Abstract # 4340

Generation of a Novel Immortalized Human Corneal Epithelial Cell Line for Reliable In Vitro Ocular Toxicity Studies

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Introduction

The corneal epithelium is a transparent cell layer that covers the cornea and allows light to enter the eye. It serves as a critical barrier against pathogens and environmental insults, protecting deeper ocular tissues. Due to its protective and regenerative functions, the corneal epithelium is widely used in studies of wound healing, drug development, permeation, and toxicity. While animal models are commonly employed in ocular toxicity testing, they often fail to accurately reflect human responses. Human primary corneal epithelial cells offer a more relevant alternative, but their short lifespan and donor-dependent variability limit their suitability in long-term, reproducible studies. To address these limitations, we developed an immortalized human corneal epithelial cell line that retains key functional characteristics of primary cells while enabling extended culture and consistent performance in ocular toxicity assays.

Methods

We established a clonal immortalized human corneal epithelial cell line (hTERT HCEC; ATCC® CRL-4067™) by stably expressing human telomerase reverse transcriptase (hTERT) and a mutant cyclin-dependent kinase 4 ($CDK4^{R24C}$) in normal primary human corneal epithelial cells (ATCC® PCS-700-010™). We assessed cell morphology, proliferation rate, epithelial marker expression, migratory capacity, and performance in in vitro eye irritation assays.

Results

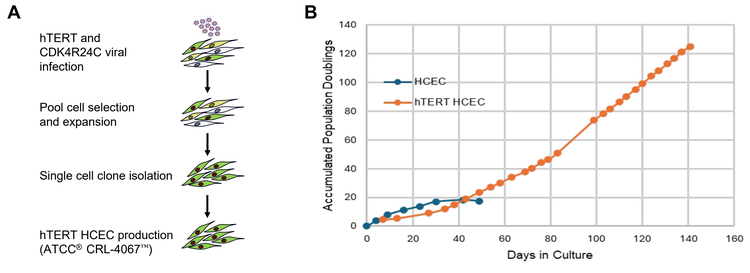

Figure 1: Immortalization of human primary corneal epithelial cells (HCEC). (A) Primary corneal epithelial cells (ATCC® PCS-700-010™) were infected with hTERT retrovirus and CDK4R24C lentivirus. The resulting hTERT HCEC (ATCC® CRL-4067™) cell line was generated with a clone. (B) hTERT HCEC cells maintained consistent growth over 125 population doublings while primary HCEC underwent senescence around 18 doublings.

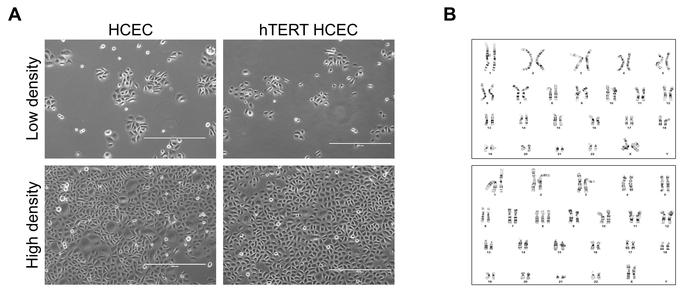

Figure 2: Evaluation of morphology and karyotype. (A) Morphology of primary HCEC and hTERT HCEC at low and high densities. hTERT HCEC showed similar morphology to that of primary cells. (B) hTERT HCEC exhibited an abnormal human female karyotype (47,XX,+8[2]/46,XX[17]).

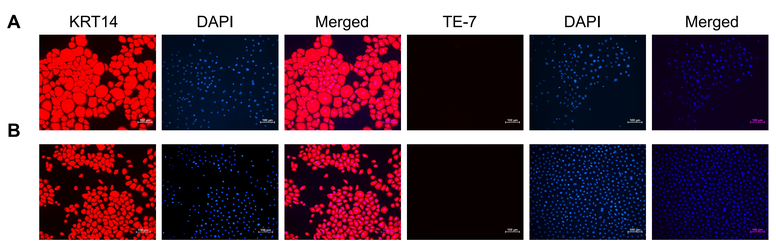

Figure 3: Marker expression in HCEC and hTERT HCEC cells. (A) HCEC and (B) hTERT HCEC cells were grown on glass chamber slides and fixed with 4% PFA. Cells were then stained with KRT14 (positive marker) and TE-7 (negative marker) antibodies (red) and then DAPI (blue). Cells were positive for KRT14 and negative for TE-7. Images were taken using a fluorescence microscope.

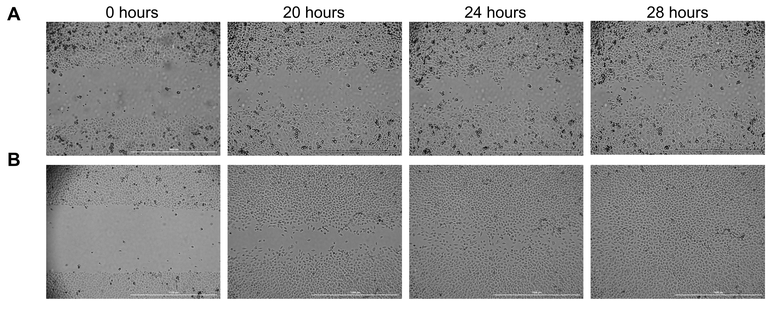

Figure 4: HCEC and hTERT HCEC in vitro wound healing assay. Representative images at different time points (0, 20, 24, and 28 hours) during the wound healing assay. (A) HCEC and (B) hTERT HCEC cells were plated in a 96-well plate and grown until confluent, then scratch wounds were made using an Incucyte Woundmaker Tool. Time-lapse phase contrast images were taken with a BioTek BioSpa Live Cell Analysis System (Agilent Technologies) every 2 hours. Cells were shown to have migration ability.

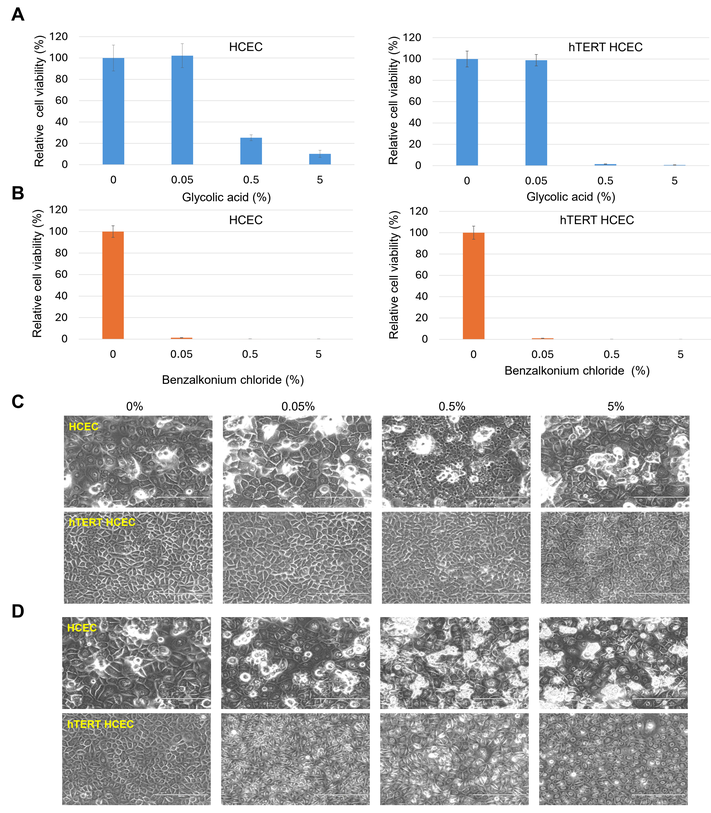

Figure 5: Toxicity of glycolic acid and benzalkonium chloride on HCEC and hTERT HCEC cells. The cells were seeded in 96-well plates and grown for 24 hours, then the fully confluent cells were exposed for 5 minutes to (A, C) 0, 0.05, 0.5, or 5% of glycolic acid in corneal epithelial cell basal medium or (B, D) 0, 0.05, 0.5, or 5% of benzalkonium chloride in corneal epithelial cell basal medium. (A, B) The cell viability was assessed using the CellTiter-Glo assay (Promega). Representative images of the cells treated with (C) glycolic acid or (D) benzalkonium chloride without adding CellTiter-Glo reagent. Cell viabilities reduced significantly when exposed to 0.5% glycolic acid and 0.05% benzalkonium chloride.

Conclusions

- The corneal epithelial cells were successfully immortalized by hTERT and mutant CDK4.
- The hTERT HCEC cells retain the functional characteristics of primary cells.
- hTERT HCEC cells provide a robust and reproducible platform for in vitro eye irritation testing and broader ocular toxicology applications.

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