Pathomorphological and Immunohistochemical Analysis Biopsy Specimens from Large Bronchi of Patients with Pulmonary Cancer

G. I. Nepomnyashchikh, V. A. Levitskii, A. K. Dolgovykh, and L. A. Naumova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 8, pp. 227-232, August, 2002
Original article submitted July 10, 2002

Biopsy specimens from large bronchi of patients with central and peripheral pulmonary cancer were studied. Central cancer was associated with hyperplasia and dysplasia of the epithelium, hyperplasia of glandular compartment, and pronounced immunocompetent cell response. Peripheral cancer was characterized by predominance of atrophic and sclerotic changes, lymph flow disorders, and tendency to lymphoid aggregation. These data suggest that not only dysplasia, but also the combination of all structural changes typical of each form of pulmonary cancer should be considered as a morphological marker of enhanced risk of tumor development.

Key Words: *pulmonary cancer; bronchobiopsy; bronchial epithelium; precancerous changes; electron microscopy; immunohistochemistry*

Pulmonary cancer is usually revealed at late stages and characterized by low survival rate [3,4,10,13]. This substantiates the necessity of examining pathological changes preceding malignant transformation, in particular, the impairment of cell regulation, proliferation, differentiation, and death [4,12], and factors promoting the progress of pulmonary cancer from invasive growth to metastatic spreading. According to M. A. Pal'tsev *et al.* [8] this process is characterized by the formation of metastatic clone of tumor cells with increased resistance to apoptosis-inducing factors.

Recent studies proved that dysplasia of the bronchial epithelium is the main morphological marker of pulmonary precancer [1]. However, interpretations of the terms "precancer" and "dysplasia" and their application to bronchial epithelium are ambiguous; no distinct morphological criteria of pulmonary precancer were elaborated [3,9]. Further study of morphological changes in the bronchi and existing data will help to propose criteria for oncological risk groups.

The purpose of the present study was pathomorphological and immunohistochemical examination of large bronchi during central and peripheral pulmonary cancer with regards to systemic structural changes during tumorigenesis.

MATERIALS AND METHODS

Biopsy specimens of large bronchi were obtained from 81 patients (66 men and 15 women, aged 23-77) with central (CPC, n=44) and peripheral (PPC, n=20) pulmonary cancer at various TMN stages according to WHO classification, and from 17 patients with chronic unspecific pulmonary diseases (8 smokers and 9 nonsmokers). Histological types of tumors were determined according to WHO classification (1999). Most patients had squamous cell carcinoma, in some patients adenocarcinoma and small and large cell carcinomas were diagnosed. Bronchus specimens were

Laboratory of Ultrastructural Bases of Pathology, Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. ***Address for correspondence:*** pathol@cyber.ma.nsc.ru. Nepomnyashchikh G. I.

dissected perifocally (proximally and distally to the neoplastic focus) and from symmetric region of the opposite lung in CPC patients. In PPC patients, specimens from symmetric large bronchi from the right and left lungs were studied. In patients with benign pulmonary diseases, specimens were obtained from the second segmental bronchus of the right lung. A BF fibroscope (Olympus) was used.

For light microscopy, the specimens were fixed in 10% neutral formaldehyde. Paraffin sections were stained with hematoxylin and eosin according to Van Gieson, elastic fibers were post-stained with Weigert's resorcin-fuchsin. Periodic acid-Schiff reaction (PAS) was performed.

The specimens for electron microscopy were fixed in 4% paraformaldehyde, postfixed in 1% OsO_4, and after standard dehydration were embedded in eponaraldite. Semithin sections were stained with 1% azure II and Schiff reagent. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM 1010 electron microscope.

Immunohistochemical study was carried out by streptavidin-biotin method on serial paraffin sections after antigen demasking by boiling in retrivagen A solution. Ki-67 biotinylated universal antigens were used as primary and secondary antibodies, respectively. The reaction product was visualized using DAV chromogen (NovoCastra Laboratories Ltd).

RESULTS

Light microscopy of the specimens from large bronchi of CPC patients revealed homogenous structural changes manifested in instability of the bronchial epithelium [6] and hyperplasia of the secretory compartment associated with pronounced reaction of immunocom-

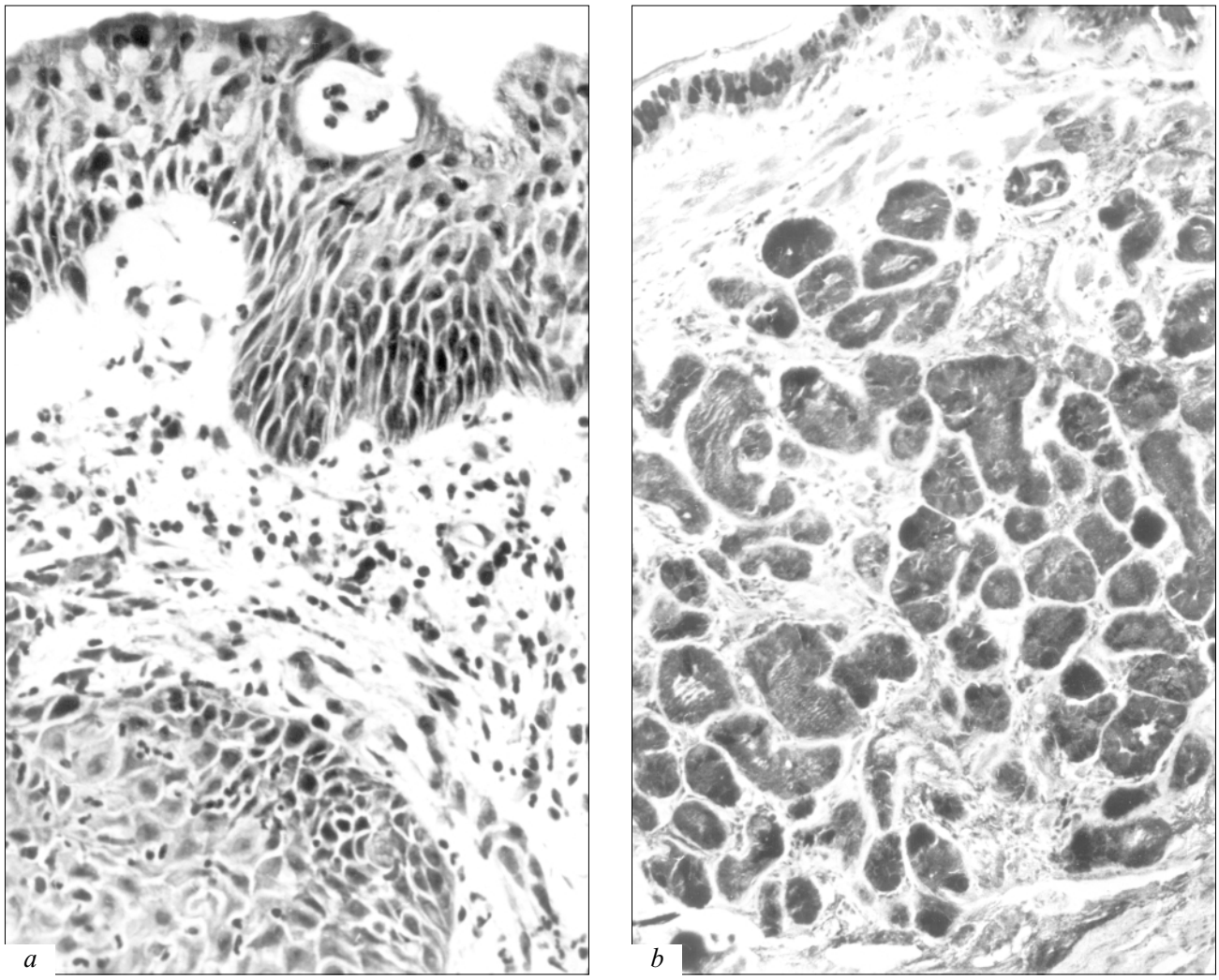

Fig. 1. Structural changes in large bronchi in patients with central pulmonary cancer. Bronchus specimens. *a*) epithelium dysplasia, intense polymorphocellular stroma infiltration. Hematoxylin and eosin staining, ×500; *b*) atrophy of bronchial epithelium and glandular hypertrophy. PAS-reaction, ×400.

petent cells (IC). Predominant changes in the epithelium were presented by goblet and basocellular hyperplasia and their combinations, dysplasia of varying degree with epitheliocyte disintegration and impairment of cell-cell contacts (Fig. 1, *a*) playing an important role in the regulation of proliferation [5,14,15]. The severity of dysplasia usually increased with approaching the tumor focus. Regions with partial or complete desquamation of the bronchial epithelium, its atrophy and squamous cell metaplasia were often seen. Damage to the ciliary apparatus observed in all specimens varied from minimum characterized by irregular arrangement of cilia (with the formation of apical cytoplasmic processes) to their complete absence. The severity of changes in ciliary apparatus correlated with dysplasia degree.

In most CPC samples, secretory function of mucus-forming elements was enhanced. The number of enlarged PAS-positive goblet glandulocytes increased and sometimes occupied 90% epithelium surface, goblet cell metaplasia, enlargement of terminal gland regions, and thickening of glandular layer of submucosa were seen (Fig. 1, *b*). These structural changes probably represent a compensatory adaptive response to damaging agents inhaled with air or during smoking.

IC presented by lymphocytes, plasma cells, and macrophages were localized intra- and subepithelially, perivascularly, and periglandularly. Sometimes they were diffusely spread in the mucosa and submucosa. Cell infiltrate sometimes contained neutrophils, eosinophils, and mast cells. The number of neutrophils and eosinophils located intraepithelially together with lymphocytes correlated with the degree of dysplasia. In severe dysplasia, the upper epithelial layer contained interepithelial cavities with dying cells, lymphocytes, neutrophils, and eosinophils (Fig. 1, *a*). Since interepithelial lymphocytes control the balance between proliferation and differentiation and possess cytotoxic activity [11], their accumulation during neoplastic process reflects both enhanced proliferation and cell death due to immune cytodestruction.

The main structural features typical of PPC were pronounced and diffuse atrophic and sclerotic changes, impaired lymph outflow, and moderate hyperplasia of the peribronchial lymphoid tissue. In all cases, partial or complete desquamation of the bronchial epithelium with exposure of basal cells or basal membrane (Fig. 2, *a*) was observed, which was regarded as an important marker of atrophy [7]. In most cases, regions with preserved epithelium were presented by fragments of ciliary epithelium consisting of 2-4 cell rows or flattened endothelium-like cells. The latter were arranged in 1-2 layers, lay far from each other, and alternated with zones of squamous cell metaplasia or cuboidal cells forming 1-2 layes without typical elements of stratified cylindric epithelium. Goblet and basocellular focal hyperplasia was weakly pronounced and observed in regions of cylindric epithelium. In some cases, degree I focal dysplasia was revealed mostly at the site of tumor (Fig. 2, *b*).

All PPC cases were characterized by significant thinning of the mucosa and submucosa associated with increased number and diameter of collagen bundles, destruction and fragmentation of elastic fibers, elastofibrosis of muscular bundles, impaired microcirculation, and cystic transformation of bronchial glands.

IC reaction in PPC patients was less pronounced than in CPC patients, however, it was associated with pronounced atrophic sclerotic changes. Hyperplasia of peribronchial lymphoid tissue was presented by intraepithelial and diffusely spread lymphocytes and their focal accumulation with the formation of subepithelial, perivascular, and periglandular lymphoid aggregates.

In most specimens from patients with PPC, coal particles localized perivascularly, along the lymphatic vessels, in interalveolar septa, and sometimes in alveolar lumens (coniophages) were found.

It should be noted that both in CPC and PPC patients, the types of structural reactions of symmetric bronchi were similar and the degree of pathological changes in the major structures varied insignificantly, which attested to multicentric character of the process. During pulmonary cancer, the content of oncomarkers in perifocal displastic tissues sometimes surpasses that in patients with unspecific pulmonary diseases [3]. Thus, our data on morphological changes in symmetric bronchi suggest that systemic instability of the bronchial epithelium can serve as a marker of oncological risk, which reflects not focal tendencies, but diffuse dysplastic reactions of the bronchopulmonary epithelium.

Microscopic analysis of the bronchial mucosa in patients with benign diseases revealed diffuse dystrophic and atrophic changes in the epithelium associated with focal hyper- and dysplasia presented by regions of basocellular, goblet, and mixed hyperplasia, dysplasia, squamous cell metaplasia, and with inflammatory cell infiltration and stroma sclerosis corresponding to the stage of the disease (Fig. 2, *c*). Changes in the epithelium indicate instability of cell populations in the bronchial mucosa reflecting a shift of dynamic balance between differentiation and proliferation.

Similar types of epithelial reactions were revealed in CPC patients and smokers, however, the level of changes in CPC was significantly higher. In nonsmokers, 2 types of structural reactions were revealed: type I was characterized by atrophic sclerotic changes combined with impaired lymph outflow, type II included increasing hyperplastic reaction and enhanced mucus formation.

Electron microscopy of bronchial epytheliocyte populations revealed heterogeneity of their ultrastuc-

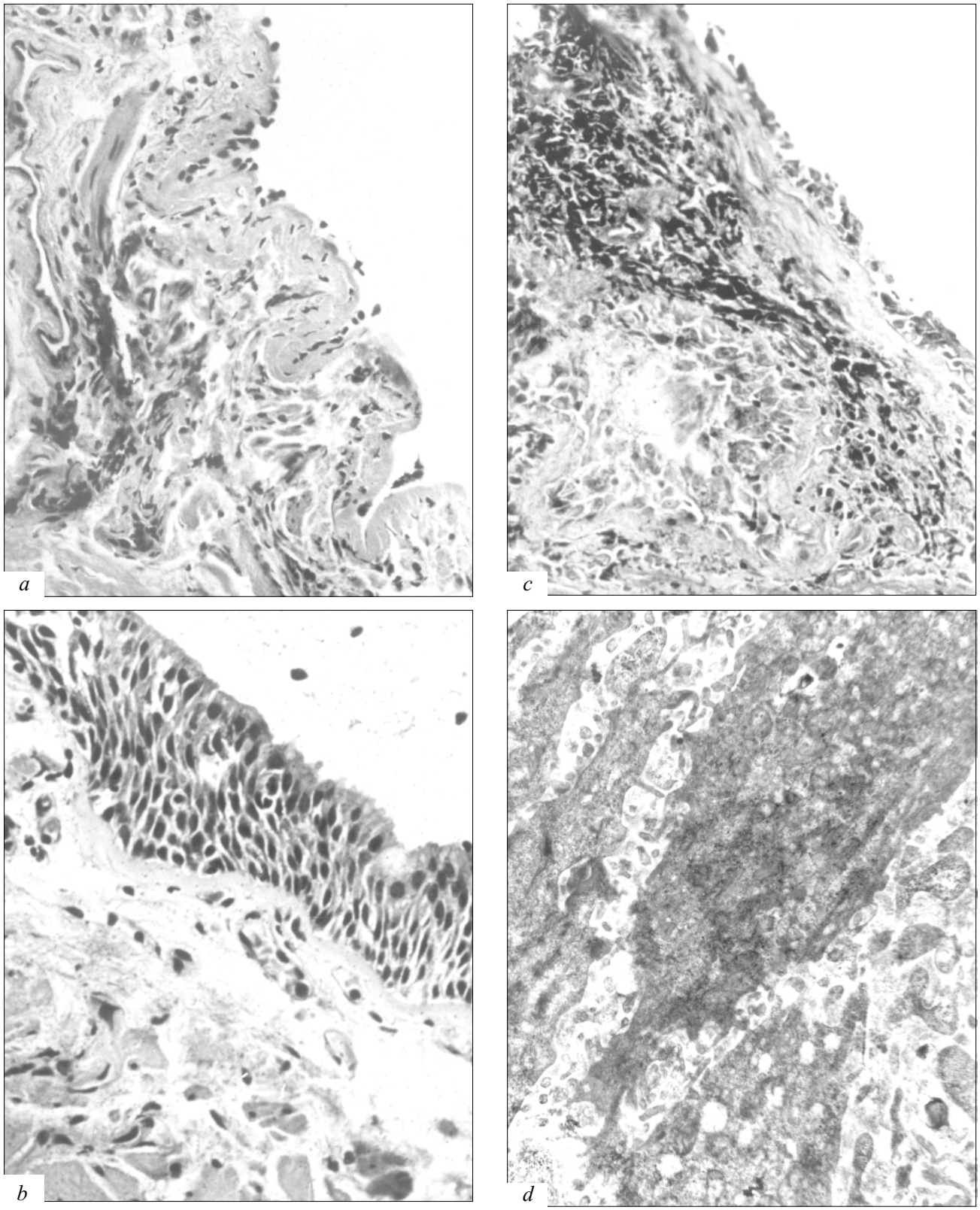

Fig. 2. Structural changes in large bronchi in patients with peripheral pulmonary cancer. Bronchus specimens. Hematoxylin and eosin staining, ×450 (*a*-*c*); electronogram, ×16,000 (*d*). *a*) atrophy and desquamation of epithelium, sclerosis of stroma; *b*) hyperplasia and dysplasia of epithelium; *c*) epithelium atrophy, mucosa hyperelastosis and sclerosis (smoker bronchus specimen); *d*) bronchial epitheliocytes: dense cytoplasmic matrix, impaired intercellular contacts.

ture. Atrophic epitheliocytes had reduced ciliary apparatus, dense cytoplasm with single elements of vacuolized and fragmented cytoplasmic reticulum, and destructed mitochondria. In the foci of basocellular hyperplasia, numerous polygonal cells with large irregular nuclei, dense cytoplasmic matrix containing single organelles, and numerous cytoplasmic processes were observed. The cells were separated by wide intercellular spaces (Fig. 2, *d*).

The state of goblet glandulocytes varied depending on epithelium changes. In zones of goblet cell hyperplasia, mucous epitheliocytes were overfilled with polymorphic secretory granules pushing the nucleus to the basal part of the cell. These cells contained hyperplastic cytoplasmic reticulum, Golgi complex, and free ribosomes. In some zones, goblet glandulocytes were less numerous, small, contained single dense, small secretory granules diffusely spread in the cytoplasm, and elements of Golgi complex and cytoplasmic reticulum.

Squamous cell metaplastic epithelium was presented by several cell layers with high number of desmosomes and interlacing cytoplasmic processes. The cytoplasm of these cells included tonofilament bundles and single keratohyaline granules. Vertically oriented basal cells had indistinctly structured cytoplasm and large nuclei. It should be noted that in all epithelium layers, intercellular spaces were sharply widened. In sites of dysplasia of the bronchial epithelium enlarged polymorph nuclei (sometimes of irregular shape), large nucleoli, high number of free polysomes, innumerous mitochondria with vacuole degeneration and crista destruction, and lesion of intercellular contacts were noted.

Analysis of proliferative activity in bronchial epithelium by Ki-67 level reflected phenotypic heterogeneity of epithelial layer. Zone of basocellular hyperplasia with atypic cells and dysplasia foci showed maximum epitheliocyte proliferative potential, while minimum proliferation was noted in basocellular hyperplastic zones without atypia and in the foci of squamous cell metaplasia. In atrophic epithelium, reaction product was revealed only in some nuclei or nucleoli.

Thus, large bronchi in CPC patients are characterized by the development of homogenous morphological reactions manifested as hyper-, dys-, and metaplasia, epithelium atrophy, and hyperplasia of glandular compartment associated with pronounced IC response. In PPC patients, predominating morphological sings were diffuse atrophic and sclerotic changes, lymph flow impairment, and tendency to lymphoid aggregation.

Polymorphic structural reactions in examined groups point to differences between CPC and PPC pathomorphogenesis and precancerous changes [2]. The obtained data suggest that in addition to dysplasia, the combination of all structural changes typical of each pulmonary cancer form should be considered as morphological marker of enhanced oncological risk. Complex approach comparing the intensity of proliferation and various types of cell death, necrosis, apoptosis, and terminal differentiation, is required for the prognosis of carcinogenesis [4].

REFERENCES

1. B. N. Zyryanov, V. S. Siyanov, S. A. Velichko, and N. A. Makarkin, *Pulmonary Cancer. New Approaches for Diagnosis and Treatment* [in Russian], Tomsk (1997).
2. E. A. Kogan, *Arch. Patol.*, No. 11, 76-83 (1989).
3. E. A. Kogan, I. A. Orlova, and G. Zhak, *Ibid.*, No. 2, 6-11 (2001).
4. E. A. Kogan and D. A. Ugryumov, *Ibid.*, No. 1, 33-37 (2002).
5. V. A. Krutovskikh, *Ibid.*, No. 1, 3-7 (2000).
6. G. I. Nepomnyashchikh, V. A. Levitskii, L. M. Nepomnyashchikh, *et al., Byull. Eksp. Biol. Med.*, **129**, No. 4, 470-474 (2000).
7. L. M. Nepomnyashchikh, V. A. Levitskii, G. I. Nepomnyashchikh, *et al., Ibid.*, No. 3, 347-351 (2000).
8. M. A. Pal'tsev, S. A. Demura, E. A. Kogan, *et al., Ibid.*, **130**, No. 7, 98-101 (2000).
9. *Pathoanatomical Diagnosis of Human Tumors*, Eds. N. A. Kraevskii, *et al*. [in Russian], Moscow (1993).
10. A. Kh. Trakhtenberg and V. I. Chissov, *Clinical Oncopulmonology* [in Russian], Moscow (2000).
11. M. Emoto, Y. Emoto, and S. Kaufman, *Immunology*, **88**, No. 4, 531-536 (1996).
12. K. Fong, Y. Sekido, and J. Minna, *J. Thorac. Cardiovasc. Surg.*, **118**, No. 6, 1136-1152 (1999).
13. F. Hirch, W. Franklin, A. Gazdar, and P. Bunn, *Clin. Cancer Res.*, **7**, No. 1, 5-22 (2001).
14. H. Yamasaki and C. Naus, *Carcinogenesis*, **17**, 1199-1213 (1996).