De Novo Production of Dermal Papilla Cells during the Anagen Phase of the Hair Cycle

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TO THE EDITOR

Although keratinocytes are the primary constituents of the hair follicle and generate the hair shaft, mesenchymal cells also have important functions. These include the follicular dermal papilla (DP) and the connective tissue sheath or dermal sheath (CTS). The DP is embedded in the hair bulb during the anagen phase and forms a compact ball during the telogen phase, whereas CTS cells line the outside of the epithelial follicle from the bulge to its base. The central function of the DP in regulating the activity of keratinocytes during hair follicle regeneration and hair shaft morphogenesis has been established by extirpation and grafting studies (Ibrahim and Wright, 1977; Jahoda *et al.*, 1984, 1993; McElwee *et al.*, 2003), and more recently by direct manipulation of gene expression in the DP *in vivo* (Enshell-Seijffers *et al.*, 2010). A strong correlation between DP size, hair bulb diameter and hair caliber has been noted (Van Scott and Ekel, 1958; Ibrahim and Wright, 1982; Elliott *et al.*, 1999). The CTS is less accessible to experimental manipulation and its function in the intact follicle is more poorly defined. However, the proximal CTS shares properties with the DP that include the capacity to reform the DP in grafting studies (McElwee *et al.*, 2003). DP cells undergo comparatively few divisions and the constituents of a DP are a largely static population when compared with the dynamic changes in the keratinocyte populations that abut them. Modest expansion and contraction of cell numbers in the DP occurs over the course of the hair cycle. Tobin *et al.* (2003b) quantified these changes for the mouse hair cycle, reporting an increase in DP cell numbers during anagen I–V and a decrease in anagen VI–telogen. They noted that the increase in DP cell number observed in early anagen precedes detectable proliferation in the DP, and suggested that it results from the migration of CTS cells into the DP.

We have generated a mouse line, *Cor-cre*, that expresses cre recombinase in the DP (Enshell-Seijffers *et al.*, 2010). When coupled with a cre-dependent reporter gene, this provides a method to trace the fate of DP cells. The reporter gene contains the sequences encoding yellow fluorescent protein (YFP) in the ubiquitously expressed *Rosa26* locus that are separated from a promoter by a transcriptional termination cassette flanked by LoxP sites (Srinivas *et al.*, 2001). When this stop cassette is excised in cells expressing cre-recombinase, YFP is expressed in the cell and its progeny regardless of their position in the tissue or changes in expression of the cell type-specific cre recombinase allele. In *Cor-cre/+*; *rYFP/+* mice, YFP is not detected until p3 and effective deletion across the DP population is not complete until p7 (Enshell-Seijffers *et al.*, 2010). By the end of the anagen phase, virtually all DP cells express YFP, while the proximal CTS is variably labeled. *Corin* is not expressed in catagen or telogen, and although *Corin* expression returns during early anagen, cre recombinase is not reliably detected until anagen V (data not shown) (Enshell-Seijffers *et al.*, 2008).

If all DP cells in a follicle are labeled with YFP at the end of one growth phase, the appearance of unlabeled cells in the subsequent anagen phase would provide evidence of recruitment of new cells to the DP. Mice of the genotype *Cor-cre/+*; *rYFP/+* were killed at p13 and the extent of labeling in the DP was determined in tissue sections (Figure 1a). In a survey of follicles from five mice, no unlabeled DP cells were observed in 94 ± 5% of the follicles scored ($n = 129$) (Figure 2). Although most DP cells were labeled in the remaining follicles, one or two cells were unlabeled. During the catagen phase, a compact ball of YFP+ cells is associated with the regressing epithelial strand. In telogen phase, the descendents of the anagen DP form a compact ball of contiguous labeled cells, surrounded by more elongated cells that are variably labeled (Figure 1b). The variable labeling of these peripheral cells is consistent with the assumption that they derive from the CTS and confirms that most are not descendents of the DP. However, definitive distinction between DP and CTS derivatives at this stage is not possible.

In contrast to p13, many unlabeled DP cells are observed during the early anagen phase of the first post-natal hair cycle (Figure 1c and d). Skins in the early anagen phase were harvested ($n = 5$), and follicles in anagen stage III–IV were analyzed (Muller-Rover *et al.*, 2001). Before stage III, distinction between DP and adjacent CTS is open to debate. At stage III, the DP is engulfed by the hair bulb, thereby allowing unambiguous definition of the boundary between DP and CTS with a line between the keratinocytes at the base of the follicle. Overall, 75% ± 8 of the follicles showed one or more DP cells that did not express YFP ($n = 256$) (Figure 2). To confirm that this was not a failure of the *rYFP* reporter transgene, mice in which *rYFP* had been activated in the germ line were

Abbreviations: CTS, connective tissue sheath or dermal sheath; DP, follicular dermal papilla; YFP, yellow fluorescent protein

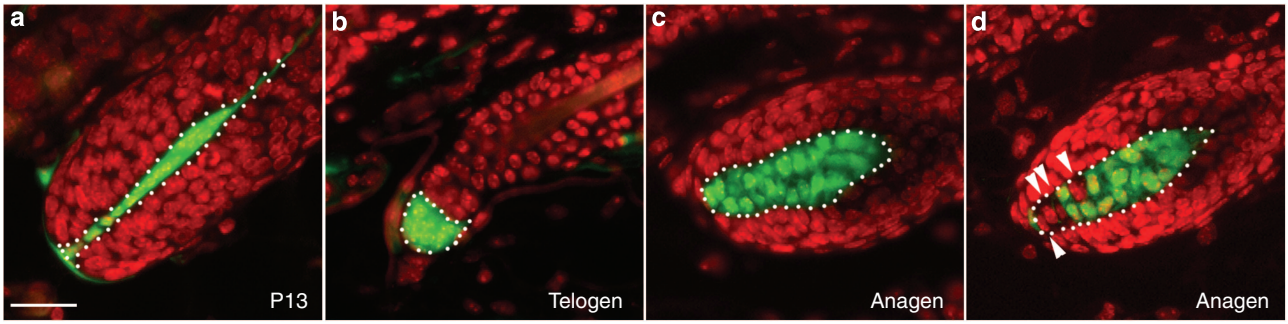

Figure 1. Recruitment of cells to the follicular dermal papilla (DP) during the early anagen phase of the hair cycle. Representative hair bulbs from (**a**) post-natal day 13, (**b**) the first telogen, and (**c**, **d**) the first post-natal anagen are shown. Although some DP are completely labeled in early anagen (**c**), unlabeled, newly recruited DP cells (arrowheads) are observed in most follicles (**d**). Nuclei are shown in red, yellow fluorescent protein is shown in green, and white dotted lines demarcate the DP. Bar = 25 μ.

examined. No unlabeled DP cells were observed ($n = 68$ follicles).

These data demonstrate either that new cells are recruited to the DP during early anagen, or that a rare unlabeled population of DP cells in each follicle escaped detection in the random section analysis at P13. To rule out this latter possibility, 10 follicles were optically sectioned at 1-μ intervals through the entire hair bulb. All of the DP cells were labeled in each of these follicles. Given the preponderance of follicles containing unlabeled DP cells in the first true anagen, this result clearly demonstrates that these unlabeled cells do not arise from unlabeled DP cells persisting from the previous hair cycle ($P < 0.0001$). We therefore conclude that most follicles incorporate new cells into the DP during the early anagen phase.

Previous work had demonstrated that engrafted DP/CTS cells could be incorporated into the DP of existing follicles (McElwee *et al.*, 2003; Biernaskie *et al.*, 2009). Induction of anagen enhanced the efficiency with which engrafted SKPs, a multipotent population that can be derived from DP in culture, are incorporated into existing hair follicles (Biernaskie *et al.*, 2009). This suggests that the early anagen environment includes conditions conducive to recruitment of new cells to the DP. The results reported here demonstrate that new cells are incorporated into the DP during the early anagen phase of a natural hair cycle.

It has been suggested that emigration from the DP to the CTS and/or dermis also occurs to achieve the reduction of DP cell numbers observed during anagen VI–telogen (Tobin *et al.*, 2003b). Both Corin and cre recombinase are detected in the proximal CTS, and sporadic labeling of the proximal CTS is observed during morphogenesis of the follicle as early as p4 (Enshell-Seijffers *et al.*, 2010), well before the mature anagen stages when export of cells from the DP is postulated. As a result, we cannot address the possibility of export to the CTS. If YFP-expressing CTS cells are incorporated into the DP during early anagen, this would lead to an underestimate of the magnitude of cellular recruitment to the DP.

When the DP was viewed as a static lineage compartment, depletion of that restricted population could be invoked as the cause of reduction in follicle size. The observation of *de novo* generation of DP cells has important implications for the way we view the consistency of hair growth across hair cycles and its decay during aging or pathological hair loss (Tobin *et al.*, 2003a). It emphasizes that regulation of DP size is an active process and that conservation of hair size is not the passive result of a fixed DP compartment, nor is decay in follicle size an inevitable consequence of damage within this compartment. Although it is reasonable to infer, based on close proximity and shared properties that the CTS is a likely source for new DP cells, identifying the pool of potential DP progenitors and the mechanisms that regulate DP cell number

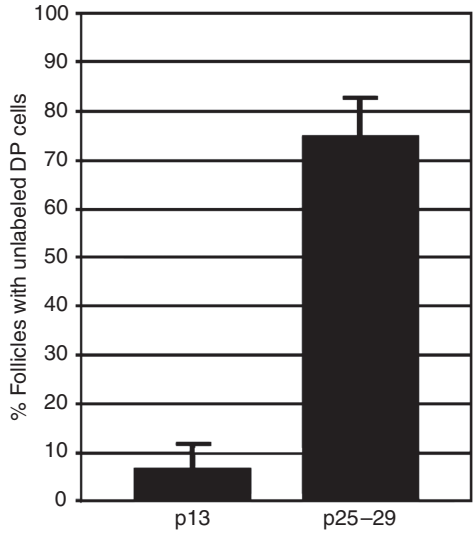

Figure 2. The frequency of follicular dermal papilla (DP) with unlabeled cells at p13 and during the first anagen (p25–29). Error bars represent SD; $n = 5$ mice per sample.

remain important steps toward improved management of hair loss.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Mutations in Sterol O-Acyltransferase 1 (*Soat1*) Result in Hair Interior Defects in AKR/J Mice

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TO THE EDITOR

Genes regulating the development and function of skin and hair often have critical roles in other organs. Accordingly, understanding the mechanisms underlying hair follicle morphogenesis and cycling may provide insight into disease processes of relevance for various organs. The hair interior defect mutation (*hid*) arose spontaneously in AKR/J mice (Trigg, 1972; Giehl *et al.*, 2009). Although AKR/J-*hid/hid* mice seem superficially normal at all ages, a microscopic analysis of internal hair morphology revealed changes in the corticomedullary boundary, a lesion that was proposed to be a failure of medullary cells to change shape during their early differentiation (Trigg, 1972). Subsequent ultrastructural studies found *hid/hid* hair to be deficient in projections of cortex cells (Rice *et al.*, 2009), which normally have a characteristic tonofilament pattern (Morioka, 2005) into adjoining medulla cells. Proteomic analyses further revealed the *hid/hid* hair to be low in trichohyalin (Rice *et al.*, 2009).

Having mapped the *hid* locus to mouse chromosome 1 (Giehl *et al.*, 2009), we generated an additional 260 AKRCByJF2 mice yielding 14.62% (38 mice) affected progeny (<25% expected), and suggesting one or more modifier genes. Crosses between AKR/J-*hid/hid* and C3H/HeJ, C57BL/6J, FVB/NJ, and CAST/EiJ mice yielded 1289 F2 progeny at the expected 25% frequency. Genotyping reduced the *hid* locus to 0.74 Mb containing nine genes. Sequencing of the corresponding complementary DNAs revealed only synonymous single-nucleotide polymorphisms, with the exception of *Soat1*, which had a 118 bp deletion that included exon 2 and ranged from nucleotides 30 to 88 of the *Soat1* open reading frame. Genomically, we confirmed a 6.8 kb deletion using whole-genome resequencing data (http://www.sanger.ac.uk/modelorgs/mousegenomes/). This mutation results in a 33 amino acid truncation at the *N*-terminus of the SOAT1 protein consisting of 540 amino acids. As no structure of any member of the SOAT family was confirmed, tertiary structural changes caused by this mutation could not be

Abbreviations: hid, hair interior defect