Differences in Expression of Specific Biomarkers Distinguish Human Beard from Scalp Dermal Papilla Cells

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Androgen exposure stimulates the growth of beard hair follicles. The follicle dermal papilla appears to be the site of androgen action; however, the molecular mechanisms that regulate this process are not well understood. In an attempt to identify genes that contribute to the androgen-responsive phenotype, we compared gene expression patterns in unstimulated and androgen-treated cultured human dermal papilla cells isolated from beard (androgen-sensitive) and occipital scalp (androgen-insensitive) hair follicles. Through this analysis, we identified three genes that are expressed at significantly higher levels in beard dermal papilla cells. One of these genes, *sfrp-2* has been identified as a dermal papilla signature gene in mouse pelage follicles. Two of these genes, *mn1* and *atp1β1*, have not been studied in the hair follicle. A fourth, *fibulin-1d*, was slightly upregulated in beard dermal papilla cells. The differences in the expression of these genes in cultured beard and scalp dermal papilla cells reflected similar differences in microdissected dermal papilla isolated from intact beard and scalp follicles. Our findings introduce potentially novel signaling pathways in dermal papilla cells. In addition, this study supports that cultured dermal papilla cells provide a cell-based model system that is reflective of the biology of *in vivo* hair follicle cells.

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INTRODUCTION

At the onset of puberty, androgens stimulate robust follicle growth on the face, while follicles on the crown of the scalp begin a process of miniaturization in susceptible individuals (Randall *et al.*, 2000; Hoffmann, 2002). The androgen receptor and 5-α-reductase II are localized to the hair follicle dermal papilla (Choudhry *et al.*, 1992; Hamada *et al.*, 1996; Eicheler *et al.*, 1998; Ando *et al.*, 1999), suggesting this is the site where androgen processing occurs. Androgen signaling is thought to result in the release of paracrine factors from the dermal papilla to the surrounding follicle epithelium (reviewed in Randall *et al.*, 2001), a process that causes a significant increase in beard follicle size (Elliott *et al.*, 1999).

The androgen responsiveness of hair follicle dermal papilla cells can be recapitulated in cell culture (reviewed in Randall *et al.*, 1992, 1994). Dermal papilla cells (DPC) isolated from beard follicles express type I and type II 5α-reductase (Itami *et al.*, 1991, 1994) and convert testosterone to 5α-dihydrotestosterone (DHT) (Randall *et al.*, 1992; Thornton *et al.*, 1993; Hamada *et al.*, 1996). These characteristics have not been observed in occipital scalp DPC (Itami *et al.*, 1991, 1994; Randall *et al.*, 1992; Thornton *et al.*, 1993; Hamada *et al.*, 1996), or dermal fibroblasts (Itami *et al.*, 1991). As the activated androgen receptor acts as a transcriptional regulator, attempts have been made to identify unique gene expression patterns and phenotypic characteristics that define androgen-sensitive DPC. Kim *et al.* (2003) identified androgen-regulated genes in SV-40 transformed DPC and published a set of five genes that were induced by DHT treatment. Seo *et al.* (2001) have identified an androgen regulated gene, AIG1, a human homolog of a hamster gene expressed in the androgen-sensitive flank organ, which is a model of human beard hair growth (Kaszynski, 1983). Cha *et al.* (2005) have identified the human homolog of the mouse URB gene as an androgen-inducible gene in DPC. Midorikawa *et al.* (2004) identified BMP2 and ephrinA3 as hair growth promoting genes that are downregulated in DPC isolated from balding scalp. Together, these studies begin to define molecular characteristics that define DPC in androgen-sensitive hair follicles.

Cultured DPC (from beard or scalp follicles) demonstrate some unique properties. In addition to the expression of smooth muscle α-actin (Jahoda *et al.*, 1991; Reynolds *et al.*, 1993; Chiu *et al.*, 1996), the ability to form aggregates

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Abbreviations: BMP, bone morphogenic protein; DHT, 5α-dihydrotestosterone; DPC, dermal papilla cells; KGF, keratinocyte growth factor; RT-PCR, reverse transcriptase-PCR; TGFβ, transforming growth factor beta

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(Jahoda and Oliver, 1984; Chiu *et al.*, 1996; Bratka-Robia *et al.*, 2002) their generally quiescent nature (Ferraris *et al.*, 1997) and production of a distinct extracellular matrix (Katsuoka *et al.*, 1988; Chiu *et al.*, 1996; Bratka-Robia *et al.*, 2002; de Almeida *et al.*, 2005), cultured DPC can retain the ability to induce hair growth when transplanted to an *in vivo* environment (Jahoda *et al.*, 1984, 1993; Reynolds and Jahoda, 1992; Matsuzaki and Yoshizato, 1998; Kishimoto *et al.*, 1999, 2000; McElwee *et al.*, 2003), or a three-dimensional model system *in vitro* (Krugluger *et al.*, 2005). Characteristics such as versican expression (Kishimoto *et al.*, 1999; Soma *et al.*, 2005), alkaline phosphatase activity (McElwee *et al.*, 2003), and activation of matrix metalloproteinases (Yuspa *et al.*, 1993) have been correlated with hair inductive ability in *in vivo* transplant models. A number of studies have been performed attempting to identify critical genes that distinguish DPC from dermal fibroblasts that are not able to support hair growth (Sleeman *et al.*, 2000; Yang *et al.*, 2004; Yu *et al.*, 2004). Molecular signatures for different compartments of mouse pelage follicles have been defined, illustrating clear differences between DPC, other types of hair follicle cells, and dermal fibroblasts (Rendl *et al.*, 2005).

Since androgen responsiveness in hair follicles appears to channel through the dermal papilla, we wished to identify differences in gene expression between cells in the dermal papilla of scalp and beard hair follicles that could contribute to the enlarged beard phenotype. Using human beard and scalp follicles as our tissue source, we have established cultures of DPC that at low passage, appear to reflect patterns of gene expression in the dermal papilla isolated from intact hair follicles. Using this model, we have defined the differential expression of four genes that have not been previously characterized in cultured human hair follicle DPC. Three of these genes show elevated expression specifically in beard cells when compared to scalp cells, and one appears to provide an additional biomaker to define the DPC phenotype.

RESULTS

Beard DPC attach more readily than nonbalding scalp cells and form dense cell outgrowths

A comparison of beard and occipital scalp DPC 9 days after plating indicates that scalp cells form outgrowths more slowly than beard cells (Figure 1). Whereas beard DPC outgrowths are very tightly packed, scalp cells have a more spread-out appearance with spaces between the cells. We have also noted that a greater percentage of scalp DPC express smooth muscle α-actin upon initial passage than is seen in cultures of beard cells, and trypsin-resistant beard DPC that remain attached to the culture well after the initial first passage tend to be enriched for smooth muscle α-actin expression (not shown). The beard and scalp cultures used in this study were obtained from the first trypsinization, and then screened for high levels of smooth muscle α-actin expression (see Figure 4).

Secreted factors from beard or nonbalding scalp DPC differentially regulate epidermal keratinocyte proliferation

In order to determine whether beard and scalp DPC secrete factors that are mitogenic or growth inhibitory to epithelial cells, co-cultures were established between epidermal keratinocytes and preparations of scalp and beard DPC (three scalp DPC preps from three individuals, one preparation of commercially available scalp DPC and seven beard DPC preparations from two individuals). Analysis of the number of keratinocytes growing on tissue culture inserts after 48 hours of co-culture indicated that some of the dermal papilla lines stimulated and some inhibited keratinocyte growth (Figure 2a). In this study, the growth stimulatory DPC were derived from male beard follicles. In contrast, DPC isolated from the non-balding scalp appeared to have either no effect or a negative effect on keratinocyte growth. Thus, DPC isolated from beard and nonbalding scalp follicles appear to secrete different cocktails of growth factors with different effects on the growth of target epithelial cells. These findings are consistent with other co-culture studies (Itami *et al.*, 1995; Pan *et al.*, 1999; Inui *et al.*, 2002; Itami and Inui, 2005), and demonstrate that our preparations of DPC show similar characteristics with androgen-sensitive DPC reported elsewhere.

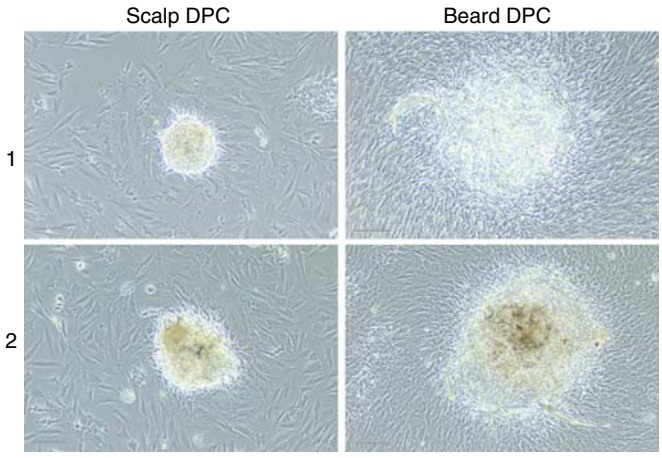

Figure 1. Dermal papilla cell outgrowths on day 9. Dermal papilla were isolated from hair follicles obtained from a 4-mm punch biopsy taken from either the occipital scalp (scalp DPC) or the chin (beard DPC) of two individuals (1 and 2). Each papilla was plated onto a well of a 24-well dish. The cells were photographed on day 9. Bar = 0.1 mm.

In an attempt to begin to characterize differences in secreted factors between scalp and beard DPC, we examined levels of transforming growth factor beta (TFGβ) in the DPC conditioned medium. TFGβ is well-known as an inhibitor of epidermal cell growth, and as a catagen-inducing factor (reviewed in Hibino and Nishiyama, 2004; Soma *et al.*, 2002). DPC from follicles undergoing androgenic alopecia secrete TGFβ_1, which inhibits the proliferation of epidermal keratinocytes in a similar model system (Inui *et al.*, 2003). In our studies, we found that scalp DPC produced higher levels of TGFβ_2 than beard DPC (Figure 2b).

Androgen receptor expression is inducible in beard DPC

Immunostaining of beard and scalp hair follicles indicated the presence of the androgen receptor in the dermal papilla and dermal sheath of beard follicles, but not follicles from male or female occipital scalp (Figure 3a). Androgen receptor