MORPHOLOGY AND PATHOMORPHOLOGY

Pathomorphological and Endoscopic Study of Large Bronchi in Lung Cancer

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Diffuse progressive atrophy of the bronchial epithelium and mucosa are the main changes detected in the biopsy specimens of large bronchi in lung cancer. Unstability of the bronchial epithelium in lung tumors (alternating atrophy, metaplasia, hyperplasia, and dysplasia) is described.

Key Words: *lung cancer; bronchial epithelium; bronchial biopsy; atrophy; instability phenomenon*

Pathomorphosis of respiratory diseases [7,8] and many new lung diseases induced by ecological factors [3, 4,6,11] are responsible for modification of pretumor processes. Reduced capacity to DNA repair and induced and spontaneous mutations facilitate malignant transformation of target cells [1]. Normal cell can be transformed into tumor cell not only due to gene mutations, but also due to epigenomic, or regulatory disorders [1,10]. Initial sign of central lung cancer is desquamation of bronchial epithelium (BE), one of the most important markers of its atrophy, denudation of the basal layer, and damage to basal cells, followed by their hyperplasia and metaplasia. Unstable tissue proliferation is believed to play an important role in this process [2]. Atrophic processes, highly prevalent in recent years, attracted our attention, because of the priority importance of early diagnosis of cancer.

Our purpose was a pathomorphological and endoscopic study of the large bronchi in lung cancer for evaluating the role of structural changes in the airways in the morphogenesis of cancer of the respiratory organs.

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MATERIALS AND METHODS

Clinical, endoscopic, and pathomorphological studies were carried out in 250 patients with central and peripheral cancer. Among histological forms of lung cancer, squamous-cell (52%) and small-cell (30%) cancer and adenocarcinoma (15%) predominated, in the rest cases mixed forms were identified. Specimens of large bronchi were examined under light optic and electron microscopes and analyzed by autoradiography. For light microscopy the fragments were fixed in 10% neutral formalin and treated routinely. Paraffin sections were stained with hematoxylin and eosin with Pearl's reaction, elastic fibers were stained by the method of Van-Gieson followed by Weigert's resorcin-fucsin poststaining, and periodic acid Schiff reaction was performed.

For electron-microscopy, small fragments (no more than 1 mm^3) were fixed in 4% paraformaldehyde and 1% OsO_4. After standard treatment for electron microscopy, the tissue was embedded in epon-araldite. Semithin sections were stained with 1% azur II and Schiff reagent. Ultrathin sections after double-contrasting were examined under a JEM 1010 electron microscope at accelerating voltage of 80 kV.

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The intensity of biosynthetic reactions of bronchial cell populations was evaluated *in vitro* by autoradiography. The specimens were incubated with radioactive RNA and DNA precursors as described previously [9]. Semithin sections were coated with type M photoemulsion, exposed for 7 and 13 days at 4°C, developed, and stained with 1% azur II. Label density (LD) and index (LI) were counted under a light microscope at ×600.

The height of the bronchial mucosa epithelium was measured on semithin sections with a MOB-1-15^x ocular micromer and coefficient of variations for this value was determined [5].

RESULTS

Bronchoscopy showed diffuse bilateral atrophy of bronchial mucosa sometimes accompanied by inflammation. Atrophic changes were more pronounced in small bronchi. Mainly local deformation of lobular and segmented bronchi was observed in the majority of cases; in some patients "dust tattoo" of bronchial mucosa was seen.

Light microscopy of large bronchi showed mosaic changes in BE and stereotypical restructuring of the lamina propria of the bronchial mucosa. In the majority of cases alternating sites of stratified cylindrical epithelium, one- and two-layer squamous, and stratified squamous epithelium were observed in a single specimen. Focal BE hyperplasia and dysplasia often developed.

Disintegration of epitheliocytes, dystrophy and hypoplasia of goblet cells developed in the stratified cylindrical epithelium. No distinct cell subpopulations were observed in sites of atrophy: epitheliocytes were monomorphous, small, cube-shaped, without ciliary apparatus, which was sometimes replaced by microvilli. Pronounced epithelial polymorphism in biopsy specimens with squamous-cell metaplasia of BE is worthy of note. The structure of epithelial layers varied from monolayer, sometimes endothelioid epithelium to stratified epithelium forming club-shaped or fungiform thickenings (Fig. 1).

Basal epithelial layer was unevenly thickened in the majority of cases, with thin diffusely sclerosed mucosa. The greater part of the lamina propria consisted of thick bundles of collagen fibers; in many cases, especially in elderly patients, hyperelastosis and degeneration of elastic fibers with alteration of their structural and tinctorial characteristics were observed. Surface vessels were few, with signs of vascular and perivascular sclerosis. Inflammatory cell infiltration detected in few biopsy specimens was presented by mononuclear cells (lymphocytes, plasmacytes, macrophages, and fibroblasts) with solitary eosinophils and neutrophils. Intraepithelial cytodiapedesis was observed in some cases.

Muscle bundles had signs of myoelastofibrosis; mucosal nerve formations were dystrophic and sclerotic. Mucous serous glands often formed cysts, which impaired the excretory function.

During electron microscopy of bronchial biopsy specimens special attention was paid to bronchial epitheliocytes and endotheliocytes of the microcirculatory bed. Ultrastructural signs of progressive atrophy of BE were detected: dilatation of intercellular spaces, destruction of cell-to-cell contacts and ciliary system. Cytoplasmatic reticulum and mitochondrial compartment were reduced in ciliary cells. Polymorphous autophagosomes including small compact secretory granules were seen in goblet cells.

In the majority of cases BE cells had no specific ultrastructural signs characteristic of differentiated bronchial epitheliocytes (Fig. 2, *a*). These cells contained hyperchromatic nuclei; electron-dense cytoplasm was characterized by the absence of anisotropy and poorly discernible organelles. Solitary endothelioid cells containing tonofilaments (Fig. 2, *b*) and elongated or stellate epitheliocytes with ultrastructure typical of squamous epithelium were seen.

Ultrastructural organization of microcirculatory endotheliocytes was characterized by alteration of mitochondria and cytoplasmatic reticulum, focal lesions of the plasma membrane, and obliteration and deformation of endothelial contacts. The majority of endotheliocytes had flattened cytoplasmatic processes and electron-dense cytoplasm without pinocytotic vesicles and almost without organelles.

Autoradiography showed mosaic structural and functional organization of DNA and RNA synthesis in BE cells. Metabolic activity was appreciably decreased in epitheliocytes with signs of degeneration, silver granules were seen in few cells with the minimum label density.

High biosynthetic activity was observed in hyperplasia foci: ^{3}H-uridine incorporation in the majority of basal and intermediate cells was characterized by relatively high label density (LI 79.8-87.2%). The intensity of metabolic processes was reduced in sites with flattened and atrophic epithelium; LI with ^{3}H-uridine was no more than 70%. Hence, the distribution of cells labeled with ^{3}H-uridine in the epithelium was different in the same preparation, but due to foci of hyperplasia LI was very high in all cases: 80.6±5.8%.

A positive correlation between the level of protein production in bronchial epitheliocytes and endotheliocytes of subepithelial microcirculatory bed was observed in all cases. Label density of endotheliocytes was not high, LI with ^{3}H-uridine was 26.3-76.5%, mean value 53.2±18.1%. Silver granules were detected in solitary perivascular fibroblasts and pericytes.

Distribution of ^{3}H-thymidine labeled epitheliocytes was also uneven: in sites with pronounced bas-

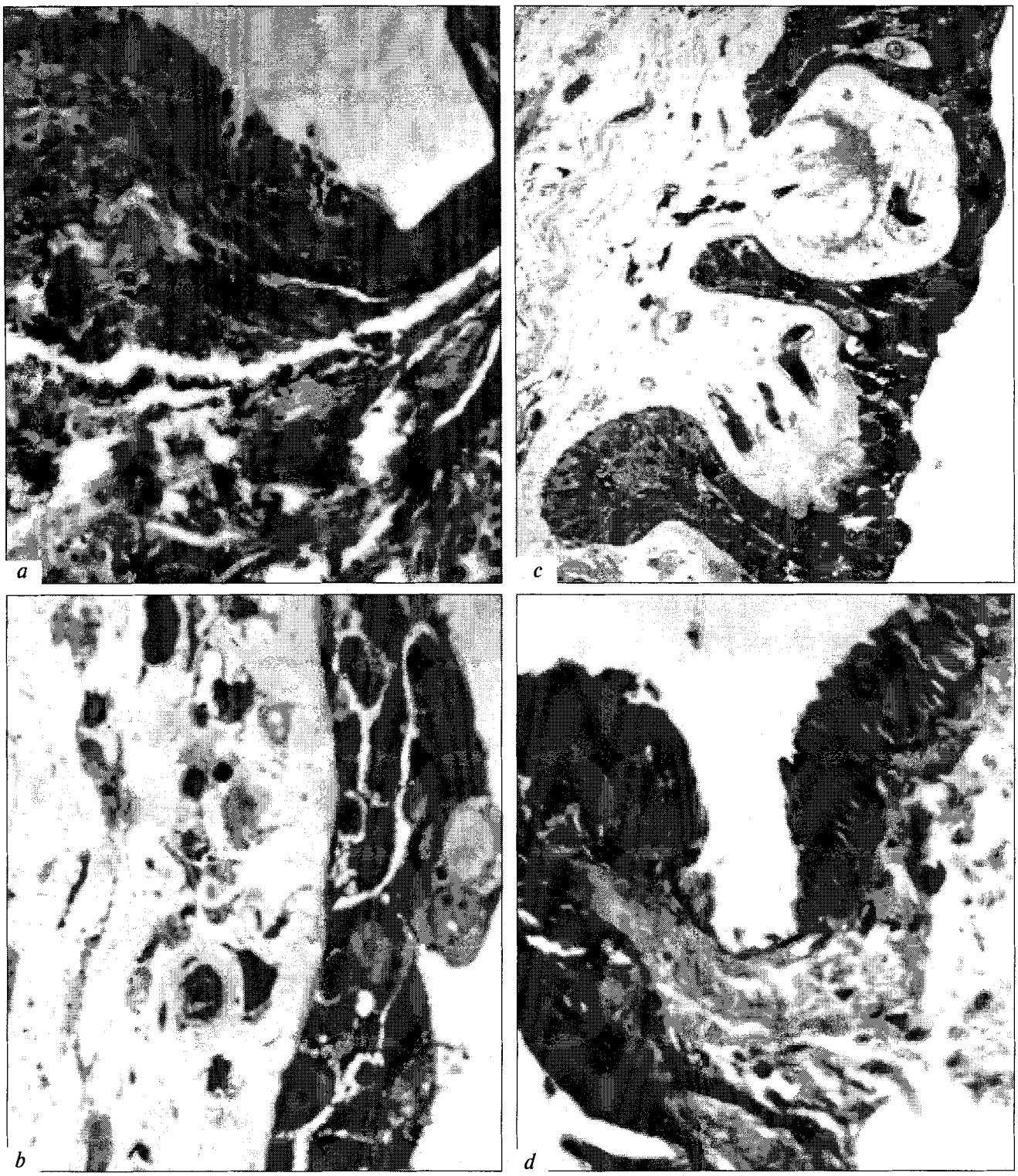

Fig. 1. Light microscopy of biopsy specimens of large bronchi in lung cancer. *a*) atrophy of bronchial epithelium (BE), thickened basal layer, Van-Gieson staining, ×160; *b*) BE transformation into stratified squamous epithelium, epitheliocyte degeneration. Semithin section, azur II staining, ×1000; *c*) epidermoid metaplasia of BE with formation of papillary processes. Semithin section, azur II staining, ×400; *d*) club-like process in the mucosa, heterogeneous BE. Periodic acid Schiff staining, ×160.

al-cell hyperplasia LI was 16.7%, in squamous-cell metaplasia no more than 4%, and in epithelial atrophy no label was detected.

The height of the bronchial epithelium greatly (coefficient of variations more than 50%), which corresponded to variations in its structural organization —

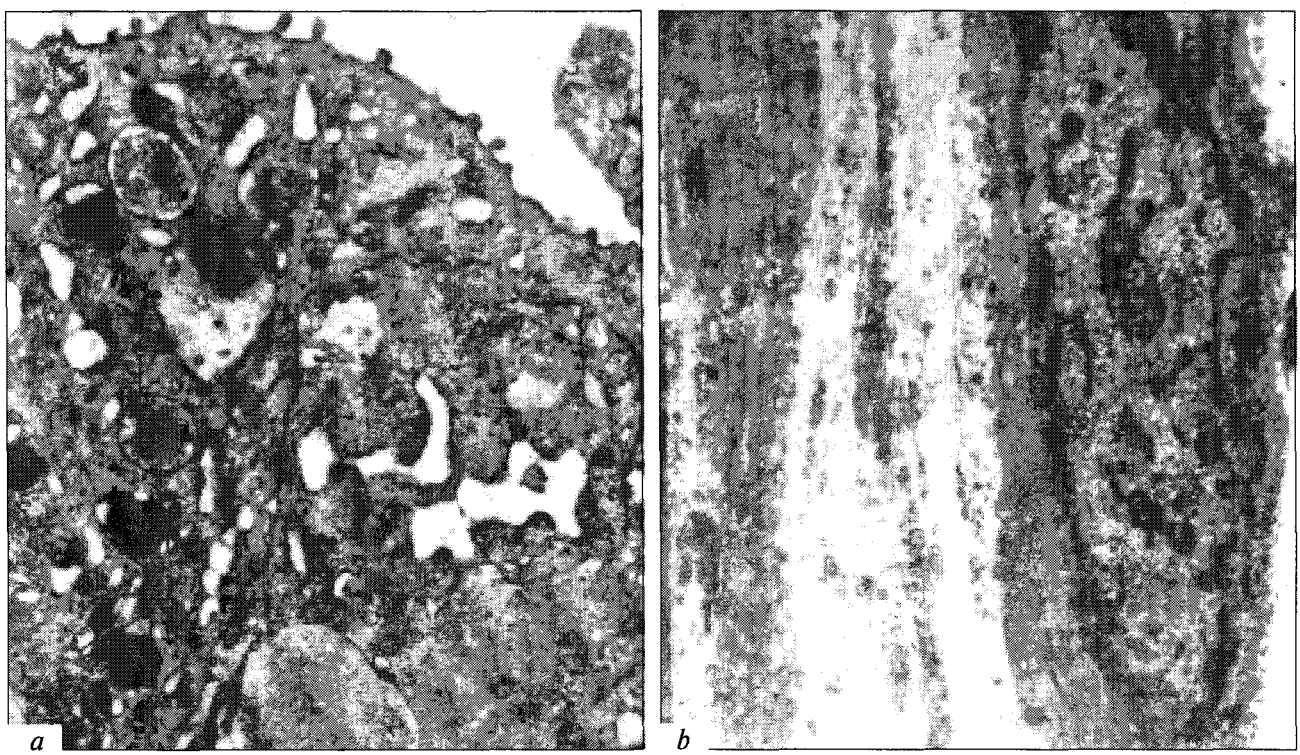

Fig. 2. Electron microscopy of bronchial epitheliocytes in lung cancer. *a*) destruction of cilia, formation of microvilli on the apical surface, alteration of cytoplasmatic organelles, ×12,000; *b*) endothelioid bronchial epitheliocyte, ×8000.

from endothelioid (7.68 μ) to squamous-cell metaplasia with maximum height of 254.72 μ (mean value 97.28±18.43 μ).

Hence, stereotypical structural reactions develop in the bronchial wall in lung cancer. These reactions are primarily progressive systemic atrophy of BE, not synchronous with ageing and epithelial "instability". This morphological phenomenon manifests by focal hyperplasia, metaplasia, atrophy, and dysplasia. The absence of inflammatory cell infiltration of the mucosa and other signs of chronic inflammation in the majority of biopsy specimens from patients with lung cancer suggest that these changes can be regarded as paraneoplastic bronchopathy. Disorders in biosynthetic reactions [7,8] associated with instability of BE differentiation and its phenotypical lability contribute to the development of this bronchopathy.

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