SCANNING ELECTRON MICROSCOPIC OBSERVATIONS ON PRENEOPLASTIC AND NEOPLASTIC LESIONS OF THE TRACHEA IN SYRIAN GOLDEN HAMSTERS INDUCED BY DIETHYLNITROSAMINE

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Abstract: Ultrastructural observations by scanning electron microscope (SEM) of pre- or neoplastic tracheal lesions in Syrian golden hamsters induced by diethylnitrosamine (DEN) were investigated. Twenty five, six-week-old, male Syrian golden hamsters were injected subcutaneously twice a week for 12 weeks with 18 mg/kg body weight of DEN. Six hamsters were injected only vehicle and served as control. At weeks 2, 4, 8, 12, and 16, 5 hamsters of DEN treated group were sacrificed. At weeks 2, 8, and 12, hamsters of vehicle control group were also sacrificed. Sequential changes of tracheal tumors were investigated by ultrastructural and histopathological observations. At week 2, a focal loss of cilia from surface cells of the trachea of DEN treated hamster were already detected by SEM. At week 4, deciliated changes of the surface became more markedly, and at week 8, various kinds of preneoplastic and neoplastic changes were observed in trachea of DEN treated hamster. Those changes could be detected by microscopy as well as SEM. By week 16, papillary tumors were found in all animals treated with DEN. No changes were observed in trachea of control animals. These results showed that scanning observations can detect the very early lesions of trachea and it is a very useful method especially for detecting the changes of tracheal lesions on hamster tracheal carcinogenesis. (J Toxicol Pathol 3: 231–237, 1990)

Key words: SEM, Hamster, Trachea, Diethylnitrosamine, Preneoplastic lesions

Introduction

In experimental carcinogenesis studies, scanning electron microscopy (SEM) is a useful method for investigating the surface structure, and the surface changes have been investigated by SEM in some organs including urinary bladder, cheek pouch, and nasal cavity1-3. The surface epithelium of these organs were altered by chemical carcinogens in the early stage, and SEM observation can detect earlier lesions than microscopical observation. Diethylnitrosamine (DEN) is known to be carcinogenic for the respiratory system of Syrian golden hamsters, especially for the upper respiratory tract4-6. Although the morphological changes of these tumors have been investigated4-6, only few sequential studies and the detailed morphological changes of the surface of tracheal epithelium have been observed by SEM on the development of tumors induced by chemical carcinogens8.

Therefore, in the present investigation, sequential studies of ultrastructural changes in the tracheal surface mucosa of hamsters induced by DEN were investigated by SEM and the results were compared with histopathological findings.
Materials and Methods

Thirty-one male Syrian golden hamsters, 6-week-old and weighing approximately 85 g, were purchased from Sankyo Labo Service (Tokyo, Japan). They were housed in wire cages under standard laboratory conditions (room temperature, 23±2°C; relative humidity, 60±5%; light/dark cycle, 12 hr/12 hr), and were given commercial diet (Oriental MF, Oriental Yeast Co., Tokyo) and tap water ad libitum.

Twenty-five hamsters were injected subcutaneously twice a week for 12 weeks with 18 mg/kg body weight of DEN (Wako Pure Chemical Ind. Ltd., Tokyo). DEN was stored at 4°C and dissolved in physiological saline at a concentration of 3.6 mg/ml just before each administration. Six male animals were injected vehicle alone as DEN treatment, and served as control group.

At weeks 2, 4, 8, 12, and 16, each five animals in DEN treated group were sacrificed for histopathological examinations. At weeks 2, 8, and 12, each two hamsters in the control group were sacrificed. At each sacrifice, the trachea of each hamster was rapidly removed under ether anesthesia and cut at the anterior wall, and then they were opened longitudinally. To remove the superficial mucus, luminal surface was washed with a gentle jet of physiological saline and then carefully examined using a stereoscopic microscope (SZH-111, Olympus Ltd., Tokyo). It was then separated into two parts longitudinally, right and left, for

Fig. 1.
(a) Light micrograph of tracheal epithelium in the control animal. ×680
(b) Irregular arrangement and slight hyperplasia of basal cells. Note the loss of superficial cilia. ×340
(c) Marked basal cell hyperplasia. The lesion folds and slightly projects into the tracheal lumen. ×215
Fig. 2. Scanning electron micrograph of luminal surface of trachea in control animal. Ciliated and non-ciliated cells are clearly defined. ×1190

Fig. 3. Shortening and/or loss of cilia from luminal surface of trachea. SEM × 2975

Fig. 4. Non-ciliated flat lesions. SEM × 680

Fig. 5. Dome-shaped area of trachea consisting of cell surface varying in shape and form. Note folds and slight projection in the tracheal lumen. SEM × 340
SEM and light microscopical observations, respectively. For SEM study, one half was cut into some blocks with a razor and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 at 4°C for 2 hours. These specimens were dehydrated through increasing concentrations of ethanol, transferred to amyl acetate, and dried by the critical point method. The tracheal tissues were sputter-coated with gold in an ion-coater (ION SPUTTER FJC-1100, JEOL, Tokyo). The specimens were examined with Hitachi S450 SEM (Hitachi, Tokyo). For light microscopic examination, another half of trachea was fixed with 10% phosphated buffered formalin, embedded in paraffin, and sections were routinely stained with hematoxylin and eosin (H.E.).

Results

In the control hamsters, trachea was covered by pseudostratified epithelium, consisting of ciliated and basal cells (Fig. 1A). By use of SEM, two cell types, ciliated and microvillus covered cells, were recognized and the number on these two cell types appeared roughly equal (Fig. 2). However, small patches of epithelium were seen consisting mainly of microvillus covered cells or of ciliated cells. Throughout this experimental period, no morphological changes were observed in the tracheal epithelium of control animals.

In the treated animals, tracheal epithelium showed a typical sequence of changes. The lesions of tracheal epithelium were classified into 4 histological types by light microscope; irregular arrangement of basal cells, basal cell hyperplasia, papillary hyperplasia, and papilloma. By SEM observations, the lesions were also classified into 4 types; focal loss of cilia, non-ciliated flat lesion, non-ciliated exophytic growth lesion and papilloma. The appearance of these changes observed
Table 1. Sequential Changes of Tracheal Epithelium in Syrian Golden Hamster Treated with Subcutaneous Injections of DEN by Stereomicroscopical, Microscopical and Scanning-Electron Microscopical Observations

<table>
<thead>
<tr>
<th>Pathological Findings</th>
<th>Experimental period (weeks)</th>
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<tbody>
<tr>
<td>Stereomicroscopical Observation</td>
<td>2</td>
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<tr>
<td>Papillary hyperplasia or papilloma</td>
<td></td>
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<tr>
<td>Microscopical Observation</td>
<td></td>
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<tr>
<td>Irregular arrangement</td>
<td></td>
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<tr>
<td>Basal cell hyperplasia</td>
<td>-</td>
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<tr>
<td>Papillary hyperplasia</td>
<td></td>
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<tr>
<td>Papilloma</td>
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<tr>
<td>Scanning-Electron Microscopical Observation</td>
<td></td>
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<tr>
<td>Focal loss of cilia</td>
<td></td>
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<tr>
<td>Non-ciliated flat lesion</td>
<td></td>
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<tr>
<td>Non-ciliated exophytic growth lesion</td>
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<tr>
<td>Papilloma</td>
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</tbody>
</table>

by stereoscopic microscope, light microscope or SEM during the experiment periods are summarized in Table 1.

At week 2, the earliest pathological alterations were detected as a focal loss of cilia from surface cells by SEM (Fig. 3). Cilia were torn off, shortened and lost from the surface of ciliated cells. No particular changes were observed by light- and stereoscopic microscopic observation.

At week 4, deciliated changes in surface-structure become more marked, and light-microscopically disarrangement of tracheal epithelium (Fig. 1B) was found in some animals. However, no changes were detected by stereomicroscopical observation.

At week 8, various kinds of preneoplastic and neoplastic changes were developed. Two kinds of patched lesions were apparent by SEM. One was the patches of non-ciliated flat lesions and they were usually completely differentiated from surrounding epithelium, but did not project into the tracheal lumen (Fig. 4). Another patch consisted of non-ciliated exophytic growth lesions. These lesions were slightly projected into the tracheal lumen with the appearance of slightly domed surface and occurrence of large folds (Fig. 5). By light microscopical observation, basal cell proliferation with slight folding into luminal surface (Fig. 1C) and papillary hyperplasia (Fig. 6) of tracheal epithelium were seen.

In 3 of 5 animals killed at week 8, papillary tumors of trachea were developed. Stereomicroscopically, tumor growth was formed papillary polyps markedly projected into the lumen and often revealed white or gray cauliflower-like nodules with a coarse granular surface. By use of SEM, the surface of tumors consisted of a lot of small knob- or globule like structures composed of cells bulging into the lumen (Fig. 7). At high magnification of SEM, these surfaces of the cells showed variable ultrastructural changes. Some surfaces were mainly covered by microvilli, and others were characterized by microridges or microridges and microvilli which indicated that these cells were undergoing squamous metaplasia, and a few distorted cilia were found.

By light microscopical observation, the papillary tumors were covered by squamous cells or cuboidal cells. Cuboidal cells sometimes secreted alcian-blue positive mucus and had large vacuoles in their cytoplasm (Fig. 8). In some instances both squamous and cuboidal cells were present and rarely a few ciliated cells were found. However, most tumors were classified as squamous cell papillomas. The stalk of these papillary growths showed a well vascularized raminiform or stem-like stroma composed of a loose, poorly cellular connective tissue.
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At weeks 12 and 16, papillary tumors were found in all animals. They were always multiple and were located throughout the tracheal lumen. Some tumors were large in size filling almost all the air space of tracheal lumen, but none of the tumors showed invasion or metastases.

Discussion

After treatment with DEN, the first change of tracheal epithelium was observed at week 2, which was only distinguished by SEM and, consisted of loss or shortening of cilia. This observation was supported by Althoff's study that loss of cilia with irregular arrangement of bronchial epithelium was firstly observed in Syrian golden hamster treated with DEN using light microscope.

Alterations of the respiratory cilia in the air conducting system, such as reduction in the number of cilia per unit surface area or deformation of the cilia, are the common lesions, induced by inhaled noxious agents. Such changes have also been reported after treatment with intratracheally and systemically administered carcinogens and are commonly found in heavy smokers and human patients with bronchial carcinoma. These observations suggest that impairment of important mucociliary defense mechanisms may pave the way for more serious damage from sequentially inhaled substances.

The second change observed by SEM study was "black" patchy lesions, flat and exophytic type. These lesions correlated with hyperplasia and/or irregular arrangement of basal cells by light microscopical observation. The proliferative character of these lesions suggested preneoplastic nature of patchy area. In the present SEM study we observed quite different cell surface patterns during carcinogenesis induced by DEN in the trachea, and these different SEM features allowed a clear-cut grading and served as a reliable classification when the light microscopic classification was dubious. Thus, SEM is a useful method for characterizing surface changes during tracheal carcinogenesis.

Transmission electron microscopical observations were also useful method, especially for investigating pulmonary carcinogenesis, because it can distinguish Type 2 alveolar epithelial cells by detecting lamellated inclusion bodies. Immunohistochemical observations are also helpful for detecting the cell origin for pulmonary carcinogenesis, and it has been reported that the histogenesis of hamster lung tumors could be detected using Type 2 epithelial cell or Clara antigens.

Together with other techniques including immunohistochemical staining, SEM is one of the very useful methods for detecting early changes of tracheal epithelium in tracheal carcinogenesis and toxicity of hamster.
Acknowledgement: This work was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, and the Ministry of Health and Welfare of Japan.

Reference


