#105850 Cytokeratin Expression in Lung Epithelial Tumors and Precursor Lesions in Unexposed Rats and Rats Exposed to Room-Aged Cigarette Sidestream Smoke or Diesel Engine Exhaust

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Objective

Corroborate the specificity of CK expression patterns for rat lung tumors and their preneoplastic precursors.

Background

The use of specific cytokeratin (CK) expression is a well-established tool not only in the differential diagnosis of human lung cancer but also in mechanistic investigations of various animal non-respiratory carcinogenesis models. Previously we analyzed CK expression changes in the rat respiratory tract following exposure to room-aged cigarette sidestream smoke (RASS) (Schlage et al., 1998a, b) and in a limited number of spontaneous lung tumors (Teredesai et al., 1999). Here we analyze CK expression as an additional mechanistic investigation to complement the tumorigenicity data from a 30-month inhalation study on the effects of RASS (a surrogate for environmental tobacco smoke) and diesel engine exhaust (DEE) in the rat respiratory tract (Haussmann et al., 2001; Teredesai et al., 2002).

Materials and Methods

Inhalation Study

- 30-month study: 24 months inhalation, 6 months postinhalation
- SPF-bred male and female Wistar rats
  - CRL: (WI)WU BR
  - 51 animals/group/sex
- Nose-only exposure to fresh air (sham), RASS, or DEE
  - 6 hours/day, 7 days/week
- Total particulate matter (TPM) conc.:
  - 3 mg/m³ (low groups)
  - 10 mg/m³ (high groups)
- Histopathology of respiratory tract organs

Immunohistology

- 131 lung tumors diagnosed in the histopathological evaluation
  - H&E stained sections with positive diagnosis for 73 tumors and 14 metastases (**Table 1**): 8 following sections obtained
  - 60 representative precursor lesions included
- Sections stained with 7 mAbs specific for different rat CK polypeptides (**Table 2**)
- Indirect ABC immunostaining procedure (for details, see Schlage et al., 1998b, c):
  - antigen retrieval step (microwave or protease, **Table 1**)
  - automated incubation (TechMate 500) with blocking solution, primary antibody (**Table 1**), 2nd antibody, peroxidase-labelled streptavidin, AEC chromogen, and hematoxylin counterstain (Dako ChemMate kit)
  - modification after microwave retrieval to suppress nonspecific staining in inflammatory regions: 2nd antibody of ChemMate kit replaced by biotin labelled anti-mouse F(ab')$_2$ (BioTrend)
- 4-stage score to evaluate distribution of CK staining--0 (no positive cells); 1 (focal, <15%); 2 (patchy, 15-85%); 3 (diffuse, >85%)--combined to create CK profiles

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Histology

Fixation
- Instillation of lungs with ethanol/acetic acid/formaldehyde/ saline followed by immersion in ethanol

Sectioning: 5- to 6-µm paraffin sections
- Lung step serial sections to increase sensitivity for detection of tumors (distance: 0.5 mm)
  - left lung: up to 9 levels (including conventional frontal section passing through pulmonary main bronchus)
  - right lung: up to 17 levels (including conventional frontal section passing through lobes with maximum volume)
- Histopathological evaluation of H&E stained sections
  - according to an international classification of proliferative changes in rats (Mohr, 1992)
  - reviewed by ACVP board-certified pathologist

Rat CK	Antibody	Supplier	Dilution	Retrieval
19	CK-E3	Sigma	1:8000	Protease
7	OVTL	BioGenex	1:1000	Protease
18	Ks 18.04	Progen	1:1000	Protease
15	8.12	Sigma	1:50	Protease
14	LL002	BioGenex	1:100	Microwave
4	6B10	Sigma	1:100	Microwave (*)
1,10/11	Ks 8.60	Sigma	1:500	Microwave

(*) additional blocking step before 1st AB: mixture of anti-rat/mouse/rabbit F(ab), diluted 1 : 200 (BioTrend)

Table 1 Monoclonal Antibodies to Rat CK and Staining Conditions

Abbreviations
BA: bronchiolo-alveolar
BKC: benign keratinizing cystic nonkerat.: non-keratinizing squam.: squamous

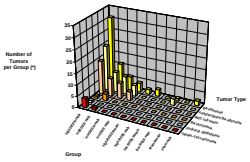
Tumor (*) or Lesion	DEE Low	DEE High	RASS Low	RASS High	Sham	Total
lung tumors						
BA adenoma	10	27	1	4	2	44
nonkerat. epithelioma	0	1	0	0	0	1
BKC tumor	3	8	0	0	0	11
BA carcinoma	0	3	0	0	0	3
squam.-cell carcinoma	0	2	0	0	0	2
metastases						
malignant mesenchymal tumor	3	1	1	3	0	8
malignant epithelial tumor	4	0	1	2	0	7
non-neoplastic lesions						
alveolar hyperplasia type "A"	2	17	0	0	1	20
alveolar hyperplasia type "B"	0	11	0	0	0	11
alveolar hyperplasia type "D"	0	7	0	0	0	7
BA metaplasia	0	13	0	0	0	13
squam. metaplasia	1	8	0	0	0	9

(*) sections for 12 tumors not evaluated because no tumor was seen in following sections

Table 2 Tumors and Lesions Evaluated

Discussion

- Epithelial lung tumors found in all groups
- All tumor types originated in bronchiolo-alveolar epithelium, not in bronchial epithelium

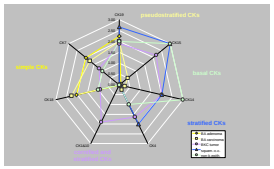


(*) includes some animals from earlier time points

- BA adenoma (most frequent type): sham, RASS, and DEE groups
- BA carcinoma, squamous-cell carcinoma, non-keratinizing epithelioma, and BKC tumors: DEE groups only

- In normal lung alveolar epithelium CK7, 18, and 19 are expressed; staining conditions adjusted so that normal epithelium only stains focally.
- CK19 was markedly expressed in all lung tumors.
- A **simple epithelial** pattern with CK18 and CK7 was seen in BA adenoma and BA carcinoma; in single BA carcinomas, CK15 or focal CK14 or CK4 was also seen.
- In different non-neoplastic precursor lesions, such as alveolar hyperplasia and BA metaplasia, simple or stratification-related CK patterns were observed; in squamous metaplasia, squamous and cornification markers were also expressed.

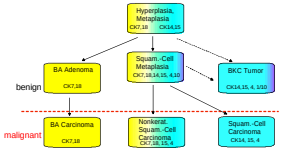
- **Squamous differentiation without cornification** was reflected by the high prevalence of basal cell/stratification markers CK15, CK14, and CK4 together with strongly reduced or lacking CK18 and CK7 non-keratinizing epithelioma and squamous-cell carcinoma.



- **Squamous differentiation with cornification** was seen in the benign well-differentiated DEE-specific BKC tumors expressing a similar but more complex CK pattern than the non-keratinizing epithelioma with less CK14 and CK15 but additional CK1, 10/11.

Conclusion

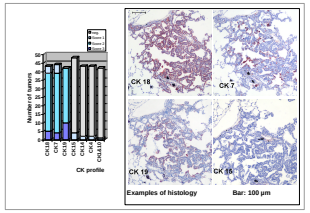
- Specific CK patterns are expressed in different rat lung tumors and related precursor lesions.
- The obvious parallels between precursor lesions and neoplastic tumors led us to the following hypothetical relationship between non-neoplastic and neoplastic lesions in the rat lung based on differential CK expression:



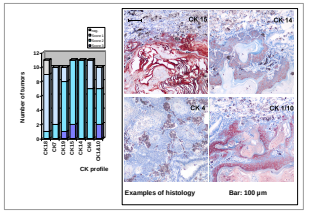
Analysis of enhanced or aberrant CK expression in the lung at earlier time points might be a useful intermediate biomarker in inhalation studies.

Results

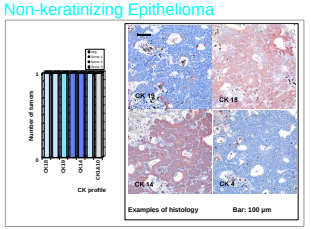
BA Adenoma



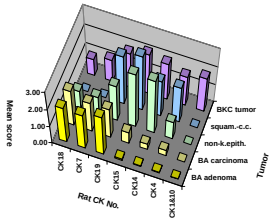
BKC Tumor



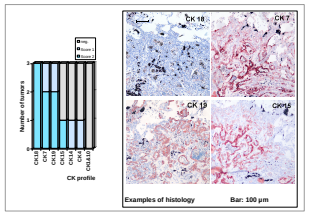
Non-keratinizing Epithelioma



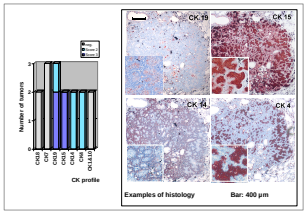
CK Expression in Lung Tumors



BA Carcinoma



Squamous-Cell Carcinoma



Metastases of Non-lung Tumors

- Malignant mesenchymal tumors: no CKs expressed
  - exception: some CK19-positive cells in a few cases
- Malignant epithelial tumors: CKs 7,18,19, and 14 or 15

Non-neoplastic Lesions

- Multiple non-neoplastic lesions in sections with lung tumors
  - possible precursors of tumors
  - most frequent in the DEE groups
  - major types: alveolar hyperplasia (which could be classified into 3 types--A, B, and D), BA metaplasia, and squamous metaplasia
- Lesions could be classified according to their CK expression patterns, which resemble the tumor-specific patterns (cf. 3-d graphs, right)
  - lesions with enhanced normal alveolar pattern (CK 18, 19, 7) found in all groups
  - lesions with squamous and/or cornification-related CKs seen in DEE groups only

CK Expression in Non-neoplastic Lesions