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Creation of differentiation-specific genomic maps of human epidermis through laser capture microdissection

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TO THE EDITOR

Keratinocytes change dramatically as they differentiate from the basal layer to spinous, granular, and cornified cells. This epidermal stratification is tightly controlled and essential to maintain the effective barrier function of the epidermis (Koster, 2009), but differences in gene expression and the controlling transcription factors are only partly understood. Some insights into differential gene expression have been obtained by laser capture microdissection (LCM) or cell separation methods, but in the case of LCM only selected genes were analyzed by real-time PCR (Percoco *et al*., 2012). Radoja *et al.* separated basal vs suprabasal cells by sorting based on β4 integrin expression and profiled genes using whole genome arrays. However, differentiation-specific genes such as loricrin and filaggrin were enriched <4-fold in the β4-negative (suprabasal) fraction (Radoja *et al.*, 2006). In this study we have coupled LCM that generated >400-fold enrichment in loricrin and filaggrin mRNAs with whole genome mRNA arrays to broadly assess differentiation-related transcription factors and other gene products in basal vs suprabasal human epidermis.

We applied LCM to normal human skin derived from three volunteers in order to isolate the following three regions: reticular dermis (as a reference), basal epidermis, and suprabasal (spinous, granular, and cornified layers) epidermis (Figure S1). We then extracted RNA from each of these regions and generated their gene expression profiles using Affymetrix HGU133 Plus 2.0 arrays as described (Supplementary Materials and Methods).

The transcriptional profiles of the three captured regions distinctly separated from each other by principal components analysis, with minimal deviation between the three biological replicates. As expected due to their vastly different cellular composition, the reticular dermis samples were especially far removed from the other two epidermal regions (Figure 1a). Whole epidermis captured by LCM was very similar to suprabasal epidermis but quite

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distinct from basal epidermis, highlighting the utility of this approach to separate out basal from suprabasal epidermis. Using cutoffs of false discovery rate (FDR)<0.01 and fold change (FCH)>1.5, we found 286 upregulated and 310 downregulated genes in basal versus suprabasal epidermis (raw data deposited in Gene Expression Omnibus accession number GSE42114). Some gene expression changes confirmed many expected differences between basal and suprabasal epidermis (Table S1, full list available on request) as well as between basal epidermis and reticular dermis (Table S2, full list available on request). For instance, keratin 5 was increased 7.7-fold in basal epidermis while filaggrin was increased 492.8-fold in suprabasal epidermis (FDR<10^{-4} for both). Furthermore, many genes associated with basal epidermis-resident melanocytes, such as dopachrome tautomerase and melan-A, were upregulated in basal epidermis (Table S1). In other words, many of the genes found to be upregulated in basal epidermis will prove to be melanocyte-specific and therefore these differentially expressed gene lists have the potential to improve our understanding of melanocyte biology.

Our data led to the identification of many transcription factors to our knowledge not previously localized in human skin, including regulatory factor X, 5 and sterol regulatory element-binding transcription factor 2 (SREBF2) (Table 1, full list in Table S3). The preferential localization of transcription factors to either basal or suprabasal epidermis was confirmed by immunohistochemical staining of normal human skin using antibodies specific to six identified factors (Figure 1b–h). The discovery of transcription factors such as those found in this study has the potential to improve our mechanistic understanding of how cells differentiate from the basal to suprabasal layers of the epidermis. The functional role played by many of these factors remains to be determined but several of them are already known to display skin phenotypes (either related to pigmentation (Nagarajan *et al*., 2009) or differentiation (Steinmayr *et al*., 1998)) when knocked out in mouse models. Combined with our data, this suggests that these transcription factors have direct, endogenous functions in epidermal cells.

By Ingenuity Pathway Analysis, SREBF2 is predicted to be activated and account for the gene expression changes observed between suprabasal epidermis and reticular dermis (bias-corrected z-score = 2.603) but not between basal epidermis and reticular dermis, therefore suggesting that this transcription factor plays a functional role in epidermal differentiation. This view is supported by the role of SREBF2 in lipid synthesis (Harris *et al*., 1998) which is known to a be a function of suprabasal keratinocytes. Also, SREBF2 has been implicated in wound healing by mouse studies (Merath *et al*., 2011).

We note that our results differ significantly with those reported by Radoja *et al*. Of the 596 genes we found to be differentially regulated between basal and suprabasal epidermis, only 72 were found by the Radoja *et al*. study (Figure S2). Two factors may account for the observed differences between studies. First, enrichment of differentiated epidermal layers is higher for LCM methods. Similar to a previous study in which loricrin and filaggrin mRNA were enhanced by 783- and 446-fold, respectively, by LCM (Kennedy-Crispin *et al.*, 2012), we had >400-fold enrichment in suprabasal vs basal epidermis in this study. Incomplete cell separation using trypsinization techniques makes reliable recovery of granular layer keratinocytes more difficult and this presumably reflects minimal enrichment of loricrin and

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filaggrin by Radoja *et al*. Second, trypsinization/brief culture of separated epidermal cells can dramatically alter gene transcription profiles (Kennedy-Crispin *et al.*, 2012). Another consideration is that melanocytes, located in basal epidermis, do not express β4 integrin and thus are aberrantly included in the suprabasal fraction by cell separation. As our study is, to our knowledge, the first to combine LCM with transcriptional profiling of different epidermal regions, it provides a unique basis of comparison for future work that will use LCM to examine specific cell populations of various disease states where the epidermis is undergoing hyperplastic or neoplastic changes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

LCM	laser capture microdissection
SREBF2	sterol regulatory element-binding transcription factor 2
FCH	fold change
FDR	false discovery rate

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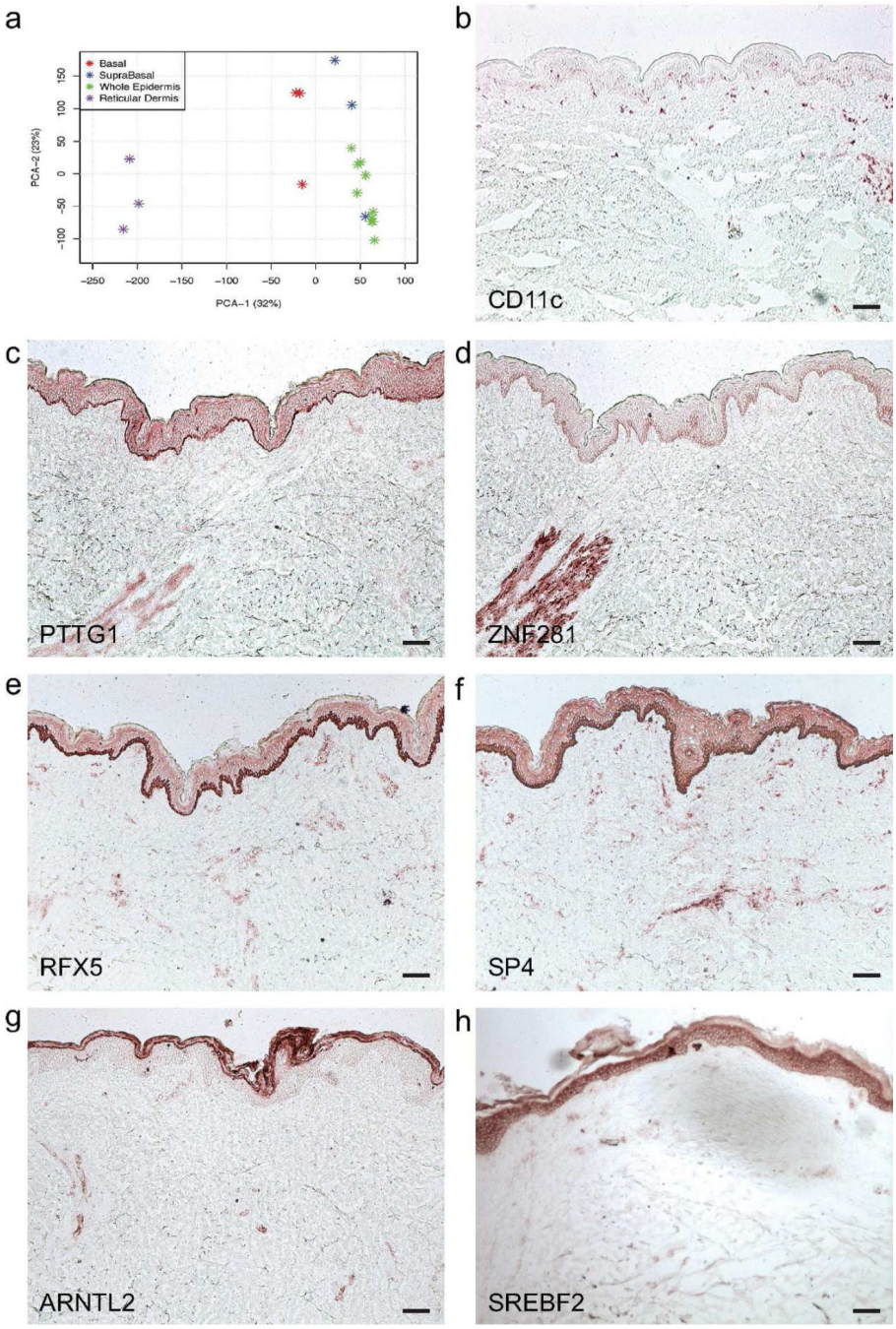

Figure 1.

Principal components analysis and immunohistochemistry confirm effective separation of different epidermal regions. (a) Principal components analysis of microarray data. The "Whole Epidermis" data points represent full epidermis LCM performed as part of a separate experiment (Kennedy-Crispin *et al*., 2012). (b–h) Immunohistochemical confirmation of transcription factor localization (see Table 1). Immunohistochemistry staining of normal skin samples for: (b) CD11c as a negative control for epidermal staining, (c) pituitary tumortr-ansforming gene 1 (PTTG1), (d) zinc finger protein 281 (ZNF281), (e)

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regulatory factor X, 5 (RFX5), (f) Sp4 transcription factor (SP4), (g) aryl hydrocarbon receptor nuclear translocator-like 2 (ARNTL2), and (h) sterol regulatory element-binding transcription factor 2 (SREBF2). Images c–f confirm a basal layer localization while images g–h confirm a suprabasal localization. Scale bar = 100 μm.

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Table 1

Selected transcription factors (identified through Gene Ontology term 0003700) differentially expressed between basal and suprabasal epidermis

Gene	Description	FCH[1]
(a) transcription factors upregulated in basal compared to suprabasal epidermis		
*PTTG1**	pituitary tumor-transforming 1	10.303
ZNF281	zinc finger protein 281	9.241
RFX5	regulatory factor X, 5 (influences HLA class II expression)	8.914
SP4	Sp4 transcription factor	6.347
(b) transcription factors upregulated in suprabasal compared to basal epidermis		
ARNTL2	aryl hydrocarbon receptor nuclear translocator-like 2	7.155
*SREBF2**	sterol regulatory element binding transcription factor 2	2.500

[1] FDR <0.05 for all genes

* reported as differentially expressed by Radoja *et al*., 2006, but not previously confirmed by immunohistochemistry