EPIDERMAL DIFFERENTIATION AND PROLIFERATION HETEROGENEITY IN SKIN OF COLOR

Sarah Girardeau-Hubert, Céline Deneuville, Hervé Pageon, Kahina Abed, Charlotte Tacheau, Nükhet Cavusoglu, Mark Donovan, Dominique Bernard, Daniel Asselineau and Inês Sequeira

L'Oréal Research and Innovation, Aulnay-sous-Bois, France

INTRODUCTION

Heterogeneity in skin surface and barrier function has been reported among skin color types. Previous studies have shown that epidermal morphogenesis is the result of the balance between keratinocyte proliferation and differentiation processes. The purpose of this study was to characterize and compare the epidermis biology from African descent skin type and with European descent skin type (usually also referring to Caucasian), using 3D *in vitro* tissues with cells and comparing these with human skin biopsies.

MATERIAL AND METHODS

Human skin samples

Normal human skin samples obtained from breast reduction of adult women were separated in two groups according to the skin color (mean age 29 years ± 7; n=10 per skin type). Skin with dark/brown color were used for African descent skin type (referred as **Af**) and light/intermediary/tan color for European descent skin type (referred as **Eu**).

Reconstructed skins

Keratinocytes and papillary fibroblasts were isolated respectively from the epidermis and the superficial layer of the dermis of skin biopsies (n=4 per skin type). Reconstructed skins (RS) were prepared using a differentiated epidermis grown with keratinocytes on a dermal equivalent lattice populated with fibroblasts (n=3 or 4 for each condition). Briefly, dermal part is prepared with bovine collagen I (Symatèse, Lyon) and fibroblasts. After 4 days, keratinocytes were seeded on dermal equivalent and cultured for 9 days. *In vitro* models were raised at the air-liquid interface and keratinocytes were allowed to differentiate during 8 days (Emersion culture phase D8). Hematoxylin eosin saffron coloration (HES) was performed on tissue sections to observe epidermal architecture.

Immunostainings of epidermal markers

Immunostainings of human skin and *in vitro* reconstructed skin were performed on frozen sections for human keratin 14, keratin 15, filaggrin 1 and proliferation marker Ki67.

Gene expression analysis

Total RNA from epidermis of *in vitro* skin was isolated after 8 days of emersion phase and mRNA were extracted. Reverse transcription of mRNA into cDNA and q-PCR were performed. Gene expressions corresponding to each samples were normalized with housekeeping genes (TBP, YWHAZ, GUSB).

ELISA assay

KGF protein expression in supernatants of reconstructed skin cultures were quantified using ELISA Quantikine kits (R&D Systems) according to the manufacturer's instructions.

Statistics

Non parametric Wilcoxon test was applied to data for statistical significance. p value ($p < 0.05$) and effect size of the difference were used to conclude.

CONCLUSION

- Balance between proliferation/differentiation appears to be different on epidermis from African and European descent skin types: increased number of proliferating cells and lower level of keratin 14 and 15 were observed in African skin and increased level of filaggrin was observed in European skin.
- *In vitro* epidermis exhibited distinct features with regard to stratification and differentiation according to cell origin in reconstructed skins.
- Our results emphasize differences in signaling between African and European papillary fibroblasts. KGF appears to be one of the regulatory factors which could explain the in vitro features. Since, cross-talk between keratinocytes and fibroblasts is a complex regulatory process, other soluble factors from the fibroblasts may also be involved.
- Taking together, this study highlights that dermal fibroblasts play an important role as regulators of epidermal processes. This report showed *in vitro* differences between fibroblasts from African and European descent skin types that could contribute to the different skin behaviors observed with aging and in specific disorders.

RESULTS

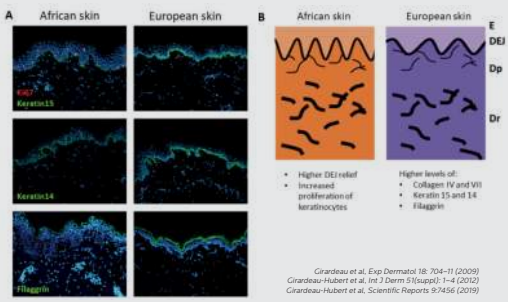

Figure 1: Pattern of proteins expression in human epidermis for basal cells: keratins 14 and 15, proliferating cells: Ki67 and differentiated cells: filaggrin.

Increased number of proliferating basal keratinocytes (Ki67) was observed in African skin when compared to European skin. On the contrary, higher levels of filaggrin, keratin 14 and 15 were observed in epidermis from human skin of European skin origin (A). Differences identified between skin samples from African and European descent skin types are summarized in (B). E, Epidermis; DEJ, Dermal-epidermal junction; Dp, Papillary dermis; Dr, Reticular Dermis.

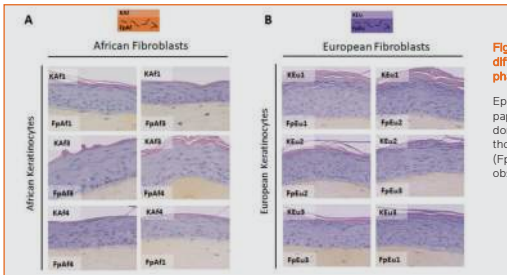

Figure 2: Epidermal morphogenesis of reconstructed skin is different according to keratinocytes origin at day 8 of emersion phase.

Epidermis with African keratinocytes (KAf) combined to African papillary fibroblasts (FpAf) from the same donor or a different donor (**A**) showed lower level of granular cells layers compared to those with European keratinocytes (KEu) and papillary fibroblasts (FpEu) (**B**). Note that accumulation of stratum corneum were observed with European keratinocytes.

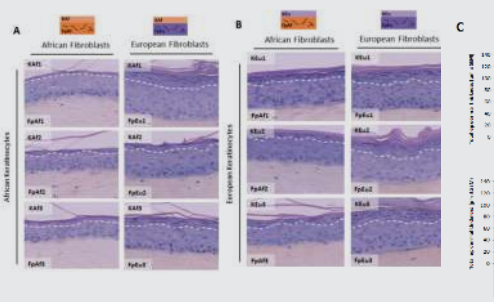

Figure 3: Terminal differentiation of epidermis is partly regulated by skin type origin of fibroblasts.

Increased number of granular layers (delimited by doted line) were observed with European fibroblasts as compared to African fibroblasts at day 8 of emersion phase. Epidermis with African keratinocytes were combined to African or European fibroblasts (**A**) and epidermis with Caucasian keratinocytes were combined to African or European fibroblasts (**B**). Thickness of living epidermis (without stratum corneum) was measured for each *in vitro* skin sample (**C**). Means are reported ± SEM (n = 3). *significant difference with Wilcoxon test.

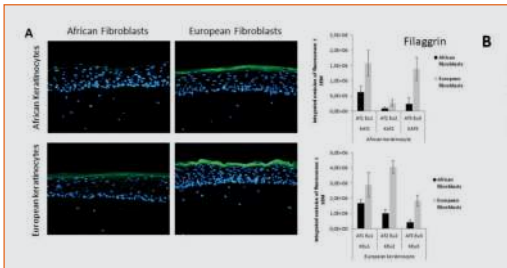

Figure 4: Epidermal markers of terminal differentiation were increased with European dermal papillary fibroblasts in reconstructed skin.

At day 8 of emersion culture phase, immunostaining of filaggrin revealed an accumulation in granular layer and stratum corneum in reconstructed skin with European fibroblasts (A). Mean are reported ± SEM (n = 3 per condition). KRT2 mRNA levels were increased in keratinocytes cultured with European fibroblasts when compared to those with African fibroblasts (B). Means are reported ± SEM (n = 4 per condition). *significant difference with Wilcoxon test.

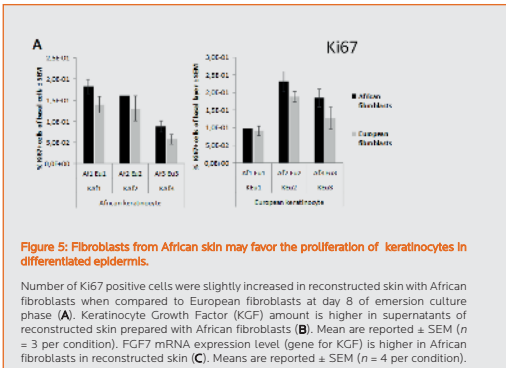

Figure 5: Fibroblasts from African skin may favor the proliferation of keratinocytes in differentiated epidermis.

Number of Ki67 positive cells were slightly increased in reconstructed skin with African fibroblasts when compared to European fibroblasts at day 8 of emersion culture phase (**A**). Keratinocyte Growth Factor (KGF) amount is higher in supernatants of reconstructed skin prepared with African fibroblasts (**B**). Mean are reported ± SEM (n = 3 per condition). FGF7 mRNA expression level (gene for KGF) is higher in African fibroblasts in reconstructed skin (**C**). Means are reported ± SEM (n = 4 per condition). *significant difference with Wilcoxon test.

L'ORÉAL
Research & Innovation