ONLINE SUPPLEMENTAL DATA for:

Title: Novel Human Airway Epithelial Cell Lines For Cystic Fibrosis Research

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Materials and Methods

Western blots. Primary cells at each passage during the time course of growth curves on plastic were lysed in RIPA buffer (100mM Tris-HCl pH 8.0, 100 mM NaCl, 5 mM NaF, 2 mM EDTA, 1% NP-40, 1 mM Na_3VO_4, 100 μM TPCK, 1 μM pepstatin A, 2 μM leupeptin, 1 mM PMSF, 100 μM quercetin), sonicated, and centrifuged at 13,800 G for 10 minutes at 4ºC to remove insoluble material. Supernatant protein concentrations were determined using the Coomassie Protein Assay Reagent (Pierce Biotechnology, Rockford, IL). After addition of β-mercaptoethanol (5%), glycerol, and tracking dye, samples were boiled, and equivalent amounts of protein were resolved by SDS-PAGE (4%-12% Bis-Tris gels, Invitrogen, Carlsbad, CA) and transferred to Immobilon-P membranes (Millipore, Billerica, MA). Blots were blocked in TBS with 0.1% Tween-20 and 5% nonfat dry milk for 1 hour, incubated with primary, then peroxidase-conjugated secondary antibodies, followed by chemiluminescent detection of peroxidase activity (Millipore).

Karyotyping. Colchicine-treated ells were trypsinized, pelleted, and resuspended in 0.075M KCl hypotonic solution at room temperature for 10 minutes, then centrifuged and suspended in freshly made 3:1 methanol:acetic acid fixative. After two changes of fixative and 18 hours at 4°C, cell suspensions were dropped onto clean, moist slides. Slides were baked at 65°C for 18 to 20 hours, banded with trypsin and Karyomax Giemsa Stain (Invitrogen), and coverslipped with Permount.

DNA fingerprinting. Eight polymorphic tetranucleotide repeat loci: CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317, and D5S818, plus amelogenin were analyzed with the

PowerPlex® 1.2 System (Promega, Madison, WI). Briefly, DNA was amplified according to manufacturer's instructions, using the provided buffers, primers, and Ampli Taq Gold® DNA polymerase (Applied Biosciences, Foster City, CA) in a Perkin Elmer GeneAmp® 9700 thermal cycler (Perkin Elmer, Waltham, MA). PCR products were evaluated on an ABI PRISM® 310 Genetic Analyzer (Applied Biosciences), using Genotyper® 3.7 software (Applied Biosciences).

Results

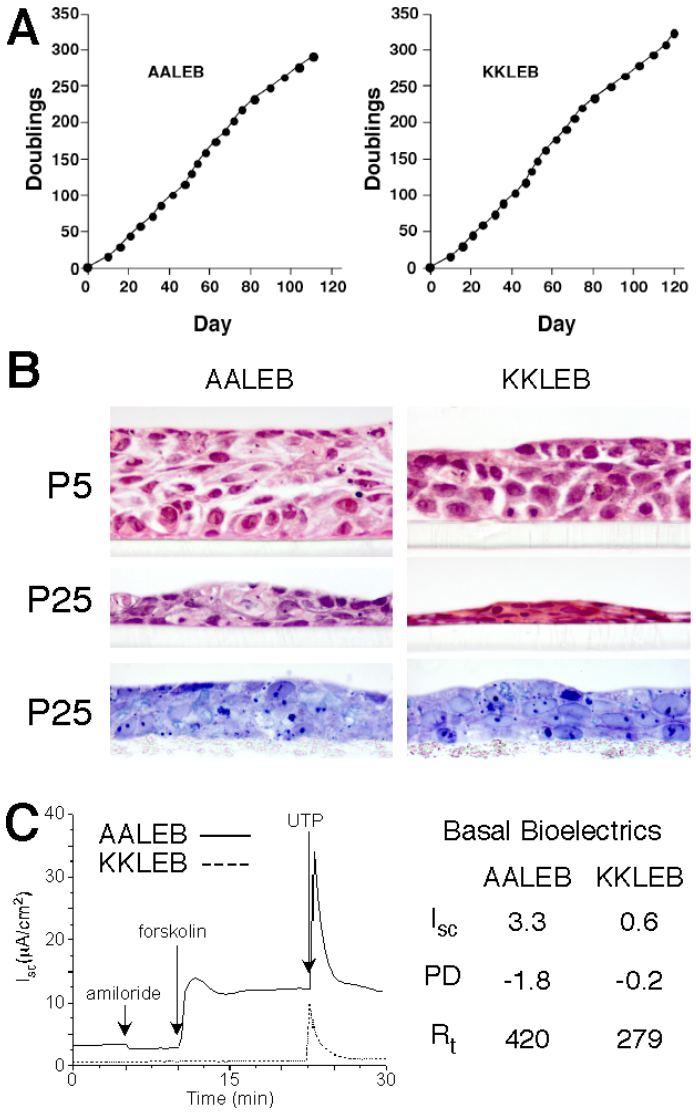

Figure S1. SV40ER/hTERT immortalized hBE cells make poor ALI cultures. Growth, morphology and electrophysiology of hBE cells immortalized with SV40ER/hTERT as described previously (Lundberg et al. Oncogene 2002 Jul 4;21(29):4577-86). AALEB=non–CF and KKLEB=CF. A) Growth curves demonstrate rapid growth and immortality. B) Morphology of early and late passage, day 21 ALI cultures, the top 4 panels are paraffin sections, H&E stain, and bottom 2 are thin plastic sections, Richardsons stain, all micrographs at the same magnification, originally 500X. Passage 5 cells of both types at day 21 appear multilayered and hyperplastic with abundant apoptotic bodies. Adjacent apical cells appear to form junctional complexes, but overall, the cells are squamous to cuboidal without cilia or mucous secretory cells. Passage 25 cultures of both cell types at day 21 are thinner, varied and jumbled with a high apoptotic fraction and apparently lesser ability to form apical junctions. C) Representative Ussing chamber recordings and mean data from triplicate passage 7-8 cultures. Similar preparations of late passage (>P15) KKLEB cells did not form electrical resistances > 50 Ω*cm^2. I_{sc} = short circuit current (μA/cm^2), PD = potential difference (mV), R_t = trans-epithelial resistance (Ω*cm^2).

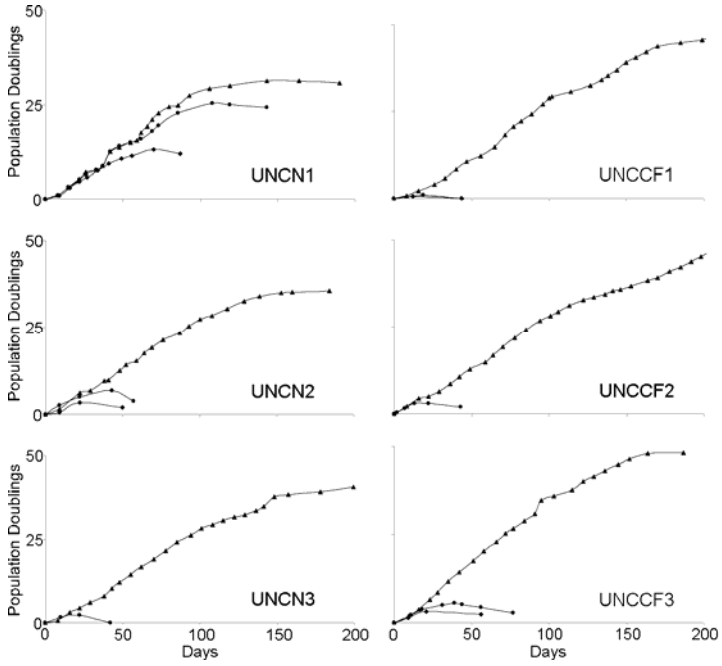

Figure S2. Bmi-1 alone enhances the growth of hBE cells but not indefinitely in most cases. Growth curves of "Bmi-1 only" cell lines on plastic. Three non-CF and 3 DF508 homozygous CF hBE cell preparations, UNCN1, UNCN2, UNCN3, and UNCCF1, UNCCF2, UNCCF3, respectively, were infected with Bmi-1 (triangles) or GFP (diamonds) expressing HIV lentiviral vectors or sham infection as a control (circles). All sham control or GFP vector infected cells senesced prior to 25 population doublings. Addition of Bmi-1 alone significantly enhanced the growth rate of all cell lines, but 4 of 6 senesced (defined as no increase in cell number in 21 days) prior to passage 30.

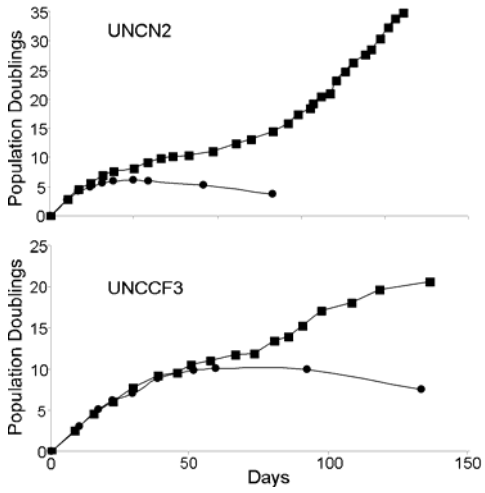

Figure S3. hTERT extends the growth of "Bmi-1 only" hBE cells. Two "Bmi-1 only" cell lines, one non-CF and one CF (UNCN2 and UNCCF3), were infected with hTERT-expressing lentivirus vectors (squares) or were exposed to sham infection as a control (circles) at passage 14 (UNCN2) or 15 (UNCCF3), five passages prior to their predicted senescence. The sham infection control cells senesced as predicted while hTERT extended cell life span.

Cell line	D5S818	D13S317	D7S820	D16S539	vWA	TH01	AMELOGENIN	TPOX	CSF1PO
UNCN1T	12	11	8,10	10,11	14,17	8,9	XY	8,10	9,10
UNCN2T	11,13	12	11	9,10	15,18	6,7	X	8,9	12
UNCN3T	11,13	11,12	10,11	9,12	15,16	9.3,10	X	8,9	12
UNCCF1T	9,11	8,13	10	10,12	18	6,7	XY	8,12	11,14
UNCCF2T	13,14	11	9,10	11,12	17,19	6,7	X	8,11	10
UNCCF3T	12	11,12	9,10	11	18,19	8,10	X	8	12,13

Figure S4. DNA fingerprinting indicates cell purity at passage 14-15. Cellular genomic DNA was extracted from each of the 6 novel cell lines at passage 14-15 to assess if any cross-contamination occurred during infection, expansion, or cell passaging. The cell-type-specific genotypes of the eight polymorphic tetranucleotide repeat loci as indicated plus amelogenin was unique in each of the novel cell lines.

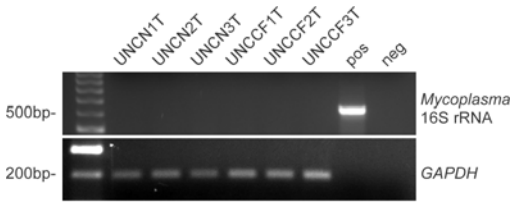

Figure S5. Mycoplasma contamination was not detected by PCR testing of novel hBE cell lines. Total DNA was extracted from each of the 6 novel cell lines between P12-16 and was subjected to PCR as per manufacturers instructions, employing positive and negative controls (MycoFind™ Version 1.0 Mycoplasma Detection PCR Reaction Kit, Clongen, Germantown, MD).