Establishment of a Spontaneously Immortalized Rat Bronchial Epithelial Cell Line with Basal Cell-Like Characteristics

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Aim

To establish an epithelial cell line from the rat lower respiratory tract as a tool for in vitro testing of inhalation toxicants

Objective

To characterize the spontaneously immortalized Rat Bronchial Epithelial (RaBE) cell line as a basis for future applications in in vitro toxicology investigations concerning the rat lung

Introduction

Rationale

Rats are commonly used for investigations of the respiratory tract following inhalation exposure. In vitro tests are often used in place of animal studies wherever possible. When in vitro results are to be compared with in vivo results (e.g., in carcinogenesis studies), it is advisable to use cells from the same tissue/area of the rat respiratory tract.

Background

- There are only a few epithelial cell lines from the respiratory tract/lung of rodents available, and these cells may not have the properties desired (e.g., growth behavior, cytokeratin pattern, colony-forming efficiency (CFE)).
- Many lung epithelial cell lines have frequently been established from fetal tissue or from tumors that were induced by chemicals.
- Immortalization is often achieved by in vitro virus transformation (e.g., Driscoll et al., 1995 [1]).
- The new, spontaneously immortalized, cell line presented here is unique concerning its origin and features: RaBE cells appear to be of bronchial epithelial origin with basal cell characteristics, and were established from untreated adult rats.

Methods

Isolation and Cell Culture

- RaBE cells were isolated by pronase digestion (0.25 %, 25 min) of the lavaged lung from one adult male Wistar rat, followed by dissection and Percoll gradient centrifugation.
- Growth medium comprised Ham's F12 medium supplemented with 5 % FBS, insulin, transferrin, EGF, aFGF, and cholera toxin, and was changed twice a week.
- From the 6th passage, cells were subcultured once a week by trypsinization (0.25 % trypsin/1 mM EDTA) and seeding of 10^4 cells per 75-cm^2 culture flask.

Criteria for Cell Characterization

(for details see legends to figures)

- Intermediate filaments (cytokeratin (CK) pattern, desmoplakin I + II, vimentin)
- Karyotype
- Growth characteristics
- Serum and growth factor dependency
- Colony-forming efficiency

Results and Discussion

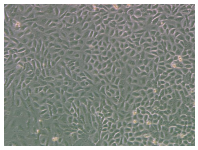

Fig. 1 Phase-contrast picture of a confluent monolayer of RaBE cells growing on plastic

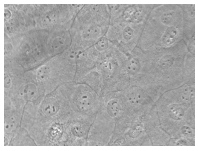

Fig. 3A,B Morphology of postconfluent RaBE cells.
(A) Phase-contrast microscopy demonstrating 2 cell layers.
(B) Immunofluorescence microscopy of desmosomes. Cell contacts of the upper squamous layer show reactivity with anti-desmoplakin monoclonal antibody.

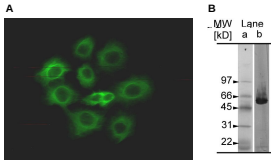

Fig. 2 Demonstration of CK 14 in RaBE cells by (A) immunofluorescence and (B) Western blotting.
(A) Cells were fixed with ethanol : aceton 1 : 1. 1st antibody: LL002 (Bio-Genex) 2nd antibody: FITC-conjugated anti-mouse IgG
(B) High salt extraction of cytoskeletal proteins separated by SDS-PAGE. Immunoblotting was performed with the same antibody as that used for immunofluorescence [2]. lane a: molecular weight marker; lane b: CK 14 blot of RaBE cell extract.

Antigen	Signal	Remarks
CK 7 CK 8 CK 18 CK 19	(+) + + +	simple epithelia
CK 14 CK 15	+++ +++	basal (bronchial) epithelia
CK 4 CK 13	++ +	squamous epithelia, noncornifying
CK 1 CK 10	+ +	squamous epithelia, cornifying
desmosomes	+	postconfluency
vimentin	--	mesenchymal cells

Remarks: - negative; (+) weak; + moderate; ++ distinct; +++ strong

Table 1 Cytoskeletal markers determined by Western blotting and/or immunofluorescence.

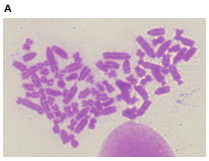

Fig. 4A Metaphase spreads of RaBE cells at passage 9. A hypotetraploid cell (N = 82) is shown. Metaphase arrest was induced by addition of colcemid (40 ng/ml final concentration) for 4 h. Chromosomes have been stained with Giemsa's stain.

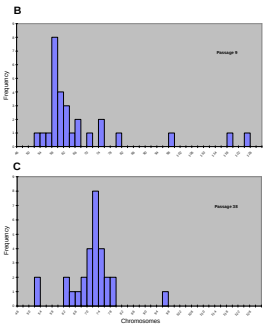

Fig. 4B,C Chromosome analysis of RaBE cells at passages 9 (N = 29) (B) and 38 (N = 30) (C). For details see Table 2.

Parameter	P9	P14	P19	P38
mean	67.4	71.4	80.0	75.1
SD	18.2	11.2	20.6	16.3
median	62	70	75	74

Table 2 Chromosome analysis of RaBE cells between passages 9 and 38 revealed a shift from 62 to 74 chromosomes (median, N = 17 to 43), which remained relatively stable between passages 19 and 38.

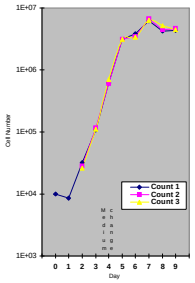

Fig. 5 Growth curve of RaBE cells. To perform a growth curve of RaBE cells, 10^4 cells were seeded in 75-cm^2 culture flasks and cells were trypsinized and counted at the time points indicated. Medium change was twice a week. The population doubling time (PDT) during exponential growth phase is 10 to 11 h.

- This epithelial cell line is adherent and grows as a monolayer until confluency (Fig. 1).
- RaBE cells react strongly with antibodies against the "basal" cytokeratins (CKs) 14 and 15, and somewhat less intensely for the "simple" CKs 7, 8, 18, 19, the "noncornifying squamous" CK 4 and CK 13, and the "cornifying squamous" CK 1 and CK 10. The relative abundance of the basal and squamous markers typical for pseudostratified epithelia is consistent with the possible origin from bronchial epithelial basal cells (Fig. 2A,B; Tab. 1).
- The epithelial origin can also be demonstrated in aging squamous cell layers by distinct staining of desmosomal structures with a desmoplakin I + II antibody (Fig. 3A,B).
- The number of chromosomes shifted from 62 in early passages to 74 at passages 19 to 38 towards a relatively stable hypotetraploid (2 N = 42) pattern (Fig. 4A to C; Tab. 2).
- RaBE cells exert a population doubling time of 10 to 11 h in growth factor and serum-supplemented medium only (Fig. 5), and a high cloning efficiency (36 ± 3.6 % before and 32.5 ± 4.4 % after freezing).

Conclusion and Outlook

(1) The Rat Bronchial Epithelial (RaBE) cell line presented here is now well-established and appears to be immortal.
(2) The strong expression of CK 14 and 15 supports their possible origin from bronchial basal cells.
(3) This cell line may be useful for inhalation toxicology or genotoxicity assays because it employs a possible target cell type of the rat lung (e.g., cell communication assay, toxicity assays, studies of lung cell metabolism, DNA adducts).
(4) Whether these cells represent pluripotent stem cells that are able to differentiate under complex culture conditions remains to be investigated.

References

[1] Driscoll et al., Establishment of immortalized alveolar type II epithelial cell lines from adult rats, In Vitro Cell Dev. Biol. 31: 516-527 (1995)
[2] Schlage et al., Cytokeratin patterns of epithelial cells of the rat nasal cavity in vivo and in vitro, Toxicol. Lett. 88: 65-73 (1996)

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