mechanisms of lateral inhibition, mediated by diffusible signals that act within the epidermal compartment, coordinate the even spacing of these placodes [20–22]. As development progresses, stabilized placodes signal to underlying dermal cells, prompting the formation of dermal condensates or clusters of DP precursor cells. Finally, these condensates are believed to signal back to the epithelial compartment to stimulate proliferation and downgrowth of hair germs [4]. Hair follicle stem cells arise from epidermal progenitors early on [23] but remain located in the upper portion of the follicle while supplying rapidly dividing cells at the tip that allow further downgrowth of the hair peg. As the epithelial component of the nascent follicle extends deep into the skin, DP precursor cells remain at the leading edge and are eventually engulfed. The dermal component of the mature hair follicle consists of these DP cells, which remain in the bulb region, and an adjoining connective tissue sheath that encircles the follicle in its entirety [4].

The first epithelial placodes appear at embryonic day E14.5, and eventually develop into primary guard hair follicles. These unique hairs comprise only 1–5% of the adult mouse coat, and are distinguished by their large follicle size and longer shaft length. Primary placodes have already progressed to form prominent downgrowths by E16.5, when a second wave of placode formation initiates. Secondary placodes appear in an even distribution between established guard follicles and give rise to awl and auchene hairs. These contribute to twenty percent of the final adult coat, with smaller follicles and shorter shaft lengths compared to primary guard hairs. A third and final wave of placode formation begins at E18.5, giving rise to zig-zag hairs that represent the vast majority of the adult coat [2,24].

2.2. Hair growth phase

After initial hair follicle downgrowth, the DP is completely encased by the lowest part of the hair bulb, although it remains separated from the epithelial compartment by an enveloping basement membrane. From this position, the DP lies adjacent to a population of transit-amplifying matrix cells and is thought to emit signals crucial for regulating their proliferation and differentiation into the hair shaft and its channel, the inner root sheath [3,16,25]. The hair shaft is in the center and consists of a medulla, cortex and a cuticle layer. The inner root sheath surrounds the hair shaft and consists of cuticle, Henley and Huxley layers. It is bordered by the outer root sheath layer that contains proliferating cells derived from stem cells in the bulge that feed into the matrix compartment of the bulb. Melanocytes reside above the DP within the epithelial compartment and provide pigmentation to the hair shaft [26]. Morphogenesis initiated during all three waves continues well into postnatal development, when hair shafts eventually erupt from the skin around postnatal day P5 and follicles reach the most advanced stage of postnatal hair growth by days P13–15 [27].

2.3. Regeneration in the hair growth cycle

Once morphogenesis is complete, follicles are prompted to enter the first hair cycle by an unknown stimulus, either presumed to emanate from the DP, or by the absence of continuous growth stimuli from the DP [7]. Fully formed follicles transition into catagen, a destructive phase characterized by profound apoptosis in the epithelial compartment of the lower follicle including the matrix cells and all differentiating layers. The DP remains intact and moves upwards toward the permanent portion of the hair follicle, which contains epithelial and melanocyte stem cells in the bulge [28,29]. Most outer root sheath cells survive as well and move upwards to give rise to a second bulge containing new stem cells and the hair germ of transit-amplifying cells [30]. Whether this movement of DP and outer root sheath is due to active migration or a passive external tug is unknown; regardless, this shift brings the DP into

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close contact with the newly formed bulge and hair germ around P19 in the first hair cycle. After a short period of rest until P21, the DP emits signals that induce stem cell activation and proliferation of hair germ cells that grow down together with the DP to generate a new complete follicle, resembling the activation of epidermal stem cells during embryonic hair follicle induction [27,31].

3. Mesenchymal–epithelial interactions during embryonic hair follicle formation

3.1. Integrative overview of inductive signals and events

The early stages of hair follicle formation involve the tight temporal and spatial regulation of inductive signals in what is thought to be a sequential process of secreted molecules alternating from epidermis and dermis [4]. However, efforts to definitively place the major players such as Wnt, Eda, Fgf, and Bmp in such a cascade are complicated by the multifactorial nature of these interactions and the limited time frame in which these exchanges occur (Fig. 1). Nevertheless, widespread Wnt ligand expression in the epidermis seems to be most upstream event (Fig. 1A) [32]. Secreted Wnts from the epidermis are thought to incite similarly broad Wnt signaling activity within the dermis [32,33], which could in turn drive expression of the elusive first dermal signal(s) necessary to bring about hair follicle induction (Fig. 1B) [4,8,16]. Given that the concept of an inductive dermis was first described many years ago [34,35], it is remarkable that the underlying molecular mechanisms remain obscure. However, a singular epithelial signal promoting dermal cell condensation has not been definitively described either; rather, a number of molecules are thought to promote condensate formation and maintenance (discussed below) [4]. Therefore, it is possible that multiple dermal factors are involved to initiate induction as well.

Multiple molecular markers such as Wnt10b, Edar, Dkk4 and K17 pattern the epidermis before any visible signs of hair placodes [36–39]. Similarly, beneath these epidermal "pre-placodes", new markers such as Sox2 and Sdc1 identify groups of specialized dermal cells [40–42]. At this point in development, parsing out the precise timing and function of each signaling molecule or other genes within the greater scheme of mesenchymal–epithelial interactions becomes difficult because they appear virtually simultaneously. As a result, a comprehensive understanding of how all pathways interact remains incomplete. In the following chapter we provide a detailed discussion of individual signaling pathways implicated in morphogenesis, while noting confirmed upstream and downstream effectors, in an attempt to piece together a model of how these molecules cooperate during hair induction. These relationships are further depicted in Fig. 1C.

The factors that specifically promote follicle growth after induction are slightly more well-defined, since several mutants exist in which hair follicles are induced, but do not mature. In this regard, epithelial Shh and Pdgfa, in addition to Fgf and Tgfb2 ligands emitted from the dermis, are central to promoting hair germ formation (Fig. 1D). A balance of dermal Inhba (activin-βA) secretion and epidermal follistatin expression is similarly important for early progression of hair peg growth (Fig. 1E). In the future, advances in molecular analysis and tools to genetically and/or inducibly target specific compartments at precise time points during hair development will be invaluable to define the subtext underlying epidermal–dermal conversations.

3.2. Inductive signals in embryonic skin

The foundations of modern skin and hair development research were established many years ago by a "cut-and-paste" approach (reviewed in [8,9]). These classic experiments employed tissue recombination techniques to explore the functional basis of mesenchymal–epithelial interactions in skin appendage formation. Epidermal and dermal layers were separated from early mouse embryos, and recombined such that dermis from the hairy back was paired with epidermis from a glabrous region (e.g. hairless foot pad) – or vice versa – before further culture and assessment of hair growth [34,35]. The results of these grafts revealed that only dermis from hairy mouse backskin induced appendage formation, but dermis from hairless regions did not, regardless of the origin of the epidermal tissue. Therefore the inductive potential lies within the dermis, since the origin of dermal tissue dictated whether skin appendages developed.

Morphologically recognizable hair placodes in backskin first appear around E14.5, along with concomitant expression of signaling genes [4,16], and many studies have looked into the roles of these factors in orchestrating follicle induction and subsequent hair formation. The functions of canonical Wnt/β-catenin signaling [43] in epidermis and dermis are especially well-characterized, and it is clear that this pathway is necessary for hair induction [44]. Mutant mice lacking the transcription factor Lef1, a β-catenin binding partner, formed only rudimentary mammary gland, tooth and hair structures providing early evidence of the central role of Wnt signaling in skin appendage development [45]. Subsequent studies confirmed Lef1 activation modulates hair growth: transgenic Lef1 overexpression in epidermis resulted in pelage follicle crowding and ectopic hair growth within other epithelial tissues [46]. Further recombination experiments using wild-type and knockout skin demonstrated a selective requirement for dermal Lef1 expression in mediating normal hair growth [47]. In direct studies of Wnt signaling, transgenic expression of stabilized β-catenin in the epidermis led to de novo hair follicle formation [48], an effect confirmed later with inducible expression of stable β-catenin or epidermal deletion of the intracellular β-catenin inhibitor APC [49–51]. Moreover, early and sustained Wnt activation by epidermal expression of constitutively active β-catenin resulted in increased dermal fibroblast proliferation, precocious placode formation and later switched the entire epidermis to a hair fate or induced excessive, ectopic follicles [52–54]. Correspondingly, selective β-catenin ablation in the epidermis entirely prevented epithelial placode formation [33,55]. Forced expression of constitutively activated β-catenin within the dermis led to major skin phenotypes as well: overproliferation of mesenchymal fibroblasts and excessive follicle morphogenesis following precocious dermal condensate establishment [32]. Thus, a role for Wnt signaling in hair induction is well-established.

Wnt signaling reporter mouse lines have been particularly helpful for defining dynamic patterns of Wnt signaling activity during skin development [33,56–58]. Broad dermal activity driven by widespread epidermal Wnt ligand secretion (Fig. 1A) [32] precedes Wnt signaling in epidermal placodes [33]. Ablation of dermal β-catenin prior to hair induction precludes the expression of any placode markers by the epidermis and results in the failure of first wave hair formation. This suggests that widespread Wnt signaling in dermal cells regulates the first signal(s) to directly or indirectly promote hair fate specification in the epidermis (Fig. 1B) [32,59,60]. Concomitant with Wnt signaling activity in pre-placodes, dermal Wnt activity becomes intensified in underlying dermal condensates. Interestingly, ablating β-catenin in placodes abrogated this focused Wnt activity and resulted in a failure of dermal condensate formation [33,55]. The mechanisms that specifically support dermal condensate formation are not yet clear; Shh and Pdgfa signaling have been proposed in the past, but epidermal Wnt ligands themselves might also play a central role [4,61]. Wnt10a and Wnt10b are upregulated in the placode as morphogenesis begins and might perpetuate focused Wnt signaling activity within both placode and condensate [36]. Both Wnt5a, produced by dermal condensates,

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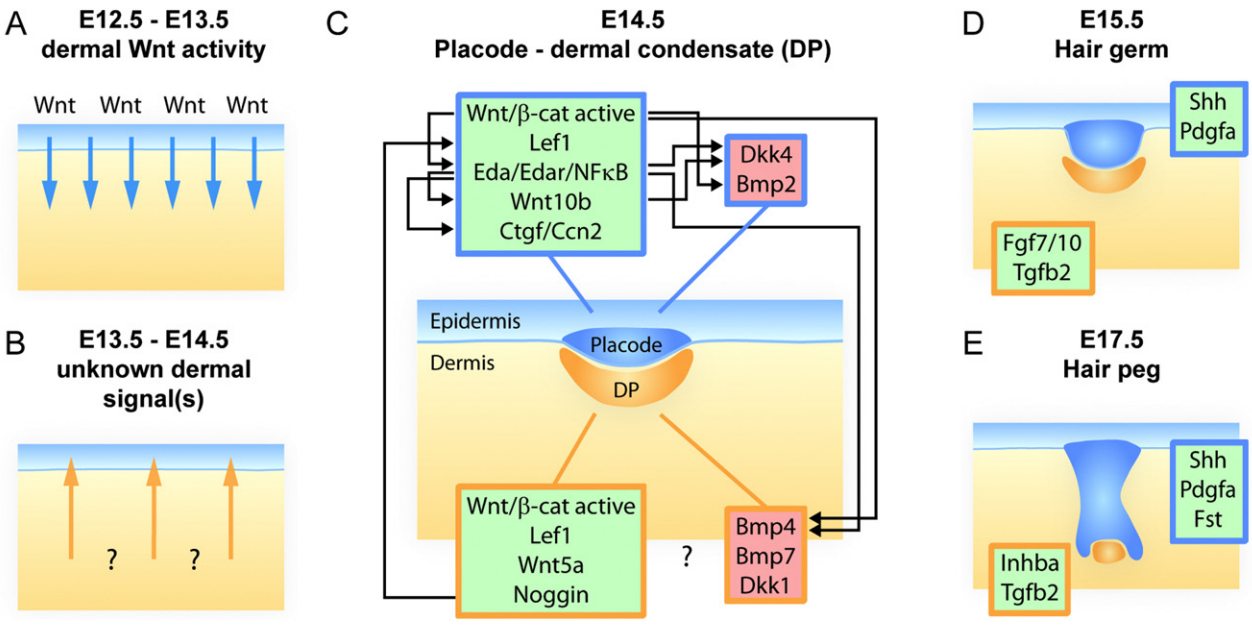

Fig. 1. Mesenchymal–epithelial signal exchange during hair follicle induction. Developmental stages (A–E) are represented schematically. (A) Epidermal Wnts activate dermal Wnt/β-catenin signaling. (B) Unknown dermal signal(s) induce an epidermal response leading to placode formation. (C) Activating (green) and inhibitory (red) signals from placodes and dermal condensates (DP precursors) consolidate pattern formation through reinforcing placode/DP fate and lateral inhibition on neighboring epidermis. The network diagram depicts known hierarchies and regulatory connections between signaling pathways (as described in text). (D and E) Signals regulating hair downgrowth at hair germ and peg stages.

and Wnt10a, turned on in dermis during downgrowth, may contribute as well.

Patterns of Wnt inhibitors in the developing skin are similarly dynamic and compartment specific, in that Dkk1 is expressed in the mesenchyme surrounding follicles during the first stages of downgrowth but is conspicuously absent from the follicle itself [20,36,62]. When this secreted Wnt inhibitor was misexpressed in transgenic epidermis, effectively blocking Wnt signaling in both adjoining epithelial and dermal compartments, the appearance of physical dermal condensates and downgrowths was completely abolished [63]. In contrast, Dkk4 is expressed in the placode of primary wave follicles [38]. It has been proposed to act in a lateral fashion along with BMP ligands to affect placode spacing (to be discussed further below). Intriguingly, overexpression of this factor affects only secondary wave hair morphogenesis while primary guard hairs form normally [64]. The role that these inhibitors play in compartmental crosstalk remains to be clarified.

In addition to Wnt, Ectodysplasin (Eda) signaling is similarly essential for hair follicle induction [6,65]. Eda is a Tnf family ligand [66] that signals through downstream NFκB transcriptional activation after binding to the corresponding Ectodysplasin receptor (Edar) [67,68]. The central role Eda signaling plays in skin appendage morphogenesis was first recognized because mutations in pathway components lead to human disorders of hair, tooth, and mammary bud formation [69]. Mouse models of mutated Edar (*downless*) or ligand Eda (*tabby*) have similar phenotypes [70,71], and are characterized by a sparse coat and absent guard hair formation [65]. During embryonic stages, Eda is widely detectable throughout the epidermis while Edar expression becomes confined to early placode structures. As development continues, Eda expression is progressively confined to the interfollicular epidermis [72]. Because both ligand and receptor are expressed only by the epidermis, Edar signaling appears to act as a purely intraepithelial method of communication, and indeed a number of studies suggest that this pathway is important for placode stabilization and patterning, but not necessarily for initial placode induction [33,73].

Recently the timing and hierarchy of Eda signaling with respect to Wnt/β-catenin signaling was clarified. Using reporter mice for both β-catenin and NFκB activity revealed that Wnt signaling precedes Edar activation, and crossing reporters with knockouts confirmed that Wnt signaling could be activated in the absence of Eda [33]. Conversely, inhibiting Wnt precludes Edar expression and NFκB activation, definitively placing Edar signaling downstream of Wnt pathway components during early hair induction (Fig. 1C). Nevertheless, placodal Wnt10b itself is a direct target of NFκB signaling likely reinforcing placode fate stabilization (Fig. 1C) [33]. Additionally, multiple studies found that the expression of Wnt inhibitor Dkk4 appears downstream of Edar signaling [38,64,74]. In terms of facilitating mesenchymal–epithelial interactions, Eda overexpression in Eda null skin explants identified both dermal Bmp4/7 and epidermal Bmp inhibitors to be downstream targets of Edar signaling [75]. This allows a model in which Dkk4 and Bmp4/7 diffuse laterally to act on surrounding interfollicular epidermis to suppress placode induction. In this reaction-diffusion model, the central placode remains unperturbed, thanks to the expression of Bmp inhibitors Ccn2 and Ctgf also downstream of Edar activation [20,22,63,75–77]. Finally, Shh has been identified as a downstream target of Edar signaling [77] which promotes initial follicle growth following induction.

Apart from Wnt and Eda signaling as promoters of hair induction, BMP signaling activity in embryonic skin has an inhibitory role. During early follicle formation, the BMP receptor Bmpr1a is expressed in the epidermal compartment along with BMP2. BMP4 expression is selectively upregulated in dermal condensates [78]. Noggin, a BMP inhibitor, is also expressed from this compartment; a balance of these contradictory signals is thought to fine-tune the dermal messages sent to an epidermal target at this stage of development (Fig. 1C) [79]. Neutralization of BMPs by noggin overexpression stimulated robust formation of excess placodes [80], while constitutive deletion of noggin impaired the induction phase of follicle generation [79,81]. Secondary follicle induction was specifically inhibited in noggin null embryos, and although primary follicles did form, they arrested at an early downgrowth stage

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lacking Lef1 and Shh expression. Interestingly, impaired epidermal BMP signaling in receptor-null mice promoted accelerated placode development, but was not sufficient to drive excessive follicle formation [82]. To add further complexity, when BMP signaling was abnormally sustained in noggin null skin, it could act back on the epidermal compartment to downregulate Lef1 and Wnt/β-catenin activity [83]. Such observations highlight the complex, overlapping nature of the signals involved in this process, and the intricate balance that needs to be maintained for successful morphogenesis.

Besides Wnt, Eda, and BMP pathways as major mediators of follicle induction, Fgf signaling has been implicated as well, although its role is less clearly defined. Multiple receptor and ligand isoforms are present during the early stages of hair development [84–88]. Transgenic mice expressing a soluble, dominant-negative Fgfr2IIIb isoform failed to develop hair [89], and Fgfr2IIIb knockout mice displayed delayed induction suggesting that Fgf ligands work to promote placode establishment [90,91]. However, more recent investigations conclude that Fgf signaling actually deters induction. Immunostaining for Fgfr2IIIb reveals widespread expression throughout E13.5 epidermis, and then subsequent downregulation in placodes [42]. The role of Fgf signaling in normal hair follicle induction thus requires further study and clarification.

3.3. Initial growth after induction

After induction, placode cells start to proliferate and generate morphologically recognizable downgrowths under the direction of two central signaling pathways: Shh and Pdgf. Shh is first expressed in the developed placode and then localized to the tip of the downgrowing bulb in contact with the DP as development proceeds [78,92]. The Shh receptor Patched is expressed by both epidermal and dermal compartments from an early stage [61]. Shh knockout mice revealed an important role for this signaling pathway in mediating early hair formation [93,94], since hair germs arrested at the early downgrowth stage. Both epidermal and dermal components of these early follicles were already recognizable suggesting that Shh signaling, while dispensable for induction, is crucial for these slightly later stages. To place this pathway in the context of mesenchymal–epithelial interactions, studies used epithelial or dermal-specific ablation of primary cilia components to effectively abrogate Shh signaling separately within each compartment [95,96]. Only dermal-specific knockout mice had a similar hair phenotype as Shh mutants, suggesting that secreted Shh activates effector pathways in a responsive dermis that directly or indirectly supports placode proliferation (Fig. 1D). Very recently, studies in which Smoothened was knocked out in early embryonic dermis have conclusively proven that Shh signaling within dermal condensate cells is crucial for DP development and subsequent hair follicle maturation [97]. Earlier studies of Shh pathway knockouts found normal Wnt10b, Lef1, and Bmp2/4 expression in arrested follicles, indicating that hedgehog signaling either lies downstream or functions independently of these inductive molecules [93,94]. Complementary analyses have confirmed abrogated Shh expression in mice lacking epithelial Wnt or Eda, thus implicating it as a target [55,73–75,77]. However, other dermal factors such as Wnt5a and Pdgf receptor Pdgfra were found to be dysregulated in Shh null follicles [36,61]. Wnt5a expression was completely missing from stalled follicles in Shh mutants, while Pdgfra expressing dermal cells were still present but abnormally dispersed [61]. Since these mice displayed normal Pdgfa ligand expression, the study concluded that downstream targets of Shh signaling within the dermis mediate Pdgf responsiveness and the effects of these two pathways are jointly important for maintenance of the DP.

The role of Pdgf signaling in hair morphogenesis was recognized because Pdgfa knockout mice have sparse coats that degenerate with age. This system provides a clear example of mesenchymal–epithelial interactions, as the ligand is secreted solely by epidermis and the Pdgfra receptor is uniquely expressed in the dermis (Fig. 1D) [61]. Pdgfa expression is initially robust and widespread in E13.5 epidermis before becoming concentrated in early stage placodes [61]. On the dermal side, Pdgfra expression is broadly present throughout the upper dermis early on, but becomes progressively restricted to cells within the DP and along the dermal sheath. A significant percentage of Pdgfa knockout mice die during embryogenesis, but those that survive display abnormally sparse hair and thin skin phenotypes due to diminished white adipose tissue stores. The hair follicles that do appear form normally, suggesting that the signaling pathway is not essential for induction, but the primary coat cannot be maintained and the secondary coat, which usually appears at the first postnatal anagen starting after day P21, is never generated [61]. Pdgfra knockouts die during embryogenesis, but analysis of early skin reveals that follicles form normally, confirming that this signaling axis is not necessarily involved in induction.

Tgfb signaling also promotes hair germ growth; in particular, mesenchymally-expressed Tgfb2 acts on epithelial receptors (Fig. 1D) [98–100]. Full Tgfb2 knockout mice displayed delayed and/or arrested follicle growth at E18.5 reminiscent of Shh null mutants. Furthermore, culturing skin explants in vitro with exogenous Tgfb2 promoted excessive follicle growth [101]. Finally, a role of Tgfb/Activin signaling in hair morphogenesis was recognized because Inhba (activin-βA) ligand knockout mice lack vibrissae at birth [102,103]. Moreover, epidermal-specific receptor knockout mice produced fewer and misshapen follicles that degenerate over time, suggesting dermally-generated ligands are needed to direct both early and late stages of differentiation within the epidermal compartment [104]. The related molecule follistatin, which inhibits activin and Bmp ligands, is expressed from the epithelial compartment and has been investigated in the context of hair growth as well. Surprisingly, full knockouts resemble Inhba knockouts, with fewer, stunted follicles at birth. These findings suggest follistatin works to fine-tune inputs from separate Tgf signaling avenues before morphogenesis can move forward [105,106].

From several of the above-mentioned studies the idea emerges that varying input from multiple signaling cascades leads to the specification of unique hair types. For example, mouse models with compromised Edar signaling lack only guard hairs, indicating that this cascade is uniquely necessary for first wave follicle induction [37]. Conversely, only guard hairs can form in the absence of noggin [81], suggesting that inhibition of Bmp signaling is distinctly required for second and third wave induction. When Shh is overexpressed in the epidermis, both first and second wave follicles are missing, and only third wave zigzag follicles are induced to form [107]. Unique gene expression profiles within the mesenchymal component of the HF specify hair type as well [40]. A differential requirement for Wnt signaling in either compartment has not yet been described, except that epidermal overexpression of Dkk4 appears to affect only second wave morphogenesis [64]. Taken together, evidence from these mutants suggests that the correct balance of morphogens is necessary for the development of discrete hair types, adding yet another layer of complexity for defining a hierarchy of the central signaling pathways implicated in hair formation.

4. Postnatal hair follicle induction capacity

4.1. Inductive capacity of mature DP

Dermal condensates in embryonic hair follicles are precursor cells of the DP in fully formed hair follicles. Although it is believed

that dermal condensates require stimuli from the placode to form, mature DP cells retain hair inducing activity independent of placodal signals. Early studies demonstrated that microdissected DPs could induce new hair growth after transplantation into glabrous skin of the foot pad [108]. Similarly, adult rat DPs from pelage follicles were microdissected, cultured as single cells and then implanted as cell clumps below foot pad epidermis to induce hair follicle formation from overlying afollicular epidermis [109]. Subsequent refinement of hair induction protocols by growing hairs at the skin surface in chamber grafts [110] or deep in the subcutaneous skin tissue [111] now allows hair induction to be assessed for hundreds of hairs simultaneously. Using such methods, pure DP cells isolated based on fluorescent markers from postnatal backskin retained hair induction capacity when transplanted together with postnatal epidermal cells [14,40].

Interestingly, the hair type origin of DP cells also determines the type of experimentally induced hair follicles; for example, whisker DP cells induce whisker-like follicles on mouse ears [109]. Recent transcriptional profiling of DP cells from pelage follicles generated a DP gene signature [14] and DPs from pelage hair cell types retain a core signature but also exhibit distinct gene expression profiles [40]. Sox2, for example, is robustly expressed in guard and awl/auchene DP, but not in zigzag DP. The functional importance of this difference was recently illustrated by isolating pelage DP based on Sox2 expression prior to using these cells in separate hair-reconstitution assays. Isolated Sox2-negative DPs, when combined with keratinocytes in chamber graft assays, produced only zigzag type hairs. These experiments highlight the importance of mesenchymal–epithelial interactions in hair formation and provide powerful evidence that such interactions help drive hair type specification during morphogenesis [40].

4.2. Adult follicle neogenesis after wounding

According to common knowledge, de novo hair follicle morphogenesis is a one-time affair that is limited to embryogenesis and early postnatal development. However, over half a century ago observations in adult rabbits, mice and even humans suggested the potential of new hair follicle formation in the context of a wound response [112–115]. Recently wounding-induced hair follicle formation was confirmed with elegant experiments in mice, in which definitive genetic fate mapping demonstrated the origin of new follicles, including their stem cells, from neighboring epidermal cells during reepithelialization [116]. Ablation of Wnt signaling in the healing wound completely abrogated new hair formation. The potential role of an inductive mesenchyme and the origin of the newly formed DPs has yet to be examined in this context.

5. Compartmental crosstalk during postnatal hair growth

After the early stages of downgrowth are complete, the DP is thought to direct neighboring epithelial matrix cells to proliferate and differentiate into the multiple cell types that form the hair shaft and its channel [3]. Several signaling programs central to induction are involved in these later stages of follicle maturation as well (Fig. 2); for example, Wnt signaling activity and nuclear Lef1 and β-catenin expression in maturing hair shaft precursors point to an important role of this pathway [56,117]. Hair shaft keratins are regulated by Wnt signaling activity [117], and forced activation of Wnt signaling drove matrix cells into differentiating hair masses resembling human benign hair tumors [48,118]. Inducible β-catenin ablation to block Wnt signaling activity in the matrix cells specifically during the hair growth phase has not yet been performed. However, active signaling in the dermal compartment is important at this stage; cultured DP cells grown in the presence

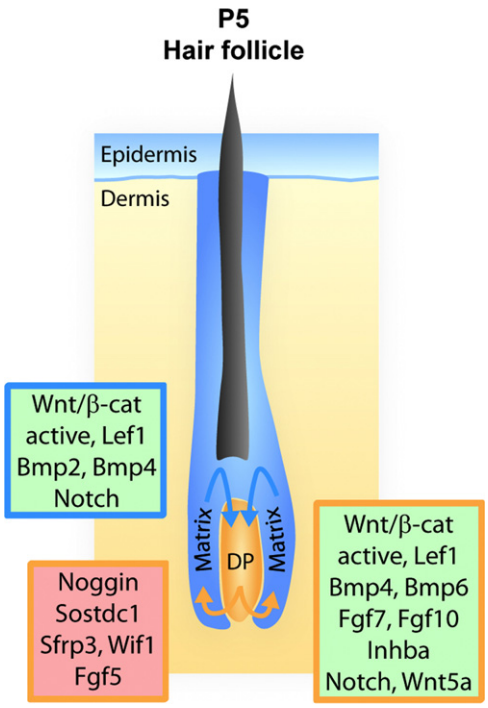

Fig. 2. Signaling between matrix and DP during hair follicle growth. Multiple positive and negative regulators are in both compartments that may also signal in an autocrine fashion.

of Wnt ligands retained hair inductive capabilities [119], and postnatal ablation of β-catenin in the DP compromised hair growth [120].

The importance of Bmp signaling is also reiterated during postnatal hair growth. Follicles formed when the Bmp receptor was selectively deleted within the epithelial compartment, but matrix cells were unable to undergo the proper program of maturation and differentiation [82,121,122]. Ultimately, highly abnormal follicles were generated because of an inherent inability of epithelial progenitors to stabilize Lef1 and activate Wnt signaling. In other investigations of Bmp signaling in postnatal growth, ligand overexpression inhibited proliferation within the outer root sheath resulting in small and misshapen follicles that were unable to regenerate [123]. Overexpression of the Bmp inhibitor noggin leads to excessive matrix cell proliferation and prevented hair shaft maturation [124]. An important role for Bmp activity within DP cells exists as well [125]. Ablation of Bmp signaling in isolated DP cells abolished their ability to organize hair growth in a chamber graft assay, suggesting that Bmp activity within the DP is required for instructive capabilities.

Another pathway important for organizing hair growth during postnatal morphogenesis is Fgf signaling through Fgf7/Fgf10 ligands [86]. Neonatal Fgfr2IIIb null skin, insensitive to both ligands, displayed cystic or misaligned follicle growth when cultured in grafting experiments [91]. Finally, Notch signaling also appears to participate in hair maturation, since mice with disrupted dermal Notch signaling developed intrinsic hair shaft defects [126]. Decreased Wnt5a in DP and reduced Foxn1 in matrix cells were part of the mechanism behind this phenotype. Notch signaling within matrix progenitors is also necessary to maintain proper terminal hair differentiation [127,128].

Several intrinsic transcriptional regulators such as Cutl1, Gata3, Hoxc13, Foxn1 and Msx2 directly affect hair shaft differentiation, structure and shape (reviewed in [2]). Whether mesenchymal–epithelial interactions are involved or these factors function in a compartment-autonomous manner remains to be

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determined. Egf, Igf and Tgfa signaling pathway activation can also affect hair shape [2].

After the anagen growth period, follicles enter the catagen destruction phase, which also seems to be regulated by mesenchymal–epithelial interactions and influences from the macroenvironment [16]. Knockout mice lacking Fgf5, which is expressed in DP, are characterized by abnormally long hair due to a prolonged anagen phase, indicating that signaling through this ligand promotes catagen entry [129]. Other examples of factors that advance the anagen/catagen transition include Bdnf, IL1b, Ntf3, Tgfb1 and Tnf, while Hgf, Igf1 and Vegf promote anagen maintenance (reviewed in [130,131]). The direct source of origin and the potential involvement of mesenchymal–epithelial interactions for many of these molecules remain to be clarified.

6. Signals during hair regeneration

6.1. Signals from the dermal papilla

During the anagen growth phase DP cells in the bulb are far removed from bulge epithelial stem cells in the upper part of the follicle, and most likely do not contribute to regulation of stem cell quiescence [15,132,133]. Other cell types in the immediate stem cell microenvironment or niche, such as endothelial cells, Schwann cells and nerve endings, and dermal sheath cells are considered to provide signals keeping the stem cells in a quiescent state [31,134]. Although tantalizing gene expression analyses in the stem cells suggest such a model [12,13,15], direct evidence is lacking. The same analyses proposed secreted factors generated by stem cells may regulate their own behavior in an autocrine fashion. In addition, bulge epithelial stem cells affect neighboring melanocyte stem cells [135,136] and muscle progenitor cells just outside the bulge that give rise to the arrector pili muscle [137], and in return these cells may influence epithelial stem cell behavior as well. On the other hand, many stem cell intrinsic factors, such as transcription factors Lhx2, Nfatc1, Runx1, Sox9, Stat3, Tcf3/Tcf4 were shown in loss of function studies to directly affect stem cell quiescence and activation, and subsequent hair regrowth during the hair cycle [23,138–144]. Again, direct regulation of these factors by interactions of the epithelial stem cells with the neighboring mesenchyme has not been established yet, leaving the possibility that these essential genes are regulated cell-autonomously and not necessarily influenced by mesenchymal–epithelial interactions.

As the hair cycle ensues, DP cells move upwards toward the skin surface during the catagen destruction phase and come to rest next to the bulge stem cells and hair germ progenitor cells during the telogen resting phase. It is not clear whether DP cells join the niche efforts to regulate stem cell quiescence, but historically the presence of DP cells next to the stem cell compartment is considered essential for activating stem/germ cells to regenerate the follicle in a new anagen growth (Fig. 3) [3]. While conceptually appealing, this model lacked substantiating evidence until very recently because of the absence of DP-specific inducible gene targeting tools to directly interrogate the role of genes in the DP for stem cell activation in the bulge. Nevertheless, without such tools, the activating role of the DP was confirmed by using laser ablation to selectively target DP cells in vivo during hair cycling [145]. After DP cells were physically disrupted corresponding follicles became quiescent while neighboring unaffected follicles continued to cycle. Other examples supporting the instructive role of DP cells during hair re-growth came from hairless (Hr) and vitamin D receptor (Vdr) mutant mice, in which DP cells fail to move upwards toward the bulge during the catagen destruction phase, leaving DP cells stranded deep in the dermis [146,147]. New hair follicle regeneration at the end of telogen is absent, suggesting that the presence of DP cells next to bulge stem cells is important for inducing new hair re-growth. More

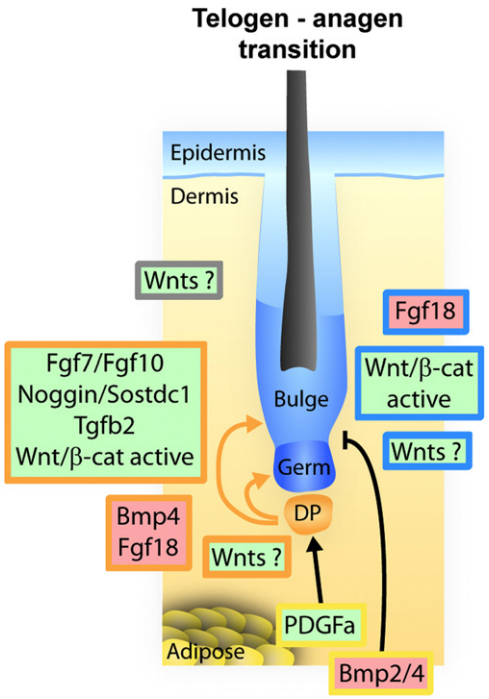

Fig. 3. Signals regulating stem cell quiescence and activation during the hair cycle. Bmp2/4 from DP/adipose tissue and Fgf18 from bulge/DP inhibit stem cell activation. Activation of Wnt signaling in the bulge and secreted Fgf7/10 and Bmp inhibitors from the DP activate stem cells to re-grow a new follicle during hair regeneration.

recent, albeit indirect evidence comes from work demonstrating that DP-derived Fgf7 and Fgf10 are involved in promoting hair follicle regeneration during the anagen to telogen transition [148]. Exogenously supplied Fgf7, normally expressed in DP cells [14], induced bulge/hair germ proliferation, suggesting that DP-derived Fgf7 could be a stem cell activating signal (Fig. 3). Another cytokine that could act on the stem cells both in an autocrine fashion and through mesenchymal–epithelial interactions is Fgf18, which was found to be expressed in bulge cells and to inhibit bulge cell proliferation in vitro [13]. More recently, Fgf18 expression was described as high in both DP and bulge cells during mid-telogen, and ablation of the factor in the stem cell compartment prompted rapid progression into active hair growth (Fig. 3). Additionally, Fgf18 could suppress hair growth in studies involving the injection of recombinant Fgf18 protein [149]. Genetic tools to selectively target genes of interest in the DP will be necessary to understand the molecular mechanisms behind DP-induced stem cell activation during hair cycling.

Many recent studies have also demonstrated a critical role for Wnt and Bmp signaling during hair regeneration in terms of controlling stem cell quiescence and activation [28,150,151]. Forced activation of Wnt signaling through expression of stabilized β-catenin led to precocious stem cell activation in the bulge [50,152,153]. Conditional and inducible ablation of β-catenin in the bulge during telogen showed a loss of quiescence and depletion of stem cells [152]. Therefore inhibition of Wnt signaling by Tcf3 within the stem cells [142] and by secreted Wnt inhibitors from the stem cells [15] and the niche [148] appear to be crucial for maintaining stem cell quiescence, while activation of Wnt signaling is required for the transition to a new hair growth phase (Fig. 3). In a reversed role to Wnt signaling, active Bmp signaling is required for stem cell quiescence, since ablation of Bmp receptors in stem cells leads to aberrant stem cell activation [154,155]. It appears that for stem cell activation and new hair follicle regrowth to occur, upregulation of Bmp inhibitors in the DP [148,156] and downregulation

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of long-range Bmp signals from deep in the dermis (see below) have to coincide with activation of Wnt signaling in the bulge (Fig. 3).

Most recent evidence also implicated an essential role of Tgfb signaling in the stem cell compartment. By selectively ablating the Tgfbr2 receptor expressed in stem cells, these studies demonstrated that Tgfb2 ligands generated in the DP act on the epithelial compartment to promote a switch from quiescence to active regeneration [157]. Downstream of activated Tgfb2 signaling, target genes suppress propagation of Bmp signaling and allow onset of a new round of follicle cycling. This is consistent with earlier studies, in which authors were able to provoke premature anagen by injecting recombinant Tgfb into skin [101].

6.2. Role of the macroenvironment

Besides influences from the local stem cell microenvironment, fat tissue deeper in the dermis was recently described as a heretofore unrecognized niche cell population, capable of secreting factors to influence hair cycling from a distance. Fat-derived Pdgf in particular was proposed to act on DP cells which in turn regulate induction of follicle regeneration in the hair cycle (Fig. 3) [158]. Mutant mice with defects in skin adipocyte precursor cells, which normally express high levels of Pdgfa ligand, lacked Pdgfra receptor activation in DP cells. Hair re-growth failed during the cycle, but could be recovered by injecting beads soaked in Pdgfa, suggesting that fat-stimulated activation of this signaling pathway in the DP niche elicits downstream events to trigger follicle regeneration.

Influences from fat may regulate the behavior of cohorts of hair follicles at once, providing macroenvironmental cues that can affect larger domains of the hair coat in which all follicles cycle together in a dynamic fashion. Such a model is supported by recent findings of cyclical Bmp expression in the fat domain [159]. High Bmp levels reach the bulge area and help to keep Wnt-repressed stem cells quiescent, thereby promoting a refractory telogen phase. Together with activation of Wnt/β-catenin signaling, widespread downregulation of long-range Bmp signals then promotes stem cell activation and new hair re-growth during an "induction competent" phase [160].

7. Concluding remarks

Hair follicle morphogenesis is an excellent model system in which to explore universal developmental themes, and studies of mesenchymal–epithelial interactions in this context have been particularly robust. As described in this review, numerous aspects of the communication between epidermis and dermis during hair induction, growth and regeneration have been uncovered. Nevertheless, despite decades of increasingly meticulous investigation, many details of the complex mechanisms driving hair follicle morphogenesis and cycling remain obscure. Studies have been hindered by multiple signaling isoforms that impart redundancy, as well as intricate pathway intersections and feedback loops that are difficult to untangle using mouse models. Two central mysteries that remain to be explored are the nature of the first dermal signal(s) during embryonic hair follicle induction and the activating signal(s) from DP cells during hair regeneration in the cycle. Clarification of timing, origins, and targets of important signaling pathway components will be necessary as well. Additionally, advances have been hampered by the absence of tools to specifically manipulate gene expression in inductive DP precursors during early formation stages and adult DP cells during regeneration. Compartment–specific genetic drivers to target the placode and lineages in the mature hair follicle will be useful as well. As our tools continue to be refined, so too will our understanding of how epithelial and mesenchymal tissues cooperate to create such elaborate and patterned structures as the hair follicle, imparting a greater understanding of developmental paradigms and potentially information about hair growth that will be useful in clinical applications.

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