American Journal of Pathology, Vol. 134, No. 1, January 1989

Immunohistochemical Demonstration of Clara Cell Antigen in Lung Tumors of Bronchiolar Origin Induced by N-nitrosodiethylamine in Syrian Golden Hamsters

Sabine Rehm,* Michihito Takahashi,† Jerrold M. Ward,* Gurmukh Singh,† Sinkandar L. Katyal,‡ and John R. Henneman§

From the Tumor Pathology and Pathogenesis Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute, Frederick, Maryland, Department of Pathology, National Institute of Hygienic Sciences, Tokyo, Japan,† Department of Pathology, University of Pittsburgh, Veterans Administration Medical Center, School of Medicine, Pittsburgh, Pennsylvania,‡ and the Biological Carcinogenesis Development Program, Program Resources, Inc., Frederick, Maryland§*

Both alveolar type II cells and Clara cells have been suggested as cells of origin of human bronchioloalveolar lung carcinomas and other pulmonary neoplasms, based on the presence of cell specific markers identified by immunocytochemical methods. Alveolar type II cell origin of solid and papillary lung tumors of the mouse has been demonstrated, and Clara cells have been suggested as cell of origin for hamster pulmonary neoplasms. Therefore, chemically induced bronchiolar hyperplasias and pulmonary neoplasms of Syrian golden hamsters were analyzed by avidin-biotin immunohistochemistry to localize a hamster-specific Clara cell antigen (CCA) and keratin. The hamsters had been treated subcutaneously with multiple doses of N-nitrosodiethylamine (NDEA). Proliferative lesions of low cuboidal, tall columnar, or pleomorphic cells were present within bronchioles or adjacent to airways in the alveolar parenchyma. Frequently areas of squamous cell differentiation were present focally or diffusely that were immunoreactive for cytokeratin. Immunoreactivity for cytokeratin was also noted for hyperplastic bronchiolar neuroepithelial bodies. Cellular hyperplasias extending out into the alveolar parenchyma contained ciliated cells and frequently consisted of cells immunoreactive for CCA, showing them to be of bronchiolar Clara cell origin. Tumors developed from bronchiolar cell hyperplasias localized within bronchioles and from bronchiolar cells lining former alveolar walls. Neoplastic growth patterns were tubulo-papillary, forming loose networks or densely cellular areas. Immunoreactivity for cytoplasmic CCA was found in 50% of the tumors and was seen most frequently in small cuboidal cells and larger, vacuolated cells scattered throughout the neoplasms. In summary, evidence is presented that NDEA-induced pulmonary tumors of the Syrian golden hamster originated from cells lining bronchioles and from extrabronchiolar Clara cell hyperplasias of the terminal bronchioles. As the pulmonary tumors of the hamsters progressed towards a squamoid cell type, CCA was no longer detectable but cells became immunoreactive for keratin. (Am J Pathol 1989, 134:79–87)

Alveolar type II cells can be detected by immunocytochemistry using antibodies against surfactant apoproteins.[1,2] Clara cells can be identified similarly by antibodies raised against Clara-cell-specific proteins.[2] Use of these techniques facilitates the recognition of the cellular origin of proliferative lesions, particularly in cases where infiltrative growth into the surrounding tissue obscures the primary site, in cases of distant metastasis, or when char-

Supported in part with Federal funds from the Department of Health and Human Services under contract number NO1-CO-74102 with Program Resources, Inc.

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Accepted for publication August 19, 1988.

Address reprint requests to S. Rehm, National Cancer Institute, FCRF Bldg. 538, Room 220, Frederick, MD 21701-1013.

acteristic morphologic features are changed during tumor progression. Application of immunocytochemical methods with specific antibodies against different cell types has shown that in humans both alveolar type II cells and bronchiolar Clara cells may give rise to bronchioloalveolar carcinomas as well as to papillary adenocarcinomas of the lung.[3-6] In mouse and rat lung tumors, alveolar type II cells are frequently found to be the cell of origin.[7-9]

The Syrian golden hamster is often used to study neoplasms of the upper and lower air conducting systems that develop in the nasal cavity, trachea, bronchi, and bronchioles.[10] Neoplasms have been classified as adenomas, papillomas, adenocarcinomas, carcinomas, and squamous cell carcinomas arising from the olfactory epithelium, nasal glands, and tracheal basal cells.[11-13] Ciliated cells have been noted in tracheal tumors as pre-existing normal cells and not as part of the neoplastic process.[14] Clara cells and neuroendocrine cells have both been suggested as the cells of origin of neoplasms found in bronchioles and pulmonary tissue.[15-17] In the present investigation, we found N-nitrosodiethylamine (NDEA)-induced pulmonary proliferative lesions immunoreactive for a specific hamster Clara cell antigen, and illustrate tumor development from extrabronchiolar Clara cell hyperplasia.

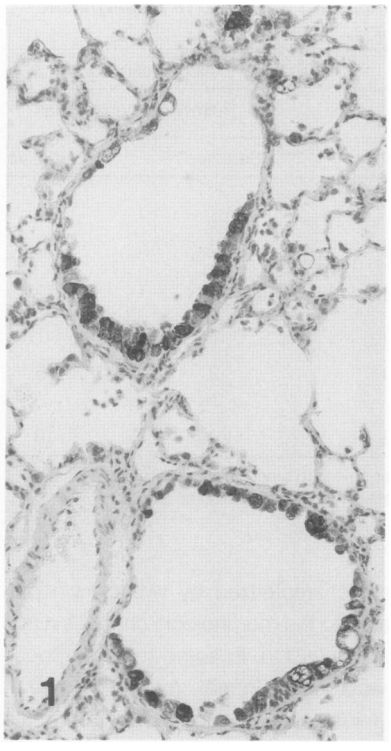
Materials and Methods

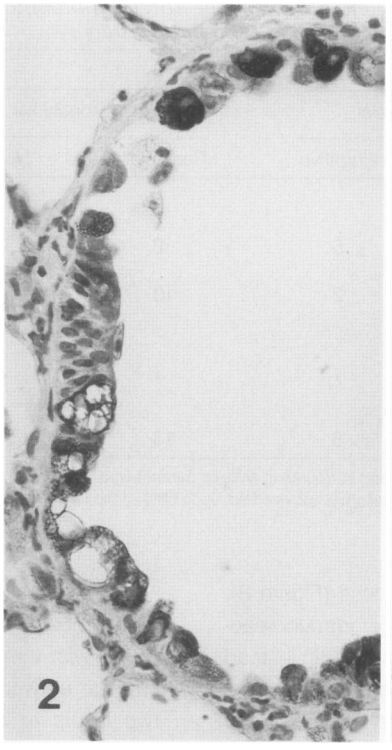
Histologic sections from lungs of male and female Syrian golden hamsters were obtained as part of two studies designed to examine preneoplastic and neoplastic lesions of the nasal cavity and trachea induced by subcutaneous injection of NDEA.[13,18] In both studies sequential age groups were examined, ie, animals were killed at different times after carcinogen exposure. Lung lesions, however, were only seen in the oldest groups. The lung sections obtained from the first study,[13] were from 32 Syrian golden hamsters (NCI, Division of Cancer Treatment, Animal Production Areas, Frederick, MD), which were treated starting at 6 weeks of age with 20 mg NDEA/kg body weight (Eastman Kodak Co. Rochester, NY) twice weekly for 7 weeks (14 injections) and killed 20–36 weeks after the first injection. In the second study,[18] 20 male and 20 female Syrian golden hamsters (Shizuoka Laboratory Center, Shizuoka, Japan) were treated with 18 mg NDEA/kg body weight twice a week, starting at the age of 7 weeks, for 12 weeks (24 injections), and were killed 16 weeks after the first injection. The lungs were fixed in 10% neutral buffered formalin, 4–6 trimmed lobe pieces were embedded in paraffin, and 5-μ thick sections were cut for studies by light microscopy using various stains: hematoxylin and eosin (H & E), alcian blue-safranin, and periodic acid Schiff's reaction (PAS) with and without prior diastase digestion. The avidin-biotin peroxidase complex immunohistochemical procedure (ABC) was used as described previously[19] for selected representative cases, to localize reactivity of a specific rabbit antiserum to hamster Clara cell antigen (CCA), diluted 1:1000, and to localize a selected range of cytokeratins by the use of rabbit anti-human keratin (DAKO Corp., Santa Barbara, CA) at a dilution of 1:200. Prior trypsinization was carried out to facilitate cytokeratin localization (0.02% Type II: crude trypsin from porcine pancreas for 30 minutes at 37 C, Sigma Chemical Co., St. Louis, MO). Preparation of CCA and the specific antibody reactivity for hamster Clara cell antigen have been demonstrated in an earlier publication.[2] The lungs from two untreated, age-matched Syrian golden hamsters were used as controls and submitted to the same procedures.

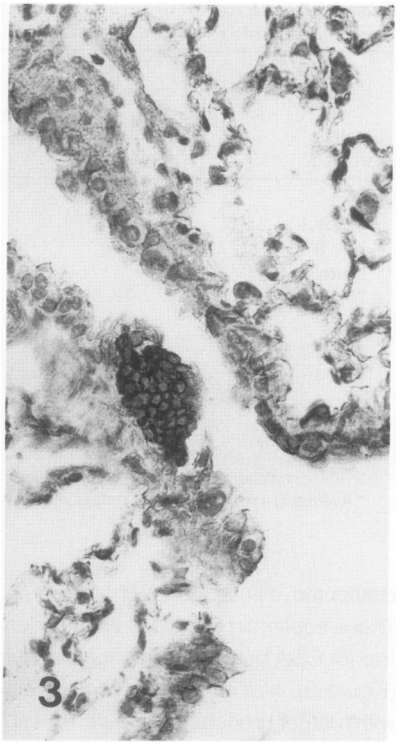
Results

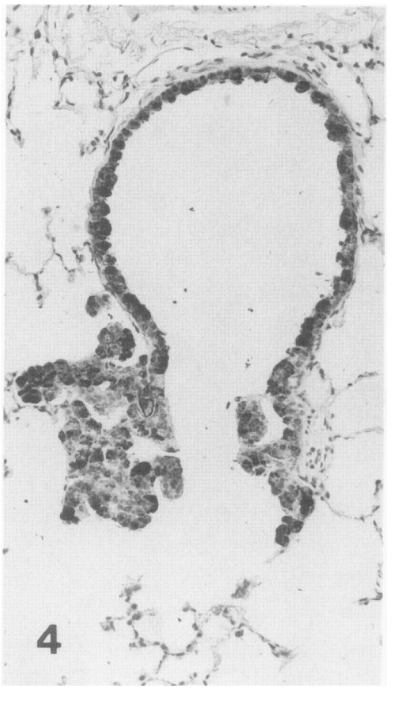
Normal bronchiolar cells immunoreactive to CCA by the ABC technique were found to be more numerous in the distal than in the proximal bronchioles, as expected for the normal distribution of Clara cells. The staining reaction was highly specific for Clara cells, as alveolar and ciliated cells were consistently negative.

Most lungs from treated hamsters in the oldest group showed bronchiolar cell hyperplasias. Hyperplasias were defined as proliferative lesions of limited size; they were noninvasive, and did not compress adjacent tissues. Hyperplastic areas were classified according to their location within the lung as intrabronchiolar, ie, being limited to the bronchiolar epithelium and lumen (Figures 1–3), or as extrabronchiolar, ie, involving the cells of the terminal bronchioles and extending beyond these into the surrounding alveolar parenchyma (Figure 4). Hyperplasias within airways appeared either diffusely, covering extensive bronchiolar surface areas, or arose focally. Pyramidally-shaped bronchiolar hyperplasias of neuroendocrine cells (dome formation) occurred as a special reaction of the Syrian golden hamster to NDEA exposure (Figures 2, 3). The endocrinelike nature of these cells has been identified previously with electron microscopy by the presence of neurosecretory granules[20,21] as well as by argyrophilia and corticotropinlike immunoreactivity.[21] Proliferative neuroepithelial bodies were seen regularly and were immunoreactive for keratin (Figure 3). Normal bronchiolar cells did not show immunoreactivity for keratin with the currently used antiserum. Frequently, Clara cells in the terminal bronchioles appeared irregularly enlarged and vacuolated (Figure 2), a reaction probably related to the NDEA treatment because these changes were not seen in untreated hamsters. Furthermore, pale intranuclear inclusions that









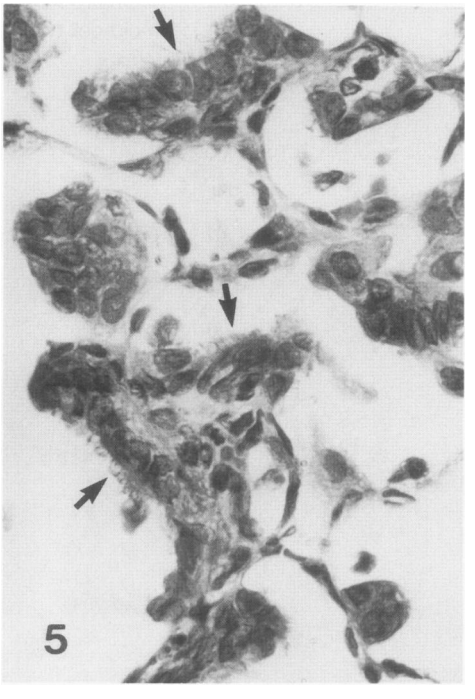


Figure 1. *Bronchiolar Clara cell hypertrophy and focal hyperplasia of neuroendocrine cells shown at higher magnification in Figure 2. (ABC immunohistochemistry for CCA, hematoxylin, ×100)* **Figure 2.** *Higher magnification of specimen shown in Figure 1 from area of focal hyperplastic neuroendocrine cells without immunoreactivity for CCA. Adjacent enlarged Clara cells, some with vacuolated cytoplasm are immunoreactive for CCA. (ABC immunohistochemistry, hematoxylin, ×250)* **Figure 3.** *Focally hyperplastic neuroendocrine cells immunoreactive for cytokeratin. (ABC immunohistochemistry, hematoxylin, ×250)* **Figure 4.** *Early Clara cell hyperplasia in NDEA-treated hamster extending from terminal bronchiole into alveolar parenchyma. (ABC immunohistochemistry for CCA, hematoxylin, ×100)* **Figure 5.** *Alveolar parenchyma occupied by ciliated cells (arrows) observed in NDEA-treated Syrian golden hamsters. (H & E, ×400)*

were PAS negative and negative for CCA were noted frequently in bronchiolar Clara cells of NDEA treated hamsters.

Bronchiolar cell hyperplasias arising from terminal bronchioles appeared to grow out into the adjacent parenchyma of alveolar walls (Figure 4). In four cases, ciliated cells could be observed in such lesions (Figure 5); however, the most frequent cell type was immunoreactive with antibodies to CCA as identified by ABC immunohistochemistry (Figure 4, Table 1). In more advanced proliferative lesions, cells showed squamous cell metaplasia arranged in small compact peripheral foci (Figures 6, 7) or

Table 1. *Localization of Hamster Clara Cell Antigen by the ABC Immunohistochemical Technique in Hyperplastic and Neoplastic Lesions Induced by NDEA in Syrian Golden Hamsters*

Hamsters examined	Hyperplastic lesions				Neoplastic lesions	
	Extrabronchiolar		Intrabronchiolar		Pulmonary carcinomas	
	Reactive*	Negative	Reactive*	Negative	Reactive*	Negative
Males						
Study 1						
N = 8	9	5	2	2	3	1
Study 2						
N = 7	12	2	10	8	15	12
Females						
Study 2						
N = 4	2	1	1	3	2	5
Total						
(N = 19)	23	8	13	13	20	18

Extrabronchiolar, involving terminal bronchioles and surrounding alveolar parenchyma. Intrabronchiolar, limited to bronchiolar epithelium and lumen.
* Averaging a minimum of two immunoreactive cells/high-power field; up to 60% of the tumor cells were immunoreactive in some tumors.

distributed diffusely throughout the process (Figure 8). These squamoid cell types were no longer immunoreactive for CCA but were immunoreactive for keratin instead (Figure 9). Half of the proliferative lesions arising from within larger bronchioles (Figure 10, Table 1) showed only a few cells immunoreactive for CCA, and several of these were interpreted as pre-existing.

Neoplasms were characterized by two growth patterns probably related to the morphologic changes associated with tumor progression. In some tumors a loose irregular network was seen centrally, formed by one to two layers of cuboidal cells supported by a delicate stroma with dense peripheral clusters of slender or squamoid cell types (Figures 6, 7). The cuboidal cells usually

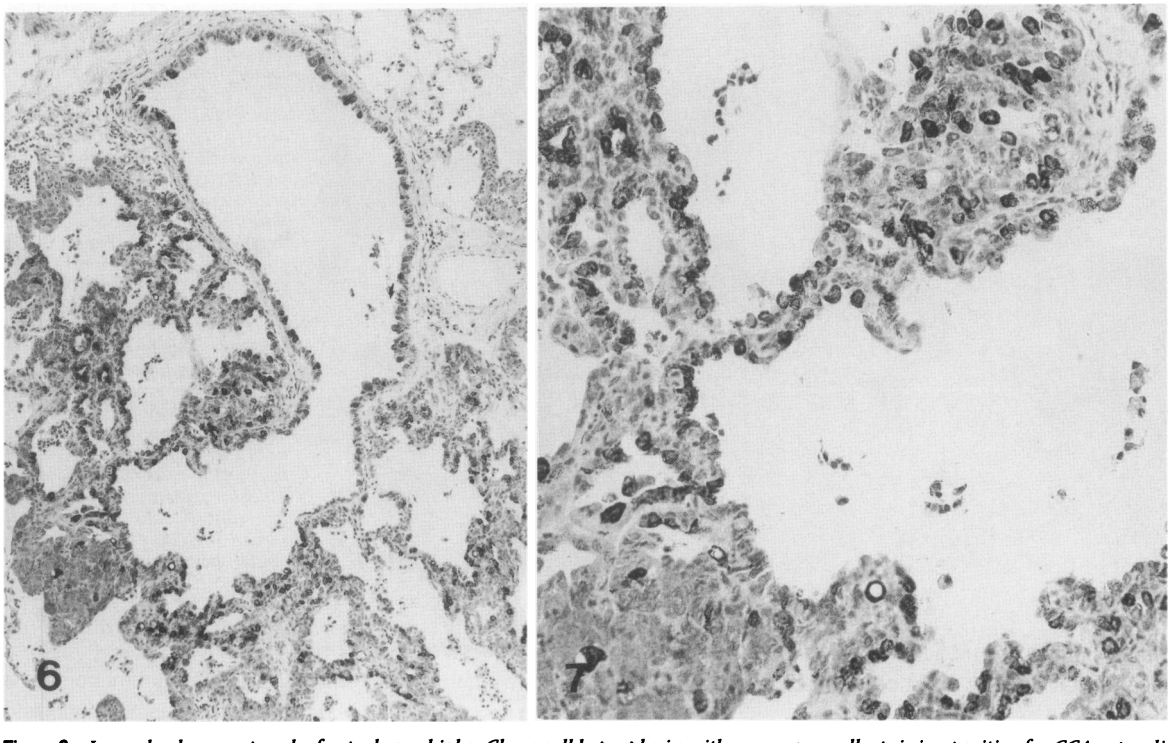
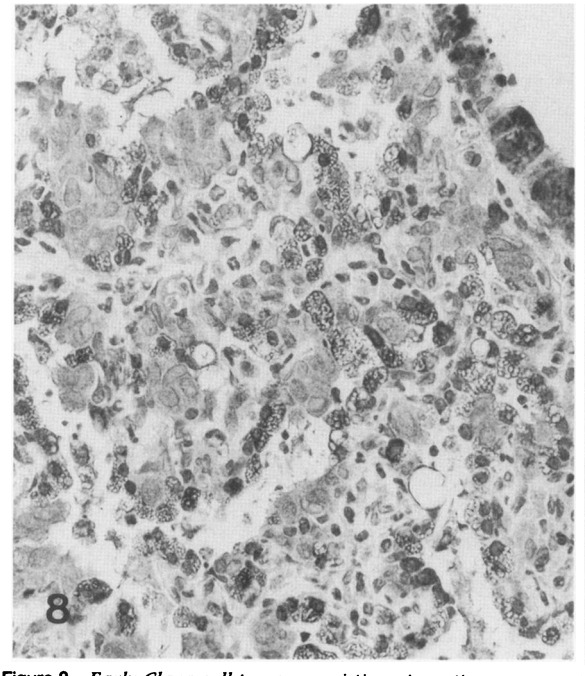


Figure 6. *Irregular loose network of extrabronchiolar Clara cell hyperplasia with numerous cells staining positive for CCA extending from terminal bronchiole. Early peripheral neoplasia characterized by loss of cytoplasmic immunoreactivity for CCA shown at higher magnification in Figure 7. (ABC immunohistochemistry, hematoxylin, ×40)* **Figure 7.** *Higher magnification of specimen shown in Figure 6. Squamous cell metaplasia in lower left corner. (ABC immunohistochemistry for CCA, hematoxylin, ×100)*



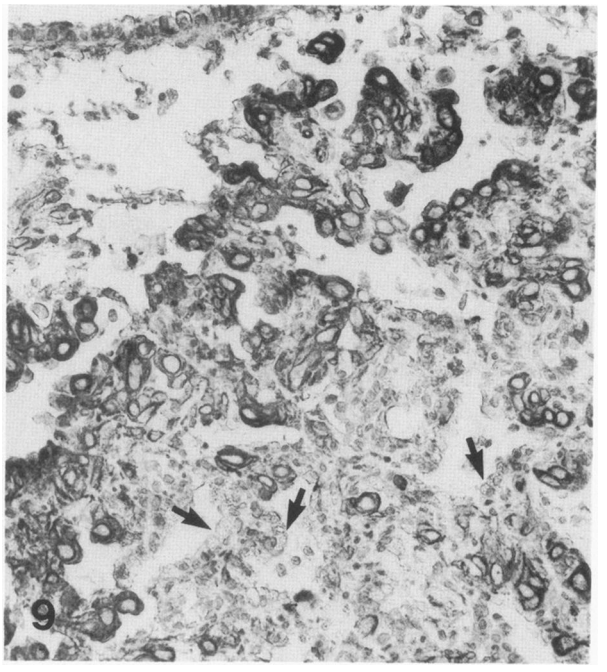


Figure 8. *Early Clara cell tumor consisting of mostly vacuolated cells immunoreactive for CCA and large squamoid cell types with negative staining for CCA. Note also immunoreactivity of bronchiolar cells. (ABC immunohistochemistry, hematoxylin, ×250)* **Figure 9.** *Early Clara cell tumor with squamoid cells immunoreactive for cytokeratin. Vacuolated cells (arrows) and bronchiolar cells show no reactivity. (ABC immunohistochemistry, hematoxyllin, ×250)*

reacted strongly with antibody to the Clara cell antigen. Solid neoplasms without loosely structured components were also seen, but the number of cells immunoreactive for Clara cell antigen varied (Figures 8, 10, 11). In some larger tumors (≥5 mm in diameter), the cells grew in dense papillary fronds or cell nests that were basally lined by one layer of basophilic cells and apically showed several layers of pale eosinophilic cells of nonkeratinizing squamous cell metaplasia (Figure 12). Cytokeratin was found to be present in the cytoplasm of these cells as shown by the ABC technique (Figure 13). Interspersed in all larger tumors were mucus-producing cells staining positive with alcian blue and PAS, and single, large vacuolated cells immunoreactive for Clara cell antigen. Whether ciliated cells were present could not be evaluated with certainty because an alcian blue positive film (eosinophilic with H & E) covered most of the free cell surfaces. In most neoplasms, neutrophilic granulocytes were transmigrating into cystic spaces and were present together with alveolar macrophages.

Discussion

The upper pulmonary airways in the hamster are mainly lined by ciliated cells and fewer nonciliated cells that, in turn, are more numerous in the distal airways and consist primarily of Clara cells in the terminal bronchioles.[22,23] Other, less frequently occurring nonciliated cells are mucous cells, neuroepithelial bodies, and rare serous and brush cells.[23] Clara cells are characterized in the hamster by prominent apical smooth endoplasmic reticulum and secretory granules that vary in numbers and electron density.[23,24]

Complete analysis of the hamster CCA (protein C) has yet to be performed. In the rat, the Clara cell antiserum reacts with Clara cell protein A, B, and C the latter of which has been shown to consist of three isotypes.[25] CCA is localized in the secretory granules of the normal Clara cell.[26]

Major morphologic problems determining the cell of origin in hamster pulmonary tumors are early squamous cell metaplasia or growth beyond the confinement of the bronchiolar lumen, so that the site of origin can no longer be determined. Using the specific antiserum to hamster Clara cells,[2] the present study shows convincingly that hamster pulmonary tumors induced by NDEA may, in part, arise from hyperplastic Clara cells present in the alveolar parenchyma. In a few cases of hyperplasia, ciliated cells were present, identifying such foci to be of bronchiolar cell origin by routine light microscopy. If the parenchymal lesion consisted entirely of cuboidal cells, a distinction between hyperplastic alveolar type II cells and Clara cells is only possible by electron microscopy or by immu-

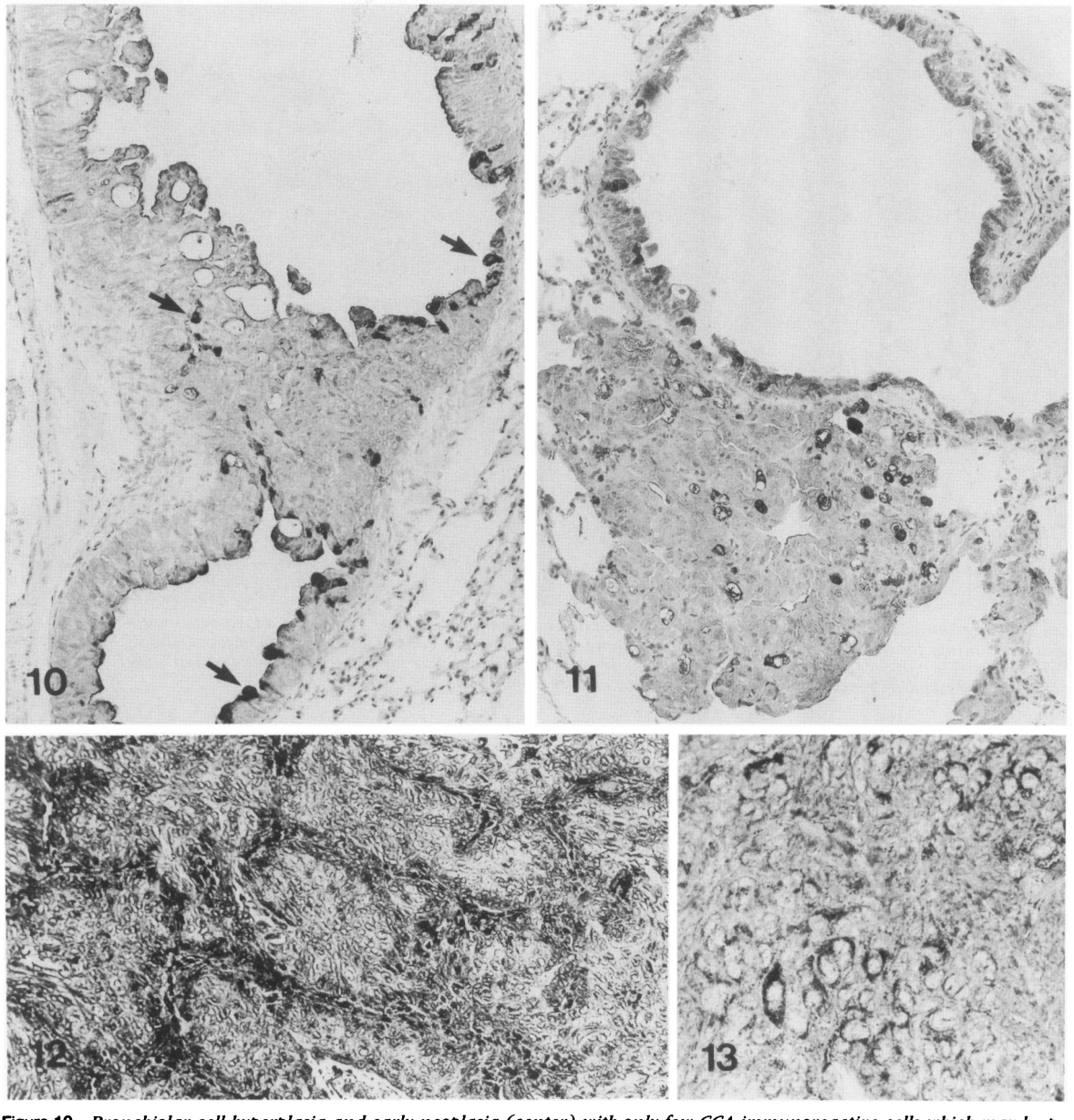


Figure 10. *Bronchiolar cell hyperplasia and early neoplasia (center) with only few CCA immunoreactive cells which may be pre-existing normal Clara cells (arrows). (ABC immunobistochemistry, hematoxylin, ×100)* **Figure 11.** *Early squamoid lung tumor of Syrian golden hamster containing scattered cells staining positive for CCA. (ABC immunohistochemistry, hematoxylin, ×100)* **Figure 12.** *Pulmonary carcinoma of Syrian golden hamster. Dense cell nests composed of a basal cell layer staining basophilic and central areas with paler eosinophilic squamoid cell types. (H & E, ×100)* **Figure 13.** *Central area of squamoid cell types of hamster lung carcinoma with cells showing immunoreactivity for cytokeratin. (ABC immunohistochemistry, hematoxylin, ×250)*

nohistochemical demonstration of specific hamster Clara cell antigen in the cytoplasm. Comparable changes, spontaneous or induced, are well known to occur in the alveolar parenchyma of many species[27–31] and have been referred to as adenomatosis, alveolar bronchiolization, epithelialization, or fetalization. These terms should be used with great care since they are often applied synonymously to alveolar type II cell hyperplasia. Bronchiolar cell hyperplasia within alveolar parenchyma is frequently associated with infections or chemical insults.[32,33] The histopathogenesis of bronchiolar cell types populating alveolar walls is a controversial issue. In many studies, this phenomenon is interpreted as migration of Clara cells through pores in airways connecting bronchioles and alveoli[34] or as regenerating bronchiolar cells growing peripherally into denuded alveolar walls.[32] It has also been suggested that

ciliated cells in alveolar parenchyma originate by metaplasia from transformed alveolar type II cells.[35] In the present study, lesions were always associated with bronchioles and if seen in a pattern of a very early stage (Figure 4), the process seems less likely to result from type II cell metaplasia but is more suggestive of hyperplastic bronchiolar cells growing beyond the limitations of the terminal bronchiole. There was no indication of alveolar type II cell damage that would result in metaplastic cells. Similarly, other investigators suggested that induced pulmonary neoplasms in hamsters arose from areas of so-called "alveolar epithelialization,"[16,36] from "bronchiolar-type cells in the alveolar region,"[37] or from "bronchiolar outgrowths."[38] In the Dzungarian dwarf hamster, lung tumors can be induced transplacentally by NDEA that may consist of undetermined cuboidal cells but frequently also show mucous cell differentiation. It was suggested that these neoplasms derive from outgrowths of the epithelium of small bronchioles and represent different metaplastic potentials of a bronchiolar stem cell.[39]

Ultrastructural studies of induced hamster lung tumors provided evidence for their Clara cell origin by the presence of secretory granules similar to those observed in normal hamster Clara cells.[16] Indirect evidence was presented in the suppression of tumor development by piperonylbutoxide, a substance that inactivates cytochrome P450 that is present in particularly high concentrations in Clara cells and is responsible for metabolizing NDEA to its active form.[40] Mucous cells and Clara cells were shown to be the major binding sites for tritiated NDEA[41] and degradation of N-^{14}C-nitrosodiethylamine is confined mainly to the cells lining the bronchial tree.[42]

The Clara cells of the untreated hamsters did not have the vacuolated appearance seen in the treated hamsters presently studied. Reports showed that induced Clara cell enlargement in the terminal bronchioles may be due to the formation of myelin figures.[15,43] Similar, although smaller, lamellated myelin figures, demonstrable ultrastructurally in normal hamster Clara cells, can be of lysosomal origin and may be morphologically indistinguishable from mature lamellar bodies seen characteristically in alveolar type II cells.[16] Ultrastructural studies alone are therefore apparently not sufficient to identify the cell of origin of pulmonary neoplasms, particularly because the tumors have been shown to contain entrapped normal alveolar type II cells.[16]

In the present study, the range of pulmonary lesions induced by NDEA in Syrian golden hamsters shows that, during tumor development, the neoplastic Clara cells may lose specific antigens and concomitantly undergo squamous cell metaplasia with expression of a subpopulation of keratins not apparent in the normal Clara cell. It had been noted that cells within bronchioles or at alveolar sites may undergo mucous or squamous cell metaplasia,[10,16,36,38,44] the original cell type, however, had not been identified. In some cases, hyperplasia and early neoplasia without evidence of squamous cell metaplasia also did not show CCA immunoreactivity, which could be related to the reduced production of secretory granules. Normal hamster Clara cells of the central pulmonary bronchioles may show considerable variation in the number of secretory granules.[23]

Neuroendocrine cell hyperplasia has been shown to coincide with loss of neurosecretory granules and simultaneous appearance of numerous intermediate filaments,[20] an explanation for the strong immunoreactivity of the neuroepithelial bodies for keratin in the present study.

The cumulative NDEA dose was higher in study 2 than in study 1, and therefore, the lesions had progressed more rapidly in study 2. Both studies combined give a representative range of lung tumor development in the Syrian golden hamster that is otherwise difficult to obtain because most hamsters succumb much earlier during the experiments to tumors developing in the upper respiratory tract.[10]

Comparative immunohistochemical studies involving human and animal tissues were very successful in localizing surfactant apoproteins to show normal embryonic lung development[1,45] and in recognizing and defining neoplasms originating from alveolar type II cells.[3,4,7] Recent studies have localized CCA in human pulmonary adenocarcinomas and "sclerosing hemangiomas."[5,46,47] The hamster has been suggested as a suitable animal model for human tumors of the respiratory tract because of similar morphology and pattern of reaction to injury[48] and the Syrian golden hamster is, so far, the only animal developing tumors that clearly arise from bronchiolar Clara cells.

References

1. Ten Have-Opbroek AAW: Immunological study of lung development in the mouse embryo. Appearance of a lung-specific antigen, localized in the great alveolar cell. Dev Biol 1975, 46:390–403
2. Singh G, Katayal SL, Ward JM, Gottron SA, Wong-Chong M-L, Riley EJ: Secretory proteins of the lung in rodents: Immunocytochemistry. J Histochem Cytochem 1985, 33:564–568
3. Singh G, Katyal SL, Torikata C: Carcinoma of type II pneumocytes. Immunodiagnosis of a subtype of "bronchioloalveolar carcinomas." Am J Pathol 1981, 102:195–208
4. Mitzutani Y, Nakajima T, Morinaga S, Gotoh M, Shimosato Y, Akino T, Suzuki A: Immunohistochemical localization of pulmonary surfactant apoproteins in various lung tumors. Cancer 1988, 61:532–537
5. Singh G, Singh J, Katyal SL, Brown WE, Kramps JA, Paradis IL, Dauber JH, MacPherson TA, Squeglia N: Identification,

cellular localization, isolation, and characterization of human Clara cell-specific 10 KD protein. J Histochem Cytochem 1988, 36:73–80

6. Linnoila RI, Whittset JA, Mulshine JL, Jensen S, Gazdar AF: Immunohistochemical (IHC) expression of surfactant associated proteins (SAP-35) in non-small cell lung cancer (NSCLC) (abstr). Modern Pathol 1988, 1:55A
7. Ward JM, Singh G, Katyal SL, Anderson LM, Kovatch RM: Immunocytochemical localization of the surfactant apoprotein and Clara cell antigen in chemically induced and naturally occurring pulmonary neoplasms of mice. Am J Pathol 1985, 118:493–499
8. Rehm S, Ward JM, Ten Have-Opbroek AAW, Anderson LM, Singh G, Katyal SL, Rice JM: Mouse papillary lung tumors transplacentally induced by N-nitrosoethylurea: evidence for alveolar type II cell origin by comparative light microscopic, ultrastructural, and immunohistochemical studies. Cancer Res 1988, 48:148–160
9. Oshima M, Ward JM, Singh G, Katyal SL: Immunocytochemical and morphological evidence for the origin of N-nitrosomethylurea-induced and naturally occurring primary lung tumors in F344/NCr rats. Cancer Res 1985, 45:2785–2792
10. Montesano R, Saffioti U: Carcinogenic response of the respiratory tract of Syrian golden hamsters to different doses of diethylnitrosamine. Cancer Res 1968, 28:2197–2210
11. Spit BJ, Feron VJ: Comparative study of the ultrastructure of tracheal and pulmonary tumours induced by multiple intratracheal instillations of diethylnitrosamine in Syrian golden hamsters. Europ J Cancer 1975, 11:867–872
12. Mohr U: Tumours of the respiratory tract. Pathology of tumours in laboratory animals, Volume III, Tumours of the Hamster. Edited by VS Turusov. Lyon, IARC, 1982, pp 115–145
13. Ward JM: Leu 7 immunoreactivity in fetal olfactory epithelium and dysplastic or neoplastic olfactory lesions induced in Syrian golden hamsters by N-nitrosodiethylamine. Am J Pathol 1986, 123:371–376
14. Wahnschaffe U, Emura M, Mohr U: Ciliated cells in diethylnitrosamine-induced tracheal papillary tumors of Syrian golden hamsters. Exp Pathol 1987, 32:169–177
15. Reznik-Schüller H: Sequential morphologic alterations in the bronchial epithelium of Syrian golden hamsters during N-nitrosomorpholine-induced pulmonary tumorigenesis. Am J Pathol 1977, 89:59–66
16. Kennedy AR, McGandy RB, Little JB: Histochemical, light and electron microscopic study of polonium-210 induced peripheral tumors in hamster lungs: Evidence implicating the Clara cell as the cell of origin. Europ J Cancer 1977, 13: 1325–1340
17. Schüller HM, Becker KL, Witschi H-P: An animal model for neuro-endocrine lung cancer. Carcinogenesis 1988, 9:293–296
18. Miyakawa Y, Takahashi M, Furukawa F, Hayashi Y: Scanning electron microscopic study on preneoplastic and neoplastic lesions of the trachea in Syrian golden hamsters induced by diethylnitrosamine (abstr). Proc Jap Cancer Assoc 1985, 44:76
19. Ward JM, Reynolds CW, Argilan F: Immunoperoxidase localization of large granular lymphocytes in normal tissues and lesions of athymic nude rats. J Immunol 1983, 131:132–139
20. Reznik-Schüller H: Ultrastructural alterations of APUD cells during nitrosamine-induced lung carcinogenesis. J Pathol 1977, 121:79–82
21. Linnoila RI, Nettesheim P, DiAugustine RP: Lung endocrine-like cells in hamsters treated with diethylnitrosamine: Alterations *in vivo* and in cell culture. Proc Natl Acad Sci 1981, 78:5170–5174
22. Becci PJ, McDowell EM, Trump BF: The respiratory epithelium. II. Hamster trachea, bronchus, and bronchioles. JNCI 1978, 61:551–561
23. Christensen TG, Breuer R, Hornstra LJ, Lucey EC, Snider GL: The ultrastructure of hamster bronchial epithelium. Exp Lung Res 1987, 13:253–277
24. Plopper CG: Comparative morphological features of bronchiolar epithelial cells. The Clara cell. Am Rev Respir Dis 1983, 128:S37–S41
25. Singh G, Singal S, Katyal SL, Brown WE, Gottron SA: Isolation and amino acid composition of the isotypes of a rat Clara cell specific protein. Exp Lung Res 1987, 13:299–309
26. Bedetti CD, Singh J, Singh G, Katyal SL, Wong-Chong M-L: Ultrastructural localization of rat Clara cell 10 KD secretory protein by the immunogold technique using polyclonal and monoclonal antibodies. J Histochem Cytochem 1987, 35: 789–794
27. Ward JM: Naturally occurring Sendai virus disease of mice. Lab Anim Sci 1974, 24:938–945
28. Zurcher C, Burek JD, Van Numen MCJ, Meihuizen SP: A naturally occurring epizootic caused by Sendai virus in breeding and aging rodent colonies. Lab Anim Sci 1977, 27: 955–962
29. Pour PM: Spontaneous respiratory tract tumors in Syrian hamsters. Comparative respiratory tract carcinogenesis, Volume I, Spontaneous Respiratory Tract Carcinogenesis. Edited by HM Reznik-Schüller. Boca Raton, CRC Press, 1983, pp 131–170
30. Boorman GA: Bronchiolar/alveolar hyperplasia, lung, rat. Respiratory system, Monographs on Pathology of Laboratory Animals. Edited by TC Jones, U Mohr, RD Hunt. Berlin, Springer-Verlag, 1985, pp 177–179
31. Ito T: An ultrastructural study of the acute effects of 4-nitroquinoline 1-oxide on the hamster lung. Exp Mol Pathol 1985, 42:220–233
32. Stinson SF, Ryan DP, Hertweck MS, Hardy JD, Hwang-Kow S-Y, Loosli CG: Epithelial and surfactant changes in influenzal pulmonary lesions. Arch Pathol Lab Med 1976, 100:147–153
33. Aso Y, Yoneda K, Kikkawa Y: Morphologic and biochemical study of pulmonary changes induced by bleomycin in mice. Lab Invest 1976, 35:558–568
34. Nettesheim P, Szakal AK: Morphogenesis of alveolar bronchiolization. Lab Invest 1972, 26:210–219
35. Adamson IYR, Bowden DH: Origin of ciliated alveolar epithelial cells in bleomycin-induced lung injury. Am J Pathol 1977, 87:569–580

36. Kennedy AR, McGandy RB, Little JB: Serial sacrifice study of pathogenesis of ^{210}Po-induced lung tumors in Syrian golden hamsters. Cancer Res 1978, 38:1127–1135
37. Shami SG, Thibodeau LA, Kennedy AR, Little JB: Proliferative and morphological changes in the pulmonary epithelium of the Syrian golden hamster during carcinogenesis initiated by ^{210}-Po α-radiation. Cancer Res 1982, 42:1405–1411
38. Saffiotti U, Cefis F, Kolb LH: A method for the experimental induction of bronchogenic carcinoma. Cancer Res 1968, 28: 104–124
39. Warzok R, Thust R: Morphology of diethylnitrosamine-induced lung tumours in Dzungarian dwarf hamsters. Exp Pathol 1977, 13:44–51
40. Schüller HM, McMahon JB: Inhibition of N-nitrosodiethylamine-induced respiratory tract carcinogenesis by piperonylbutoxide in hamsters. Cancer Res 1985, 45:2807–2812
41. Reznik-Schüller HM, Hague BF: Autoradiographic study of the distribution of bound radioactivity in the respiratory tract of Syrian hamsters given N-[^{3}H]nitrosodiethylamine. Cancer Res 1981, 41:2147–2150
42. Löfberg B, Tjälve H: The disposition and metabolism of N-nitrosodiethylamine in adult, infant and foetal tissues of the Syrian golden hamster. Acta Pharmacol Toxicol 1984, 54:104–114
43. Reznik-Schüller H: Ultrastructural alterations of nonciliated cells after nitrosamine treatment and their significance for pulmonary carcinogenesis. Am J Pathol 1976, 85:549–554
44. Christensen TG, Breuer R, Hornstra LJ, Lucey EC, Stone PJ, Snider GL: An ultrastructural study of the response of hamster bronchial epithelium to human neutrophil elastase. Exp Lung Res 1987, 13:279–297
45. Otto-Verberne CJM, Ten Have-Opbroek AAW, Balkema JJ, Franken C: Detection of the type II cell or its precursor before week 20 of human gestation, using antibodies against surfactant-associated proteins. Anat Embryol 1988, 178:29–39
46. Nagata N, Dairaku M, Sueishi K, Tanaka K: Sclerosing hemangioma of the lung. An epithelial tumor composed of immunohistochemically heterogenous cells. Am J Clin Pathol 1987, 88:552–559
47. Wick MR, Yousem SA, Mills SE, Legier J, Singh G, Katyal S, Manivel JC: Pulmonary "sclerosing hemangioma": An immunohistochemical study of eight cases (abstr). Modern Pathol 1988, 1:103A
48. Becci PJ, McDowell EM, Trump BF: The respiratory epithelium. IV. Histogenesis of epidermoid metaplasia and carcinoma in situ in the hamster. JNCI 1978, 61:577–586

Acknowledgment

The authors acknowledge the excellent technical assistance of Cindy Harris, Barbara Kasprzak, and Shirley Hale.