Plasticity of Rodent and Human Hair Follicle Dermal Cells: Implications for Cell Therapy and Tissue Engineering

Gavin D. Richardson,* Elisabeth C. Arnott,* Claire J. Whitehouse,* Clifford M. Lawrence,† Amanda J. Reynolds,* Nicholas Hole,* and Colin A. B. Jahoda*

*School of Biological & Biomedical Sciences, University of Durham, Durham, NC, USA; †Department of Dermatology, University of Newcastle upon Tyne, Newcastle upon Tyne, UK

The dermal components of the hair follicle exhibit a number of stem cell properties, including regenerative potential, roles in wound healing and the ability to produce a functional dermis. Here we examine the stem cell phenomenon of plasticity, focusing on recent observations of *in vitro* plasticity of dermal papilla and sheath cells, including previously unpublished data of neuronal-like differentiation. We then briefly address the implications of the stem cell potential of hair follicle dermal cells for the field of tissue engineering.

Hair Follicle Dermal Stem Cells

Hair follicles contain discrete populations of mesenchymal (dermal) and epithelial cells, and over recent years the stem cell capabilities of the latter have generated intense interest in relation to follicle cycling; skin renewal; and tumor biology (e.g., Taylor et al, 2000; Callahan and Oro, 2001; Niemann and Watt, 2002; Alonso and Fuchs, 2003; Tumbar et al, 2004). One of the dermal compartments of the follicle, the dermal papilla (DP), is widely recognized as the key signaling center, responsible for maintaining hair growth and controlling the complex system of hair follicle cycling. Papilla cells, and those from the adjacent dermal region, the dermal sheath (DS), have been shown to have powerful inductive properties in experimental assays (Jahoda and Reynolds, 1996; Reynolds et al, 1999; Kishimoto et al, 2000). Until recently, however, the stem cell properties of the follicle dermis have largely been overlooked. Based on the many features that these dermal cells share with follicle epithelial stem cells, including regenerative potential (Oliver, 1966); a role in wound healing (Jahoda and Reynolds, 2001); and the ability to form a fully functional skin dermis (Gharzi et al, 2003), we recently suggested that follicle papilla and sheath cells may act as stem cells for both follicular and interfollicular dermis (Jahoda et al., 2003). Now we have evidence that the stem cell potential of follicle dermal cells extends beyond the skin.

Stem Cell Plasticity

Little is known about the phenotype of mammalian adult or somatic stem cells; therefore, the definition of a stem cell remains largely functional. Until fairly recently, the prevailing dogma stated was that whereas embryonic stem (ES) cells are able to generate most, or all cell lineages found within an organism, in contrast, adult stem cells are more restricted in their differentiation potential, and therefore are only able to differentiate into lineages associated with the tissues from which they are derived. Although the subject of considerable debate and controversy, evidence is accumulating that adult stem cells can exhibit greater plasticity than previously believed (Blanpain et al, 2004). Through the addition of growth factors known to induce stem cell differentiation from other tissue systems, or by the relocation of adult stem cells into a new niche, such cells have displayed the ability to be "reprogrammed" down lineages or become cells that are characteristic of the new environment (Jiang et al, 2002; Wagers and Weissman, 2004). In addition, many other sources of adult stem cells have been discovered, including from skeletal muscle, skin, adipose tissue, and the central nervous system (reviewed in Anderson et al, 2001; Blau et al, 2001; Wulf et al, 2001). As a source of multipotent stem cells, skin dermis has received much attention because of its accessibility, and skin dermal-derived stem cells have been shown to have broad differentiation capabilities in culture, giving rise to muscle- and neuronal-type cells (Toma et al, 2001; Fernandes et al, 2004); however, As the initial dermal population was heterogeneous, the origin of this stem cell population remains unclear, and we have hypothesized that the origin of much of this stem cell activity could be the follicle dermis (Jahoda et al, 2003).

## Plasticity of Rodent Hair Follicle Dermal Stem Cells

Our own observations of the sporadic appearance of muscle-, lipid- and bone-type cells in discretely isolated rat follicle DP and DS cell primary cultures initially led us to investigate the stem cell capabilities of specific hair follicle dermal cells. Utilizing clonally derived rodent papilla and

sheath cell lines established in our laboratory, we recently published data demonstrating that these cells could be directed toward adipocyte and osteocyte phenotypes in culture via the administration of the appropriate media (Jahoda *et al*, 2003). Moreover, we have demonstrated that the hair follicle dermal compartment harbors cells that have hematopoietic stem cell activity (Lako *et al*, 2002). Most recently, based on work carried out by Toma *et al* (2001), we have investigated whether it is possible to induce a neural phenotype in discretely isolated DP and DS populations. We have found that the administration of FGF2 to primary dermal cell explants grown on a laminin substrate results in adherent cells displaying a classical neural morphology with rounded cell bodies and multiple process-like extensions (Fig 1*a*). These cells were most evident on the peripheral zones of the explants; the cells in the central zones maintained a more rounded phenotype than the control explants. Immunohistochemistry also identified an increase in the expression of the pan-neurological markers, βIII-tubulin and NF200, in all treated cultures regardless of zone, when compared with controls grown under normal culture con-

ditions (MEM/20% FCS) (Fig 1*a*). After 7–9 d within the primary cultures, we observed a number of small floating cell spheres that no longer adhered to the culture dish (Fig 1*b*), similar to the cell "neurospheres" formed by skin-derived precursors (SKP), observed by Toma *et al* (2001). These floating sphere cells could be transferred to new culture dishes adhered, then aggregated to form spheres, which expressed the highest levels of βIII-tubulin within the culture. Five to seven d post–transfer, the non-adherent spheres could again be seen. Although these results point to follicle dermal stem cells being able to be directed toward neuronal lineage, a great deal of caution must be applied to these observations. First, the pan-neural markers that we have used are not specific to neural cells. We need to show that specific neural cell types can be produced much more definitively. Also, we observed a low level of expression of some of these neural-associated markers in our control cultures after as little as 3 d, suggesting that their expression could, in part, be a culture effect. For example, one group (Neuhuber *et al*, 2004) recently reported that the treatment of mesenchymal stem cells (MSC) with DMSO results in the rapid expression of some neuronal markers; however, other essential neuronal proteins are absent. They suggest that it is possible that the upregulation of neuronal markers may be because of aberrant gene expression rather than *bonafide* neuronal differentiation, and caution that *in vitro,* differentiation protocols may have non-specific effects. In support of our current observations of neural differentiation, Fernandes *et al* (2004) have characterized a population of cells capable of both neural and mesodermal differentiation. Termed SKP, these cells have been identified within the skin dermis. Based on expression of neural crest-associated markers, it is possible that these cells are neural crest derived. In the hair follicle, SKP are located in the area comprising the DP and express proteins associated with this hair follicle structure including versican and nexin. The results described above, together with our previous observations of spontaneous skeletal muscle and beating myocardiocytes in rodent dermal cell cultures (Jahoda *et al*,
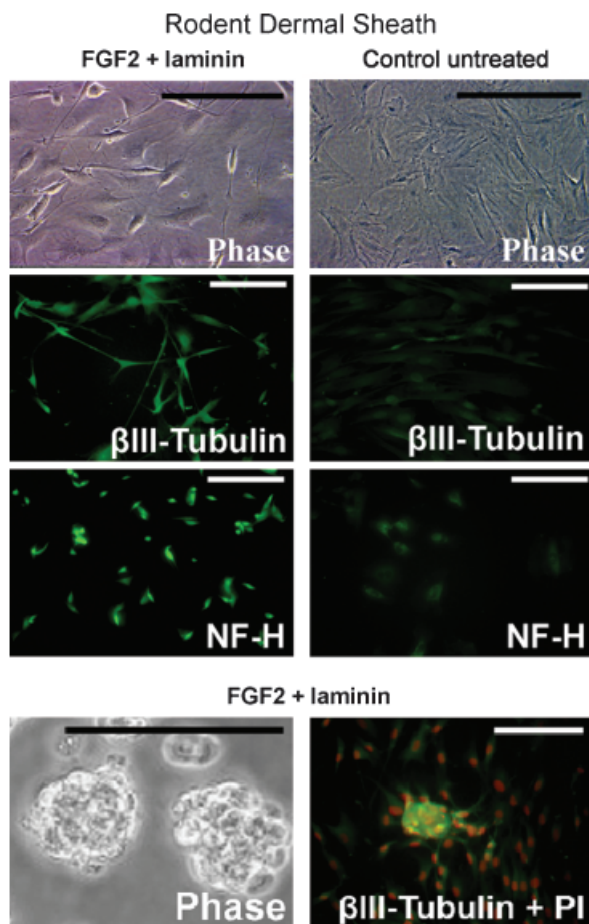
Figure 1
Primary rodent follicle dermal explants grown on laminin substrate can be induced by FGF2 (40 ng per mL) to express neural cell-associated markers and form spheres in culture. (*a*) *Top panel*: Phase contrast image of treated and control cells. *Middle panel*: FGF2-treated cell show greater immunoreactivity for neuron-specific βIII-tubulin (green). *Bottom panel*: FGF2-treated cell show greater expression of neuron-specific neurofilament (green). (b) *Left*: phase contrast image of non-adherent floating sphere. *Right*: cell aggregate with labeled nuclei PI (red) and βIII-tubulin (green). *Scale bar*: 10 μm.
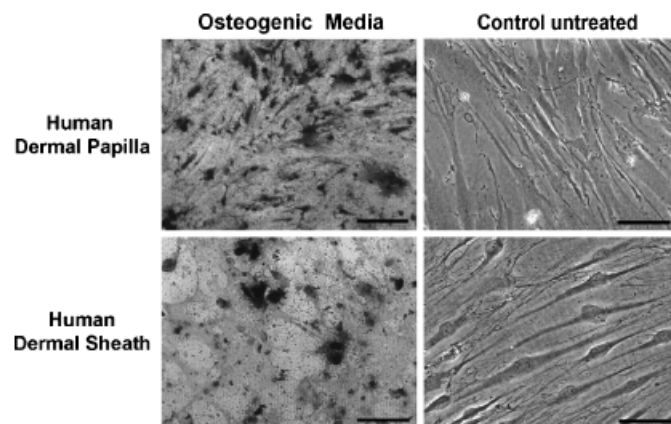
Figure 2
Human dermal sheath and papilla directed towards osteogenic differentiation. Cells cultured in osteogenic medium (MEM plus 10% FBS, 100 nM of dexamethasone and 10 mM of β-glycerophosphate, supplemented every 3 d with 50 μM L-ascorbate-2-phosphate) were subsequently Von Kossa stained for the detection of calcification seen as dark deposits. *Scale bar*: 20 μm. (Figures adapted from Richardson *et al*, 2005).

2003), suggest that rodent hair follicle-derived dermal stem cells may have a broad differentiation potential.

Plasticity of Human Hair Follicle Dermal Stem Cells

Although the investigation of rodent stem cell plasticity provides a model to increase our understanding of stem cell biology, ultimately, investigations must be carried out using human cells. We have repeated our plasticity experiments using dermal cell populations isolated directly from the human hair follicle. Both human DP and DS cell cultures could be directed toward adipogenesis or osteogenesis depending on the culture media. When supplemented with adipogenic media (modified from Zuk *et al*, 2001), DP and DS cell populations produced large lipid deposits, which stained positive with oil red-O within 10 d of culture. When subjected to osteogeneic media, formation of nodules was seen in both human DS and DP cell culture after approximately 4 d. Subsequently, both were fixed and stained via the Von Kossa method to confirm the presence of calcified deposits (Fig 2). The above observations demonstrate for the first time the capacity of cells from human hair follicle DP and DS to be directed along other mesodermal lineages *in vitro*.

Human Hair Follicle Dermal Stem Cells in Cell Therapy and Tissue Engineering

Until recently, adult hair follicle dermal cells were only studied in the context of hair growth induction, and cyclic regeneration. We now consider the same cells to have a much broader range of activities, and stem cell capabilities, as shown in Fig 3. In the context of skin, the properties of the hair follicle dermis readily convert into cell therapy and tissue-engineering approaches. The inherent inductive properties of these cells obviously translate to new follicle creation, the wound healing and dermal stem cell capabil-
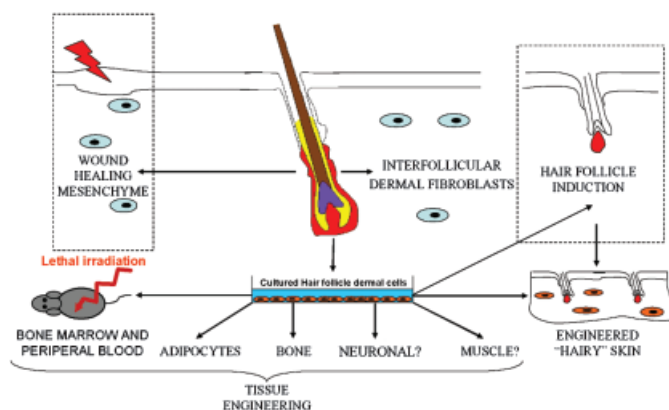
Figure 3
Diagram, summarizing the stem cell activities of hair follicle dermal stem cells, and some possible directions for their use in cell therapy and tissue engineering. It has been identified that hair follicle dermal cells have a role in wound healing, can be used to produce skin equivalents and have the potential to induce new follicle formation *in vivo*. The observed plasticity of hair follicle dermal cells suggests a wider potential for applications in the field of tissue engineering.

ities into dermal and skin replacement, and a combination of the two could provide the much sought after goal—tissue-engineered skin containing hair follicles (Gharzi *et al*, 2003). But, the possibility of utilizing the plasticity of hair follicle dermal cells as an easily accessible source of stem cells for other areas of cell therapy and tissue engineering is also worth considering. As illustrated in Fig 3, stem cells from the follicle dermis have the potential to be used within a wide array of areas within the field of tissue engineering. Hair follicle dermal cells are identified as participants in wound healing and have successfully been used to create skin equivalents (Lenoir *et al*, 1988; Jahoda and Reynolds, 2001; Gharzi *et al*, 2003; Cho *et al*, 2004). Moreover, given the inductive potential of the DP cell population, it may even be possible to induce the formation of hair follicles within tissue grafts. It is becoming clear that the hair follicle dermal populations may have further differentiation potential, which will allow their use in areas where single-cell therapies are being researched (nerve and blood), as well as for tissues like bone, where tissue-engineering approaches are more complex. Globally, it remains to be seen how successful these strategies will be. At this early stage, hair follicle dermis-derived stem cells appear to satisfy at least some of the criteria for use in tissue engineering (Ringe *et al*, 2002). They are easy to isolate, responsive to distinct environmental cues, and may have some immuno-privileged properties (Reynolds *et al*, 1999). We have therefore begun to examine the ability of these cells to grow and differentiate on bio-compatible scaffolds.

Future Questions

The observations on stem cell activity within the hair follicle dermis leaves open some intriguing questions. The rodent cell clones used in these experiments have been screened and display no gross chromosomal abnormalities. Extensive or prolonged culturing of ES cells, however, has been shown to increase the frequency of chromosomal abnormalities because of cells adapting to culture conditions (Draper *et al*, 2004). If the culturing techniques of hair follicle dermal cells for tissue engineering are found to increase the incidence of aneuplodies, this may render the cells unsuitable for *in vivo* transplantation. By studying these cells *in vitro*, however, it may be possible to identify the genetic, molecular, and cellular events involved in congenital disease and neoplasm. Problems that arise from the spontaneous multilineage differentiation of MSC and ES cell following transplantation are well documented (Mackenzie and Flake, 2001; Odorico *et al*, 2001). Although we have yet to establish whether the same is true for hair follicle dermal cells, this possibility needs to be addressed. It appears that the follicle dermis is a stem cell "niche" within the skin dermis in general, but if so, how does the stem cell activity of follicle dermal cells differ from that of the stem cells already described from the skin dermis? (Toma *et al*, 2001) It is possible that the bulk of the stem cell activity reported from the skin dermis is actually derived from the hair dermal compartments. It would also be interesting to know the developmental origin of these stem cell phenomena. Particularly intriguing is the recent evidence that the DP contains a

neural crest-derived cell population (Fernandes *et al*, 2004). We also need to determine whether there are differences in stem cell capability from cells in different parts of the follicle DS and papilla, and how these change during the course of the hair cycle. Finally, we can ask the interesting biological question of whether these cells can be in stem cell mode and inductive mode at the same time. In other words, whether a DP cell displaying a stem cell phenotype is compatible with or mutually exclusive from one capable of inducing a new hair follicle, when analyzed at the molecular level.

Address correspondence to: Colin A. B. Jahoda, School of Biological & Biomedical Sciences, University of Durham, South Road, Durham DH1 3LE, UK. Email: colin.jahoda@durham.ac.uk

References

Alonso L, Fuchs E: Stem cells of the skin epithelium. Proc Natl Acad Sci USA 100 (Suppl 1):11830–11835, 2003

Anderson DJ, Gage FH, Weissman IL: Can stem cells cross lineage boundaries? Nat Med 7:393–395, 2001

Blanpain C, Lowry WE, Geoghegan A, Polak L, Fuchs E: Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. Cell 118:635–648, 2004

Blau HM, Brazelton TR, Weimann JM: The evolving concept of a stem cell: Entity or function? Cell 105:829–841, 2001

Callahan CA, Oro AE: Monstrous attempts at adnexogenesis: Regulating hair follicle progenitors through Sonic hedgehog signaling. Curr Opin Genet Dev 11:541–546, 2001

Cho HJ, Bae IH, Chung HJ, *et al*: Effects of hair follicle dermal sheath cells in the reconstruction of skin equivalents. J Dermatol Sci 35:74–77, 2004

Draper JS, Moore HD, Ruban LN, Gokhale PJ, Andrews PW: Culture and characterization of human embryonic stem cells. Stem Cells Dev 13:325–336, 2004

Fernandes KJ, McKenzie IA, Mill P, *et al*: A dermal niche for multipotent adult skin-derived precursor cells. Nat Cell Biol 6:1082–1093, 2004

Gharzi A, Reynolds AJ, Jahoda CA: Plasticity of hair follicle dermal cells in wound healing and induction. Exp Dermatol 12:126–136, 2003

Jahoda CA: Cell movement in the hair follicle dermis—more than a two-way street? J Invest Dermatol 121:ix–xi, 2003

Jahoda CA, Reynolds AJ: Dermal-epidermal interactions. Adult follicle-derived cell populations and hair growth. Dermatol Clin 14:573–583, 1996

Jahoda CA, Reynolds AJ: Hair follicle dermal sheath cells: Unsung participants in wound healing. Lancet 358:1445–1448, 2001

Jahoda CA, Whitehouse J, Reynolds AJ, Hole N: Hair follicle dermal cells differentiate into adipogenic and osteogenic lineages. Exp Dermatol 12: 849–859, 2003

Jiang Y, Jahagirdar BN, Reinhardt RL, *et al*: Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418:41–49, 2002

Kishimoto J, Burgeson RE, Morgan BA: Wnt signaling maintains the hair-inducing activity of the dermal papilla. Genes Dev 14:1181–1185, 2000

Lako M, Armstrong L, Cairns PM, Harris S, Hole N, Jahoda CA: Hair follicle dermal cells repopulate the mouse haematopoietic system. J Cell Sci 115:3967–3974, 2002

Lenoir MC, Bernard BA, Pautrat G, Darmon M, Shroot B: Outer root sheath cells of human hair follicle are able to regenerate a fully differentiated epidermis *in vitro*. Dev Biol 130:610–620, 1988

Mackenzie TC, Flake AW: Multilineage differentiation of human MSC after *in utero* transplantation. Cytotherapy 3:403–405, 2001

Neuhuber B, Gallo G, Howard L, Kostura L, Mackay A, Fischer I: Reevaluation of *in vitro* differentiation protocols for bone marrow stromal cells: Disruption of actin cytoskeleton induces rapid morphological changes and mimics neuronal phenotype. J Neurosci Res 77:192–204, 2004

Niemann C, Watt FM: Designer skin: Lineage commitment in postnatal epidermis. Trends Cell Biol 12:185–192, 2002

Odorico JS, Kaufman DS, Thomson JA: Multilineage differentiation from human embryonic stem cell lines. Stem Cells 19:193–204, 2001

Oliver RF: Whisker growth after removal of the dermal papilla and lengths of follicle in the hooded rat. J Embryol Exp Morphol 15:331–347, 1966

Reynolds AJ, Lawrence C, Cserhalmi-Friedman PB, Christiano AM, Jahoda CAB: Trans-gender induction of hair follicles. Nature 402:33–34, 1999

Richardson GD, Arnott EC, Whitehouse CJ, Lawrence CM, Hole N, Jahoda CA: Cultured cells from the adult human hair follicle dermis can be directed toward adipogenic and osteogenic differentiation. J Invest Dermatol 124:1091–1092, 2005

Ringe J, Kaps C, Burmester GR, Sittinger M: Stem cells for regenerative medicine: Advances in the engineering of tissues and organs. Naturwissenschaften 89:338–351, 2002

Taylor G, Lehrer MS, Jensen PJ, Sun TT, Lavker RM: Involvement of follicular stem cells in forming not only the follicle but also the epidermis. Cell 102:451–461, 2000

Toma JG, Akhavan M, Fernandes KJ, Barnabe-Heider F, Sadikot A, Kaplan DR, Miller FD: Isolation of multipotent adult stem cells from the dermis of mammalian skin. Nat Cell Biol 3:778–784, 2001

Tumbar T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, Fuchs E: Defining the epithelial stem cell niche in skin. Science 303:359–363, 2004

Wagers AJ, Weissman IL: Plasticity of adult stem cells. Cell 116:639–648, 2004

Wulf GG, Jackson KA, Goodell MA: Somatic stem cell plasticity: Current evidence and emerging concepts. Exp Hematol 29:1361–1370, 2001

Zuk PA, Zhu M, Mizuno H, *et al*: Multilineage cells from human adipose tissue: Implications for cell-based therapies. Tissue Eng 7:211–228, 2001