Cultured dermal papilla cells induce follicle formation and hair growth by transdifferentiation of an adult epidermis

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Summary

Adult rat pelage follicle dermal papilla cells induced follicle neogenesis and external hair growth when associated with adult footpad skin epidermis. They thus demonstrated a capacity to completely change the structural arrangement and gene expression of adult epidermis - an ability previously undocumented for cultured adult cells. Isolation chambers ensured that de novo follicle formation must have occurred by eliminating the possibility of cellular contributions, and/or inductive influences, from local skin follicles. These findings argue against previous suggestions of vibrissa follicle specificity, and imply that the potential for hair follicle induction may be common to all adult papilla cells.

Key words: hair growth, dermal papilla, cell culture, follicle neogenesis, dermal-epidermal interaction, epidermal differentiation.

Introduction

Dermal-epidermal interactions are a crucial element of skin appendage morphogenesis, and during embryonic development the respective roles of the tissues have been well defined by an enlightening series of recombination experiments (Dhouailly, 1977; Sengel, 1986 - for reviews). Certain skin appendages remain dynamic in maturity, like most hair follicles in which the fibre is repeatedly renewed. Thus, hair follicle components can be used to investigate the largely unknown mechanisms that operate in adult dermal-epidermal interactions. More specifically, they provide a tool to tackle important questions about the inductive capabilities of adult dermal cells and the extent to which epidermal differentiation can be modified, even in maturity.

In this context, the adult rat vibrissa follicle has long been employed as an experimental model (Cohen, 1961). The crucial work of Oliver (1980, review) demonstrated that local dermal-epidermal interactions control hair growth, and that the dermal papilla component has particular inductive properties, both within follicles and when associated with different epithelia. Cultured vibrissa papilla cells have also been shown to actively promote follicle induction and hair fibre growth (Jahoda et al., 1984; Horne et al., 1986; Jahoda et al., 1987; Oliver and Jahoda, 1989; Reynolds, 1989). However, all of the above protocols left open the possibility of additional influences, or cellular contributions, being made from established hair follicles - either via the introduced tissue, or from the host body site. Therefore, de novo follicle induction has not been proved in the strictest terms - which would require that the epidermis of the induced structures was all derived from a mature, glabrous source, and produced in a non-appendageal environment.

Rodent pelage- and vibrissa-type hair follicles are morphologically and functionally variable: they are considerably different in size; produce their own distinctive kinds of fibre, and, while the former (sub-categorized into at least five morphologically distinct types; Dry, 1926; Montagna and Van Scott, 1958) are widely distributed across most of the body, the latter are very restricted in accordance with their specialized sensory function (Park, 1970). A long standing question relates to the general applicability of work involving the vibrissa follicle. Indeed, it has been strongly suggested that the behaviours exhibited by vibrissa follicle component tissues are unique, being attributable to their highly specialised function, and therefore unrepresentative of the behavioural capabilities of tissues from other types of follicle (Montagna, 1980, 1984). In this context, it has not yet been experimentally demonstrated that dermal papilla cells from other kinds of adult hair follicle possess the embryonic-type abilities that are suggested for vibrissa-type follicles - specifically, the capacity to induce new hair follicle formation.

Previous embryological recombination studies have exploited the footpad region as a source of glabrous skin (Kollar, 1966, 1970; Kollar and Baird, 1970), and,
in maturity, epidermis from this region is not only afofacicular but also expresses a different set of keratins from those that are found in interfolicular epidermis. In the present study, serially passaged dermal papilla cells from adult pelage follicles were associated with adult footpad skin to investigate whether the former were capable of hair follicle induction. To exclude contributory influences from follicular skin, the associations were carried out within modified isolation chambers (Fusenig et al., 1981; Worst et al., 1982). The resultant neogenesis of pelage-type, fibre-producing hair follicles provides strong evidence for inductive capabilities being a generalized phenomenon amongst all forms of this appendage. Moreover, the unambiguous response of the adult footpad epidermis (in becoming induced hair follicle epidermis), gives an important insight into tissue specificity and questions pertaining to epidermal transdifferentiation.

**Materials and methods**

**Animals**

Male and female animals from an inbred colony of PVG/C hooded rats (Dundee University) were used in this study.

**Preparation of dermal populations**

**Pelage papilla cells**

Skin biopsies were obtained from the mid-flank region of dead, adult hooded rats aged three to twelve months. Fat and connective tissue was cleared from the undersurface of each biopsy to expose the follicle end bulbs, under a dissecting microscope (x40 magnification), which also aided subsequent manipulations. The largest monotrich-type follicles were selected and their proximal-most tips, consisting of less than 4% of their entire length, removed and placed in individual drops of Eagle’s Minimal Essential Medium (MEM) with antibiotics (penicillin 50 U/ml, streptomycin 50 U/ml, kanamycin 150 μg/ml and fungizone 2.5 μg/ml; Gibco) at 4°C. Fine pointed tungsten needles were used to manipulate the outer dermal sheath layers, and part the epidermal matrix component, from around the dermal papilla (Fig. 1A) in each end bulb. Isolated papillae were then transferred to 35 mm plastic Petri dishes (Nunc) where they were fragmented and forced to adhere. Approximately 20-30 dermal papillae were used to initiate each culture dish (Reynolds and Jahoda, 1992, unpublished data).

**Skin fibroblasts**

Adult rat body skin fibroblasts were cultured by explant outgrowth from small pieces of dermal tissue, which were taken from skin sites in the location of the follicles employed above.

**Cell maintenance**

Initial explants were maintained at 37°C in 5% CO₂/95% air, in MEM containing 20% foetal calf serum (FCS, Gibco), with antibiotic supplements and 1% L-glutamine at a final concentration of 2 mM; after 10 days, FCS concentration was reduced by half. Subsequently, medium was replenished every 4 days. Passageing was performed at 1-2 week intervals - cells were washed with phosphate-buffered saline (Dulbecco A., Oxoid) containing 0.2 mg/ml of ethylenediamine tetracetic acid (PBS/EDTA), and released by digestion in 2.5% trypsin at 37°C. Trypsin digestion was inhibited by addition of an equal volume of culture medium. Cells were then pelleted by centrifugation (500 g), resuspended and counted by haemocytometer before being plated at densities of 5 x 10⁴ cells per 35 mm dish.

The skin fibroblasts and pelage papilla cells (Fig. 1B) were observed with a Nikon diaphot inverted microscope and representative photographs taken at periodic intervals.

**Operational protocol**

**Host site preparation**

Each animal was anaesthetised by intramuscular injection with 0.4 ml of hypnorm (Janssen Pharmaceuticals Ltd), followed by 0.5 ml of valium intraperitoneally (Roche Products Ltd) per kg body weight. Hair was shaved from the mid-dorsum and any remaining external hair stubs removed with depilatory cream. A horizontal incision was made across the lumbar region and a 2.5 cm diameter, rough surfaced glass disc inserted (Fig. 1C, i). The wound was sutured with 5/0 coated Vicryl sutures (Ethicon) and the glass disc secured using surgical thread, before applying a protective dressing.

**Recombination procedure**

After 10 days, footpad skin was cut from the central (and thus completely hairless) sole region of a dead, adult rat hind paw using a number 11 scalpel blade. It was scraped on its internal surface to remove any remnants of adherent connective tissue, cut to an appropriate size (approximately 3 mm x 4 mm), and split into dermal and epidermal components by dispase treatment (0.25%, 4°C, 1 hour). Confluent cultures of second or third passage pelage papilla cells were scraped into clumps with a rubber policeman, then transferred to the centre of the dermis and the epidermis repositioned over them (Fig. 1C, ii). Although close examination revealed no evidence of epidermal cell contamination in the passed dermal cell cultures, for three of the operations the papilla cells were put through Percoll density gradient centrifugation prior to use. Since epidermal germinative cells are considerably smaller than, and differ in density from, dermal papilla cells, they accumulate at a completely different level when spun in a Percoll gradient column, allowing the dermal populations to be removed from the gradients in isolation (Reynolds, 1989). The reconstituted footpad skin specimens were then incubated overnight at 37°C in rat serum.

Identical procedures were performed with equivalent ages of rat skin fibroblasts, for control purposes.

**Implantation**

On the following day, a single horizontal cut was made in the dorsal skin of the adult rat which had been operated on eleven days earlier. The incision was made directly above the glass disc, which was then removed. The lower portion of a silicone chamber was positioned inside the granulation tissue pocket that had formed, and a pre-prepared footpad sandwich placed at its centre. It was then covered with a silicone chamber upper section before the whole structure was secured in place by stitching together the edges of the incision around the chamber. This arrangement was further stabilised using metal clips over the top of the sown regions (Fig. 1C iii). The operated area was sprayed with Op-site wound dressing (Smith and Nephew Ltd) and sterile gauze laid over the clipped regions of the incision. Strips of Gypsona plaster of Paris bandage (Smith and Nephew Ltd) were then loosely, but securely, wrapped around the trunk of the rat in a manner that left the top of chamber exposed to the air. Six operations were performed for each cell type.
After 8 weeks the animals were killed and the implantation sites biopsied, photographed and fixed in formol saline, prior to their processing for standard wax histology.

Results

Pelage papilla cells

Close examinations were made of all of the recombinations and their surrounding sites, eight weeks after their preparation. These revealed that the firmly positioned silicone chambers had provided an effective barrier to any possible migratory contamination from local skin or hair follicles, since the edges of this host skin had been held well outside of the chambers. On removing the upper domed portion of each chamber, pale, moist regions of raised material, consisting of granulation tissue and the implanted footpad skin, were observed to the centre of the lower section. Removal, and close examination of this lower portion of the chamber, as well as the tissue directly underneath it, confirmed that there could have been no influx from the surrounding local tissue.

Pelage-type hair fibres (average length 8 mm) were observed to have emerged from 3 of the 6 pieces of footpad skin which had received papilla cell implantations (Fig. 2A). All of these induced fibres were broad centrally and became progressively narrower towards their base, as is usual for pelage hairs (Fig. 2B).

Histological examination of the 6 recombinations revealed that 5 contained groups of pelage-type follicles in anagen (Fig. 3A-D). The other specimen was disregarded as it had been displaced during the experimental period and, by the time of biopsy, the tissue had necrosed. Induced follicles in any one group tended to run in parallel with each other, whereas the groups themselves appeared to be quite shallow within the skin and randomly orientated (Fig. 3B-D). Sebaceous glands, which are a normal feature of hair follicles in situ, were not observed. The induced dermal papillae and epidermal matrices (both pigmented and unpigmented) were generally more robust and elongated than the equivalent structures in the donor follicles, from which the experimental pelage papilla cell cultures had been established (Fig. 3A, B and C). The larger oval dermal papillae displayed about 40 nuclei in median section (Fig. 3A), and in the majority of instances the pigmented fibres had distinctly banded medullae (Fig. 3D).

Skin fibroblasts

The implants of skin fibroblasts, which served as controls for the pelage papilla cells, could not be recognised in histological sections through the post-experimental tissue, and there was never any sign of structure formation (Fig. 4). The morphology of this footpad skin appeared to be quite normal and retained its typically deep ridged epidermis (Fig. 4).

Fig. 1. Cell culture and implantation protocols. (A) An oval-shaped dermal papilla (dp, phase contrast, x340) isolated from an adult rat pelage hair follicle: it is still attached to a small portion of dermal sheath tissue at its base. (B) A monolayer culture of pelage dermal papilla cells (phase contrast, x80) displaying only a limited tendency to overlap, or clump (in contrast to the pronounced aggregation behaviour previously observed in cultures of vibrissa follicle-derived dermal papilla cells). (C) Sequence of operational procedures involved in the implantation of cultured rat pelage papilla cells (p) between footpad epidermis (e) and dermis (d): (i) granulation tissue beds were prepared by subcutaneous insertion of glass discs (gd); (ii) footpad associations (fa) containing passage two pelage papilla cells (p) were prepared and then, (iii) placed inside silicone chambers (sc) which were positioned within the previously prepared subdermal granulation tissue pockets (gt) on the rat dorsum.
Fig. 2. Appearance of emergent fibres following papilla cell implantation into footpad skin. (A) Macrograph of an isolated piece of post-experimental footpad skin in which irregularly orientated fibres can be seen emerging from well separated sites. (B) An example of an induced pigmented fibre with a banded central medulla - it is broad in the middle of the hair and becomes progressively narrower towards the base (arrow), as is characteristic for pelage (monotrich) hairs.

Discussion

Experimental hair follicle neogenesis in adult skin has long been a subject of debate, with a recurring problem being the interpretation of observations from experiments whose protocols did not rule out local follicular influences (Muller, 1971). During this study, we have elicited follicle formation and hair growth in afollicular adult footpad skin. We provide strong evidence that complete follicle neogenesis must have occurred, since use of an isolation chamber technique prevented populations from surrounding follicles from contributing to the recombinants. Furthermore, the Percoll density gradient centrifugation of papilla cell cultures before their recombination in half of the experiments, removed any likelihood of unobserved contamination by hair matrix germinative epidermal cells (Reynolds and Jahoda, 1991). We do not know if the footpad dermis made a direct contribution (cellular or otherwise) to the follicle inductions: or whether it simply provided physical support for the interactions. The experiments could be repeated with labelled papilla cells, or using freeze-thaw treated footpad dermis in order to determine exactly which cell populations are directly involved, and what proportion of the introduced cells contribute to follicular structures. Repeat experiments would also permit monitoring of early morphogenetic events with, for example, changes in keratin expression. Maintaining material in chambers over well-vascularized granulation tissue sites, has previously been shown to be effective in allowing the reformation of organized epidermal structure in skin-derived cell cultures (Fusenig et al., 1981; Worst et al., 1982). The present study now shows that this chamber methodology is also suitable for work involving small pieces of whole skin.

Cultured rat vibrissa dermal papilla cells are capable of stimulating hair growth when implanted back into inactivated portions of vibrissa follicle (Jahoda et al., 1984). However, the experimental induction of a complete fibre-producing follicle from non-follicular adult epidermis is a more profound event, requiring morphogenetic activities and inductive signals of a complexity similar to those involved in embryonic hair follicle development (Holbrook et al., 1989). In this context, Oliver (1970) demonstrated that vibrissa dermal papillae wrapped in pieces of epidermis from hairless scrotal skin (which is nevertheless hair follicle-bearing skin) could induce follicle, or follicle-type, formations in the rat ear site. Furthermore, while oral epithelium normally only contributes towards dental appendages, Oliver (1973) also demonstrated that whole vibrissa dermal papillae could induce follicle-type formations in association with this tissue, once again in the rat ear site. Similarly, cultured vibrissa papilla cells in contact with ear skin wound epidermis in situ, can induce vibrissa-type follicles and fibres (Jahoda et al., 1987; Reynolds, 1989). However, wound epidermis is known to derive substantially from hair follicles (Eisen et al., 1955; Sandford et al., 1965), so that in the latter experiments in particular, indirect follicular influences could be involved. Moreover, footpad skin has an obvious advantage over all of the above alternatives as a potential site for hair follicle induction, because none of its constituent cells ever contribute to any form of appendage. The present finding that pelage papilla cells can instructively induce new pelage-type hair follicles, contradicts the idea that vibrissa follicle properties may be generally unrepresentative or unique (Montagna, 1980, 1984), and reinforces the validity of the vibrissa follicle as a model system for hair growth studies. Furthermore, it supports the proposition that embryonic-like inductive potential may be a widespread property shared by all adult hair follicle dermal papilla cells. This has possible implications for human hair loss reversal, and shows that
Fig. 3. Histological appearance of pelage papilla cell induced follicles within footpad skin. (A) The end bulb region of a hair-producing follicle in median section, showing an elongated dermal papilla (p) displaying around 30 nuclei and a pigmented epidermal matrix (m). (Magnification ×360). (B) Lower-power view of footpad skin containing a group of three active pelage-type follicles. An oval-shaped dermal papilla (p) and pigmented matrix (m) are visible in the largest of these follicles. (Magnification ×320). (C) Another small group of pelage-type hair follicles: one of them displays a kinked region (arrow), as was observed to a greater or lesser degree in about 40% of the induced appendages. (Magnification ×160). (D) Detail of two fibres in a group of induced follicles: one has pelage-typical banding of the medulla (arrow), while the other represents one of the few fibres seen to have a solid medulla. (Magnification ×310). All specimens were fixed in formol saline, embedded in paraffin wax, sectioned at 8 μm, and stained with a combination of Alcian blue, Weigert’s haematoxylin and Curtis’s Ponceau S.

different papilla cell types can be employed in the search for hair growth induction mechanisms using biochemical or molecular approaches. It also reinforces previous findings that adult papilla cells retain follicle-type specificity, and the capacity to manifest this information phenotypically by determining the particular type of follicle (in this case pelage) which is produced (Jahoda et al., 1987; Reynolds, 1989).

In this study the pelage cells were placed within the footpad skin as small agglomerations and it appears likely that the retention of some of these aggregates contributed to the observed formation of small groups of induced follicles. While vibrissa papilla cells display pronounced aggregative behaviour in culture (Jahoda and Oliver, 1984), the trait is less clear in pelage papilla cell cultures (Withers, 1987; Reynolds, 1989). Thus, in vitro clumping behaviour appears to relate to the relative size of induced appendages, in that here pelage papilla cells induced small pelage-type follicles, while vibrissa papilla cells induce large follicles, and vibrissa-type fibres (Jahoda et al., 1987; Reynolds, 1989). Hence, as in embryonic appendages (Sengel, 1986; Holbrook et al., 1989), dermal cell aggregation almost certainly has a bearing on follicle size (Jahoda, 1982; Reynolds, 1989) and aspects of pattern formation.

One justification for suggesting that the adult papilla cell influences represent retained embryonic properties, is that our findings are in close agreement with embryonic dermal-epidermal recombination results - both in relation to the direction of influence and the
nature of the response. More specifically, it has recently been shown that postnatal footpad epidermis can be induced to form hair follicles when recombined with embryonic dermis (Delorme, 1989). While footpad skin has classically been used as a non-appendageal site in embryonic manipulations (Kollar, 1966, 1970), our choice of this region as host skin may have been particularly beneficial in the context of the adult recombinations we performed. A high proportion of footpad epidermal germinative cells are thought to be localised at the base of the pronounced epidermal ridges (Lavker and Sun, 1983), paralleling the arrangement of germinative epidermal cells at the base of the adult hair follicle, and thus possibly aiding the interactive process. It was interesting that sebaceous glands were never seen in association with the induced hair follicles in this experiment, since we have observed them with hair follicles that have been induced in adult rat ear skin sites during some of our previous studies (Jahoda et al., 1987; Reynolds, 1989). While the significance of this finding is unknown, it does appear to demonstrate that the pilosebaceous unit does not have to form as a whole entity, and suggests that ear skin provides some contribution to sebaceous gland formation that is absent from the footpad skin site.

In relation to the epidermis, the pelage papilla cells instructively induced a specific form of epidermal cell differentiation that was profoundly different from that which would normally be exhibited by adult footpad skin. The important point is that, while some specific hair-type keratins have been found in regions as diverse as tongue (Dhouailly et al., 1989) and thymus (Heid et al., 1988), they have never been shown to be present in footpad epidermis at any time during development or maturity (Delorme et al., 1987; Delorme, 1989). Therefore, since the expression of genes for hair-specific proteins must have been elicited in the footpad epidermis in this experiment, footpad epidermis must have been induced to switch from its normal gene expression. Hence, it would appear that our results can only be explained if a proportion of adult basal epidermal cells are multipotent. The implications relating to this are far-reaching, with a crucial enigmatic aspect of general epithelial cell biology revolving around the extent to which adult cells retain the capacity to fill an alternative niche if provided with the appropriate environmental and 'developmental-type' cues (Wessells, 1970). A previously documented example of mesenchymally induced epithelial transdifferentiation in mammals, is that of embryonic mouse urogenital mesenchyme inducing prostate-like acini in adult mouse bladder epithelium (Cunha et al., 1980). Our recombinations differ in that both the dermis and epidermis were of adult origin, and the changes were brought about by cultured dermal cells rather than mesenchymal tissue.

In conclusion, we highlight the inductive powers of adult hair follicle dermal papilla cells, and show that adult footpad epidermal cells retain the embryonic-like competence to respond to specific dermal morphogenetic influence/s by switching to an alternative developmental fate. This finding provides a striking illustration of how dermal-epidermal interactions have the capacity, even in adulthood, to control epidermal genome expression.

We thank The Royal Society for support to C.A.B.J., who held a 1983 University Research Fellowship, and the Wellcome Trust for equipment funding. A.J.R. was supported by a University of Dundee studentship. We gratefully acknowledge the kind help and constructive criticism of Professor Danielle Dhouailly and Dr. John Riley, and thank Bruce Pert for his photographic assistance.

References


Jahoda, C. A. B., Home, K. A. and Oliver, R. F. (1984). Induction of electrolysis and the problem of hair re-
Montagna, W. J. 1-9.


(Accepted 18 March 1992)