Keratinocyte expression of the cell-surface glycoprotein CDCP1 during epidermogenesis and its role in keratinocyte migration

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Introduction

CDCP1 is a transmembrane glycoprotein that is differentially tyrosine phosphorylated during changes in cell adhesion and survival signaling, and is expressed by keratinocytes in native human skin, as well as in primary cultures. We utilised Real-time Cell migration analysis (xCELLigence RTCA-DP system, manufactured by ACEA Biosciences Inc.) and an in-vitro human skin reconstruct to investigate the expression of CDCP1 during epidermogenesis and its role in keratinocyte migration.

Advantages of Quantitative Cell Invasion/migration analysis using the xCELLigence System

Primary human cells are notoriously variable in their responses. The peak rate of cell migration of primary keratinocytes from different patients varies from 12-22 hours. Thus, the use of single time-point Transwell assays is unreliable, costly and time consuming. The real-time aspect of the xCELLigence system enabled us to monitor the cell migration rates continuously. This removed the variability from our experiments and provided fast, high quality results for less time and consumable costs.

Cell-migration through the membrane from the upper chamber to the lower chamber. Cells that have migrated to the underside of the lower chamber are now in direct contact with the gold microelectrodes that detect the attachment and quantify the rate of cell migration.

Antibody targeting of CDCP1 reduces keratinocyte chemotactic cell migration in vitro.

Keratinocyte migration from an upper chamber containing cells to a lower chamber containing FCS as a chemoattractant was monitored in real time by following changes in impedance in an electrode located on the underside of a Transwell using the xCELLigence DP system and CIM plates. Primary human keratinocytes (5 x10⁴) were serum starved for 4 h then seeded into the upper chamber, and cell migration was monitored every 15 min for 24 h.

(a) Effect of increasing FCS concentration on keratinocyte migration. Black, 1.25% FCS; blue, 2.5% FCS; red, 5% FCS; green, 10% FCS. Averages of quadruplicate wells are shown ± SEM.

(b–d) Keratinocytes from three individuals were seeded in serum-free medium alone (red) or serum-free medium supplemented with 20 ug/mL anti-CDCP1 function-blocking antibody (green). The lower chamber contained 2.5% FCS-supplemented medium.

The rate of cell migration was quantified by measuring the slope of each curve during the linear phase (indicated by the vertical lines) using the system software. Real-time impedance measurements are presented as individual traces of duplicate wells, except for panel (c) control, where only one measurement is displayed.

Human skin equivalents were constructed by seeding keratinocytes onto a de-epidermised dermis and allowing them to grow for 3 days. The HSE was then bought to the air-liquid interface where the epidermis stratifies over the next 9 days. We have shown the morphology between the DEE-HSE and native human skin to be comparable (Dawson et al., 2008 and Xie et al., 2010).

CDCP1 is localized to the stratum basale and stratum spinosum in native human skin.

CDCP1 is localized to the cell–cell contacts of stratum basale keratinocytes in epidermogenesis.

Conclusions

These findings delineate the expression of CDCP1 in human epidermal keratinocytes during epidermogenesis and demonstrate that CDCP1 is involved in keratinocyte migration. This work has been published in the Bri Derm – McGovern et al., 2013.

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