hTERT-immortalized Adult Dermal Melanocytes: An *In Vitro* Cell Model for the Study of Skin Pigmentation

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Abstract #2692

Abstract

Skin pigmentation is a complex process; melanocytes produce melanin and package it into melanosomes that are in turn exocytosed into the surrounding extracellular matrix and adjacent cells. Numerous genes play a role in controlling pigmentation at various levels of melanin production. Mutations in these genes are characteristic of multiple skin disorders, including hyperpigmentation, hypopigmentation, and mixed hyper/hypopigmentation. Additionally, extrinsic factors secreted by the surrounding resident cell types also regulate the melanin expression in adult melanocytes. Human primary cells can be a useful model for elucidating melanocyte biology. However, primary cells have their limitations such as donor variability, a limited lifespan and loss of melanin. Therefore, there is a need for a more robust human cell model system for studying skin pigmentation.

In this study, we immortalized primary dermal melanocytes by expressing human telomerase reverse transcriptase (hTERT) in cells that were isolated from an adult donor. The immortalized primary melanocytes were cultured continuously for more than 40 population doublings without any signs of replicative senescence, yet retaining melanin production. The immortalized primary melanocytes maintained a consistent expression of the melanocyte-specific marker TYRP-1, and lacked expression of the fibroblast-specific marker TE7. In addition, we demonstrate the ability to modulate melanogenesis with specific stimulators and inhibitors and the capacity of immortalized melanocytes to incorporate into a functional 3D organotypic skin culture. Taken together, the hTERT-immortalized primary melanocytes described here provide a versatile *in vitro* cell model for studying melanin production and melanocyte:keratinocyte interactions in the dermal environment.

Results

I. Cell Immortalization of hTERT Melanocytes

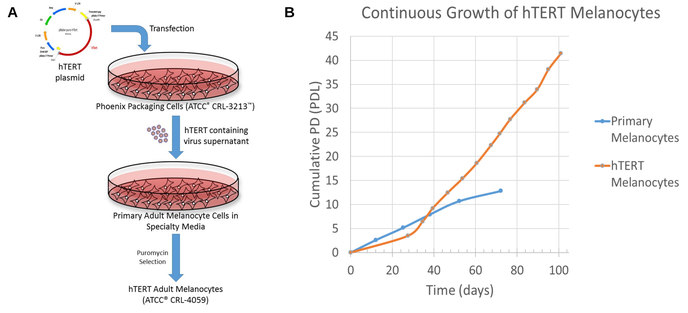

Figure 1. **Immortalization of Skin-derived Melanocyte (ATCC® CRL-4059™) cells with human telomerase (hTERT).** (A) Primary Epidermal Melanocytes (ATCC® PCS-200-013™) were retrovirally transduced with hTERT viral particles in a specialty medium that promoted both melanin production and growth. The stable presence of hTERT was confirmed with a telomeric repeat amplification protocol (TRAP) assay (data not shown). (B) Melanocytes were grown continuously for greater than 40 population doublings without signs of replicative senescence. (C) Dermal-derived melanocytes demonstrate a normal fibroblast-like morphology similar to the primary melanocytes from which they were derived. Scale bar, 1000 µm.

II. Immortalized Melanocyte Cells Maintain Melanin Production

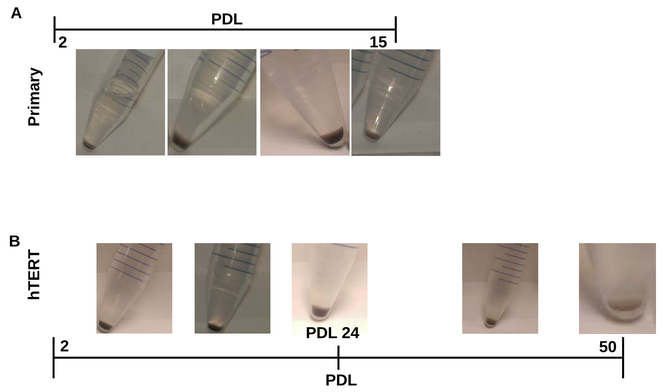

Figure 2. **hTERT melanocytes maintain prolonged production of melanin.** During routine continuous culturing of dermal melanocytes, cells were collected and pelleted by centrifugation. The resulting pellets were imaged with a standard digital camera. (A, B) Melanin containing cell pellets were observed throughout the longevity of cultures. (B) For hTERT melanocytes, melanin content began to decrease after 45 population doublings, which may indicate aging of the cell.

III. hTERT Melanocyte Cells maintain Melanocyte Specific marker TYRP1

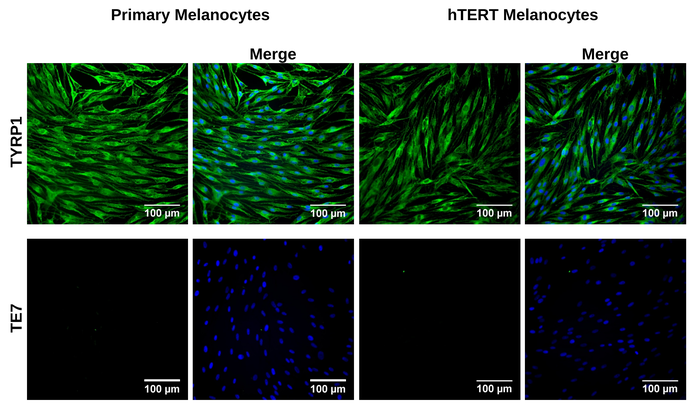

Figure 3. **Melanocytes express a melanosomal enzyme, but do not express a fibroblast marker**. Primary and hTERT melanocytes were fixed with 4% paraformaldehyde, immunostained with primary antibodies to tyrosinase related protein 1 (TYRP1) and anti-human fibroblast (TE7), and then stained with a secondary fluorescent antibody (green). The nuclei were stained with DAPI (blue). Cells were imaged with a fluorescent high-content screening system, and a composite image was generated (merge). Melanocytes show expression of TYRP1, but do not express TE7, suggesting their ability to produce melanin. Scale bar, 100 µm.

IV. hTERT Melanocytes Respond to Stimulators and an Inhibitor of Melanogenesis

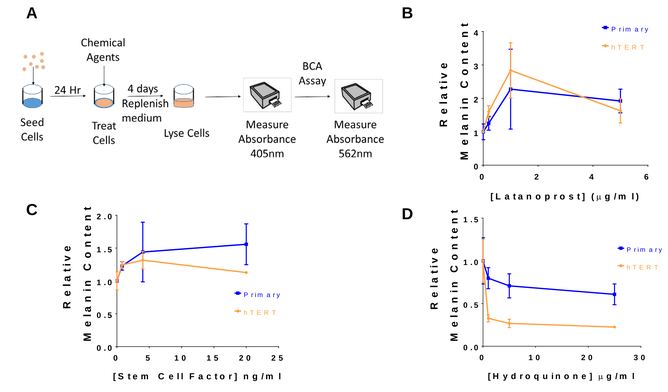

Figure 4. Stimulation of melanocytes with latanoprost, stem cell factor, and hydroquinone. (A) Primary and hTERT melanocytes were seeded into a 96-well plate and were treated with (B,C) known stimulators and an (D) inhibitor of melanogenesis. Cells were then lysed by using 1N NaOH, and melanin content was measured via absorbance at 405nm. Total protein content was determined by BCA assay from an aliquot of the lysate. Melanin content values were adjusted per microgram of total protein for each sample. The relative melanin content was determined by normalizing to the melanin content of untreated cells for each treatment. Both latanoprost and stem cell factor (known stimulators of melanogenesis) increased melanin content of the lysates, while hydroquinone (a known inhibitor of melanogenesis) decreased melanin content of the lysates in a concentration-dependent manner. Data represents the average ± SD of two independent experiments done in triplicate (N=2).

V. hTERT Melanocytes Pigment a 3D Organotypic Skin Culture

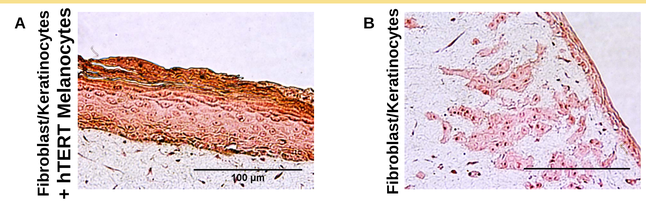

Figure 5. 3D organotypic skin culture. hTERT-immortalized BJ-5ta (ATCC® CRL-4001™) fibroblasts were embedded into a rat collagen matrix. Next, hTERT-immortalized Ker-CT (ATCC® CRL-4048™) keratinocytes in the (A) presence or (B) absence of hTERT melanocytes were added to the apex of the collagen matrix. After 12 days, cultures were fixed with 4% paraformaldehyde and matrices were embedded in paraffin and sectioned. Sections were stained for melanin (black) with a Fontana-Masson stain kit. Organotypic cultures lacking hTERT melanocytes failed to develop as robustly as melanocyte-containing cultures, demonstrating the synergistic role of the three cell types. Additionally, skin cultures containing hTERT melanocytes appear darker than skin cultures lacking melanocytes, indicating the deposit of melanin into keratinocytes (40x, scale bar = 100 µm).

Summary

- Epidermal-derived melanocytes were successfully immortalized with the catalytic subunit of hTERT.
- hTERT-immortalized melanocytes can grow continuously and maintain the fibroblast morphology of the parental primary cell.
- hTERT and primary melanocytes retain a key marker for melanin biosynthesis, TYRP1, but lack the presence of a fibroblast marker.
- Both primary and hTERT melanocytes are responsive to stimulators and inhibitors of melanogenesis.
- hTERT melanocytes incorporate into a 3D organotypic skin culture and pigment keratinocytes.
- Taken together, hTERT adult melanocytes are ideal for the study of skin pigmentation.

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