Smooth muscle alpha-actin is a marker for hair follicle dermis in vivo and in vitro


Abstract

We have examined the expression of smooth muscle alpha-actin in hair follicles in situ, and in hair follicle dermal cells in culture by means of immunohistochemistry. Smooth muscle alpha-actin was present in the dermal sheath component of rat vibrissa, rat pelage and human follicles. Dermal papilla cells within all types of follicles did not express the antigen. However, in culture a large percentage of both hair dermal papilla and dermal sheath cells were stained by this antibody. The same cells were negative when tested with an antibody to desmin. Overall, explant-derived skin fibroblasts had relatively low numbers of positively marked cells, but those from skin regions of high hair-follicle density displayed more smooth muscle alpha-actin expression than fibroblasts from areas with fewer follicles. 2-D SDS-PAGE confirmed that, unlike fibroblasts, cultured papilla cells contained significant quantities of the alpha-actin isoform. The rapid switching on of smooth muscle alpha-actin expression by dermal papilla cells in early culture, contrasts with the behaviour of smooth muscle cells in vitro, and has implications for [...]
Smooth muscle α-actin is a marker for hair follicle dermis in vivo and in vitro

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Summary

We have examined the expression of smooth muscle α-actin in hair follicles in situ, and in hair follicle dermal cells in culture by means of immunohistochemistry. Smooth muscle α-actin was present in the dermal sheath component of rat vibrissa, rat pelage and human follicles. Dermal papilla cells within all types of follicles did not express the antigen. However, in culture a large percentage of both hair dermal papilla and dermal sheath cells were stained by this antibody. The same cells were negative when tested with an antibody to desmin. Overall, explant-derived skin fibroblasts had relatively low numbers of positively marked cells, but those from skin regions of high hair-follicle density displayed more smooth muscle α-actin expression than fibroblasts from areas with fewer follicles. 2-D SDS–PAGE confirmed that, unlike fibroblasts, cultured papilla cells contained significant quantities of the α-actin isoform.

The rapid switching on of smooth muscle α-actin expression by dermal papilla cells in early culture, contrasts with the behaviour of smooth muscle cells in vitro, and has implications for control of expression of the antigen in normal adult systems. The very high percentage of positively marked cultured papilla and sheath cells also provides a novel marker of cells from follicle dermis, and reinforces the idea that they represent a specialized cell population, contributing to the heterogeneity of fibroblast cell types in the skin dermis, and possibly acting as a source of myofibroblasts during wound healing.

Key words: smooth muscle α-actin, hair follicle, fibroblasts, dermal heterogeneity.

Introduction

Skin appendage development is engineered through a series of interactions involving dermal mesenchyme and epithelial-derived epidermis (Sengel, 1986, for review). In hair follicle formation, the mesenchymal cells that form the dermal component of the structure first become visible as a cell agglomeration just below a primary epidermal thickening. At an early stage, cell division within the mesenchymal aggregation apparently ceases (Wessels and Roessel, 1965), and these cells form the dermal papilla component during follicular downgrowth. Connected with the base of the adult dermal papilla, and separated from the outermost epidermal layer of the follicle by a thick basement membrane, is the external element of the follicle mesenchyme, termed the dermal sheath.

Microsurgical manipulation of the rat vibrissa follicle model system has shown that the adult dermal papilla is a prerequisite for hair growth initiation and maintenance (Oliver, 1966a,b, 1967). Moreover, the adult dermal papilla retains powerful inductive capabilities in that it induces new hair follicle formation when associated with alopecic or wound epidermis (Oliver, 1970; Reynolds, 1989). The vibrissa follicle dermal sheath cells also possess distinctive properties, since they can be the source of regenerated dermal papillae after papilla removal, or lower follicle amputation (Oliver, 1966b; Jahoda et al. 1991).

The finding that cultured rat vibrissa dermal papilla cells (Jahoda and Oliver, 1981) retain their capacity to stimulate hair growth (Jahoda et al. 1984; Horne et al. 1986) distinguishes them as a culture system with particular relevance to known biological function. Other investigations have used cultured dermal components from human hair follicles (Messenger, 1989; Arai et al. 1989 for reviews). The morphology, behaviour and biosynthetic activities of papilla cells reflect their distinct in situ properties (Jahoda and Oliver, 1984; Messenger et al. 1986; Couchman, 1986), and it has been suggested that papilla cells represent a specialized skin fibroblast population (Jahoda and Oliver, 1984). This view is supported by the finding that fibroblasts derived from skin explant cultures are incapable of hair growth stimulation (Jahoda et al. 1984; Horne et al. 1986). However, while certain features such as the synthesis of basement membrane extracellular components (Couchman, 1986) help to distinguish papilla cells from skin fibroblasts, none can be expressly described as a specific papilla cell marker.

This question fringes on the broader problem of categorization of the cells that are present in the skin dermis, and which commonly appear fibroblast-like when
put into culture. It has become increasingly apparent that dermal subpopulations exist within adult skin, and that explant culture in particular may produce heterogeneous fibroblast outgrowth for investigative purposes. One group has recently attempted to tackle this problem by adopting a classification system that separates fibroblasts into a series of differentiation states according to their morphological and behavioural properties (Bayreuther et al. 1988).

A distinctive fibroblastic cell type first described in granulation tissue, and associated with retractive processes of soft tissues, is the myofibroblast. These cells possess a microfilamentous system akin to that observed in cultured fibroblasts or smooth muscle cells. More recently, myofibroblasts have been described in normal tissues, where it has been proposed that they exert contractile activities (Sappino et al. 1990). The use of cytoskeletal markers such as desmin, an intermediate filament protein typical of most muscle cells; smooth muscle α-actin, an actin isoform found in smooth muscle cells; and smooth muscle myosin, has enabled several myofibroblastic subpopulations in normal and pathological tissues to be defined. Using a monoclonal antibody that specifically recognizes smooth muscle α-actin Skalli et al. (1986) noted that some hair follicle dermis stained positively. This, and resemblances between cultured dermal papilla cells and myofibroblasts prompted us to investigate whether hair follicle mesenchyme had smooth-muscle cell cytoskeletal characteristics, an idea previously mentioned by Couchman (1986). In the current study we used immunolabelling methods to examine human and rat follicular elements in vivo and in vitro, and report that the two principal dermal components, the dermal papilla and the dermal sheath, both express vascular smooth muscle α-actin in vitro.

Materials and methods

Rat tissues were derived from an inbred strain of PVG rats (Colony Dundee University), and from Wistar rats. Human material came from groin, scalp and scrotal skin biopsies as a result of surgical procedures.

Pieces of rat skin were taken from body regions with high hair follicle density (mystacial pad), reduced hair follicle density (ear skin), and without follicles (footpad skin). Isolated vibrissa follicles were dissected from the mystacial pad region as previously described (Jahoda and Oliver, 1981), and human follicles were dissected from groin, scalp and scrotal skin with watchmakers’ forceps and curved iridectomy scissors.

For tissue immunohistochemistry, specimens were embedded in Tissue tek III (Miles Scientific) water-based mounting medium and snap frozen over liquid nitrogen. Cryostat sections of 6 μm were cut at −20°C and air dried prior to labelling.

Cell cultures

Dermal papilla cultures were established from intact papilla explants (Fig. 1) dissected from vibrissae, and human follicles, as

Fig. 1. An isolated vibrissa dermal papilla prior to being put into culture. Note how papillae can be cleanly separated from the follicle epidermis, and the dermal sheath. Phase-contrast; ×360.

Fig. 2. A human dermal papilla still attached to dermal sheath (ds) that has been inverted during the dissection procedure. The two are then separated at the narrow basal stalk region (arrowed). Phase-contrast; ×170.

Fig. 3. Dermal papilla cell culture treated with pre-immune serum in the staining procedure as one of the immunohistochemical controls.
previously described (Jahoda and Oliver, 1981; Messenger, 1984). Vibriissa dermal sheath cultures were initiated from the thin circular envelope that remains attached to the dermal papilla after removal of the epidermal component during dissection. Human dermal sheath cells were cultured from all of the bulb dermis remaining after the papilla and epidermal matrix has been excised (Jahoda et al. unpublished). Briefly, the bases of anagen follicles were cleared of any adherent tissue, under a binocular dissecting microscope (×20) in Minimal Essential Medium containing penicillin/streptomycin at 50 units ml⁻¹ (both Gibco), and their bulb regions were excised and transferred to dishes with fresh medium. The follicle bulbs were then inverted using a combination of finely tipped watchmakers’ forceps and sharp tungsten needles. This invariably led to separation of the epidermal component from the dermal papilla and attached sheath of follicular dermis (Fig. 2). The sheath was then easily separated from the papilla with tungsten needles.

Rat skin fibroblast cultures came from explants from the three above mentioned regions of skin: mystacial pad, ear and footpad. Where possible, fibroblast cultures were set up from the same individuals as were used to provide papilla and dermal sheath cells. Areas of skin were cleaned, and surface hair fibres removed, before the tissue was chopped into small pieces. Fibroblast cultures were set up from explants of human skin in a similar manner.

For immunolabelling of cells during early outgrowth, explants were established directly on top of glass coverslips in 35 mm culture dishes. For later observation, passaged cells were seeded at various densities on top of coverslips in 35 mm dishes. All cultures were fixed and permeabilized by exposure to methanol for 30 min at −20 °C before marking.

Immunohistochemistry

Cultured cells and sections were incubated with a monoclonal antibody to smooth muscle α-actin (anti-asm-1, Skalli et al. 1986) diluted 1:10 in phosphate-buffered saline (PBS), pH 7.4, at room temperature for one hour. After thorough washing in PBS, material was exposed to biotinylated anti-mouse antibody (BRL), diluted 1:90, for one hour, washed again, and incubated with fluorescein-linked streptavidin, diluted 1:100, for the same length of time. Specimens were mounted in Citifluor (Agar aids) medium, and examined and photographed on a Zeiss ICM 406 inverted microscope equipped with epifluorescence. Alternatively, for more permanent preparation the material was stained as described above, but with horseradish peroxidase-linked streptavidin. Estimations of positive smooth muscle actin marking in cell cultures, were carried out by examining one hundred fields in randomly chosen fields. Rat tissues and cell cultures were also labelled with a polyclonal antibody that recognizes all actin isoforms (Skalli et al. 1986). The same material was tested for desmin with an affinity-purified rabbit anti-desmin polyclonal antibody (Kocher et al. 1984) and a monoclonal antibody to vimentin (Transformation Research, Inc. Framingham, MA, USA). As one of the control procedures, specimens were treated with pre-immune sera. None of the control sections or cultures treated in this fashion showed any significant fluorescence (Fig. 9).

Gel electrophoresis

For 2-D PAGE, passage-two cultured dermal papilla cells and fibroblasts were dissolved in sample buffer containing 1% sodium dodecyl sulphate, 1% dithiothreitol, 1 mM N'-p-tosyl-L-arginine methyl ester and 1 mM phenylmethylsulphonyl fluoride. In the first dimension, actin isoforms were separated by isoelectric focusing according to O’Farrell (1975). The pH gradient was established with 2% preblended Ampholines. pH 4.0–6.8 (LKB Co., Lucerne, Switzerland). The second dimension was run on SDS–10% PAGE. For the different cell types, equal amounts of sample protein were loaded, following protein determination by the Bradford (1976) method.

Electron microscopy

Specimens were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) and 0.05% Ruthenium Red (to enhance visualization of extracellular material) for two hours. Postfixation was in 1% osmium tetroxide, with 0.1 M cacodylate buffer and 0.025% Ruthenium Red, for three hours at 4°C. Material was dehydrated in increasing concentrations of ethanol and propylene oxide before being embedded in Epon resin. Ultrathin sections were stained with uranyl acetate, followed by lead citrate, and examined with a Zeiss EM 109 electron microscope.

Results

Staining of normal tissue sections

Rat vibrissa follicles. In anagen vibrissa follicles, overall actin distribution, as revealed by the general antibody, was widespread, with the outer root sheath of the follicle epithelium strongly marked (Fig. 4).

Smooth muscle α-actin labelling of mid-anagen vibrissa follicles revealed that the dermal papilla component was virtually unmarked. However, the lower dermal sheath of the follicle was clearly labelled, except for a region surrounding part of the bulb at the base of the follicle (Fig. 5). This marking clearly delineated a line of dermal sheath cells just exterior to the glassy membrane (Fig. 5) but from about half way up the follicle, staining became patchy, and then petered out (Fig. 6). Follicles were not labelled by the antibody to desmin but vibrissa sections acted as an excellent positive control for this antibody, because follicles could be left with some of their surrounding muscle tissue attached, and these muscle blocks always displayed intense staining (Fig. 7).

Rat skin. Foot pad skin was virtually unmarked, with only dermal vasculature and Meissner corpuscles positively labelled. Ear and whisker pad skin both showed widespread follicular staining with the total actin antibody (Fig. 8). As in vibrissa follicles, smooth muscle α-actin label was restricted to the dermal sheath, with no expression at all inside the dermal papilla except for occasional marking of blood vessels (Fig. 9). However, the smaller pelage follicles differed from vibrissae in that labelling extended right around the follicle base. There was also an indication that staining of the dermal sheath was absent or reduced in follicles that had shortened, and were in the telogen phase of the hair cycle. Intriguingly, longitudinal sections cut at the outside edge of follicles revealed a highly organized pattern of smooth muscle α-actin within the dermal sheath. The label showed up as a stack of concentric horizontal rings around the follicle circumference (Fig. 10). This tied in with parallel observations from median sections where the outer line of dermal sheath fluorescence was punctuated rather than continuous.

Human follicles. Anagen human hair follicles were also marked around the dermal sheath and, like rat vibrissa follicles, label was not always strong outside the lowest part of the follicle bulb. In some specimens the basal stalk below the papilla was strongly marked (Fig. 11), but papillae were never stained. Unlike vibrissa follicles, dermal sheath labelling extended into the upper half of appendages. Detailed examination showed that the antibody was only staining the internal cell layer of the dermal sheath. When this was cut tangentially, it showed the same circular horizontal labelling observed in the dermal sheath of rat pelage follicles. The outer connective tissue sheath remained unmarked, although blood vessels peripheral to the follicle were often fluorescent (Fig. 12).

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Passaged cell culture labelling

Smooth muscle α-actin. In early-passage rat dermal cells (passages 1 to 3), hair follicle-derived dermal papilla and dermal sheath cells were both highly positive for smooth muscle α-actin; inside prominent stress fibres (Figs 13, 14). Although the amount of positive staining was slightly variable between different lines, it was estimated that over 75% of papilla cells, and more than 95% of dermal sheath cells, were marked in all these cultures. Indeed, several dermal sheath cell cultures appeared to have nearly every cell stained positively, although within groups individual cells displayed different intensities of label. The distribution of marker within cells reflected previous observations, in that marking of stress fibres did not always extend to the periphery of the cell projections. However, intricate patterns of cytoskeletal architecture were visible near the periphery of many larger flattened cells, perhaps associated with
adhesion plaques. Further passaging reduced the estimated number of papilla or sheath cells with positive marking, and with subculture the intensity of label also diminished within individual cells; however, passage six or seven papilla and sheath cells still remained over 60% marked.

All of the fibroblast cell lines from the various regions of skin had less than 20% of their cells positively stained by smooth muscle a-actin. Foot pad and ear skin fibroblasts contained 10% or less of positive cells, and these were often distributed in patches, presumably as a result of colony formation around a single parent cell (Fig. 15). Large areas of these cultures were often devoid of label, in stark contrast to the uniform staining of fibroblasts by the total actin antibody (Fig. 16). Fibroblasts from the mystacial region displayed a slightly higher number (up to 15%) of positively labelled cells. Once more, serial passaging did not alter the basic staining pattern, so that the fibroblasts retained small colonies of cells that contained smooth muscle a-actin after extended passaging, but again with considerable variation in staining intensity between cells.

Desmin and vimentin. Vibrissa dermal papilla and sheath cells, and rat skin fibroblasts, were all negative to the anti-desmin antibody, but positively marked by the antibody to vimentin (Fig. 17).

Explant outgrowth labelling

Vibrissa dermal papilla and dermal sheath. Cells around fragments of dermal sheath tissue were universally marked by the smooth muscle a-actin antibody as soon as they emerged from the explants. By contrast, cells from dermal papilla explants that had attached and flattened were initially negative. Then, between the first and second week in culture as cells began to move and spread out, their appearance altered. Cells at the periphery of the explants became positive for the smooth muscle a-actin antibody, while the central mass still remained negative (Figs 18, 19). In papilla explant cultures that had been established for 14 days or more, when cells had further dispersed, an increasingly high proportion of them were marked.

Mystacial pad skin. In the cell-covered substratum surrounding pieces of mystacial pad skin, whole regions were completely unmarked, and it was clear that positive staining among the fibroblast cells was highly localized. Further scrutiny of areas with labelled cells revealed that labelling was usually found around pelage hair follicles. Follicles were visible in the skin explants and groups of marked cells were seen around the outline of these follicular 'contaminants' (Fig. 20).

Human cells. Between passages 1 and 3, widespread staining of the dermal papilla and dermal sheath cells with smooth muscle a-actin was evident. Around 90% of dermal sheath cells and 90 to 80% of dermal papilla cells were positively labelled (Fig. 21), but once again individual cells showed different staining intensity. Later, passage human dermal papilla and dermal sheath cultures from groin skin follicles had reduced numbers of strongly marked cells – in one passage 7 papilla culture as low as 40%. This phenomenon was less apparent with papilla cells from scrotal skin follicles. Human skin fibroblast cultures from all of the studied regions contained very small numbers of marked cells (Fig. 22): in some cases less than 2%.

Explant cultures showed precisely the same trends as previously described for rat follicles. At an early stage in culture, dermal sheath explants produced arrays of cells in parallel lines, all positively marked and emanating from the central tissue (Fig. 23). At the same time, dermal papilla cultures were again unmarked in the central papilla core, and only sparse labelling was seen where cells had initially settled and spread outwards (Fig. 24). Fibroblast cells that emerged from pieces of skin in explant culture were again almost totally negative. As with rat material, positively stained cells were localized most clearly in the vicinity of hair follicles present in the skin.

2-D PAGE. When the pattern of actin isoforms was examined by 2-D PAGE, cultured dermal papilla and dermal sheath cells were found to contain three spots corresponding to a-, b- and g-actins, as previously described (Kocher et al. 1984; Skalli et al. 1987) (Fig. 25A). Only two spots, identified as b- and g-actins, were seen in gels prepared from equivalent skin fibroblast samples (Fig. 25B).

Transmission electron microscopy. Under phase-contrast microscopy, stress fibres are prominent in rat dermal papilla and dermal sheath cell monolayers. A behavioural feature of these cell types is their propensity to form aggregates, and ultrastructural examination of cells on plastic substrata, or on top of other cells within clumps, revealed thick microfilament bundles (Fig. 26). More intriguing is the finding that in situ dermal papilla cells...
displayed extensive cytoplasmic processes, many of which were packed with microfilaments. Sometimes, elongated cells were observed with bulbous regions of cytoplasm bridged by narrower sections rich in microfilaments. These cells were undoubtedly in the process of moving (Figs 27, 28), and appeared most often during periods of changing activity, at the beginning or end of the hair cycle.

Figs 13–17. Staining of passaged rat cells.
Fig. 13. Passage 3 rat dermal papilla cells, many of which are overlapping, show widespread labelling with smooth muscle α-actin. x90.
Fig. 14. Rat dermal sheath cells after three passages display strong marking with the smooth muscle α-actin antibody. Note the broad flattened morphology of this cell type. x90.
Fig. 15. One α-actin-labelled cell against a field of unstained cells in a passage 2 ear skin fibroblast culture. x56.
Fig. 16. Antibody to total actin marks stress fibres in every cell in this passage 2 skin fibroblast culture. x220.
Fig. 17. Vimentin filaments labelled in a passage 2 dermal papilla cell. x380.

Fig. 18. A dermal papilla explant after 11 days. The centre of the explant (e) has collapsed, and papilla cells have attached to the substratum but are still relatively small and tightly bunched. There is almost no smooth muscle α-actin staining in this central area but some cells are marked towards the edge of the field. x90.

Fig. 19. Lower magnification view of the same explant culture. Cells at the core of the explant are unmarked, but towards the periphery, where they have become more spaced out and flattened, there is widespread marking. x56.

Fig. 20. Low magnification view, showing two hair follicles at the edge of a skin explant, and two clumps of smooth muscle α-actin-labelled cells in close proximity (arrowed). In fibroblast explant culture, the only large groups of positively stained cells were invariably associated with hair follicles, and were usually in aggregated form. x56.

Fig. 21. Human groin follicle dermal papilla cells after their second passage, marked with smooth muscle α-actin. Note the relatively broad morphology of some cells. x90.

Fig. 22. A second-passage groin skin fibroblast culture, showing small numbers of labelled cells, against an unstained background of confluent cells. Many parts of human skin fibroblast cultures were completely unmarked. x90.

Fig. 23. Groin dermal sheath cells positively stained for α-actin streaming outwards from a central tissue explant, which is itself well marked. Ten day culture. x90.

Fig. 24. A human groin dermal papilla explant (p) equivalent to that in Fig. 23, is not positively labelled by the α-actin antibody at ten days. Of the cells that have emerged and settled in the vicinity, a relatively small proportion are marked. x225.

Fig. 25. (A),(B) Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) of second-passage cultured cells. Dermal papilla cells contain α-, β- and γ-actins (A), whereas skin fibroblasts only show β- and γ-actin isoforms (B).
Discussion

It appears increasingly clear that the cells that are collectively called fibroblasts or stromal cells display a phenotypic diversity that was not previously suspected on morphological grounds (Sappino et al. 1990). As the predominant cell type in connective tissue, the fibroblast fulfils a variety of essential functions, and plays a pivotal role in tissue repair. It is also implicated in mesenchymal–epithelial interactions that are known to govern organogenesis. The recognition of heterogeneity in the cytoskeletal differentiation programme of fibroblastic cells may therefore help us to understand further the specialized activities of the different subpopulations. Cytoskeletal markers are useful for this purpose, and are particularly suited to identifying potential contractile capacities of fibroblastic cells.

As briefly observed previously (Skalli et al. 1986), the
dermal sheath region of the hair follicle labels positively with smooth muscle \( \alpha \)-actin, and present work shows that in vibrissa follicles dermal sheath marking is often restricted to the lower half of the follicle. This observation may relate to experimental work where, on removal of the lower third of vibrissa follicles, a new papilla is re-formed from lower dermal sheath cells. The latter are observed by light and electron microscopy migrating from the sheath region through the thick specialized basement membrane of the follicle to the site of papilla formation (Oliver, 1966b: Jahoda et al. 1991). When the follicle is ablated higher up, papilla regeneration does not take place (Oliver, 1966a). While sheath cells can definitely alter their phenotype to become papilla cells, it is less clear whether the reverse is true, although characteristic Alcian Blue marking of papilla cells suggests that they at least partially assume the role of dermal sheath in new follicles induced by papilla interaction with skin epidermis (Oliver, 1970). Human follicle papilla regeneration can also be elicited (Jahoda et al. 1989), which suggests that a similar papilla/sheath cell relationship exists in the lower human follicle. The finding that only the internal, horizontally aligned layer of the human dermal sheath has smooth muscle \( \alpha \)-actin marking, emphasizes that this structure essentially consists of two layers (Kligman, 1988), each of which may have a separate function. This may also hold true for the rodent pelage follicle sheath, and indeed for other follicle types.

Here we report for the first time that cultured cells from both the dermal papilla and the dermal sheath are marked positively with smooth muscle \( \alpha \)-actin-specific antibodies, but react negatively with desmin antibody. Like skin fibroblasts, both sheath and papilla cells contained vimentin. While counts of numbers of smooth muscle \( \alpha \)-actin positive cells acted as a useful indicator, they could not be regarded as accurate assessments of overall content because of individual variation in labelling intensity. However, 2-D PAGE analysis confirmed that the smooth muscle \( \alpha \)-actin isoform is a major component in the cytoskeletal make up of follicle dermal cells, but not an appreciable element in skin fibroblasts.

The change of papilla cells from negatively stained \textit{in vivo} to positively marked \textit{in vitro} has a parallel in cultured eye lens cells (Schnitt-Graff et al. 1990), and it distinguishes these special types of fibroblastic cells from adult smooth muscle cells whose smooth muscle \( \alpha \)-actin content decreases when placed in culture. During atherosclerosis, smooth muscle cells also lose smooth muscle \( \alpha \)-actin and revert to having a \( \beta \)-isoform predominance, similar to that seen in embryonic tissue (Gabbiani et al. 1984; Kocher and Gabbiani, 1987). While dermal sheath cells did not alter their smooth muscle \( \alpha \)-actin expression in culture, a feature of the current work was the very rapid switch on of smooth muscle \( \alpha \)-actin expression by papilla cells around the second week of explant culture. The finding that cells that moved into spaces away from the original papilla mass immediately started to express smooth muscle \( \alpha \)-actin, and that the whole culture could transform in a matter of days, depending on the speed of dispersal, was intriguing. It suggests that perhaps the papilla cells are held in a state of non-expression by modulatory or inhibitory factors within the follicle, which are lost in the culture environment; this is unusual in relation to control of smooth muscle differentiation. On this point it is worth noting that the dermal papilla has a glycosaminoglycan (GAG)-rich extracellular matrix, and that heparin is one extracellular component that is known to influence smooth muscle \( \alpha \)-actin expression \textit{in vivo} (Clowes et al. 1988), and \textit{in vitro} (Desmouliere et al. 1990). The sudden change in marking also provided a possible link between smooth muscle \( \alpha \)-actin expression and cell motility, since papilla cells took up this label at the time when they began to move. In this context, ultrastructural observations of migratory papilla cells \textit{in situ} stress the need to consider the papilla cell population as dynamic and not static, particularly during periods of morphogenetic activity.

Another striking finding was the relative homogeneity of the dermal sheath cell cultures, where an exceptionally high proportion of cells stained positively with smooth muscle \( \alpha \)-actin antibody. This means that dermal sheath could become a valuable source of culture material for...
studies of smooth muscle α-actin as a cell cytoskeletal component.

In relation to papilla cell cultures, the reduction in numbers of positively stained cells over time could be a reflection of the ageing of the cell lines. It has previously been observed that human papilla cells have a relatively short lifespan before they become 'senescent' (Messenger, 1989). The low numbers of marked cells seen in a passage 7 human papilla cell culture coincided with a slowing down in cell division and other signs of senescence at the time of testing.

Although rat skin fibroblast cultures generally had low numbers of cells containing smooth muscle α-actin, those from hairy skin (whisker pad) appeared to have more labelled cells than cultures from less hairy regions (footpad or ear). This tied in with the observation that early on in skin explant culture positively stained cells had a patchy distribution, and were seen grouped at the periphery of hair follicles. In rat fibroblast cell lines we have observed heterogeneity of morphology, and from time to time have noted aggregations of cells, similar to those observed in dermal papilla (Jahoda and Oliver, 1984) or dermal sheath (Jahoda et al. unpublished) cultures. From these observations, one idea is that fibroblast cell lines from skin explants that incorporate hair follicles are 'contaminated' by follicular mesenchyme cells. This could have significance with regard to fibroblast lineage relationships. It also might need to be taken into consideration in experimental work on skin fibroblasts, where the original tissue source is hair-bearing skin. However, the finding of the same phenomenon in human explants raises a potentially more important idea. Myofibroblasts that are involved in skin wounding have specific properties, some of which resemble smooth muscle cells (Skalli and Gabbiani, 1988). Their origin in wounds appears to be local (Ross et al. 1970), and they are thought to be derived from fibroblasts (Skalli and Gabbiani, 1988, for review). A recent study of open cutaneous wounds in the rat has suggested that fibroblasts in granulation tissue transform gradually into myofibroblasts, and during a restricted period express a smooth muscle marker (Darby et al. 1990).

In superficial cutaneous wounds, a principal source of replacement skin epidermis comes from outer root sheath epidermal cells of the hair follicle (Eisen et al. 1955; Bullough, 1970; Krawczyk, 1971; Pang et al. 1978). Given the similarity between myofibroblast and dermal sheath cell characteristics, it is reasonable to suggest that the dermal sheath cell layer (previously shown to be a smooth muscle characteristic, it is reasonable to suggest that the dermal sheath of normal organs have been described in the fibroblasts of normal organs where some tissue reorganization occurs either continuously or periodically (Sappino et al. 1990, for a review), and where contractile function might be involved. One of us (Reynolds et al. unpublished data) has observed changes in smooth muscle α-actin expression in the dermal sheath during the hair growth cycle, and has suggested that the smooth muscle characteristics of dermal sheath cells could relate to contractile processes that might control hair follicle shortening and upward fibre movement during the hair growth cycle. In this context, the observed alignment of smooth muscle α-actin in the inner follicle dermal sheath as a series of concentric circular rings clearly implies functional importance, particularly as this organization appears to be common to many follicle types.

This study provides a new marker for hair follicle dermis, and possibly an opportunity of examining the hair follicle from a slightly different evolutionary and developmental point of view. It will certainly assist in those investigations where hair follicle cells need to be distinguished.

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References


