Abstract # 3124

Development and Characterization of an Immortalized Human Dermal Fibroblast Cell Line That Retains Primary Cell Functionality

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Introduction

Dermal fibroblasts (DFs) are indispensable for skin biology, playing a critical role in extracellular matrix production, wound healing, and skin regeneration. Primary DFs have long been the gold-standard model system for studying skin biology; however, donor variability and the limited lifespan of primary cells restrict their potential. While the traditional method of immortalizing primary cells through SV40 can overcome these restrictions, it often leads to the loss of normal cell biofunction. Here, we present a new method for fully immortalizing human DFs that preserves the physiological attributes of the original primary cells. In this study, we used this novel method to develop an immortalized DF cell line that retains the functional characteristics of primary cells, thereby providing an unlimited source of cells that will help improve reproducibility in toxicology research.

Methods

We generated the clonal cell line hTERT HDFa (ATCC® CRL-4066™), which was immortalized by stably expressing hTERT and mutant CDK4 (CDK4R24C) in normal human primary DFs (ATCC® PCS-201-012™). The cell morphology, growth rate, cell markers, and functionality of the immortalized cell line were analyzed and compared to that of the parental primary cells.

Results

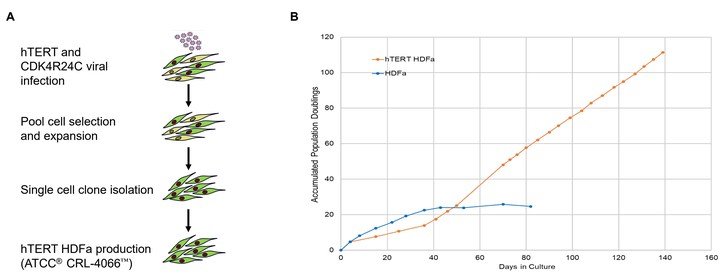

Figure 1: Immortalization of human primary dermal fibroblasts (HDFa). (A) Primary dermal fibroblasts (ATCC® PCS-201-012™) were infected with hTERT retrovirus and CDK4R24C lentivirus. The hTERT HDFa (ATCC® CRL-4066™) cell line was generated with a clone. (B) hTERT HDFa cells maintained consistent growth over 111 population doublings while primary HDFa underwent senescence around 26 doublings.

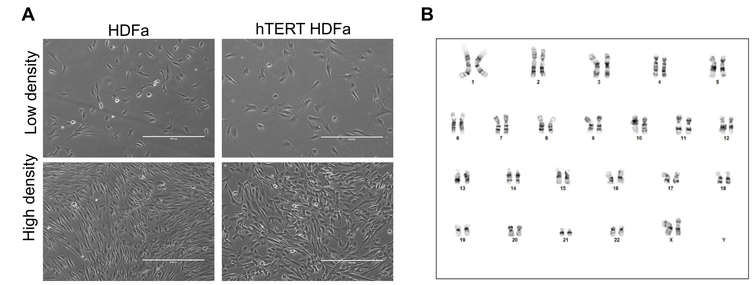

Figure 2: Evaluation of morphology and karyotype. (A) Morphology of primary HDFa and hTERT HDFa at low and high densities. hTERT HDFa showed similar morphology to that of primary cells. (B) hTERT HDFa retained a normal diploid karyotype.

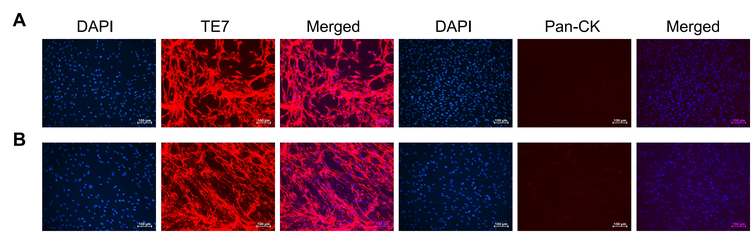

Figure 3: Marker expression in HDFa and hTERT HDFa cells. (A) hTERT HDFa and (B) HDFa cells were grown on glass chamber slides and fixed with 4% PFA. Cells were then stained with TE-7 (positive marker) and Pan-cytokeratin (Pan-CK, negative marker) antibodies (red) and then DAPI (blue). Images were taken using a fluorescence microscope.

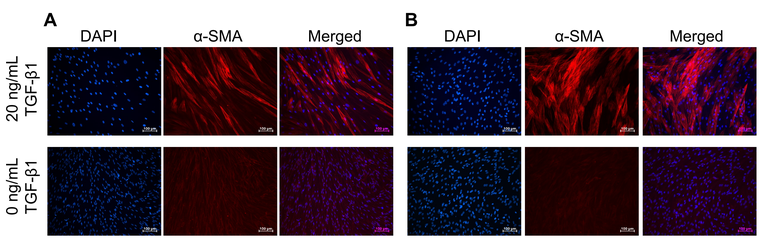

Figure 4: Differentiation of HDFa and hTERT HDFa into myofibroblasts that express alpha-smooth muscle actin. (A) hTERT HDFa and (B) HDFa cells were seeded onto glass chamber slides and cultured to confluence. Cells were starved for 24 hours. Then the cells were treated with 20 ng/mL TGFβ1 in fibroblast basal medium for 3 days. After, cells were fixed with 4% PFA and stained with α-SMA antibody (red) and then DAPI (blue). Cells were only treated with fibroblast basal medium without TGFβ1 as a negative control. Images were taken using a fluorescence microscope.

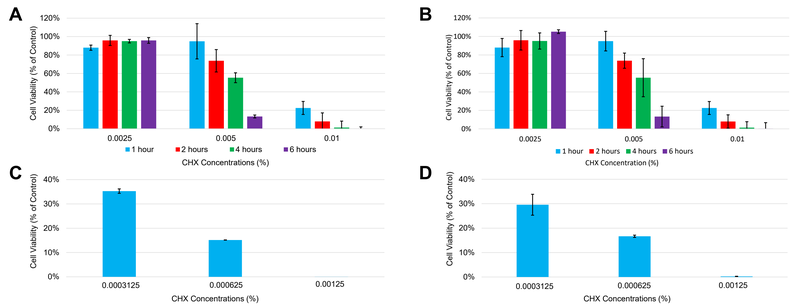

Figure 5: Chlorhexidine (CHX) inhibition effect on cell viability. (A, C) hTERT HDFa and (B,D) HDFa cells were seeded in a 96-well plate and cultured overnight. Three concentrations of CHX media (2.5 x 10^{-3} , 5.0 x 10^{-3} , 1.0 x 10^{-2} %) were added to (A) hTERT HDFa and (B) HDFa cells and treated for 1, 2, 4, and 6 hours. Three concentrations of CHX media (3.125 x 10^{-4}, 6.25 x 10^{-4} , and 1.25 x 10^{-3} %) were added to (C) hTERT HDFa and (D) HDFa cells for 3 days. Cell viabilities were measured using CellTiter-Glo (Promega Corporation) in a luminometer. The viabilities of the CHX treated cells were compared to untreated cell viability (percent of control).

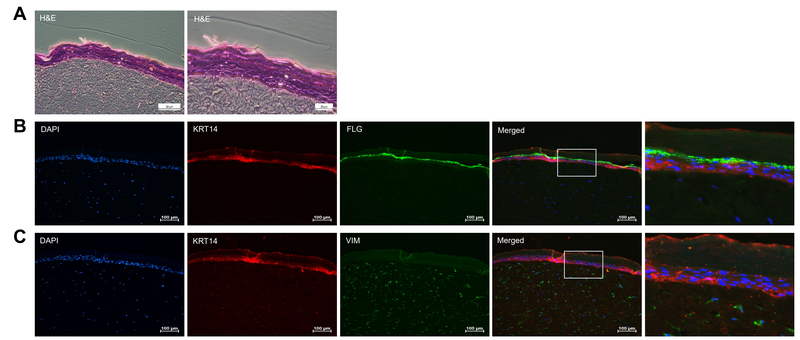

Figure 6: Formation and characterization of 3-D skin model. Immortalized keratinocytes (ATCC® CRL-4048™) cocultured with Immortalized dermal fibroblasts (ATCC® CRL-4066™) for 21 days post air-lift. (A) Representative H&E staining images. (B) Immunofluorescence staining of 3-D skin model with keratin 14 (KRT14), filaggrin (FLG), and DAPI. (C) Immunofluorescence staining of 3-D skin model with keratin 14 (KRT14), vimentin (VIM), and DAPI.

Conclusions

- The dermal fibroblast cells were successfully immortalized by hTERT and mutant CDK4.
- The hTERT HDFa cells retain the functional characteristics of primary cells.
- hTERT HDFa can be used as a valuable tool for toxicology research.

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