Characterization of a Three-Dimensional (3D) Organotypic Skin Model using Keratinocytes and Mesenchymal Stem Cells Immortalized by hTERT

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Abstract

In this study, we compared primary keratinocytes (ATCC® No. PCS-200-010) to hTERT immortalized keratinocytes (Ker-CT, ATCC® No. CRL-4046™), co-cultured with either primary fibroblasts (ATCC® No. PCS-201-010), primary adipose-derived mesenchymal stem cells (MSCs, ATCC® No. PCS-500-011), hTERT-immortalized fibroblasts (BJ-3Fla, ATCC® No. CRL-4001™), or hTERT-immortalized MSCs (hTERT-SCs, ATCC® No. SCRC-4000™). We confirmed that both primary keratinocytes and Ker-CT are able to fully differentiate into skin equivalents in a 3D culture model when co-cultured with primary fibroblasts, primary MSCs, BJ-3Fla, or hTERT-SCs. To confirm the functionality of the co-culture models, both the primary keratinocytes and the Ker-CT air-liquid interface (ALI) cocultures were subjected to scratch assay. Epithelialization occurred in both cell lines, and interleukin 8 (IL-8) showed an increase in expression from day 6 to day 1 and 3, corresponding to migration of cells into the wound. The continuous nature of the Ker-CT cell line makes it an invaluable model for the research of keratinocyte biology, as it eliminates the issue of short life span and donor variation seen with primary cells.

Introduction

• Keratinocytes undergo a program of terminal differentiation that results in a potent barrier against microbial infection, water loss, and chemical attack.
• Primary keratinocyte cultures are important cell models for the study of normal and pathological biology of the cutaneous epithelium.
• Keratinocyte differentiation is promoted by factors secreted by cells in the dermis.
• Primary keratinocytes can form skin equivalents that mimic the architectural features and behavior of normal skin in a 3D organotypic culture model in an ALI.
• Primary keratinocytes have finite lifespan in culture, which greatly restricts their use as an in-vitro cellular model.
• The constitutive expression of the catalytic subunit of telomerase, hTERT, allows for extended passage of cells with the benefit of little loss of normal cellular physiology.
• hTERT-immortalized keratinocytes cultured with primary fibroblasts, primary MSCs, BJ-3Fla, or hTERT-immortalized MSCs comprise a 3D model of the epidermis with high value for investigating drug delivery, toxicity of cosmetics, and the pathology of psoriasis.

Results

hTERT-immortalized and primary stroma-secreting cells directly and indirectly promote primary keratinocytes to form a stratified, 3D, differentiated epidermis

Figure 1. Hematoxylin and Eosin (HE)-stained cross sections of primary keratinocytes co-cultured with hTERT-immortalized and primary MSCs or fibroblasts differentiated for 21 days. Primary fibroblasts, MSCs, or their hTERT-immortalized counterparts were seeded onto A) the Transwell™ (Corning) apical chamber or B) the underside of the Transwell

Figure 2. HE-stained cross sections of Ker-CT co-cultured with hTERT and primary MSCs or fibroblasts, then differentiated for 21 days. Primary fibroblasts, MSCs, or their hTERT-immortalized counterparts were seeded onto A) the Transwell apical chamber or B) the underside of the Transwell

Figure 3. Ker-CT co-cultured with hTERT-MSC killed after scratch test. Ker-CT cells were seeded onto Transwells containing hTERT-MSCs in the basal chamber as described in figure 1. After 21 days of differentiation, the apical chamber of the Transwells were scratched using a P1000 tip. Media (EMPD) was changed every 2-3 days for a total of 14 days. Phase images at a x10 magnification were taken at the indicated days to observe the wound healing. Black lines indicate the size of the wound at day 0.

Primary keratinocytes and Ker-CT provide an in vitro model of epidermal inflammation

Figure 4. Concentration of IL-8 secreted into the basal media of the primary and immortalized keratinocytes during wound healing. Basal medium of organotypic A) primary keratinocytes and B) Ker-CT at day 2 (implant wounding) and after wounding were collected. Concentrations were then analyzed using the Luminex™(TM) (Luminex) platform (n=4, performed in triplicate, *represents p<0.05 vs day 0, ** represents p<0.005 vs day 0).

Summary

• In vitro epidermal assay models were established using an ALI Transwell system, primary epidermal keratinocyte cells or Ker-CT, and primary fibroblasts, MSCs, BJ-3Fla, or hTERT-SCs.
• Primary keratinocytes and Ker-CT displayed stratification and terminal differentiation.
• Ker-CT Black in wounds after being subjected to a scratch assay.
• Ker-CT displayed a similar inflammatory response as compared to the primary keratinocytes after wounding.
• Immortalized keratinocyte co-culture model provides a consistent and robust in vitro system which increases consistency between assays by decreasing the lot to list variability of primary cells.

References