Abstract # 4670

Novel Immortalized Pulmonary Artery Endothelial Cell Line Recapitulates the Characteristics of Primary Cells

Nicholas Lee, BS; Larisa Saari, BS; Xiangshan Zhao, PhD; Fang Tian, PhD
ATCC, Manassas, VA 20110

ATCC®
Credible leads to Incredible®

Introduction

Primary endothelial cells have been used as in vitro models to study cardiovascular diseases and cancer development. However, cellular senescence and donor availability restrict the potential usefulness of primary endothelial cells. Cell immortalization technology enables the development of new cell models that represent normal physiology while gaining the capability of an extensive lifespan. The objective of this study was to develop an immortalized pulmonary artery endothelial cell line that retains the functional characteristics of primary cells.

Methods

In this study, we generated the immortalized clonal cell line HPAEC-BMI1 (ATCC® CRL-4065™) by stably expressing the human BMI1 gene in normal human primary pulmonary artery endothelial cells (HPAEC; ATCC® PCS-100-022™). Cell morphology, growth rate, cell surface biomarkers, and bio-functions were analyzed, and the data were used to compare the immortalized cell line to the parental primary cell line.

Results

Cell immortalization

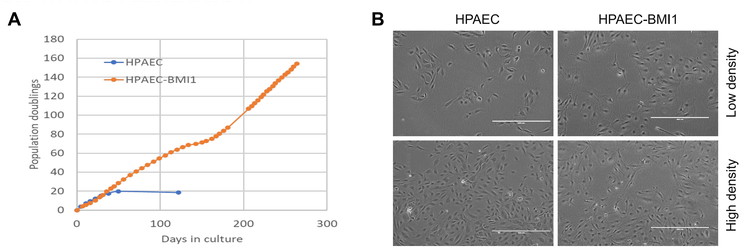

Figure 1: Immortalization of primary pulmonary artery endothelial cells (HPAEC) with BMI1. (A) HPAEC-BMI1 (ATCC® CRL-4065™) cells maintained consistent growth over >150 population doublings while primary HPAEC cells underwent senescence around 20 doublings. (B) Morphology of primary HPAEC and HPAEC-BMI1 cells at low and high densities. HPAEC-BMI1 cells showed a similar morphology to that of primary cells.

CD31 expression

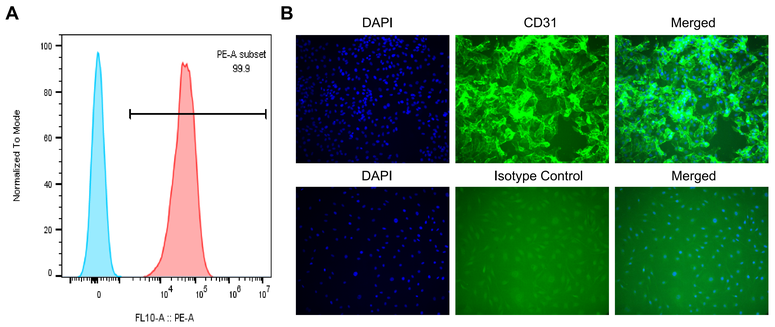

Figure 2: HPAEC-BMI1 cells are positive for endothelial cell marker CD31. (A) Flow cytometry analysis of CD31. Cells were harvested and stained with either PE Mouse Anti-Human CD31 antibody (red histogram) or PE Mouse IgG1, κ Isotype Control antibody (blue histogram). Flow cytometry was performed using a Beckman Coulter CytoFLEX Cytometer. (B) Immunocytochemistry analysis of CD31 expression in HPAEC-BMI1 cells. Cells were stained with either Mouse Anti-Human CD31 antibody or Mouse IgG Isotype Control antibody, then stained with Alexa Fluor™ 488 Goat Anti-Mouse IgG. Images were taken using a Nikon fluorescence microscope.

AcLDL uptake

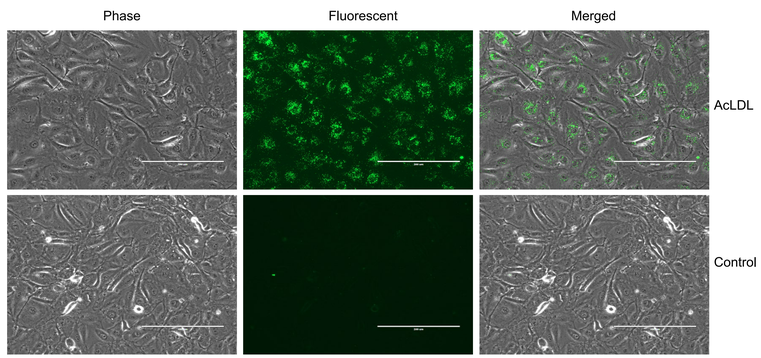

Figure 3: Uptake of AcLDL by HPAEC-BMI1 cells. Cells were cultured on four-well chamber slides and incubated with Alexa Fluor™ 488 AcLDL for 4 hours. After, cells were washed with culture medium. Then, while in culture medium, the cells were viewed and photographed using an EVOS fluorescence microscope under GFP fluorescence and transmitted light. Cells in culture medium without Alexa Fluor™ 488 AcLDL were used as a negative control.

Effect of sunitinib on capillary-like tube formation

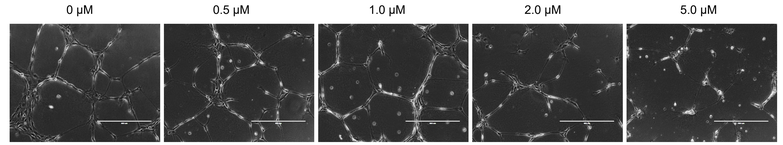

Figure 4: Sunitinib inhibits the formation of capillary-like tubes of HPAEC-BMI1 cells. The tube formation assay was performed in a 24-well plate. Cells in medium containing 0, 0.5, 1.0, 2.0, and 5.0 µM sunitinib were seeded onto a basement membrane matrix (Cell Basement Membrane; ATCC® ACS-3035™). After incubation at 37°C for 24 hours, the capillary-like tubes were viewed and photographed using an EVOS fluorescence microscope under transmitted light.

Effect of sunitinib on cell migration

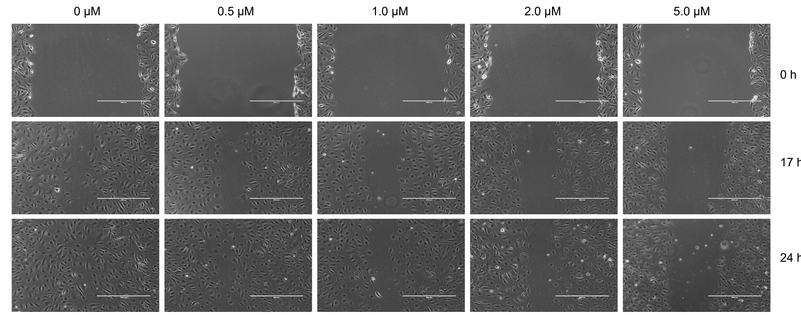

Figure 5: Sunitinib inhibits the migration of HPAEC-BMI1 cells. Cells were grown to full confluence in 12-well plates and then wounded with a sterile pipette tip. Medium containing 0, 0.5, 1.0, 2.0 , and 5.0 µM sunitinib were added to the wells. The wound gap was viewed and photographed at 0, 17, and 24h using an EVOS fluorescence microscope under transmitted light.

Conclusions

- Human primary pulmonary artery endothelial cells were successfully immortalized by BMI1.
- Immortalized HPAEC-BMI1 cells maintain endothelial cell morphology, CD31 expression, and AcLDL uptake ability.
- Sunitinib inhibits capillary-like tube formation and migration of HPAEC-BMI1 cells.
- HPAEC-BMI1 provides a valuable tool for angiogenesis research, drug screening, and toxicology studies.

ATCC 10801 University Boulevard, Manassas, Virginia 20110-2209 **Phone:** 800.638.6597 **Email:** sales@atcc.org **Web:** www.atcc.org