USING THE PAPILLOMA VIRUS E6/E7 GENES TO GENERATE WELL-DIFFERENTIATED EPITHELIAL CELL LINES.

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INTRODUCTION

The study of normal cell growth and differentiation would be greatly augmented by the development of an efficient method for obtaining human immortalized cell lines which would retain their ability to differentiate and respond to external regulatory signals. One critical research area which would greatly benefit from such an approach would be the study of cystic fibrosis (CF). Not only would CF cell lines permit the analysis of the altered ion permeability properties of these cells and their alteration by pharmacologic agents, but they would also serve as a substrate for future gene therapy experiments. In an attempt to generate such cell lines, the SV40 large T antigen has been used to immortalize CF cells. Unfortunately, the derived cell lines lose many of their differentiated properties and are inadequate for biochemical, physiological, and molecular analysis. Recently the E6/E7 genes of the human papillomaviruses (HPV’s) have been shown capable of immortalizing human epithelial cells [1]. Interestingly, these E6/E7 immortalized cell lines remain non-tumorigenic in nude mice and often display normal responses to negative regulators of cell growth (e.g. TGF-beta) [2]. When injected subcutaneously into nude mice, these cells form well-differentiated epithelial cysts which mimic normal epithelial cells [3]. To determine whether the HPV E6/E7 genes would be useful for generating well-differentiated cell lines from CF patients, these genes were transfected into primary cultures of tracheal epithelial cells from a patient with cystic fibrosis.
Primary culture. Donor tissue was obtained postmortem from a 24 year old man with cystic fibrosis who was homozygous for the phenylalanine 508 deletion in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. The trachea was cut into 2 x 2 cm pieces and washed with Joklik's modified essential medium (MEM) containing antibiotics, dithiothreitol (0.5 mg/ml), and DNAse (10μg/ml) at 4 degrees C for 3 hours. The tissues were then incubated in fresh supplemented MEM plus protease (Sigma Type XIV, 0.1 μg/ml) at 4 degrees for 18 hours. The epithelial cells were dislodged by gentle agitation and plated in hormone-supplemented F12 medium (F12 + 7x; supplements: insulin 5 μg/ml, endothelial cell growth supplement 3.7 μg/ml, epidermal growth factor 25 ng/ml, triiodothyronine 3 x 10-8 M, hydrocortisone 1 x 10-6 M, transferrin 5 μg/ml, and cholera toxin 10 ng/ml, plus ceftazidime, tobramycin, and amphotericin B).

Transfection with HPV-18 E6 and E7 genes. A pUC19-based plasmid containing the HPV-18 nucleotides 6273-2440 encoding the intact E6 and E7 open reading frames, a partial E1 open reading frame, and the upstream regulatory region [4] was transfected by lipofection as described [5]. After a 2 hr incubation at 37, 12 ml of fresh F12 + 7x medium was added. On the following day the cells were fed with fresh medium.

Culture and Clonal selection. At 14-18 days post-seeding, clusters of 30-200 dividing cells of apparent clonal origin developed and were isolated using cloning cylinders. Between passages 1-4, most subclones were co-cultured with lethally irradiated NIH3T3 fibroblasts, which were removed by differential trypsinization at passage 4. Eleven clones were isolated and developed a polygonal morphology typical of airway epithelial cells in primary culture.

Presence and expression of HPV genes in immortalized cell lines. The presence of the HPV-18 genome in selected clones was assayed using polymerase chain reaction (PCR) technology with oligonucleotide primers specific for the HPV-18 E6-E7 region. The 5' primer corresponds to HPV-18 nucleotides 105-124 and the 3' primer to nucleotides 888-907 of the HPV-18 DNA sequence. Extracts of 6 x 10³ cells of selected clones were analyzed by PCR for 30 cycles with the following conditions: 94 C for 1 min, 50 C for 2 min, and 72 C for 3 min. An HPV-18 transformed human keratinocyte cell line (18Nco) and an SV40-transformed keratinocyte