Exp Oncol 2020
42, 4, 310–313

Experimental ONCOLOGY

HISTOGENESIS OF CENTRAL LUNG CANCER: CYTOLOGICAL INVESTIGATION

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***Aim*: To study the features of the bronchial mucosa lesion in relation to centrally growing lung cancer (LC) according to diagnostic fibrobronchoscopy in comparison with the results of cytomorphological data to determine the possible origin of tumor growth (histogenesis). *Patients and Methods*: The data of fibrobronchoscopy and cytological findings based on the materials of 75 patients with a clinical diagnosis of LC were studied and compared. By the sum of the numerous cytomorphological features of epithelial cells and their components, the cells of the cylindrical epithelium and LC of various histological types were identified. The cells in bronchial smears and bronchial lavage were stained by Pappenheim and Papanicolau. Diagnostic material was examined by light microscopy. *Results:* We have found that in a part of the patients (49%), the tumors with exophytic growth in the bronchus are covered with a cylindrical epithelium, which indirectly indicates the origin of cancer growth under the epithelial layer. In cytological preparations of 51% patients, cancer cells were found, which confirms the tumor invasion into the bronchial mucosa. In 48 (64%) patients, fibrobronchoscopy revealed that the examined bronchus was compressed from 50% to pinpoint width, evidencing that tumor growth develops from the outside, peribronchially. *Conclusion*: The obtained data indirectly confirm the development of central LC from type II pneumocytes, which are found in the glands of the submucous membrane of the bronchus. However, it does not exlude the development of this type of LC from the basal cell of the bronchial epithelium.**

***Key Words*: cytological investigation, bronchial epithelium, fibrobronchoscopy, central lung cancer, histogenesis.**

DOI: 10.32471/exp-oncology.2312-8852.vol-42-no-4.15232

The consistent study of the onset, development, and treatment of cancer begins, as a rule, from experimental studies. This also applies to lung cancer (LC). This disease is the most frequent neoplasm in men in economically developed countries. According to updated data, the LC incidence in Ukraine reaches 60.3 and 13.5 per 100 thousand in men and women respectively [1]. It is known that currently LC is diagnosed most often at advanced stages III–IV, when a full complex of special treatment is difficult to apply. Therefore, the studies aimed at the issues of histogenesis can accelerate the development of prevention programs, LC diagnostics, effective therapy, and improved prognosis, which undoubtedly have important scientific, medical, and social significance.

The morphological aspects of LC development *in vivo* have been studied by many scientists [2–8]. The authors of the Moscow school of experimental oncology [7, 8] showed that when animals were injected with carcinogenic substances in different ways — through the respiratory tract, intravenously or intraperitoneally, tumors developed mainly in peripheral parts of the lung, under the pleura, that is, where the alveolar epithelium is localized. Consequently, it is the cells of the alveolar epithelium that are the source of the LC development. Nevertheless, in a few cases, tumors develop in the central bronchi.

Currently, the issues of stem cells (SC) of the normal lung and lung tumors, are widely studied [6, 9]. The structural-morphological complex of lung epithelial cells of the so-called "niche" has been studied. It includes the bronchoalveolar canal junction, the tracheal submucosal glands and the bronchial neuroendocrine tissue [10, 11]. The niches of epithelial cells attract particular attention when studying tumor processes in the lungs. According to one model, certain disturbances in these niches led to overexpression of the factors stimulating the SC to proliferate [12–14].

Sneddon and Werb [10] have proposed another model of SC — niche interactions. This model reflects the ability of the transformed SC themselves to activate a niche. Thus, there is a feedback, which leads to the proliferation of transformed SC. Researchers point on the possibility of transformed SC to activate not only their own niche, but also cells of neighboring niches, which also leads to the activation of tumor growth.

Experimental data allow clarifying the fact of the origin of the LC development from epithelial cells of the so-called niche. Scientists have concluded that type II pneumocytes are likely to belong to such cells. However, these literature data are not always unambiguous and often contradictory [14, 15].

Previously, we carried out complex multifactorial macroscopic, histological, immunohistochemical, cytological, cytogenetic, immunocytochemical and electron microscopy studies of the surgical material of LC patients. We have studied the structural changes of epithelial cells in bronchi and in peritumoral region [16–18]. Since the patients were operated mainly at stage III, it was not possible to conclude reliably on the onset of LC development, i.e. to follow LC his-

Submitted: August 15, 2019.
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Abbreviations used: LC – lung cancer; SC – stem cells.

togenesis. However, according to Willis concept [19], which is axiomatic in oncology, the tumor is surrounded by the tumor field where the different stages of development of pre-cancerous and cancerous lesions could be revealed. When we have attempted to study such a tumor field, that is, the peritumoral part of the resected tumors, our findings did not fit into the generally accepted concept on LC development from bronchial epithelium. Therefore, we required more complete information about the morphological and functional properties of the epithelial cells of conductive part of the lung: the bronchi and its respiratory region — alveolar epithelium.

Several fundamental studies [20–23] devoted to solving this question found that type II pneumocytes are one of the most intensively proliferating cell populations of the respiratory portion of the lung, which play a crucial role in the regeneration, dysplasia and atypia of the alveolar lining of the lung. In addition, the kinetics of transformation of type II pneumocytes into type I pneumocytes has been proven and documented in the research with the use of electron microscopic examination and autoradiography [23]. It was also noted that the respiratory portion of the lungs of humans and mammals is an actively secreting gland [24].

In other words, type II pneumocytes are germinative cells, which modern authors call SC. It has also been established that mitotic activity of interalveolar septum cells, which include type II pneumocytes, is 5–6 times higher than that in the bronchial epithelium [21]. The findings also confirm that type II pneumocytes were the most actively proliferating cell population, accounting for more than 50% of all mitoses in lung epithelial tissue [21, 22]. On the other hand, the time of renewal of the cylindrical epithelium of the trachea was found to be 47.6 days, the large bronchi — 18.1 days, medium bronchi — 7–10 days, small bronchi — 167–200 days, and in the interalveolar septum cells, that is, type II pneumocytes, was minimal — about 3–7 days [21].

The vast amount of experimental studies evidenced that it is type II pneumocytes that take a major role in the renewal and regeneration of the alveolar lining. This gives grounds to assume their high potential not only for proliferation, but also, under certain conditions, for dysplasia and atypia and for the development of LC. This applies to tumors that develop in the peripheral parts of the lung. The obtained convincing experimental data can be extrapolated to the LC development in humans.

The development of LC in the central parts of the lung is widely known but the cells of its origin remain obscure. This pattern of growth is widely recognized by pathologists, radiologists, and clinicians and is demonstrated in all pathological anatomy manuals. At the same time, the lesion area in the bronchi is small while peribronchial area is many (3–40) times larger [16].

Given the morphofunctional characteristics of type II pneumocytes, it was reasonable to assume the presence of this type of cells in the central regions of the lung. Our assumption is confirmed by the works of Esipova [20], who argues that the structure of the lung has similarities in its peripheral and central parts. This position is confirmed by Giangreco *et al.* [11]. The scheme presented by the authors shows that glands containing type II pneumocytes and Clara cells are located in the submucosa of the large bronchi. Given the above properties of type II pneumocytes (and they are always detected close to Clara cells), it is logical to assume that in the central parts of the lung LC could develop from type II pneumocytes. The cells of the glands of the submucosa may correspond to type II pneumocytes in morphological and functional characteristics. Based on histochemical studies, Kovalenko [25] came to the conclusion that all histological types of LC originate from the cells of the submucous bronchial glands. The author emphasized that the histochemical characteristics of tumor cells of a highly differentiated adenocarcinoma basically coincides with the metabolic characteristics of alveolar cells (type II pneumocytes) and this suggests that both central and peripheral LC develops from cells corresponding to the morphofunctional characteristics of type II pneumocytes.

The obtained experimental data are consistent with the results of our previous studies related to the SCs in the lung [16]. Therefore, the aim of our study was to analyze the afflicted zones of the bronchial mucosa in relation to centrally growing LC comparing the findings of diagnostic fibrobronchoscopy and cytomorphological data for elucidating the probable cell origin of central LC.

MATERIALS AND METHODS

The data of fibrobronchoscopy and cytological findings on the materials of 75 patients with a clinical diagnosis of LC were studied and compared. All patients provided an informed consent on the use of their materials for diagnostic and therapeutic purposes. The cytomorphological, morphofunctional and structural features of the cells were studied. The sizes, shape and staining of cells, cytoplasm, nuclei and nucleoli, the nucleus-to-cytoplasm ratio were taken into account. Particular attention was paid to the structure of the chromatin and nuclear envelope. Taking into account all these signs and the degree of their expression, the cells of a cylindrical epithelium and various histological types of LC were identified. Depending on the character of macroscopic changes and their prevalence detected by fibrobronchoscopy, as well as the results of cytological studies, the patients were distributed into five groups.

Group I — 18 patients with minimal changes in the bronchi — deformation of the wall of the bronchus, infiltration of the mucous membrane.

Group II — 4 patients in whom the pathological changes in the mucous membrane extended to 2–3 bronchi.

Group III — 24 patients with bronchus narrowed from the outside up to 50% of its lumen and infiltration of the mucous membrane.

Group IV — 24 patients with the most pronounced changes in the bronchial mucosa — narrowing of the bronchus from the outside up to 60% of its lumen or to pinpoint lumen, and infiltration of the mucous membrane of 2–3 bronchi.

Group V — 5 patients in whom only an exophytic tumor in the bronchus was clearly defined.

The surgical specimens of all patients were verified histologically. Cytological preparations were stained by Papanicolau and Pappenheim methods and studied by light microscopy using OLYMPUS BX41, OLYMPUS CX41 microscopes at different magnifications: × 100; × 200; × 400; × 1000.

RESULTS AND DISCUSSION

The procedure of fibrobronchoscopy involves the study of changes in the bronchial mucosa, the absence or presence of a tumor lesion, its macroscopic appearance, extension, density, change in the diameter of the examined bronchi depending on the size of the tumor and its topography, as well as obtaining informative material from the surface of the identified tumor or affected mucosa and preparing the specimen for cytological research.

In stained cytological specimens, the cells of the bronchial epithelium and cancer cells of tumors of different histological type and differentiation grade, sometimes with various dystrophic changes, could be identified (Figure).

However, not in all cases when tumor in the bronchus is ascertained macroscopically, cancer cells could be detected in cytological smears prepared from fibrobronchoscopical samples. In these cases, only cells of the bronchial epithelium could be found that indirectly may indicate the growth of the tumor under the mucous membrane of the bronchus.

The percentage of different patterns of the changes in the bronchial mucosa, the presence of exophytic and endophytic tumors, growing peribronchially and significantly narrowing the lumen of the bronchus from 50% to pinpoint width, as well as the results of cytological studies are given in the Table.

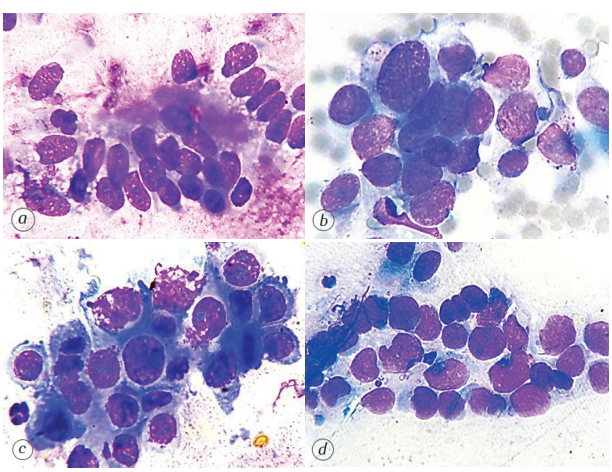

Figure. Cytological preparations of the material obtained by fibrobronchoscopy. Cells of the bronchial epithelium (*a*), glandular cancer (*b*), squamous cell carcinoma (*c*), and undifferentiated small cell LC (*d*). Staining by Pappenheim. × 1000

Table. The results of cytological studies on exfoliative material of fibrobronchoscopy

Fibrobronchoscopy findings	Number of patients	Tumor cells found
Deformation of the bronchus, minimal changes in the mucous membrane of the bronchus	18	2 (11%)
A common change in the loosened mucous membrane in several bronchi	4	3 (75%)
Constriction of the bronchus due to compression by a new formation from the outside, mucosal infiltration	24	16 (67%)
The narrowing of the bronchus due to compression by a new formation from the outside, exophyte and mucosal infiltration	24	16 (67%)
Exophytic tumor in the bronchus	5	1 (20%)

As can be seen from the Table, tumor cells in the smears from the lesions in bronchial mucosa are detected only in part of the cases with pronounced gross changes in the bronchial mucosa such as presence of an exophytic tumor, compression of the bronchus from the outside, infiltration of the bronchus mucosa detected by fibrobronchoscopy. In particular, in the presence of only an exophytic tumor in the bronchus, cancer cells were detected only in one from five observations in a smear from such a surface (Table). In the remaining 80% of observations, it turned out that the tumor is covered with cells of the bronchial epithelium, which are defined in the cytological preparation and may indirectly indicate that the surface of the exophytic tumor is covered by the bronchial epithelium. Therefore, the underlying tumor does not penetrate through the layer of the bronchial epithelium.

Upon studying 75 cases with different gross alterations revealed by fibrobronchoscopy, cancer cells in cytological smears were detected only in 38 (51%) cases, where the tumor grew through the bronchial mucosa and cancer cells could be detected in a smear from its surface. At the same time, in 37 (49%) patients in smears from the visibly changed mucous membrane of the bronchus, only cells of the bronchial epithelium could be detected, evidencing on an absence of LC penetration through epithelium. The data obtained may indicate that in this group of patients, the bronchial mucosa is covered with typical cylindrical epithelium and the tumor grows below the epithelial layer.

Analyzing another parameter of tumor growth, namely the pattern of growth relative to the bronchial wall, we have found out that in 48 (64%) of 75 patients the examined bronchus was compressed from 50% of its lumen to a pinpoint width, indicating peribronchial growth of the tumor. These data confirm indirectly the development of the central LC from the cells that are related to type II pneumocytes by their morphofunctional features and are found in the submucosal glands of the bronchus. This is confirmed in experimental and pathoanatomical studies by several authors [11, 21, 26].

Therefore, based on our findings, central LC might well originate from type II pneumocytes [11, 21]. These cells are characterized by the highest mitotic activity, proliferation, regenerative capacity, and the shorter renewal periods as compared with the cylindrical epithelium of the bronchial tree [22, 23]. Given the listed properties of type II pneumocytes, many authors at-

tribute them to SC [10–12, 21]. The results of our study are corroborated by several independent experimental studies strictly demonstrating the location of II type pneumocytes in the submucous glands of the bronchi and in the alveoli from which LC can develop [10–12].

In the aspect of studying the LC histogenesis, we do not understand the reasons why the bronchoalveolar type of LC was excluded from the list of nosological forms in the new edition of International Histological Classification of LC [26]. Moreover, the term "adenocarcinoma lepidic" (8250/3*) proposed in this new edition does not reflect the histogenesis of this LC type [26].

To sum up, this study allows us to state that the central LC does not develop from the bronchial epithelium, which is the most stable epithelial structure of the lung. As evidenced by convincing histological studies by Nepomniachtchi *et al.* [27, 28], the central LC originates from the epithelium of the submucous glands of the bronchi [25]. These cells are characterized by the morphofunctional and proliferative features resembling type II pneumocytes, which is also confirmed by the results of several experimental studies [2, 11].

The results of our studies comparing fibrobronchoscopic and cytological data, confirm the development of central LC from the cells of the basal (germinal) cells of the bronchial mucosa or its submucous glands, since scraping the surface of an exophytic tumor contains only cells of mature columnar epithelium. The obtained results underlie the possible strategy of LC prevention and treatment. We hope that our studies will be interesting for scientists and useful for pathologists, radiologists, clinicians and, possibly, for international experts in compiling the relevant international histological classifications of LC.

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