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LETTER TO THE EDITOR

Proliferation and differentiation biomarkers in normal human breast skin organotypic cultures

KEYWORDS
Involucrin; Bromodeoxyuridine; Keratin 10; Desmosomal cadherins

For basic research in skin biology, organotypic cultures of normal human skin represent a pertinent model. The main advantages consist in the preservation of the three-dimensional arrangement and the epidermal/dermal cross talking needed for keratinocyte differentiation. Pioneering works [1,2] reported quantitative parameters on cultured human normal skin, but the introduction of new experimental settings, such as the air—liquid interface, requires a redefinition of some of these parameters. Up to now, epidermal proliferation in organotypic normal human skin within the first days of culture was only qualitatively described [3].

We performed a time-course study focused on:

(1) Quantitative analysis of proliferation after 5-bromo-2′-deoxyuridine (BrdU) incorporation;
(2) qualitative analysis of the expression of biomarkers of epidermal terminal differentiation (TD) (involucrin) and adhesion (desmocollin 1, Dsc1; desmoglein 1, Dsg1);

in organotypic cultures of normal human breast skin.

Human breast skin biopsies were obtained from eight healthy 30—40-year-old women after cosmetic surgery, who all gave their written informed consent. Samples were harvested immediately after the excision (baseline, B) or 6, 24 and 72 h after overnight incubation (T6, T24, T72). Each subject was represented at all time points. Skin biopsies were cultured in triplicate as previously described [4]. All samples were processed for paraffin embedding. In baseline and T72 semithin sections were also obtained after glutharaldheyde fixation, araldite embedding and toluidine blue staining. Anti-human monoclonal antibodies for BrdU (Becton Dickinson), involucrin, Dsc1 and Dsg1 (Progen Biotechnik) were used and revealed with a FITC-conjugated goat anti-mouse antibody (Jackson Immunoresearch Laboratories Inc.). For quantitative proliferation study, seven immunofluorescence experiments were performed on each sample. Two slides/sample/experiment were examined by two investigators. BrdU positive cell number was normalized to the area of living epidermis using the software Image Pro-Plus (Version 4.5.019; Media Cybernetics Inc.). At T6, T24 and T72 the proliferation was calculated as mean percentage of baseline [4].

Epidermal stratification was unaffected within the first 72 h of culture (Fig. 1A and B). At T72 intercellular spaces appeared slightly widened in the spinous layer; pyknotic keratinocytes were never observed (Fig. 1B).

At baseline involucrin expression started from the upper spinous layer (Fig. 1C), while at T72 a suprabasal immunoreactivity was detected (Fig. 1D). A clear membrane Dsc1 labeling was always found from the upper spinous layer and disappeared in corneodesmosomes (Fig. 1E and F). Dsg1 staining was distributed throughout all epidermis (Fig. 1G). At T72 the profound epidermal layers showed an uneven expression of Dsg1 (Fig. 1H).

In all samples BrdU nuclear staining was detected in the basal layer, with scattered proliferating cells in the suprabasal compartment. At all considered time points the mean percentage of epidermal proliferation versus baseline significantly reduced (one-way analysis of variance, $p < 0.01$) (Fig. 2). Keratinocyte proliferation progressively decreased during the time-course study, and significant differences were found among time points (Tukey's honestly significant differences: T6 versus T72, T24 versus T72, $p < 0.05$).

In skin organotypic cultures the viability and the structural integrity of the epidermal compartment

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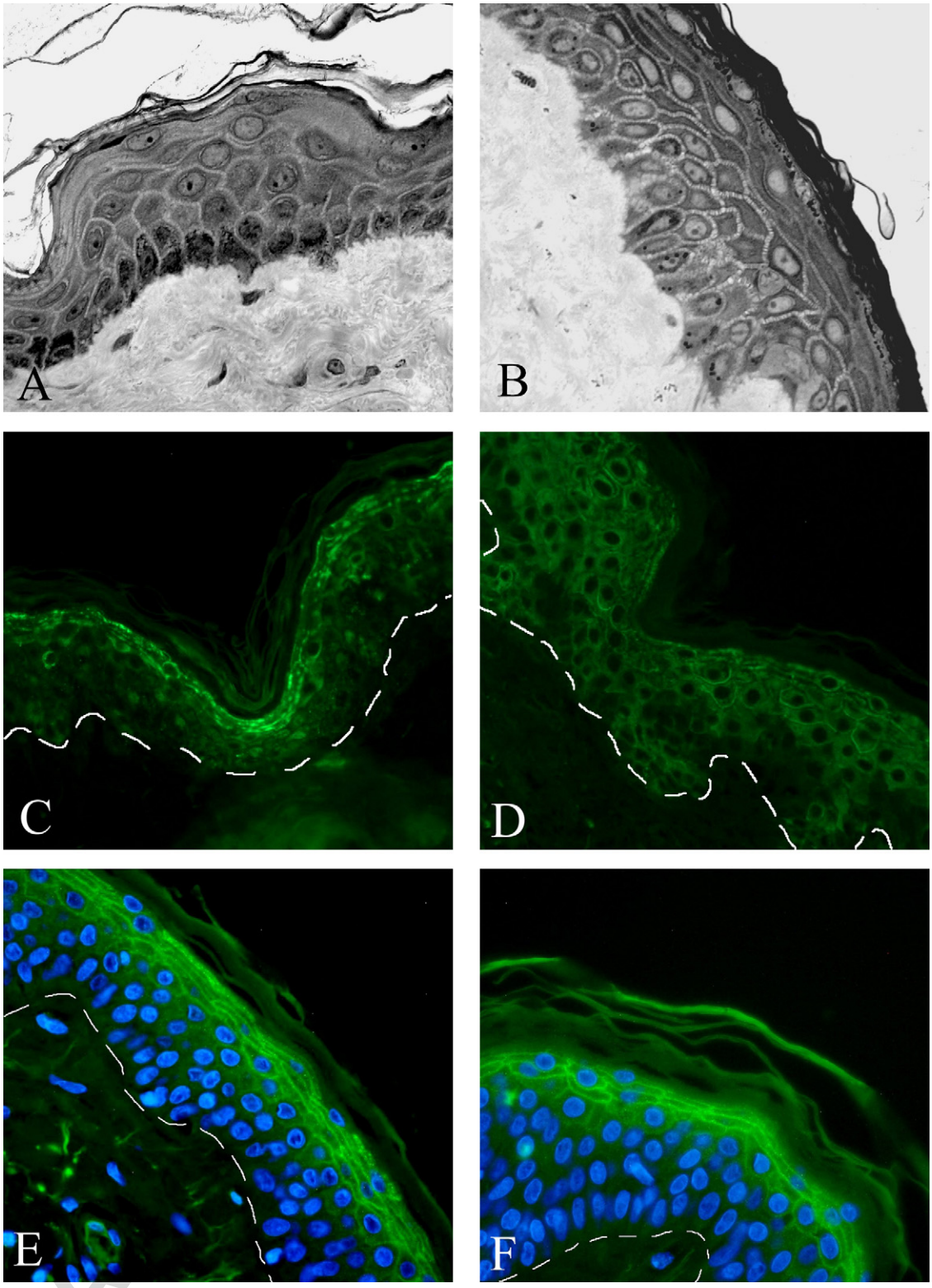

Fig. 1 Normal human breast skin in organotypic cultures. (A, C, E, G): baseline; (B, D, F, H): T72. (A and B) Toluidine blue staining on araldite semithin sections; (C and D) involucrin; (E and F) desmocollin 1; (G and H) desmoglein 1 immunofluorescence on paraffin sections. Dashed lines: basement membrane. See text for details. Original magnification: 40×.

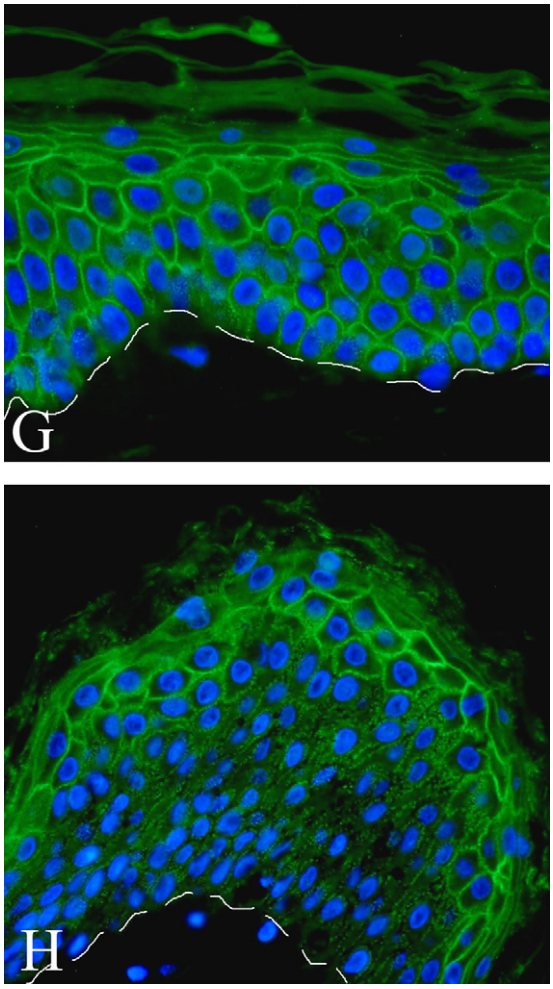

Figure 1. (*Continued*).

are maintained up to 3 days. The tissue pattern of the considered biomarkers suggests that a modification of keratinocyte TD/adhesion starts to occur at T72.

Although the epidermal response after exposure to exogenous stimuli was previously investigated in full-thickness biopsies of human skin [3—9], the characterization of this model in basal conditions was lacking. Our data represent the first morphological study of the proliferative and main differentiative features of TD and adhesion in normal human breast skin organotypic cultures in an experimental setting as close as possible to the physiological condition.

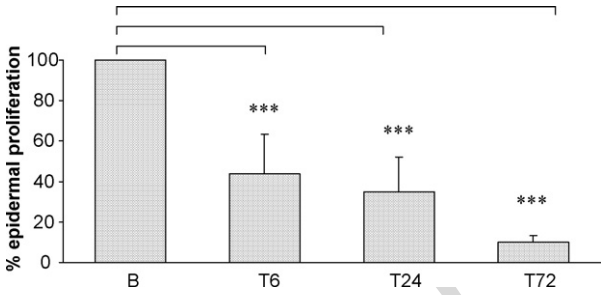

Fig. 2 Time-course quantitative analysis of cell proliferation expressed as mean percentage of cell proliferation versus baseline ($n = 8$). See text for details. (mean + 1S.D.). $^{***}p < 0.01$ vs. B.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jdermsci.2006.12.005

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