Authenticated Lung and Gingival Fibroblasts Cell Models for *In Vitro* Toxicity Testing

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Abstract

There is an increasing demand for *in vitro* models to replace animal models in toxicity testing. Drivers for this change include decreased overall cost for cell-based models and the ability to do more high-throughput screening. The development of cell-based *in vitro* models for toxicity testing is a challenging task. Primary cells can best represent the *in vivo* situation; however, donor variability and replicative senescence restrict the potential usefulness of this cell model in the study of toxicity. Conversely, continuous cell lines often have altered genomes and do not fully represent the parental cells as a result of their altered genomic state. Human telomerase reverse transcriptase (hTERT)-immortalized primary cells provide a better solution—these cells can be continuously cultured while retaining the physiological characteristics of the parental primary cell.

In this study, we established two clonal hTERT-immortalized cell lines by expressing hTERT in lung fibroblasts and gingival fibroblasts isolated from normal donors. Both lines have been cultured continuously for more than 35 population doublings without any signs of replicative senescence. Further, both hTERT-immortalized fibroblasts retained a normal diploid karyotype over extended culture period and maintained typical fibroblastic characteristics, including positive staining for a fibroblast marker and negative staining for an epithelial cell marker. Additionally, the lung fibroblast cells responded to TGF-beta treatment with elevated smooth muscle actin expression as did the parental cells. Notably, both cell lines are sensitive to the toxicological agent chlorhexidine in a dose-dependent manner, similarly to the primary cells. These results show that the hTERT-immortalized lung fibroblasts and gingival fibroblasts retain the important physiological characteristics of the primary cells from which they were derived and provide a very useful *in vitro* cell model for toxicity screening.

Results

I. Cell Immortalization, Morphology, and Karyotype of hTERT- immortalized Fibroblasts Cell Lines

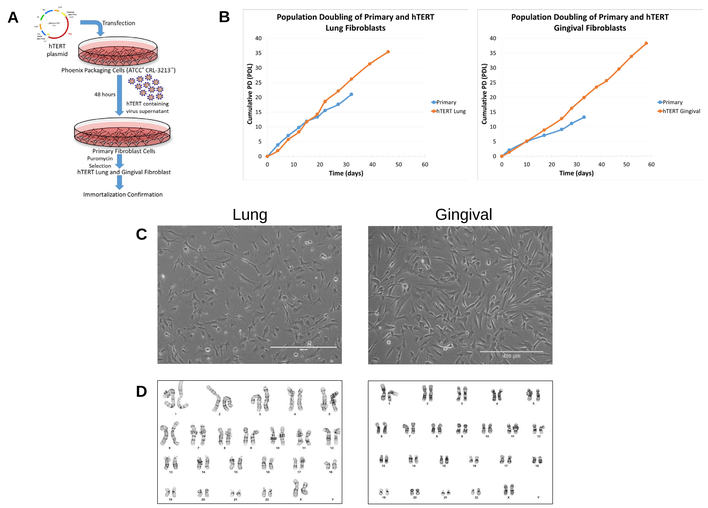


Figure 1. Lung Fibroblasts (ATCC® CRL-4058™) and Gingival Fibroblasts (ATCC® CRL-4060™) immortalized with human telomerase (hTERT). (A) Primary fibroblasts were retrovirally transduced with hTERT constructs produced in Phoenix Ampho (ATCC® CRL-3213™) packaging cells. The stable presence of hTERT was confirmed with a telomeric repeat amplification protocol (TRAP) assay (data not shown). (B) Fibroblast cells were grown continuously for greater than 35 population doublings without signs of replicative senescence. (C) Both fibroblast cell lines demonstrated a typical fibroblastic morphology and (D) retained a normal diploid karyotype. Scale bar, 400 μm.

II. Immortalized Fibroblasts Maintain a Fibroblast-specific Marker

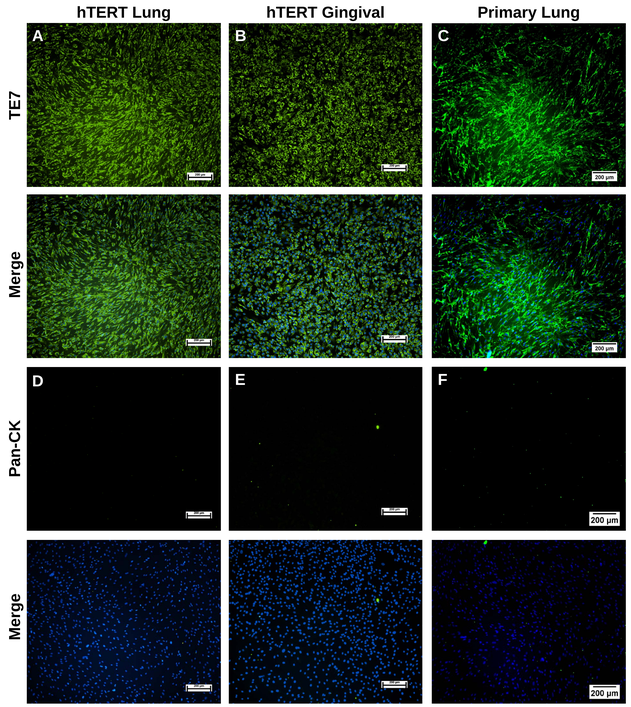
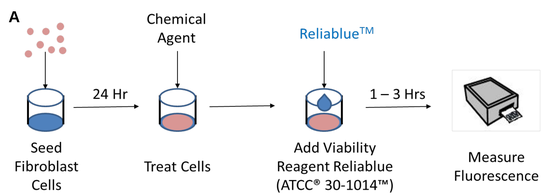


Figure 2. Lung and Gingival Fibroblasts express a fibroblasts specific marker, but do not express cytokeratin. (A, D) hTERT lung fibroblasts, (B, E) hTERT gingival fibroblasts, and (C, F) primary lung fibroblasts (ATCC® PCS-201-013™) were fixed with 4% paraformaldehyde, immunostained with primary antibodies to anti-human fibroblasts (TE7) or pan-cytokeratin (Pan-CK), and were then stained with a secondary fluorescent antibody (green). The nuclei were stained with DAPI (blue). Cells were imaged with a standard epi-fluorescent microscope and a composite image was generated (merge). Fibroblasts show the expression of the anti-human fibroblast marker but not cytokeratin, demonstrating that the cells are of mesodermal origin from which fibroblasts are derived. Scale bar, 200 μm.

III. Primary and hTERT-immortalized Fibroblast Cell Lines Respond to a Toxic Agent.



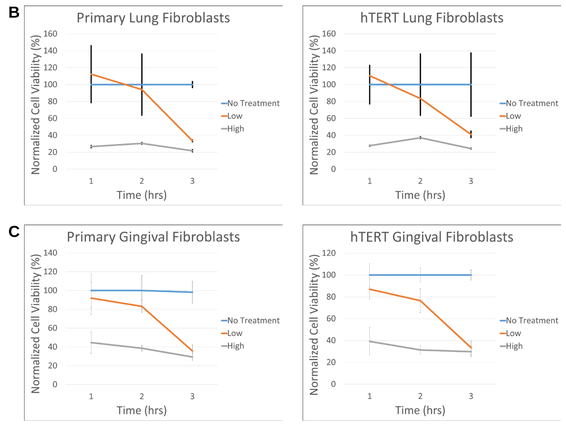


Figure 3. hTERT Fibroblast viability is reduced in the presence of chlorhexidine (CHX). (A) Fibroblasts were treated with low (1 μM) and high (10 μM) doses of CHX for 1 to 3 hours. After treatment, cells were washed and treated with Reliablue™ (ATCC® 30-1014™) reagent. Viability was assessed using a fluorescence plate reader. Data was normalized to untreated cells. (B, C) Both primary and hTERT-immortalized fibroblasts respond to CHX in a dose- and time-dependent manner. Data represents the average ± SD of two independent experiments done in triplicate (N=2).

IV. hTERT-immortalized Lung Fibroblasts Respond to TGF-b1

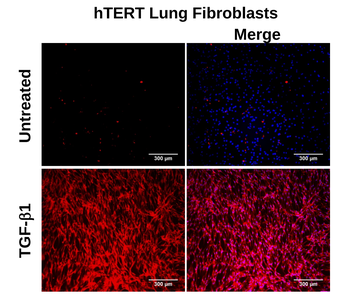


Figure 4. hTERT fibroblasts respond to TGF-β1. Lung fibroblasts were either unstimulated or stimulated with 1 ng/mL TGF-b1 for 1 hour. After exposure to TGF-β1, cells were fixed with 4% paraformaldehyde for 15 minutes and subsequently immunostained with anti-α-smooth muscle actin (α-SMA, red). Cells were imaged with a standard epi-fluorescent microscope and a composite image was generated (merge). hTERT lung fibroblasts exhibit α-SMA expression, demonstrating the differentiation potential from naïve fibroblasts to myofibroblasts. Scale bar, 300 μm.

Summary

- Lung and gingival fibroblasts were successfully immortalized with the catalytic subunit of hTERT.
- hTERT-immortalized fibroblasts retained the ability to grow continually and maintained a fibroblast morphology and a normal diploid karyotype.
- Immortalized lung and gingival fibroblasts retained the fibroblasts positive marker to the anti-human fibroblasts antibody TE7, but lacked the presence of an epithelial marker.
- Both primary and immortalized fibroblasts were responsive to toxic and differentiation molecules.
- hTERT Lung Fibroblasts and hTERT Gingival Fibroblasts provide researchers characterized tissue-specific fibroblasts lines that retain their primary cell functionality.
- These cells are useful tools for multiple applications, including high-throughput toxicity screening.

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