

The mesenchymal component of hair follicle neogenesis: background, methods and molecular characterization

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Abstract: Hair follicle morphogenesis and regeneration occur by an extensive and collaborative crosstalk between epithelial and mesenchymal skin components. A series of pioneering studies, which revealed an indispensable role of follicular dermal papilla and dermal sheath cells in this crosstalk, has led workers in the field to study in detail the anatomical distribution, functional properties, and molecular signature of the trichogenic dermal cells. The purpose of this paper was to provide a practical summary of the development and recent advances in the study of trichogenic dermal cells. Following a short review of the relevant

literature, the methods for isolating and culturing these cells are summarized. Next, the bioassays, both *in vivo* and *in vitro*, that enable the evaluation of trichogenic properties of tested dermal cells are described in detail. A list of trichogenic molecular markers identified by those assays is also provided. Finally, this methods review is completed by defining some of the major questions needing resolution.

Key words: dermal papilla – dermal sheath – hair follicle – mesenchyme – trichogenicity

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Background: The key role of mesenchyme in hair follicle neogenesis

Skin appendages including hair follicles, tooth, feather and mammary glands develop and regenerate by means of extensive epithelial and mesenchymal interactions (1–7). The inductive role of mesenchymal cells in skin appendage formation has been demonstrated experimentally by disrupting epithelial–mesenchymal interaction or by testing heterotypic tissue recombinations [e.g. (8–12) see Review by Refs (5,13–15)].

The indispensable role of the follicular dermal papilla and its related dermal sheath cells in hair follicle development and regeneration was demonstrated near 50 years ago (16–19). Since then, numerous follow-up studies have helped to characterize the molecular, cellular and biological nature of the trichogenic dermal cells as well as the mechanisms how these specialized fibroblast populations interact with trichogenic epithelium. In this methods review, we briefly summarize the development of this field of research and define our use of terminology. We then review the methods for isolating and culturing trichogenic dermal

cells, and for sustaining and probing their trichogenic properties with instructive bioassays, both *in vivo* and *in vitro*. After reviewing currently recognized molecular markers of trichogenicity, we conclude by delineating some major open questions.

At the outset we define the hair follicle (HF), as Chuong et al. (20) suggest: ‘The proximal end of the skin appendages shows a follicle configuration, with epithelial filament coming out of the distal end of the follicle and dermal papilla sitting at the base of the follicle’ (for other criteria see Ref. 20). The ‘trichogenicity’ of a cell refers to its ability to induce a hair follicle and/or to promote its morphogenesis, i.e. ‘folliculogenesis’. By ‘dermal papilla’ or, more appropriately, ‘follicular dermal papilla (FDP)’ (so as to distinguish it from the papillae of the papillary dermis that insert between two epidermal rete ridges), we refer to that mesenchymal cell condensation at the deep, i.e. proximal, end of the HF (Fig. 1a). These FDP cells are in close communication and exchange with another trichogenic fibroblast population, the cells of the dermal sheath (DS), also called connective tissue sheath (21–23) (Fig. 1a).

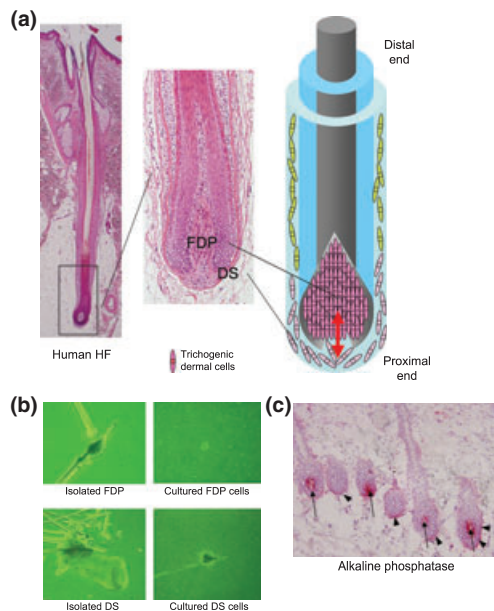


Figure 1. Trichogenic dermal cells in hair follicles and their biochemical distinctiveness. (a) Follicular dermal papilla (FDP) cells and dermal sheath (DS) cells at the lower suprabulbar to bulbar level of hair follicles (HFs) were shown to possess hair inductive capacity. Hair cycle dependent dermal cell trafficking between FDP and DS cells via the stalk of FDP has been demonstrated (ref. 21, 23; red arrow). (b) Cultured FDP and DS cells spontaneously aggregated and were indistinguishable morphologically (human samples). (c) Both FDP (arrows) and trichogenic DS cells (arrowheads) could be distinguished by up-regulation of biomarkers such as alkaline phosphatase.

Both FDP and trichogenic DS fibroblasts are distinguished from other dermal cells (fibroblasts) by their *in vitro* biological properties [e.g. support for epidermal cell growth (24–27), aggregative behaviour in culture (28) (Fig. 1b) and up-regulation of biomarkers such as alkaline phosphatase (AP) (29–31) (Fig. 1c), alpha-smooth muscle actin (32), (Fig. 1c), Id3 (33), epimorphin (34) and protease-activated receptor-1 (35)]. The cell trafficking between FDP and DS during the hair cycle is an intriguing, but still not fully understood phenomenon, which will be discussed later in this paper. The overall structure of the HF and the anatomical location of trichogenic dermal cells are illustrated in Fig. 1a.

Historical evidence for the trichogenicity of FDP and DS cells

Trichogenic capacity of the FDP in mature HFs was experimentally demonstrated by a series of seminal studies using adult rat vibrissa or whisker follicles (16–18,36,37) (for review, see Refs 38,39). In those studies, microdissected bulb region of follicles that contained the FDP, or an isolated FDP, were either implanted into the afollicular skin or into amputated vibrissa follicles without the bulb.

Implanted dermal components induced new hair formation or re-activated hair production in epithelial components (16–18,36,37).

At least in the adult HF, DS cells also have hair inductive properties. When the end-bulb with less than one-third of the lower vibrissae follicle was amputated, or the FDP alone was surgically removed from the follicular bulb, a new FDP formed (40,41), suggesting that DS cells contributed to and, probably reconstituted, the new FDP (41). Hair inductive capacity of DS cells was more directly demonstrated by implantation of microdissected DS into the bottom of a bisected upper half of rat vibrissa follicle in contact with outer root sheath (42). In implanted hair follicles, not only did shaft elongation restart, but in addition, FDP appeared to regenerate from implanted DS. Though not yet repeated by other investigators, in a landmark study, Reynolds et al. (43), implanted dermal sheath from a male human scalp follicle into the arm of a female recipient with the resultant formation, or transformation, of a new hair follicle. In aggregate, the above observations suggest that DS cells possess trichogenic activity just like the papilla (Fig. 1a). Since the above allotransplant experiment suggested that DS and FDP could also escape immunological rejection, these mesenchymal structures may enjoy a relative level of immune privilege [even though they are unlikely to represent an immunoprivileged tissue site themselves; for discussion, see Ref. (44)].

Methods for isolating FDP and DS cells (Table 1)

Surgical microdissection of FDP from the rat whisker follicles was first established by the laboratories of Cohen and Oliver (16,40). Later, Messenger isolated human FDP using similar methods (45). Since then, microdissecting approaches have been the gold standard for obtaining FDP (for details see Ref. 46) (Fig. 2).

In a more simplified method, the DS capsule of amputated bulbs is transected at the level of the stalk to remove the proximal DS (47). Pressure from the gripping forceps facilitates the release of the FDP from the hair bulb matrix. In this preparation excessive mechanical traumatization of the FDP must be scrupulously avoided.

To enhance the yield and to further simplify the methodology for the isolation of human FDP cells, enzymatic digestion can be employed (48). In this protocol, the proximal portion of HFs was enzymatically digested to retain the protease resistant FDP. A major caveat of this approach is that enzymatic digestion of collagen IV and fibronectin deprives the FDP of its natural and distinctive extracellular matrix environment (15,21,49–52), possibly unstabilizing it. For that reason, enzymatic isolation techniques should be avoided, if maximal preservation of the natural 'habitat' of

Table 1. Methods for isolating FDP cells

Method	Description	Species	Advantage	Disadvantage	Culture Conditions	Reference
Surgical Micro-dissection	Dissect bulb of HF; cut the DS to expose the FDP; release the FDP by pressure or cutting the stalk	Mouse, rat, human	Preserves the intact FDP	Labor intensive	Outgrowth from FDP, monolayer in subsequent culture or as spheres in suspension (mouse)	16, 40, 45, 46
Enzymatic Dissociation	Collagenase treatment of the proximal portion of HF; stop the reaction when DS is digested	Human	Reduced labor, more efficient	Destroys the natural habitat of FDP	Outgrowth from FDP, monolayer in subsequent culture	48
Fluorescence Sorting	Sort FDP cells with fluoresce labeled surface markers	Mouse	Efficient with high purity	Identifying proper markers for human FDP	As monolayer	54,55,57

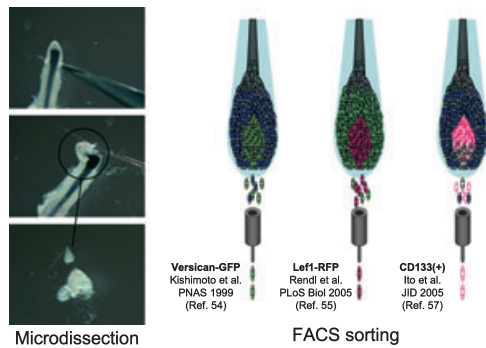


Figure 2. Directed isolation of living FDP cells. FDP and DS have been isolated by manual microdissection; however, elegant transgenic approaches or a newly identified cell surface marker enabled high-throughput isolation of living FDP cells in mice.

FDP cells is experimentally desired. In this preparation, minor contamination of DS cells is possible. Such techniques as toluidine blue staining (48) or the detection of intranuclear rodlets (53) could be applied to confirm the purity of isolated FDP cells in culture.

FDP cell isolation could be more efficient, when using fluorescence activated cell sorting (FACS). Taking advantage of the up-regulation of versican or Lef1 in the mouse FDP, Kishimoto and Rendl bioengineered transgenic mice that expressed fluorescent proteins under the control of versican or Lef1 promoters in FDP (54,55) (Fig. 2). By FACS isolation, they were able to isolate trichogenic GFP or RFP positive murine FDP cells (54,56).

Recently, Ito et al. identified CD133 as a surface marker for mouse FDP cells during the early anagen phase (57) (Fig. 2). FACS sorted CD133 positive FDP cells exhibited trichogenicity *in vivo* (57). Unfortunately, CD133 was not detected in human FDP cells (57). Robust cell surface markers for FACS-sorting of human FDP or DS cells remain to be defined. Recent finding that human HF can be transiently transfected with a GFP construct driven by the keratin 15 promoter (see abstract: Tiede S et al. In situ targeting of epithelial stem cell populations in the human

hair follicle by use of the keratin15 promotor. *Exp. Dermatol.* 2007 16, 278), suggest that similar methods may be useful for demarcating and sorting human FDP cells.

Compared to the FDP, methodologies to isolate other trichogenic mesenchymal cell populations such as embryonic mesenchymal cells or mature DS cells still rely on classic combinations of microdissection and enzymatic digestion (30,42,43,58,59).

Methods for culturing trichogenic FDP and DS cells (Table 1)

In early attempts to establish culture condition for hair follicle cells, very heterogeneous mixtures of HF cell populations were used after isolation by enzymatic digestion (60,61). In 1981, Jahoda and Oliver first reported *in vitro* cultivation of microdissected rat vibrissa FDP cells (62). Later, Messenger et al. demonstrated that human FDP cells could also be cultured successfully (45). Characteristically, cultured rat FDP cells demonstrated significant aggregative behaviour and a slow growth rate compared to interfollicular fibroblasts (Fig. 1b) (28).

Moreover, and most important, serially cultured FDP cells were able to induce the growth of hairs, when implanted into the base of surgically truncated vibrissa follicles (63,64). In addition, low-passage, cultured rat vibrissa FDP cells induced vibrissa-type hair follicles in the pelage area (65). In the same manner, DS cells could be cultured (13,30) (Fig. 1b) and their *in vivo* hair inductive capacity was demonstrated in mouse (30).

Stabilizing trichogenic activity of cultured dermal papilla cells

Since common culture conditions do not replicate the biological niche, the characteristic properties of most cells in artificial tissue culture milieu will be challenged, if not lost. Indeed, cultured FDP cells were reported to lose their hair inductive capacity in culture with passage (63,64,66,67). Considering that FDP cells *in vivo* are in intimate contact

via native extracellular matrix to the epithelial bulb (15,49–51,54,68–70), it is logical to assume that the co-culture of FDP cells with epithelial cells should stabilize the trichogenic activity of hair follicle-derived mesenchyme.

Indeed, Inamatsu et al. established rat FDP cell lines that reportedly sustain trichogenic capacity for at least 70 passages by culturing them in keratinocyte conditioned medium (71). In line with this notion, when non-hair inductive high-passage FDP cells or low-passage DS cells were implanted with hair matrix germinative epithelial cells, dermal inductive activity was restored as demonstrated by the induction of hair follicles in ear skin wounds (72). The full list of keratinocyte-derived factors that are responsible for the maintenance of hair inductive potency is yet to be identified. However, recent reports suggested that WNT signalling molecules and bone morphogenetic proteins (BMPs) are among those key factors for maintenance of hair inductive capacity (56,73–75).

The aggregative behaviour of FDP in culture ('mounting', see Fig. 1b) is one of their most characteristic features, which is somewhat analogous to the formation of the HF dermal condensate in folliculogenesis (5,15,28,45,62,76). Interestingly, late passage FDP cells, which showed no trichogenic capacity as dissociated cells, could induce hair follicle neogenesis after they were aggregated to form spheres (77). Thus, sphere formation would appear to be one of the potential approaches to restore the trichogenicity to cultured papilla cells.

The specialized extracellular matrix environment of the FDP, which is rich in laminin, type IV collagen, fibronectin, olfactomedin and versican, and the basement membrane zone covering the papilla play a crucial role in epithelial–mesenchymal interaction (49–51,54,68,69). When one of these extracellular matrix proteins is mutated, as in the laminin-511 null mouse, early hair follicle morphogenesis is impaired (78). Presumably, exogenous supplementation or the enhancement of endogenous production of such extracellular matrix could stabilize or enhance trichogenic capacity of cultured dermal papilla cells.

Collectively, mimicking the natural FDP or DS microenvironment as closely as possible *in vitro* should optimize growth conditions. Additionally, it may also be important to recreate the topobiological characteristics of the FDP, namely its prominent expression of certain cell adhesion molecules, such as NCAM (79,80).

Methods for assaying trichogenicity

Principles

Despite the variety of trichogenic cell assays reported in the literature, all assays are based on the same principle: combining responder epithelial cells with inducer mesenchymal cells that are placed into a permissive environment.

Each type of assay can be distinguished by:

- (1) The origin of the experimental sample (neonatal dermis, adult FDP or DS, etc.),
 - (2) the condition of the tested dermal population (cell assembly or dissociated cells, cultured or not),
 - (3) the epithelial counterpart used for combination (e.g. amputated HFs, whole epidermis, epithelial sheet, or dissociated keratinocytes, etc.),
 - (4) the environment surrounding the recombined test sample (if *in vivo*, e.g. under the subcutaneous tissue or kidney capsule or into a more artificial condition such as grafted silicone chamber or trachea; if *in vitro*, in liquid culture or 3D culture with scaffold, etc.),
 - (5) the readout parameters employed (e.g. hair shaft elongation, complete HF induction or reconstitution, partial reproduction of HF neogenesis).
- Previously reported trichogenic assays are summarized below (for historical assays not in frequent use any longer, see above).

***In vivo* assay to measure trichogenicity of dissociated dermal cells (Table 2)**

Chamber assay

Yuspa et al. reported that the mixture of cultured neonatal epidermal and dermal cells could reconstitute skin with hair follicles, when transplanted onto the subcutis of immunoincompetent host mice (81). In this system, the transplanted cells are confined by a silicon chamber, which maintains the humidity and restricts the assay space (Fig. 3). Following this study, separately prepared epithelial and dermal cells were variously combined and transplanted into the chamber upon the dorsal fascial plane (82,83). In all experiments, successful reconstitution of hair follicles was not possible without a dermal component, underscoring the indispensable role of mesenchyme to hair follicle induction.

Later, Weinberg, Lichti and colleagues used this method to assess the trichogenic capacity of selected dermal cell populations. By combining a fixed number of epithelial cells with tested dermal components, they demonstrated that neonatal dermal cells and low-passage, or immortalized, papilla cells, but not high-passage papilla cells or fibroblasts, could reconstitute hair follicles (66,67). Today, the chamber system represents the most widely used experimental method for assessing mesenchyme trichogenicity (54,56,73,74,84–87). The beauty of this assay is that hair follicles are reconstituted in the proper anatomical location (on the dorsal surface of the mouse) with outgrowing, easily observable shafts. Nevertheless, the assay is expensive in time (around 35 days), in number of cells (10 million), in mice (one chamber/mouse), and in overall cost; moreover, the set up is complex to perform and challenging

Table 2. *In vivo* assay of trichogenicity

Method	Materials/Species	Location	Advantage	Disadvantage	References
Chamber Assay	Mixture of dissociated dermal and epidermal cells from mouse, cultured or fresh isolated	Dorsal skin of athymic mice	Outgrowing shafts	Time & resource-consuming, technically demanding	54,56,73,74,84–87
Patch Assay	Mixture of dissociated dermal and epidermal cells from mouse, rat, pig, dog and human, cultured or fresh isolated	Hypodermis of athymic mice	Easy to perform, uses less cells, multiple injections per mouse	Shafts underneath the epidermis, difficult for observation without harvesting the skin	57,77,88, unpublished data from Zheng et al
Tracheal Transplant	Same as Chamber Assay	Deepithelialized trachea and subcutis	Grafted cells well protected by trachea structure	Special tissue required	89,90
Sandwich and Flap Assay	Dissociated dermal cells or aggregates from mouse or rat; with epidermis sheet	Affollicular skin or dorsal skin of athymic mice	Outgrowing shafts, less cells used than chamber assay	Two surgical procedures involved	58,91

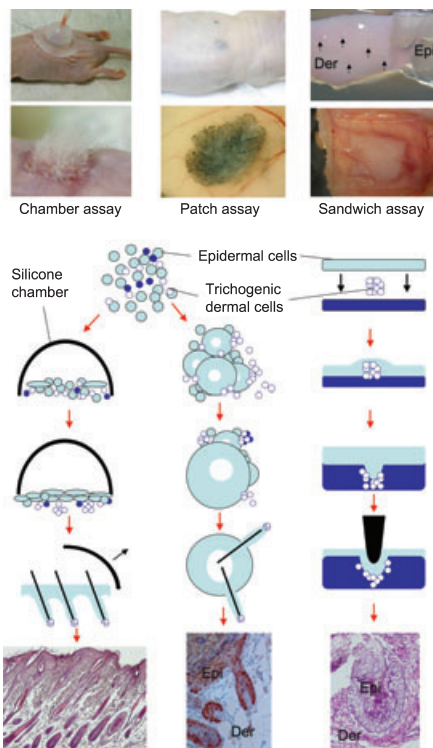


Figure 3. Assays for the evaluation of trichogenicity. In the chamber assay, the mixture of keratinocytes and dermal cells was added into the construct surgically fixed onto the dorsum of a nude mouse, while in the patch assay, the cell mixture were directly introduced into dermal, subcutaneous space or kidney capsule. Hair induction was observed on the back skin or within the microcyst, respectively. In the sandwich assay, aggregated dermal cells (arrows) were placed between the epidermis (Epi) and the dermis (Der) of affollicular foot pad skin of rodents and the tissue was implanted into subcutaneous space of an immunodeficient mice. Formation of hair follicle like structures could be observed (HE staining). The middle panel in the bottom row was reproduced from Fig. 5J of Zheng et al. *J Invest Dermatol* 2005: 871 with permission from Wiley-Blackwell.

to teach (88). Thus, there was an incentive to develop a simpler assay.

Patch assay

In an attempt to set up more rapid and small-scale hair reconstitution assays, Zheng and co-workers established the protocol for the 'Hair Patch Assay' (88) (Fig. 3). In this system, dissociated epithelial and mesenchymal cell populations are mixed and directly injected into the hypodermis of the mouse. This assay uses far less cells (1 million), animals (6–8 assays/mouse), and time (around 10 days). Detailed investigation revealed that hair follicle neogenesis took place in the subcutaneous space and that the process faithfully recapitulated embryonic hair follicle morphogenesis (88) (Fig. 3). Thus, the Hair Patch Assay provides a simple and reliable method to measure trichogenic ability of a dermal or an epidermal cell population. Using this assay, the hair inductive capacity of murine CD133 positive papilla cells, or the spheres of high-passage dermal papilla cells, has been successfully demonstrated (57,77). Zheng et al. reported that the success of this assay depends on the placement of the cells into a small space, which enables and fosters the interaction between the component epithelial and dermal cells (88). If the same cell preparation were placed onto the subcutaneous fascial plane, the cells often did not self-associate and did not form follicles (88). The limitation of the assay is that (1) the formed follicle are, in general, intradermal and thus the site of trichogenesis cannot be directly observed from the outside; and (2) the process cannot be followed for a long time (not greater than two cycles) since the shafts themselves stimulate a foreign body reaction which eventually vitiates the follicles themselves.

Tracheal xenotransplantation

In the tracheal xenotransplantation assay for hair reconstitution (89,90) a mixture of epithelial and trichogenic

dermal cells are 'packed' into the rat trachea and transplanted as a package into the subcutis of athymic mice. While also useful this method could be regarded as a variation of the Hair Patch Assay, but in this case, special tissues are needed and the time for assay development is longer (4 weeks for tracheal hair regeneration versus 10 days for the Hair Patch Assay).

Sandwich and flap graft assays

Hair inductive capacity of dermal cells could also be evaluated by the 'sandwich' assay, in which mesenchymal cell aggregates are placed between enzymatically dissociated afollicular murine sole epidermis and dermis (Fig. 3) (58). Next, the combined tissue is grafted into subcutaneous space to assess hair inductive capacity of tested dermal cells. Hair-producing bulb structures were successfully induced, when trichogenic mesenchymal cells were implanted (58). Recently, Qiao et al. refined this technique and reported the 'flap graft assay' (91). In this assay, mouse embryonic epidermis is placed on a silicone sheet with its basal side up and loaded on its basal side with mesenchymal cells to be tested. The sandwiched sample is then transplanted, silicone-side down, on the underside of a skin flap made on the back of the mouse. Four weeks after the initial surgery, the flap is reopened and inverted to expose the graft to the exterior environment. The wound end is sutured to generate a three-sided skin flap. Similar to the chamber assay, complete HF structures are formed. The induced HFs are easily observed in this method and those hair follicles which form survive for more than 12 months. In addition, the number of required transplanted dermal cells can be as low as 1×10^{-4} cells per site. However, two surgical procedures are required for this assay and the preparation of an embryonic epidermal sheet is a laborious step.

***In vitro* assays for the evaluation of dermal–epithelial interaction**

Various attempts have been made to establish *in vitro* assays for the evaluation of dermal–epithelial interactions, including hair inductive capacity. Rogers et al. reported that isolated hair follicles could sustain functional integrity, when cultured inside a collagen matrix (83). Using this system, the interactions between hair matrix keratinocytes and FDP was assessed by comparing the *in vitro* behaviour between intact hair follicles and papilla-removed follicles (24). In this experiment, the proliferation of matrix keratinocytes and hair shaft elongation was detected only when FDP was directly attached to the hair bulb matrix, suggesting an indispensable and continuous trichogenic role of the dermal component. Transwell co-culture system for HF keratinocytes and FDP enabled the monitoring of HF-related genes or protein expression in epithelial cells and

provided a valuable tool to dissect dermal–epithelial interaction in HF (92).

***In vitro* culture technique to maintain a proper *in vivo* anatomical relationship between dermo–epidermal components**

The anatomical relationship between dermal–epithelial components is important for a properly directed crosstalk. Thus, the technique to reproduce *in vivo* positioning of epithelial and dermal elements would be beneficial for an *in vitro* hair induction assay. Hardy adopted a hanging drop culture method to maintain the positioning between the epidermis and the dermis (93). Recently, this technique was used to evaluate trichogenicity of cultured human papilla aggregates (see abstract: Soma T et al. Generation and characterization of three-dimensional human hair follicle model using hanging drop culture system. *J Invest Dermatol* 2008 128 S150). An organotypic culture system in which outer root sheath keratinocytes were layered over the dermal papilla cells on a pseudo-dermis (fibroblasts and collagen gel) was designed to simulate *in vivo* anatomical positioning between dermal–epithelial components (94–96). Recently, microspheres have been developed, which incorporate both human outer root sheath keratinocytes and human FDP cells, with the latter being embedded in a collagen I/matrigel mixture, where both the HF fibroblasts and the HF keratinocytes retained at least some of the marker expression and characteristics they manifest *in situ* (97). Assay systems like this should facilitate the *in vitro*-recreation of normal FDP (and DS) cell topobiology so as to enhance, maintain, and/or restore the trichogenicity of these cells even in long-term culture.

In vitro reconstitution of hair follicles was demonstrated by the co-injection of cultured outer root sheath keratinocytes and dermal papilla cells into human skin organ culture (98). However, only miniaturized HFs were obtained after 8 weeks in culture and the unequivocal demonstration of new HF formation was not shown. At this time, *in vivo* hair reconstitution assays surpass *in vitro* assays for the evaluation of hair inductive capacity of dermal cells.

Molecular characterization trichogenic dermal cells

In search of a molecular indicator of trichogenicity

For many years, investigators have sought those molecules which define the biological characteristics of FDP, especially their trichogenic capacity (for review, 4, 5, 99, 100). Several molecules including those involved in WNT, BMP, NOTCH and SHH signalling pathways have been identified as crucial to hair morphogenesis and cycling, however, considering the complexity of the hair inductive processes, a high-throughput approach is required to fully elucidate the list of mole-

cules that govern all events involved. Adopting differential screening of cDNA libraries, microarray analyses, or proteomics, several labs have identified the molecules up-regulated in rat (101) and human (102–105) FDP cells.

However, a major limitation and concern with these studies is that the cells studied were cultured FDP cells, whose biological characteristics could have been profoundly altered compared to FDP residing in their natural 'habitat' *in vivo*. Recognizing this problem, Rendl et al. used an elegant transgenic fluorescent labelling approach and successfully collected freshly isolated mouse dermal papilla cells and elucidated their molecular signature (55,56). Developing such an approach may lead to further discovery of novel molecules that reflect hair inductive potential.

Molecules whose expression correlates with trichogenicity (Table 3)

Listed below are previously reported molecules whose expression levels in the papilla cells correlate with trichogenic capacity (Fig. 4).

Alkaline phosphatase

High expression of AP in the pilosebaceous unit, especially in FDP, has been described in the literature (29,31,106–108). The linear correlation between hair inductive capacity and AP expression in cultured dermal cells has been demonstrated by *in vivo* hair induction experiments (30,56).

Versican

Versican expression localizes to the dermal papilla of anagen hair follicles and the dermal condensate of developing follicles (54,109). A transgenic mouse expressing GFP

under the control of the versican promoter enabled the FACS isolation of living FDP cells (54). In chamber assays, the success rate of hair reconstitution correlated well with GFP signal intensity of isolated FDP cells (54,74,77).

WNT signalling molecules

FDP cells preferentially express Wnt5a, Wnt downstream transcription factor Lef1, and Wnt inhibitory factor 1 (Wif1) (55,73,110). It has been suggested that the activated state of Wnt signalling reflects the trichogenic activity of FDP cells (56,73,74).

BMP signalling molecule

BMP2,4,6 and a BMP inhibitory molecule Noggin have been reported to be expressed in FDP (56,100,111). Hair follicle induction was significantly retarded in noggin-knockout mouse (111). Recently, Rendl et al. demonstrated BMP6-treated papilla cells, expressing a high level of noggin, efficiently reconstituted hair follicles in the chamber assays (56).

Nexin 1, a serine protease inhibitor

Nexin 1, a potent protease inhibitor, is strongly expressed in the FDP of anagen HF's (112). Interestingly, nexin 1 mRNA levels in immortalized FDP cell lines correlate with their ability to reconstitute hair follicles *in vivo* (112).

CD133

CD133/prominin-1, a putative marker for tissue stem/progenitor cells, is expressed in early anagen murine FDP cells (57). CD133 enabled FACS sorting of living

Table 3. Molecular markers/pathways that correlate with trichogenicity

Marker/Pathway	Location <i>in vivo</i>	Property of positive cells	Species	Comments	References
Alkaline Phosphatase (AP)	FDP	Cultured AP+ cells can produce hair <i>in vivo</i>	Mouse	A possible indicator trichogenicity of cultured cells	29–31,56,106–108
Versican	FDP, dermal condensate	GFP cells under versican promoter can produce hair <i>in vivo</i>	Mouse	Application to human not known	54,74,77,109
Wnt molecules	FDP	Wnt signaling maintains cell inductivity	Mouse	Used as culture additives to maintain trichogenicity	55,56,73,74,110
BMP molecules	FDP	BMP6 treated FDP cells in culture can produce hair <i>in vivo</i>	Mouse	Used as culture additives to maintain trichogenicity	56,100,111
Nexin	FDP	Nexin mRNA level correlates with hair producing ability <i>in vivo</i>	Mouse	A possible indicator of trichogenicity of cultured cells	112
CD133	FDP, stem cells	Sorted CD133 cells can produce hair <i>in vivo</i>	Mouse	Used to sort out trichogenic FDP cells in mouse, however it is not found in human FDP cells	57
ET-I/SCF	Early passage cultured FDP	Expression level correlates with inductive activity	Human	Possible indicators of trichogenicity of cultured cells	113
Epimorphin	HF mesenchyme, FDP, DS	Treating mouse skin with epimorphin accelerates telogen-anagen progression	Mouse	Possible use in optimizing recipient environment for trichogenic cells	34,114,115
Others (NCAM, Alx4, Hayl, etc)	FDP	Possible indicators of cell trichogenicity	Mouse	Possible marker for cell sorting (NCAM) or indicators of trichogenicity of cultured cells	56,77,80,111

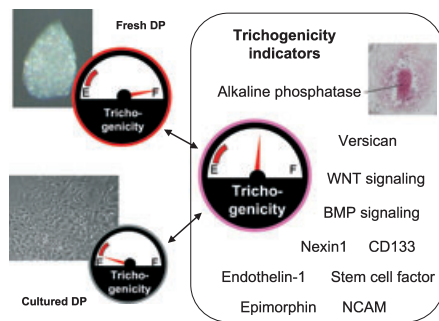


Figure 4. Molecules whose expression correlates with trichogenicity. Correlation between the expression levels of some molecules in dermal cells and trichogenicity was demonstrated using the *in vivo* hair reconstitution assays. Such molecules may be used as trichogenicity indicators.

FDP cells. Sorted CD133 positive FDP cells expressed other dermal papilla markers, including versican; moreover, the isolated FDP cells were positive in the patch assay (57). Unfortunately, CD133 was not detected in human FDPs (57).

Endothelin-1 and stem cell factor

Enhanced expression of endothelin-1 and stem cell factor in early passage cultured FDP cells has been reported (113). Endothelin-1 and stem cell factor expression levels correlated with the inductive potential of cultured FDP cells in the hair reconstitution assay (113).

Epimorphin

Epimorphin is a mesenchymal protein that plays a critical role in epithelial–mesenchymal interactions in development, including HF morphogenesis (114,115). Epimorphin was detected in HF mesenchyme during human (115) folliculogenesis and in FDP as well as DS in adult mouse anagen HFs (34). Treating mouse skin with recombinant epimorphin accelerates telogen-anagen progression (34). These findings suggest that epimorphin expression level could reflect trichogenic capacity of hair follicle dermal cells.

Other trichogenicity indicators

Several studies indicate that gene expression levels of NCAM (77,80,111), Alx4 (56), FP-1 (69) and Hey1 in FDP cells (56,77) could predict trichogenic capacity.

Summary and conclusions

Heterogeneity of cells housed in the FDP

From ultra-structural investigations we know that FDP fibroblasts are morphologically homogeneous in neither mouse nor human (21,22,116). In addition, marker stains

are not uniform: for example, AP location may vary from follicle to follicle in the same individual, and CD133+ cells in the mouse FDP are not diffusely spread but localize to the most distal FDP (30,31,57). These findings suggest that trichogenicity might not be equal among FDP cells and that more than one FDP cell type is active in the inductive process. Thus, heterogeneity and hierarchy among distinct FDP cell populations rank among the most important, unanswered questions in HF biology, and await careful characterization.

The relationship between DS and FDP cells

Tissue regeneration and implantation experiments strongly suggest that DS cells could repopulate, or even be the source of, FDP cells (41–43,117). Detailed cytogenetic analysis suggests that bidirectional cell trafficking between the DS and the FDP occur during every normal hair cycle in mouse and human HFs (21–23). These observations support the presence of ‘FDP cell progenitors’ in DS, especially in the lower, proximal, third of hair follicles (30,41), and likely even within the FDP itself (118). Considering that, in aging human skin, proximal DS cells may even demonstrate stronger hair inductive properties than FDP cells (43), these putative FDP cell progenitor populations might provide a more potent source of trichogenic mesenchymal cells. Identification and characterization of such a subset of dermal cells will represent an important next step in understanding hair inductive mesenchyme.

Other potential trichogenic populations in the skin

Recently, multipotent cell populations, such as skin-derived precursors (SKPs) (119,120) and nestin positive cells (121,122) were isolated from mouse, rat and human skin (123). Interestingly, SKPs were shown to reside in FDP in adult skin (124). In human scalp skin, nestin+ cells, contrary to a widespread misconception, are not located in the epithelial hair follicle bulge, but in the DS and other specialized stroma of skin appendages (125). To what extent these SKPs or nestin+ cells contribute to hair inductive capacity of the FDP or proximal DS remains to be elucidated. However, taking into account their remarkable plasticity (122,126–131), these multipotent cells might be able to provide an alternative cell source for the bioengineering of trichogenic mesenchymal component from autologous tissue.

Recommendations

The trichogenic assay is one of the most critical issues in dermal papilla studies. For mouse cell studies, excellent *in vivo* hair reconstitution assays are readily available where *de novo* hair follicle formation predictably occurs and can

be qualitative and quantitatively measured (56,58,67,88) (Table 2). In contrast, a robust assay for human HF regeneration has not yet been established for human cells, except in a chimeric situation (132). Because instructive bioassay are available, we believe it is critical that all reports on such studies include a measure of functional activity, which would include, for example, evidence for the induction of follicle like structures (132) or definitive hair follicle molecule expression (95,97).

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