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Modelling the hair follicle dermal papilla using spheroid cell cultures

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Abstract: Human dermal papilla (DP) cells grown in two-dimensional (2D) culture have been studied extensively. However, key differences exist between DP cell activities *in vivo* and *in vitro*. Using a suspension method of cell culture to maintain DP cells, we created three-dimensional (3D) dermal spheres morphologically akin to intact (anagen) DPs. Analysis of these spheres using immunocytochemistry demonstrates that they have expression profiles different from papilla cells cultured in 2D but with many similarities to intact DPs. This method of DP cell culture may provide us with a tool to elucidate our understanding of signalling within the DP as it relates to induction, maintenance or even inhibition of hair growth.

Key words: cell culture – dermal papilla – hair follicle

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Introduction

Dermal papilla cells have been widely exploited in work aimed at identifying and understanding the signalling within the DP, and between the epithelial and mesenchymal compartments of the hair follicle. DP cells are a specialised cell population, distinguishable from interfollicular dermal fibroblasts by unique characteristics such as aggregative behaviour (1–3) and an ability to induce new hair follicle formation (4,5). The morphology, biosynthetic activities and behaviour of DP cells *in vitro* have been studied extensively although there are key differences between 2D cultured DP cells and intact DP. For example, the mitotic activity of DP cells *in vitro*, albeit lower than dermal fibroblasts (2) is very different from DP cells *in vivo*, where only 2% of papilla cells are proliferative (6). Additionally, *in vivo* alpha smooth muscle actin (αSMA) is expressed within the dermal sheath only, although in culture DP cells switch on strong αSMA expression (7). Perhaps most relevant in terms of hair biology is the loss of inductive potency of primary DP cells, observed after a period of time in 2D culture (8). Loss of expression of the extracellular matrix protein versican, and additionally the enzyme alkaline phosphatase at higher passage numbers in DP cell cultures, have both been correlated with a loss of inductive capabilities of DP cells (9,10). Studies have been presented that detail the maintenance of versican expression *in vitro* (11,12), thought to be a way of maintaining the anagen characteristics and inductive capabilities of these cells (12).

Three-dimensional culture has been successfully developed for embryonic stem cells using a hanging drop method (13). The close aggregative conformation of DP cells within the DP *in vivo* suggests that growth in 2D culture is particularly 'unnatural'. We therefore created hanging drop cultures in which cultured DP cells aggregate together and form spherical structures, which appear morphologically akin to *in vivo* DP. Given the differences between 2D cultured DP cells, and intact DP, in this study we set out to analyse whether spherical cultures of DP cells were more representative of *in vivo* DP.

Abbreviations: DP, dermal papilla; 2D, two dimensional; 3D, three dimensional; αSMA, alpha smooth muscle actin; PCNA, proliferating cell nuclear antigen.

Experimental outline

Eight cell strains of human DP cells, established from scalp follicles, were cultured until passage 4 and above. Cells were trypsinized and plated in either 35-mm dishes, or in hanging drop cultures. Hanging drops consisted of 3000 cells in 10 μl of DMEM containing 10% FBS. Cultures were maintained for between 30 and 72 h after which time spheres were collected for immunofluorescent or transcrip-

tional comparison with 2D cultures. Detailed methods are in Appendix S1.

Results

Dermal papilla spheres formed rapidly from 2D cultures (Fig. 1a) and after 20 h were approximately 150 μm in diameter (Fig. 1b), with spheres established together being of equal proportions. Spheres were viable, and when plated back down in culture dishes, cells grew out from the spheres. Proliferating cell nuclear antigen (PCNA) was detected in the nuclei of 2D cells (Fig. 1c) whilst in contrast, PCNA expression was absent in spheres (Fig. 1d). αSMA was present in 2D cultures in its distinct fibrillar pattern (Fig. 1e), although in spheres, whereas protein expression was observed after 30 h in hanging drop culture, by 50 h all αSMA expression had disappeared (Fig. 1f). No other observations were time dependent. Although absent from 2D cultures (Fig. 1g), versican was re-expressed in dermal spheres from three of the eight cell strains (Fig. 1h). Other extracellular matrix proteins such as perlecan were prominently expressed in both 2D and 3D cultures (Fig. 1i,j).

We also analysed the activity of alkaline phosphatase, the expression of which, like versican, has been correlated with the inductive potential of DP cells. We found that while enzyme activity was absent in 2D cultures (Fig. 1k), activity within spheres was high in all cell strains analysed (Fig. 1l).

We analysed junctional expression within DP cells and spheres, and observed that in 2D cultures, β-catenin was expressed at points of contact between cells (Fig. 1m). In spheroids, prominent β-catenin expression was observed due to the increased cell–cell contact in the spherical conformation (Fig. 1n). Connexin 43, a gap junction protein expressed in the DP *in vivo*, was also expressed in both 2D and 3D cultures (Fig. 1o,p) suggesting intercellular contact and communication.

At the transcriptional level, we analysed the expression of DP markers (Fig. 1q). We found that expression of Lef1, and Axin2, members of the Wnt signalling family were partially restored in spheres comparable with 2D cultures. However, APC and GSK3B also remained high in spheres. Expression of Corin and Prostaglandin endoperoxidase synthase 1 (14,15), which are diminished *in vitro* comparative to the *vivo* DP, are restored in spheres whereas the DP marker NCAM (16) has transcript levels that increase in culture, but decrease to a level similar to an intact DP in spheroid culture. Lastly, Nexin expression remains constant in both 2D and 3D cultures comparative to the intact DP.

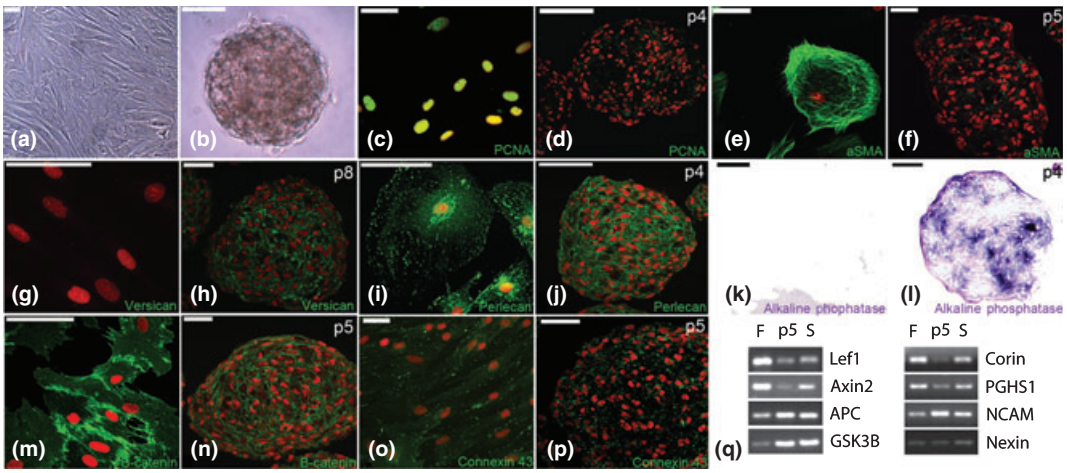

Figure 1. (a) Conventional culture systems enable the growth of cells in a 2D format. (b) Growth of dermal papilla cells in hanging drop cultures results in the formation of dermal spheres. (c) In conventional culture, dermal papilla cells are mitotically active as indicated by PCNA expression. (d) 3D spherical cultures do not express PCNA, and in terms of proliferative potential are similar to intact dermal papillae. (e) αSMA is expressed in a distinct fibrillar pattern in 2D cell cultures of dermal papilla cells. (f) Once grown in spheres, αSMA expression is lost after 50 h in culture. (g) Versican, a proteoglycan expressed in the dermal papilla during the anagen phase of the hair cycle, is absent in dermal papilla cells after a couple of passages in culture. (h) Expression of Versican is restored in dermal spheres from dermal papilla cells. (i) Perlecan, an extracellular matrix protein expressed throughout the hair cycle in the dermal papilla, is also expressed in conventionally cultured cells at all passages. (j) Expression of perlecan is maintained in dermal sphere cultures. (k) Alkaline phosphatase activity is not detected in passage 4 cultured dermal papilla cells. (l) In dermal spheres, alkaline phosphatase activity is strongly observed. (m) Expression of β-catenin is expressed at points of cell contact in 2D cultures of dermal papillae. (n) In 3D cultures, β-catenin is strongly expressed at cell membranes within the sphere. (o) Connexin 43 expression in dermal papilla cells is punctuate, localised at gap junctions between cells. (p) In dermal spheres expression of connexin 43 remains at the cell surface. (q) PCR of dermal papilla markers comparing freshly isolated dermal papillae (F), cultured dermal papillae at passage 5 (p5), and spherical cultures (S) indicates partial restoration of an intact dermal papilla profile in spherical cultures. Scale bars: 50 μm. Propidium Iodide: Red; Antigen of interest: Green.

Discussion

Dermal papilla spheres not only resemble DP morphologically, but are also similar to them on a molecular level, representing an advance over conventional culture systems. The dermal spheres that we describe here contained 3000 cells, which is comparable with male facial papillae (17), although spheres of all sizes can be generated. DP spheres showed an absence of proliferation, in addition to showing a loss of αSMA expression compared with conventionally cultured cells. The switching on of αSMA expression in conventional 2D DP cell culture has been suggested to represent a de-differentiation event (7), indicating that in a sphere configuration, cells are re-differentiating to a more papilla like phenotype.

The expression profile of proteoglycans in DP spheres did not consistently match that of an intact DP, as versican expression was only restored in three of the eight cell strains used, compared with perlecan that was consistently expressed throughout all cell strains. The restoration of versican expression is perhaps indicative of partial restoration of inductive capacity of DP cells, although as this was not observed in all cell strains, a change in structure and contact between cells is not necessarily the only requirement for rescued versican expression. Within cell strains, if versican expression was recovered, it occurred at all passages analysed. Therefore, it appears that there are large differences between cell strains derived from different donors.

Previously, the formation of spheroids has been described in both rat and mouse DP cells grown on plates with low cell binding capacities, which promote cellular aggregation (18,19). These spheroids showed a reduced proliferative capacity, but maintained αSMA expression. Comparatively, we have for the first time exploited the hanging drop method for human DP cell growth. These new methods of cell culture are exciting as they present an opportunity to isolate and expand a population of DP cells, then grow them in a 3D microenvironment that is useful for simulation of *in vivo* DP activity. The partial restoration of an intact DP signature, with the markers described here, shows promise for inductive potential of human dermal spheres.

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Supporting information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Supplementary Methods.

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