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LETTER TO THE EDITOR

Common features of umbilical cord epithelial cells and epidermal keratinocytes

Numerous experimental approaches in the field of dermatological research necessarily require the usage of human primary keratinocytes as immortal cell lines generally do not appropriately reflect the *in vivo* situation. However, the utilization of freshly isolated cells is often hampered by the limited accessibility of human skin biopsies and their derivation from donors of different age, gender and pathological background, as well as from different body sites. Recently it has been reported that the umbilical cord epithelium (UCE) expresses a cytokeratin pattern similar to human epidermis [1], although the general architecture of the umbilical cord epithelium significantly differs from the mammalian epidermis [2]. Furthermore, cells derived from UCE are capable of forming a stratified epithelium when seeded on fibroblast populated collagen gels [3,4]. We therefore asked whether the umbilical cord could possibly serve as an alternative source for primary neonatal keratinocytes and investigated the similarity of UCE cells and neonatal primary keratinocytes at the molecular and functional level.

For all experiments, Cord Lining Epithelial Cells (CLEC) that were isolated by CellResearch Corp. (Singapore) were used. Isolation was performed as previously described (international publication number WO 2006/019357 A1). Briefly, umbilical cord samples were obtained after normal delivery and the amniotic membrane was divided into small pieces. The samples were enzymatically digested in 0.1% collagenase type 1 solution (Roche Diagnostics) for 8 h at 37 °C. Cells were harvested by centrifugation (30 min, 3500 × *g*) and resuspended in supplemented EpiLife Medium (Cascade Biologics). When grown in monolayer culture, CLEC showed a typical epithelial morphology with a polygonal shape and a small nuclear cytoplasmic ratio (see Fig. 2G). At any degree of cell density, we were not able to detect morphological differences between CLEC and neonatal NHEK (Lonza, grown in KGM-2).

For that reason, we extended the comparison of CLEC and NHEK to the molecular level using real-time RT-PCR with a customized TaqMan® Low-Density PCR array (Applied Biosystems) for gene expression analysis of 20 skin specific genes. The expression patterns of cell surface (CD) markers show a remarkable similarity of CLEC and NHEK (Fig. 1A). In detail, both cell types share the typical keratinocyte cell surface profile $CD44^{++}$, $CD166^{++}$, $CD13^{+}$, $CD14^{+}$, $CD71^{+}$, $CD90^{+}$, $CD105^{+}$, $CD34^{-}$ and $CD45^{-}$. These results unequivocally demonstrate that CLEC possess considerable characteristics of epidermal keratinocytes. However, keratinocytes comprise cells of all differentiation levels, from basal progenitor to terminally differentiated cells. Therefore, the differentiation status of CLEC was characterized by performing expression analysis of differentiation-specific genes. As CLEC display morphological features of basal undifferentiated keratinocytes, we first investigated the expression of various subtypes of integrins, which display a characteristic expression pattern in basal undifferentiated keratinocytes. Our analysis again confirmed the similarity of CLEC and NHEK (Fig. 1B), as the expression intensities for all integrin variants was identical in both cell types.

This conclusion could be further confirmed by measuring the gene expression levels of five keratin subtypes (Fig. 1C). Keratin 15, which is exclusively expressed in keratinocytes adjacent to the basal membrane *in vivo*, exhibits similar expression levels in cultured CLEC and NHEK. Additionally, the expression level of the basal keratin pair 5/14 strongly exceeds the expression of the suprabasal keratins 1 and 10 in both cell types.

Further evidence for an early differentiation status of CLEC was provided by the expression analysis of the ΔN splice variant of the transcription factor p63 that has been shown to be exclusively expressed in basal keratinocytes [5], but at higher levels in the progenitor cell fraction [6]. Both cell types showed

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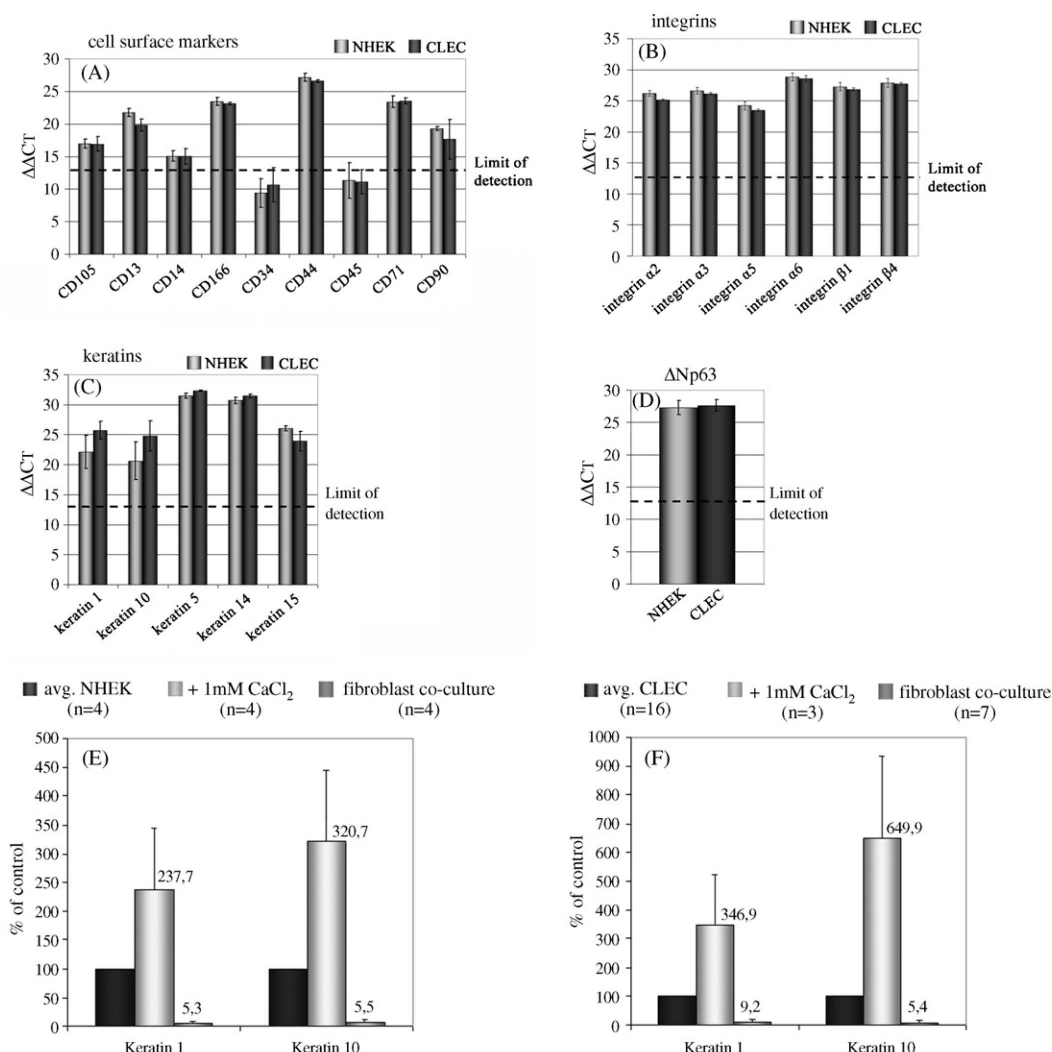

Fig. 1 mRNA expression levels of keratinocyte specific genes are equal in CLEC and NHEK. Cultured cells from two CLEC donors (passage 2 and 3, $n = 4$) and three NHEK batches (passage 2 and 3, $n = 6$) were subjected to real-time RT PCR analysis with the TaqMan® low-density array technique. Expression levels were calculated with the $\Delta\Delta C_T$-method of the ABI SDS 2.2.2 software. Target genes that did not reach the signal threshold after 35 PCR cycles were regarded as not expressed or not detectable. The corresponding average $\Delta\Delta C_T$-value (12.7) is indicated by the dashed line. The expression levels of all genes in the subgroups of CD-markers (A), integrins (B), keratins (C) as well as the expression level of ΔNp63 (D) were nearly identical in CLEC and NHEK. In both cell types $CaCl_2$ treatment led to a substantial increase in keratin 1 and 10 expression whereas an even more pronounced decrease could be observed after co-culture with dermal fibroblasts (E and F).

surprisingly high levels of Δ*N*p63 expression (Fig. 1D), indicating a large progenitor cell proportion in CLEC and NHEK monolayer cultures.

To further verify the results of the real-time RT-PCR experiments, a subset of the analyzed genes was also examined at the protein level by immunofluorescence staining on fixed CLEC monolayer cultures. In all cases the staining intensities generally reflected the results of the gene expression analysis (Fig. 2A—F).

The results above demonstrate that CLEC and NHEK are indistinguishable by expression profiling of keratinocyte specific genes. In a more functional approach we examined whether CLEC also respond to signals that typically induce or prevent differentiation of NHEK. As expected, NHEK showed a significant increase of the expression of the early differentiation markers keratin 1 and 10 after treatment with 1 mM Calcium for 24 h (Fig. 1E). On the other hand, it has been shown that the interaction with dermal fibroblasts retains keratinocytes in a proliferative state [7,8] and that cell cycle arrest can be mediated by K10 overexpression [9]. In agreement with these observations, K1 and K10

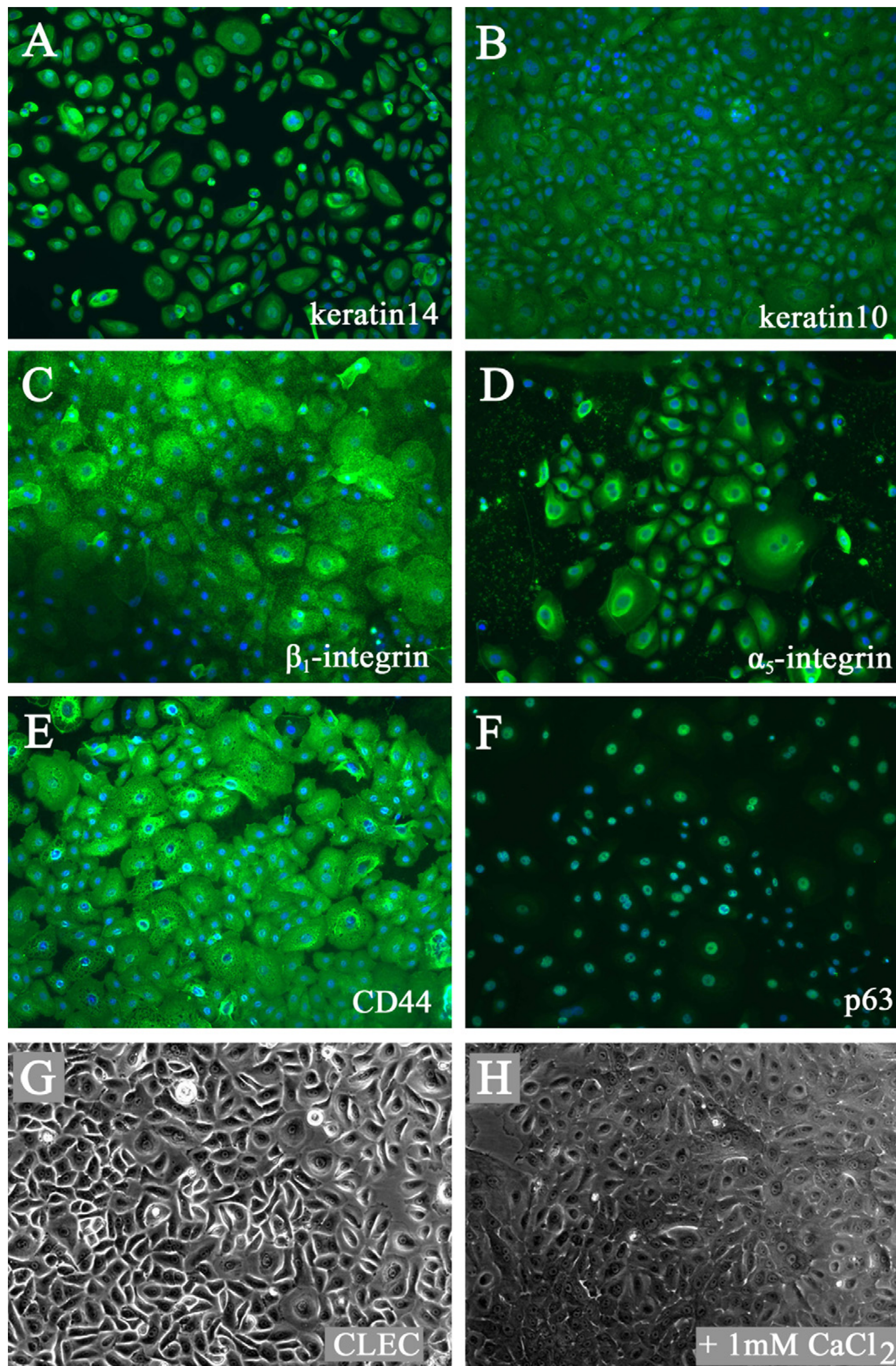

Fig. 2 CLEC express proteins that are typically found in keratinocytes. CLEC were seeded on Permanox®-coated LabTek® chamber slides and immunostained for various keratinocyte specific proteins. CLEC show a strong expression of keratin 14 (A), β_1-integrin (C) and CD44 (E) whereas stainings for keratin 10 (B) exhibits a lower intensity. This corresponds to the mRNA expression values of the same genes (Fig. 1). Staining for p63 (F) shows nuclear expression that varies on the single cell level. All staining patterns and intensities are identical to what is typically observed by immunofluorescence staining of keratinocytes (data not shown). CLEC treated with 1 mM $CaCl_2$ for 24 h acquire a flattened phenotype (H) as compared to untreated cells (G).

expression levels were dramatically decreased when NHEK were co-cultured with primary dermal fibroblasts (cultured in 6 well cell culture insert companion plates, BD Falcon). Remarkably, CLEC showed an identical regulation of K1 and K10 expression in response to both stimuli (Fig. 1F). Additionally, calcium treated CLEC appeared much more flattened compared to control cells (Fig. 2H)—a phenotype that can also be observed upon Ca^{2+}-induced differentiation of NHEK.

In summary, all of our experiments did not reveal any difference between neonatal NHEK and CLEC at the morphological, molecular and functional level. Additionally we also observed similar growth kinetics referring to growth rate and total population doublings for both cell types. These observations are especially surprising with respect to the different environments CLEC and epidermal keratinocytes are isolated from. Notably CLEC are derived from a tissue that is monolayered, surrounded by amniotic fluid and presumably subjected to a mesenchymal crosstalk with cells that significantly differ from dermal fibroblasts [10]. Nevertheless our results clearly imply that cord lining epithelial cells closely resemble primary neonatal epidermal keratinocytes. Regarding the huge amounts of umbilical cords that are routinely discarded after birth, the usage of CLEC could therefore be a considerable alternative whenever other sources of neonatal keratinocytes are not accessible.

Appendix A. Appendix A

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jdermsci.2007.12.006.

Appendix B. Supplementary data

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