41* Human adult airway epithelial basal cells are stem/progenitor cells of airway surface epithelium

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In numerous airway diseases such as cystic fibrosis, the epithelium is severely damaged and must regenerate to restore its defense functions. Although the human airway epithelial progenitor/stem cells have not been clearly identified yet, it has been recently suggested that epithelial progenitors exist among both fetal basal and suprabasal cell subsets in the human airways.

We analyzed the capacity of human adult basal cells, isolated from nasal polyps, to restore a well-differentiated airway epithelium. Cells were seeded in epithelium-denuded rat tracheae that were grafted subcutaneously in nude mice or on collagen-coated porous membranes and grown at the air-liquid interface. We used tissue factor (TF) and tetraspanin CD151, expressed specifically on the surface of basal cells of human airway epithelium, to isolate positive cells with a FACSAria cell sorter. Sorted positive and negative populations were also analyzed for telomerase activity by the telomeric repeat amplification protocol.

After cell sorting, pure (>99%) viable TF/CD151-positive basal cell population adhered and proliferated on epithelium-denuded rat tracheae and on collagen-coated porous membranes and was able to restore a fully-differentiated mucociliary airway epithelium, whereas viable negative population (purity >97%) did not. The stem cell telomerase activity was detected in the TF/CD151-positive population, but not in TF/CD151-negative cells.

Our results demonstrate that human adult basal cells are airway epithelial progenitors. Telomerase activity suggests the presence of a stem cell population among the sorted basal cells.

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42 Nasal epithelial brushing: a valuable method to study airway epithelial cells in CF and non-CF infants

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To better understand the early stages of cystic fibrosis disease (CF), it is of major interest to study respiratory epithelial cells obtained as early as possible in humans. Nasal epithelial brushing, a much less invasive technique than broncho-alveolar lavage to collect respiratory epithelial cells, has never been used in infants. The purpose of the present study was to assess its feasibility in CF infants.

In 5 CF (range 1–18 months) and 10 non-CF control infants (range 1–17 months), a nasal brushing was performed. Samples were used for microbiology, epithelial cell viability, ciliary beating frequency analysis, study of c-AMP dependent chloride efflux, and analysis of inflammatory mediators expression and release and expression by cultured cells under basal conditions and after 24 h incubation with *P. aeruginosa*.

Toleration of the procedure by infants was excellent. Freshly obtained samples could be successfully used for studies of cell ciliary beating frequency and cell culture. The feasibility of studying protein release and mRNA expression of IL-8, IL-6 and TNF-α, under basal conditions and after stimulation by *P. aeruginosa*, was demonstrated.

The present study shows that a simple nasal brushing technique, easily performed in and well tolerated by infants allows to collect respiratory cells in sufficient number to study the airway epithelial functions in CF infants.

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43 Culture of respiratory epithelial cells on copolymer-coated wires

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Tissue engineering can be used to produce artificial tissues to replace injured or defective natural tissue. Engineered tracheal tissue could potentially be used to repair embryological defects of the airway wall such as tracheal atresia, or tracheoesophageal fistula, or disease-induced defects such as in cystic fibrosis. To this end, airway epithelial cells have to be grown on a solid biocompatible surface that can be implanted in the trachea. We used 16HBE airway epithelial cells, an SV40-virus immortalized cell line, and Calu-3 cells, a cell line derived from a submucosal gland tumor. The cells were cultured on a substrate consisting of steel wires coated with different Slipskin® coatings (copolymers of hydrophilic 1-vinyl-2-pyrrolidinone, NVP, and hydrophobic n-butyl-methacrylate, BMA). In some experiments, first a layer of human fibroblasts was grown on the coated wire, prior to seeding the airway epithelial cells. The airway epithelial cells were grown in EMEM medium supplemented with 10% bovine serum albumin and antibiotics, for 16 days. Then the wires were prepared for scanning and transmission electron microscopy. Different proportions of NVP and BMA were tested, and the best cell growth was obtained at the proportions 10–30% NVP/70–90% BMA. At higher NVP concentrations, cracks between the cells could be noticed on microscopical evaluation. At the optimal proportion of NVP and BMA, the cells had a normal ultrastructure with small microvilli protruding from the cell membrane. It is concluded that this tissue culture system holds promise for developing a replacement for injured or defective airway tissue.