

Defining Hair Follicles in the Age of Stem Cell Bioengineering

Cheng-Ming Chuong¹, George Cotsarelis² and Kurt Stenn³

One challenge faced by stem cell biologists is the bioengineering of an organ. Ehama *et al.* (2007, this issue) used cells derived from human and rodent epidermis and dermal papilla to reconstitute hair-follicle mini-organs. Some result in hair follicles; others are hair follicle-like. The challenge calls for the development of a set of criteria to define a hair follicle so that bioengineered products in the future can be evaluated.

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Hair follicles result from epithelial–mesenchymal interactions. Ehama *et al.* (2007, this issue) present their engineered hair follicle-like structures using cells derived from human and rodent epidermis and dermal papillae (DP). They attempted to regenerate hair follicles by combining dissociated keratinocytes and DP cells from different sources and implanting them into grafting chambers on the backs of nude mice (Weinberg *et al.*, 1993). Results were analyzed 3 to 4 weeks later. When both epidermal and DP cells were derived from mice or rats, hair follicles with an acceptable morphology appeared, similar to that previously reported (Weinberg *et al.*, 1993; Kamimura *et al.*, 1997; Kishimoto *et al.*, 2000). When human keratinocytes and mouse DP cells were mixed, “hair follicle-like” structures formed that are clearly different from normal epidermis in that they are downgrowths with central keratinization and there is an apparent periodicity to their placement. But are they hair follicles? If not, what do we call them? As scientists engage more in organ bioengineering, such as of hair, tooth, and mammary glands (Stenn and Cotsarelis, 2005; Chuong *et al.*, 2006; Nakao *et al.*, 2007), we will be compelled to state what we mean by a given organ. In this case, what elements are essential to the definition of a hair follicle?

In embryonic development, hair follicles are built stepwise (Millar, 2002; Fuchs, 2007; Plikus *et al.*, 2007). During this process, molecular signals interact among tissues and multiple morphogenetic events occur; some are regulated by the mesenchyme and some by the epithelium. If we accept the fact that hair-follicle development involves many molecular and cellular events embedded in discrete morphogenetic steps, then it would not be so surprising to encounter incomplete and imperfect structures as we engineer hair follicles—it is hard to get every step right in this dawn of bioengineering. The salient events of hair-follicle morphogenesis can be summarized as follows: formation of dermal condensations → epithelial invagination to form the follicular wall → formation of DP at the base of the follicle → topologic arrangement of localized stem, transient amplifying (TA), and differentiated cell clusters → morphogenesis to build the architecture of different hair types in the differentiating zone → molecular differentiation of hair-shaft components → ability to shed hairs while preserving stem cells and DP for the next cycle → ability to regenerate. Failure of any of these events will lead to disrupted hair-follicle structures, resulting in various degrees of incomplete hair-follicle formation.

Based on the developmental steps, we can consider a set of defining criteria (Table 1) that may be used to evaluate the bioengineered products described in the article by Ehama *et al.* (2007). The reconstituted hair follicles using homospecific mouse or rat cells clearly generate normal-appearing hairs. Heterospecific mouse–rat combinations are also good. Homospecific human cells did not work in Ehama and colleagues’ experiments, but human–mouse combinations gave results that formed the basis of the current report. The authors reported that epidermal invaginations from the reformed epidermis occur. A cluster of dermal condensation cells was located adjacent to the follicle but was not engulfed by the follicle base. It was alkaline-phosphatase-positive, but versican-negative. Whether it can be considered a bona fide DP is debatable, but the structure was distinct from the surrounding cells and

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appeared closer to DP than other tissues. Proliferating cells were distributed in the basal epidermal layer but not localized to the matrix region. There were no K15-positive bulge cells, nor did they demonstrate the presence of stem cells by any other methods (Cotsarelis, 2006). The topologic arrangement of stem, TA, and differentiated cells seen in normal hair follicles did not appear to form. Follicular epidermal cells were layered, but the distinct differentiation of medullar, cortex, and inner and outer root sheath was unclear. The poorly formed hair shaft-like structure is better described as a keratin plug than as a hair shaft. None of the structures had associated sebaceous glands. As for biochemical differentiation, hair keratin Hb1 and AE 13 were weakly positive, and transglutaminase 1 was not found. The authors did not demonstrate cycling behavior of the hair follicle-like structures either, which is why the newly formed structures can only be called “hair follicle-like.” Although they did

¹Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, California, USA; ²Department of Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, USA; and ³Aderans Research Institute, Inc., Philadelphia, Pennsylvania, USA

Correspondence: Dr Cheng-Ming Chuong, Department of Pathology, Keck School of Medicine, University of Southern California, 2011 Zonal Avenue, HMR 313, Los Angeles, California 9003. E-mail: cheng-ming.chuong@keck.usc.edu

not achieve the engineering of a “real” human hair follicle, the article demonstrates work in progress toward this goal.

In a recent work, dissociated neonatal mouse epidermal and dermal cells were reconstituted to form hair follicles (Zheng *et al.*, 2005). The authors demonstrated that such hair follicles formed in a stepwise sequence similar to embryonic development, showed proper follicular layering, formed a bulge and sebaceous gland, showed proper biochemical differentiation markers, and demonstrated cycling. Moreover, when K15 EGFP-positive newborn mouse-hair bulge cells were isolated and tested for stem cell properties, the new hair follicles formed from these cells fulfilled the criteria listed in Table 1 (Morris *et al.*, 2004). Similarly, when two populations of putative stem cells were isolated from the hair-follicle bulge, they were able to form hairs and cycle (Blanpain *et al.*, 2004). True hair follicles can form from dissociated cells, as Ehama *et al.* showed partially.

Because cells derived from mice and rats form reasonable follicles among themselves, Ehama *et al.* (2007) attributed the failure to form better differentiated and organized follicles in a rodent/human chimera to the lack of communication between species. That is one possibility. Some molecular signal(s) may be able to crosstalk to conserved receptors while others may not, leading to an imbalanced morphogenesis. Indeed, scientists took advantage of epithelial–mesenchymal recombination experiments long ago to ask fundamental biologic questions. Epithelial–mesenchymal recombination among different classes (mouse, chicken, and lizard) was carried out in classic experiments (Dhouailly, 1973), and the results showed that the locations of ectodermal organs were determined by the mesenchyme. The epithelia can do only what it is competent to do based on its genome. Therefore, an epithelium (mouse)/mesenchyme (chicken) recombination will lead to hairs arranged in feather patterns. Within the same species, recombination between different skin regions showed that, in the early stages, epidermal cells can be molded into different types of skin-appendage structures by the mesenchyme. However, if the experiments were carried out at later stages, epidermal cells

Table 1. Developing a definition for hair follicles

1.	The proximal end of the skin appendages shows a follicle configuration, with epithelial filament coming out of the distal end of the follicle and dermal papilla sitting at the base of the follicle.
2.	It has proliferating cells (TA cells) positioned proximally and differentiating cells positioned distally, forming a proximal–distal growth mode.
3.	The follicle is made of concentric layers of outer and inner root sheath, cuticle, cortex, and medulla. Although in different hair types variations can occur with the basic design, all follicles have a distinct internal root sheath (Henle and Huxley) and companion layers.
4.	The product of a follicle, the shaft is made of unique molecular constitution.
5.	The follicle is associated with sebaceous glands.
6.	A follicle has the machinery to shed an old shaft while preserving stem cells and DP for the next cycle.
7.	Inherent in the follicle is the ability to regenerate a new hair organ through repeated hair cycles.

were committed to certain lineages and would no longer respond to the inducing signals from the mesenchyme (Sengel, 1976). In adult feather follicles, feather stem cells are real stem cells and can be molded into different phenotypes (e.g., flight versus down feathers) according to the source of DP (Yue *et al.*, 2006).

On the other hand, different epithelial tissues may have different potential, leading them to respond differently to the same mesenchymal stimulus. *Bona fide* pilosebaceous units have been induced by rabbit or rat mesenchyme using cultured human foreskin epidermal cells (Ferraris *et al.*, 1997). A surprising result was obtained when rabbit corneal epithelium was recombined with mouse hair-follicle mesenchyme. A transdifferentiation event occurred, and hair follicles formed (Pearton *et al.*, 2005). Similarly, when mouse amnion epithelium encountered embryonic dermis, skin and hair follicles with sebaceous glands formed (Fliniaux *et al.*, 2004). Unexpected results occurred when epithelia and mesenchyme of other ectodermal organs were recombined. When rat-tooth papilla cells were implanted under ear skin, hair formation was induced (Reynolds and Jahoda, 2004). When chicken oral mucosa was apposed to feather mesenchyme, tooth-like follicles formed instead of smooth oral mucosa (Chen *et al.*, 2000). Are these follicles considered tooth follicles or tooth-like, feather follicles or feather-like, hair follicles or hair-like, or simply ectoderm organ follicles?

Skin appendages from different species have different morphologic characteristics (Wu *et al.*, 2004; Sokolov and Chernova, 2001). Epithelial–mesenchy-

mal recombination between chicken and duck showed that the location of feather appendage is determined by the mesenchyme, but the filamentous structures are determined by the epithelia (Sengel, 1976). During evolution, novel characteristics for feathers appear sequentially. When feather-like structures were discovered in fossils, they had to be labeled proto-feathers, and there was a need to develop a definition for feather follicles (Chuong *et al.*, 2003). In mammals, hairs from diverse species can exhibit different shapes, ratio of cortex/medulla, etc., and it would be interesting to see what regulates the morphologic characters. Chimeric hairs may be engineered to answer these basic questions. In that case, the chimeric hairs may fulfill the definition of hair follicles, but not of the hair follicle of a specific species. In Table 1, we list the basic criteria for hair follicles, which can be easily modified to include follicles of other mammalian hairs.

The developmental biologic experiments were designed to answer fundamental questions and the resulting follicles may not meet the rigorous definition required for the purpose of translational medicine. However, the concepts of competence, plasticity, potentiality, commitment, transdifferentiation, etc., help establish modern-day stem cell biology. Thus, although the advent of stem cell biology gives new life to the classic epithelial–mesenchymal recombination experiments, today’s stem cell biologists also can gain insights from these classic experiments in terms of the regulation of the potentiality of stem cells (epithelium) and the specificity of the microenvironment (mesenchyme).

We now can return to the theme of bioengineering stem cells to form hair follicles. With the advent of stem cell biology, we anticipate that many attempts to engineer hair follicles will be made in the near future. The epidermal stem cells or progenitor cells may derive from many sources: embryonic stem cells, engineered embryonic stem cells or cell lines, interfollicular epidermal stem cells, bulge stem cells, or even bone marrow stem cells. Because the cells may have different competences, the "hair follicles" built with them may be different. In the future, we must ask whether they fulfill all the criteria of a bona fide hair follicle. Work that has had partial success is nonetheless valuable because it helps in the analysis of distinct events during hair-follicle morphogenesis. The results here highlight the challenge faced by all who aspire to engineer human organs, including hair follicles.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

Blanpain C, Lowry WE, Geoghegan A, Polak L, Fuchs E (2004) Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. *Cell* 118:635–48

Chen YP, Zhang Y, Jiang TX, Barlow A, Amand TR, Hu Y *et al.* (2000) Conservation of early odontogenic signaling pathway in Aves. *Proc Natl Acad Sci USA* 97:10044–9

Chuong CM, Wu P, Zhang FC, Xu X, Yu M, WidELITZ RB *et al.* (2003) Adaptation to the sky: Defining the feather with integument fossils from mesozoic China and experimental evidence from molecular laboratories. *J Exp Zool* 298B:42–56

Chuong CM, Wu P, Plikus MV, Jiang TX, WidELITZ RB (2006) Engineering stem cells into organs: Topobiological transformations demonstrated by beak, feather and other ectodermal organ morphogenesis. *Curr Topics Dev Biol* 72:237–74

Cotsarelis G (2006) Epithelial stem cells: A folliculocentric view. *J Invest Dermatol* 126:1459–68

Dhouailly D (1973) Dermo-epidermal interactions between birds and mammals: differentiation of cutaneous appendages. *J Embryol Exp Morphol* 30:587–603

Ehama R, Ishimatsu-Tsuji Y, Iriyama S, Ideta R, Soma T, Yano K *et al.* (2007) Hair follicle regeneration using grafted rodent and human cells. *J Invest Dermatol* 127:2106–15

Ferraris C, Bernard BA, Dhouailly D (1997) Adult epidermal keratinocytes are endowed with

pilosebaceous forming abilities. *Int J Dev Biol* 41:491–8

Fliniaux I, Viallet JP, Dhouailly D, Jahoda CA (2004) Transformation of amnion epithelium into skin and hair follicles. *Differentiation* 72:558–65

Fuchs E (2007) Scratching the surface of skin development. *Nature* 445:834–42

Kamimura J, Lee D, Baden HP, Brissette J, Dotto GP (1997) Primary mouse keratinocyte cultures contain hair follicle progenitor cells with multiple differentiation potential. *J Invest Dermatol* 109:534–40. Erratum in *J Invest Dermatol* 110:102.

Kishimoto J, Burgeson RE, Morgan BA (2000) Wnt signaling maintains the hair-inducing activity of the dermal papilla. *Genes Dev* 14:1181–5

Millar SE (2002) Molecular mechanisms regulating hair follicle development. *J Invest Dermatol* 118:216–25

Morris RJ, Liu Y, Marles L, Yang Z, Trempus C, Li S *et al.* (2004) Capturing and profiling adult hair follicle stem cells. *Nat Biotechnol* 22:411–7

Nakao K, Morita R, Saji Y, Ishida K, Tomita Y, Ogawa M *et al.* (2007) The development of a bioengineered organ germ method. *Nat Methods* 4:227–30

Pearnton DJ, Yang Y, Dhouailly D (2005) Transdifferentiation of corneal epithelium into epidermis occurs by means of a multistep process triggered by dermal developmental signals. *Proc Natl Acad Sci USA* 102:3714–9

Plikus MV, Sundberg JP, Chuong C-M (2007) Mouse skin ectodermal organs. In: *The Mouse*

in Biomedical Research (Fox J, Barthold S, Davisson M, Newcomer C, Quimby F, Smith A, eds), 2nd edn, Vol 3, Academic Press: Amsterdam

Reynolds AJ, Jahoda CA (2004) Cultured human and rat tooth papilla cells induce hair follicle regeneration and fiber growth. *Differentiation* 72:566–75

Sengel P (1976) *Morphogenesis of Skin*. Cambridge University Press: Cambridge, UK

Sokolov VE, Chernova OF (2001) Skin appendages of mammals (in Russian). GEDS: Moscow

Stenn KS, Cotsarelis G (2005) Bioengineering the hair follicle: fringe benefits of stem cell technology. *Curr Opin Biotechnol* 16:493–7

Weinberg WC, Goodman LV, George C, Morgan DL, Ledbetter S, Yuspa SH *et al.* (1993) Reconstitution of hair follicle development *in vivo*: determination of follicle formation, hair growth, and hair quality by dermal cells. *J Invest Dermatol* 100:229–36

Wu P, Hou L, Plikus M, Hughes M., Schemet J, Suksaweang S, *et al.* (2004) Evo-devo of amniote integuments and appendages. *Int J Dev Biol* 48:249–70

Yue Z, Jiang TX, WidELITZ RB, Chuong CM (2006) Wnt 3a gradient converts radial to bilateral feather symmetry via topological arrangement of epithelia. *Proc Natl Acad Sci USA* 103:951–5

Zheng Y, Du X, Wang W, Boucher M, Parimoo S, Stenn K (2005) Organogenesis from dissociated cells: Generation of mature cycling hair follicles from skin-derived cells. *J Invest Dermatol* 124:867–76

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Merkel Cell Carcinoma: More Deaths but Still No Pathway to Blame

Bianca Lemos¹ and Paul Nghiem¹

Merkel cell carcinoma (MCC) is a neuroendocrine skin cancer with a rising incidence (1500 U.S. cases per year) that now exceeds that of cutaneous T-cell lymphoma and a mortality (33%) exceeding that of melanoma. Despite this impact, little is known about its biology. Recent studies have shown that Ras/MAP kinase activity is absent and possibly detrimental to this cancer. This makes MCC distinct from other UV-induced skin cancers and highlights the question of what drives this malignancy.

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Merkel cell carcinoma (MCC) is an aggressive neuroendocrine carcinoma of the skin associated with ultraviolet exposure. Although uncommon, its

incidence is increasing and has in fact tripled in the past 15 years (Hodgson, 2005). The rise in incidence is due in part to improved diagnosis through the

¹University of Washington Dermatology/Medicine, Fred Hutchinson Cancer Research Center, Seattle Cancer Care Alliance, Seattle, Washington 98109, USA.

Correspondence: Dr Paul Nghiem, 815 Mercer Street, Seattle, Washington 98109, USA. E-mail: pngnhiem@u.washington.edu