Immortalized N/TERT keratinocytes: An excellent and versatile alternative for primary keratinocytes in experimental dermatological research

Jos P.H. Smits¹, Hanna Niehues¹, Gijs Rikken¹, Ivonne M.J.J. van Vlijmen-Willems¹, Guillaume W.H.J.F.L. van de Zande², Patrick L.J.M. Zeeuwen¹, Joost Schalkwijk¹, Ellen H. van den Bogaard¹

¹Department of Dermatology, Radboud Institute for Molecular Life Sciences (RIMLS), Radboudumc, Nijmegen, The Netherlands

²Department of Human Genetics, Radboud University Medical Center (Radboudumc), Nijmegen, The Netherlands

Introduction

- In vitro* 3D **human epidermal equivalents (HEEs)** are generally made using adult primary keratinocytes to mimick native skin.
- Disadvantages of human primary keratinocytes are:
 - **limited availability.**
 - **short lifespan.**
 - **inter-donor variation.**
- Only few immortalized keratinocyte cell lines are available.
- The widely used **HaCaT** cell line shows **aneuploidy**, abnormal **epidermal differentiation**, aberrant **epidermal stratification** and is less suitable for developing 3D skin models.

Objective:

To illustrate the potential of human keratinocyte cell lines (N/TERT1 and N/TERT2G) for studies on epidermal biology, inflammatory skin disease pathogenesis and therapeutics.

Results

N/TERT keratinocyte gene expression corresponds to that of human primary keratinocytes in monolayer cultures:

N/TERT keratinocyte cell lines are diploid:

Results

High quality HEEs are generated from N/TERT keratinocytes:

HEEs from N/TERT keratinocytes form a functional barrier:

Atopic Dermatitis (AD) hallmarks were induced in HEEs by Th2 cytokines and subsequently rescued by drug treatment:

N/TERT keratinocytes...

- ...show high quality HEE morphology. Gene and protein expression is highly comparable to that of human primary keratinocytes.
- ...HEEs are useful to study epidermal biology, inflammatory skin disease pathogenesis, and therapeutics.
- ...have a great potential for implementation of advanced techniques (e.g. CRISPR-Cas9).