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CYTOLOGICAL RESEARCH OF EXOPHYTIC TUMORS OF THE BRONCHI AND THE GROWTH PATTERN OF LUNG CANCER

Background. *The study of the growth of lung cancer (LC) has an important clinical significance for morphological verification, the choice of the treatment method, and the determining of prognosis. Investigation of this question allows to clarify the histogenesis of LC. The* ***aim*** *of our study was to compare the results of cytological studies of the material obtained during flexible bronchoscopy (FBS) and scrapings from the operated tumors of the bronchi to clarify the nature of LC growth.* ***Design.*** *To study the growth of tumors in the bronchi in relation to the bronchial mucosa, the cytological examination of the material obtained by FBS and scrapings from the surface of the operated bronchial tumors of 31 patients has been performed.* ***Results.*** *In the preoperative period, in the material of FBS, tumor cells were found only in 1/3 of patients. To obtain the optimal material from exophytic tumors of the bronchus, scrapings were carried out from the entire surface surgical material of the same patients. Cytological preparations of the obtained scrapings confirmed the presence of cancer cells in more than 1/3 of patients. In the rest of the observations, scrapings from the tumor surface contained only cells of the cylindrical epithelium.* ***Conclusions.*** *The cytological investigations made it possible to state that in 2/3 scrapings from surfaces of exophytic tumors of the bronchus contained only cells of the cylindrical epithelium; therefore, the tumors grow under it.*

Keywords: cytological research, flexible bronchoscopy studies, scrapings from operated tumors, growth pattern of lung cancer.

Introduction

The morphological diagnosis of tumors begins from a cytological examination of exfoliative or the punctured materials of the neoplasm. The diagnosis of lung cancer (LC) utilizes the method of flexible bronchoscopy (FBS), which makes it possible to determine the spread of the pathological process in the bronchi or to suggest a peribronchial growth. Wherein, the mucous surface of the bronchus is not often changed macroscopically, but the diameter of the bronchial lumen is narrowed at varying degrees. In such a case, the doctor notes the narrowing of the bronchus due to the peribronchial growth of the tumor. In all cases, during FBS the lavage is taken from the bronchi for cytological diagnosis, and in the presence of an exophytic tumor, a scraping is taken from the surface of the neoplasm.

For many years the diagnostic investigations of bronchial lavage and smears from the tumor visible by FBS have shown that cancer cells were not always detected in cytological smears [1]. Therefore, it was necessary to clarify the issues of the morphofunctional structure of the lung in the normal state and during the process of LC development.

The published data indicate that the structure of the lung is similar to a complex alveolar-tubular gland, which predetermines the development of LC from cells of the glandular epithelium [2,3].

In the lung, the cells of glandular genesis have a different morphofunctional structure, which depends on their location and function. A number of authors have described and shown on schemes the structure of the lung at its anatomical root, which morphologically resembles its peripheral part. Bronchioles with acini branch off the large bronchi, the latter are most typical for the peripheral parts of the lung [4,5].

The fundamental research carried out allowed to establish that a terminal bronchiole is a functional unit of the lung, which integrally reacts to various pathological processes. This definition was approved by an international nomenclature [6]. A similar structural type of the lung parenchyma lesion is confirmed by pathological investigation [7].

A comprehensive histological and enzymohistochemical study of the central LC has revealed that all its histological types have one origin in the form of the glandular epithelial cells [8]. Recently, scientists have been studying a significant issue – the presence of stem cells (SC) in the lung, from which the lung epithelium can be restored in case of its damage. This is also important for clarifying the origin of various pathological proliferative processes, particularly LC [9–12].

The electron microscopic studies in combination with autoradiography method have shown that type II pneumocytes (AE2) are lung SCs [3,11].

For a more complete characterization of AE2, we have studied special literature. Several authors have found that AE2 is characterized by the highest mitotic activity and renewal in comparison with other lung epithelial cells [13,14]. It is the SCs of the lung that are characterized by the signs of proliferation and the ability of active overgrowth, which can lead to the LC development [9,10,15–18].

The structural and morphological complex of lung epithelial cells, the so-called "niches", in which AE2 are located, has also been studied [19–21]. The prolonged exposure to a toxic chemical or biological factor can lead to the development of hyperplasia, unrestrained multiplication, growth, and atypia of AE2, which is observed during carcinogenesis [21,22].

The features of the SCs in a certain niche and their influence on neighboring cells have been investigated. Scientists point to the possibility of transformed SCs to activate not only the cells of their niche, but also of neighboring niches, which leads to the onset and spread of proliferation, and further development of various pathological processes and LC [12,19,21,22]. There are also known morphological studies of several authors who received more specific additional information about the SCs. They argue that AE2 are pluripotent SCs, from which adenocarcinoma and squamous cell LC can develop [23].

Also, the conducted genetic studies allowed to determine the most common triple aberrations in the cells of the main histological LC types, which can also confirm their common origin [18,24].

Interesting data were obtained during the morphological study of undifferentiated LC forms. Scientists have concluded that the studied histological preparations contain cells with complex double, and in some cases – triple phenotypic differentiation [25]. This fact also confirms a common origin of various histological LC types.

Moreover, well-known morphologists have noted that the exact origin of squamous cell LC has not been established. The authors suggest that the source of LC is a pluripotent precursor cell of the bronchial mucosa, which can differentiate into any histological type of LC [26]. Besides, glandular-squamous cell LC is found in 10 % of cases, and it is natural to assume a single origin of its development [27].

We are impressed by the hypothesis on the existence of a single pluripotent cell, from which the main histological LC types develop, but there is still no unambiguous opinion on this issue. In this regard, additional research is required.

Up-to-date, one could founda term in the literature – bronchogenic LC, which implies its development from the bronchial epithelial cells. At the same time, special studies have shown that the cells of the cylindrical epithelium of the bronchi are the most differentiated and resistant to external influences [3,28].

Credibly, this applies to the mature cells of the cylindrical epithelium. But its basal cells, which can be called germinal cells, grow and differentiate, and could give start to such well-known processes as proliferation, metaplasia, dysplasia, and malignant transformation. It is increasingly suggested that bronchial epithelial cells possess the SC potential [26].

Most likely, the toxic substances present in the inhaled air can have an insignificant effect on the mature columnar epithelium, but diffusing through it or the intercellular spaces, they can exert a stimulating effect on the basal cells, which could be attributed to the SC of the bronchial epithelium [19].

On the other hand, a large area (from 20 to 100 m^2) of the alveolar lining of the lung, including the lung SC, is constantly exposed to chemical or biological harmful substances inhaled from the air, causing reactive changes in the form of hyperplasia, dysplasia, and neoplasia.

Considering the literature information concerning the structural features of the lung, the characteristics and localization of SCs, as well as the possibility of LC development from a pluripotent cell, one could agree that pathological processes and LC may develop from the bronchiolo-alveolar structures in which SCs are located. The thesis of Esipova I. [6] that all pathological processes in the lung originate from the terminal bronchiole confirms such a statement.

In relation to the mentioned data, indicating different points of view on the origin of LC, the aim of the study was to compare the results of cytological studies of the material obtained during FBS and scrapings from the operated tumors of the bronchi to clarify the nature of LC growth.

Materials and methods

We have investigated cytological smears of 31 LC patients who were examined and treated at the National Cancer Institute of the Ministry of Health of Ukraine. After a diagnostic FBS and a cytological assessment of the material, all patients underwent surgical treatment. The results of cytological studies of materials obtained by FBS were compared with

scrapings from the surface of the operated tumors. All observations were verified by the histological method following the International Histological Classification [27]. Materials of patients with different histological LC types were studied. Squamous cell carcinoma was verified in 19 patients, glandular carcinoma in 10 patients, and small cell (undifferentiated) cancer in 2 patients. Cytological preparations were stained by Papanicolaou and Pappenheim methods. Microscopic studies were carried out using an Olympus BX41 microscope: X100; X200; X400; X1000.

Results

At the preoperative period, cytological studies of the material obtained by FBS from 31 patients were performed. Lavage from the bronchi has been taken, as well as the smears from the exophytic tumors. Exophytic neoplasms were revealed in 25 patients: only exophytic growth was present in 17 cases, endo-peribronchial growth – in 8 cases. In 6 patients, only the peribronchial LC growth was determined with a moderate roughness of the pale pink mucous membrane. Based on FBS studies, tumor cells were found in cytological preparations of 9 (29 %) patients. In the material of 22 (71 %) patients, tumor cells were not found; the cylindrical epithelium of the bronchus with the signs of proliferation was determined (Table).

During the cytological examination of the FBS material of 17 patients with exophytic tumors, cancer cells were detected only in 4 (23.5 %) patients. In a case of endo-peribronchial tumor growth (8 patients), cancer cells were found in cytological smears of 3 (37.5 %) patients. Peribronchial tumor growth was detected in 6 patients, and in 2 (33 %) cases tumor cells were found in cytological preparations from the lavage material. That is, in the absence of visible exophytic lesions, tumor cells were identified in bronchial secretions.

Thus, we have revealed the tumor cells in cytological preparations obtained by FBS only in 7 from 25 (28 %) patients with an endobronchial tumor, and in 18 (72 %) patients cancer cells were not detected, just the cylindrical epithelium of the bronchi. This results in a low level of cytological diagnostics of LC despite the presence of an exophytic tumor in the bronchi (Table).

In this regard, the results of cytological studies of the FBS material and scrapings from the surface of the operated exophytic tumors of the bronchi have been compared. A macroscopic examination of the surgical material of 31 patients has been conducted. In 25 cases, an exophytic component of the tumor in the bronchus with dimensions from 0.3 to 1.3 cm in diameter was revealed, which is confirmed by the results of FBS. Scrapings were made from the surface of all exophytic tumors, and cytological preparations were prepared and examined. The Table shows the results of cytological studies of the operated material (Table).

When studying the materials with the presence of exophytic tumor growth in the bronchus (17 cases), in scrapings from the operated tumors, cancer cells were found only in 5 (29 %) patients, and in the remaining 12 (71 %) patients, only cylindrical epithelium cells were found. The cells of the bronchi show the signs of proliferation and dystrophy.

In the scrapings from the exophytic surface of 4 (50 %) from 8 patients with endo-peribronchial tumor growth, the tumor cells were found, and in the remaining 4 (50 %) cases only bronchial epithelial cells were detected.

In the scrapings from the rough surface of the bronchial mucosa of 6 patients with absent exophytic tumor growth (just the peribronchial component), cancer cells were found in 3 (50 %) patients; in the cytological preparations of the remaining 3 (50 %) patients only the cells of cylindrical epithelium were found. The results indicate that a slightly altered mucosa without an obvious exophytic tumor contains cancer cells possibly originating from a tumor which is not macroscopically determined yet.

In the presence of obvious exophytic tumor components in the operated tumor scrapings of

Table. **The results of cytological research of FBS and scrapings from the operated exophytic tumors**

№	Tumor growth in relation to the bronchial wall	Number of patients		The presence of tumor cells			
				Flexible bronchoscopy materials		Surgical materials (the scraping from exophytic tumors)	
		Abs.	%	Abs.	%	Abs.	%
1	Exophytic	17	55	4	23.5	5	29
2	Endo-peribronchial	8	26	3	37.5	4	50
3	Peribronchial	6	19	2	33.3	3	50
	Total:	31	100	9		12	

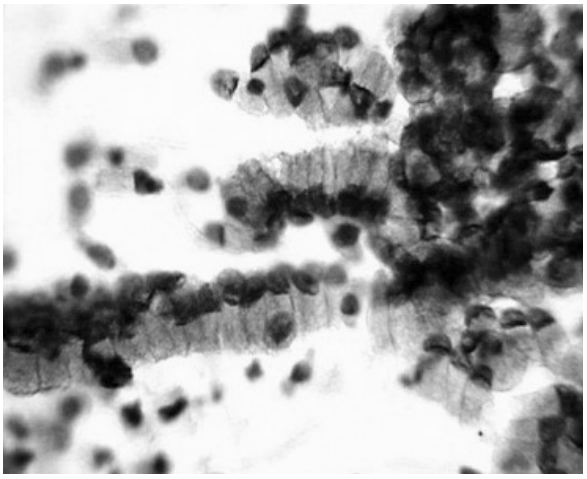

Fig. 1. Cells of cylindrical epithelium, Papanicolaou staining, X200

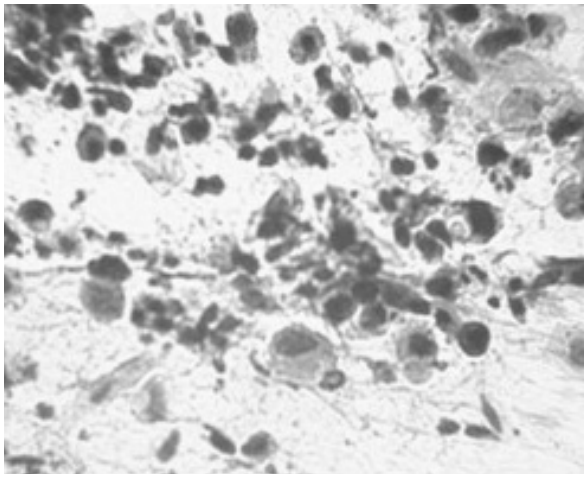

Fig. 2. Squamous cell carcinoma, Papanicolaou staining, X200

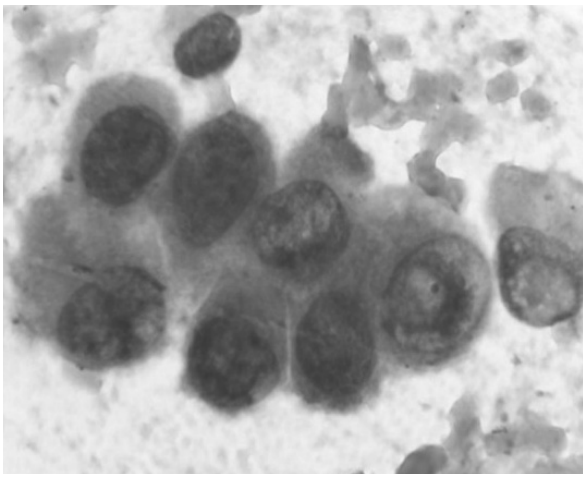

Fig. 3. Adenocarcinoma, Pappenheim staining, X400

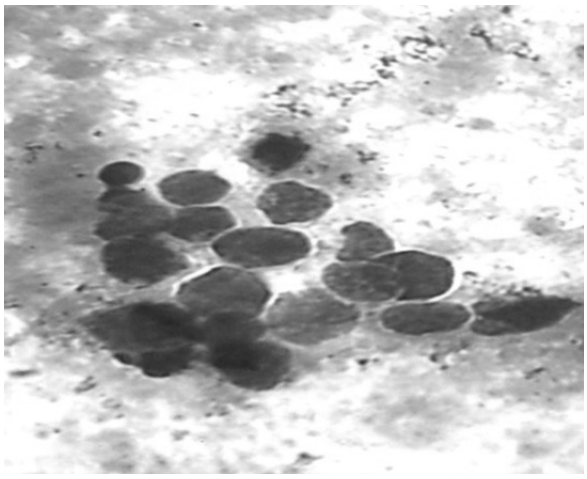

Fig. 4. Small cell carcinoma, Pappenheim staining, X400

Figures: Scrapings from the surface of exophytic cancerous tumors in the bronchus

25 patients, in 16 (64 %) cases the tumor surface was covered with unchanged cells of the cylindrical epithelium, as evidenced by cytological studies. The obtained data explain the low percentage of positive results of cytological LC diagnostics based on FBS materials due to tumor growth under the cylindrical epithelium.

The results of the study allow to conclude that a tumor invading the bronchial mucosa was present only in 12 (39 %) of 31 patients. This is evidenced by cancer cells found in cytological preparations from the scrapings from the operated tumors (Table).

In the remaining 19 (61 %) cases, in the scrapings from the operated exophytic tumors only the cells of bronchial epithelium with some signs of proliferation and dystrophy were found as well as a slightly altered or macroscopically unchanged bronchial mucosa. Cytomorphological features are presented in cylindrical epithelium cells (Fig. 1), squamous (Fig. 2), glandular (Fig. 3), and small cell cancer (Fig. 4).

Discussion

It has now been accepted that lung stem cells (SC) are AE2, characterized by the fastest renewal and the highest mitotic activity in comparison with other epithelial cells of the lung [3,11,14]. The well-known statement of I. Esipova [5,6] is that all processes in the lung begin from bronchiolo-alveolar structures which grow in lung parenchyma and at its lung root. The bronchiolo-alveolar structures in which the SC are located branch off the large bronchi, and it can be assumed that LC develops precisely from the stem cells that are located in these structures.

However, central LC, as it is claimed by several authors, develops from the epithelium of the bronchi [23,26]. Morphologists know that the cylindrical epithelium of the bronchi is an epithelial structure the most resistant to external influences [28]. Based on this, it is logical to assume that the central LC can develop from the basal cells. Using macroscopic

examination, we have established that the endobronchial part of the tumor is 0.3-1.3 cm in diameter, while the peribronchial part is by tens times larger [16]. A similar pattern is described in special monographs, in the International Histological Classification [27], in pathological anatomy textbooks. How can these data be explained?

In this investigation, we have found that out of 25 exophytic tumors in the bronchus, cancer cells in the FBS material were found only in 7 (28 %) cases, and in 9 (36 %) cases of the scrapings from operated tumors. In the rest of the cases, only slightly changed cells of the cylindrical epithelium were revealed in the scrapings, which indicated the growth of the tumor under the cells of the cylindrical epithelium. These data are consistent with the results of pathological studies of A.A. Have-Opbroek et al., confirming two sources of LC development – pluripotent cells (AE2), and bronchial epithelial cells. The results of our study confirm the opinion of W.D. Travis et al. [26] that the origin of squamous cell LC remains unknown, however the presence of a pluripotent cell of the bronchial epithelium, which may be a precursor differentiating into any histological type of LC, could be supposed.

We have obtained reliable data on the growth of LC in relation to the bronchial wall, contributing to the study of LC histogenesis and justifying the necessity of further in-depth morphological studies.

Conclusions

1. The centrally growing LC, regardless of its histological type, in 25 (81 %) of 31 patients is manifested by exophytic growth in the bronchus. At the same time, in cytological preparations obtained by FBS, only in 7 (28 %) patients it was possible to verify LC.

2. In the preparations of 18 (72 %) patients only the cells of the cylindrical epithelium, partially with the signs of proliferation, have been detected. The results of the study have substantiated the limitations of LC verification in exfoliative FBS materials. It could be explained by the growth of a tumor under the intact cylindrical epithelium of the bronchus.

3. To clarify the growth of LC in the bronchus relative to its mucous membrane, scrapings were performed from the surface of the operated tumors. In cytological preparations of 9 (36 %) of 25 cases of exophytic tumor growth, cancer cells were found, while in 16 (64 %) cases only the cells of the cylindrical epithelium were determined. All tumors were verified by the histological method.

Statements. The study was performed within the project of the Ministry of Health of Ukraine "To Study the Histogenesis of Lung Cancer Using a Set of Modern Morphological Research Methods to Improve the Efficiency of Diagnosis".

Statement of Ethics. Compliance of the study with bioethical standards in accordance with 1964 Helsinki declaration and its later amendments was approved by the Ethics Committee of the National Cancer Institute, Kyiv, Ukraine. All patients provided their informed consent on the participation in the study.

Conflict of Interest Statement. The authors declare no conflicts of interests.

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Author Contributions

Lidiya Bolgova has studied special literature, collected, evaluated, and compared the surgical material of the LC patients and examined the materials obtained by the flexible bronchoscopic method. She wrote the article body and conclusions.

Tamara Tuganova has studied the special literature, performed research on cytological material obtained by flexible bronchoscopy, and participated in the formulation of the conclusions.

Anna Ponomarenko has analyzed the results of cytological examinations of flexible bronchoscopic and surgical material, created the Table, and was responsible for the design of illustrative material.

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ЦИТОЛОГІЧНІ ДОСЛІДЖЕННЯ ЕКЗОФІТНИХ ПУХЛИН БРОНХІВ І РІСТ РАКУ ЛЕГЕНІ

Вступ. Дослідження росту раку легені (РЛ) має важливе клінічне значення для морфологічної верифікації, вибору методу лікування та визначення прогнозу. Дослідження цього питання дає змогу уточнити гістогенез РЛ. **Мета** цього дослідження – порівняти результати цитологічних досліджень матеріалу, отриманого під час гнучкої бронхоскопії (ГБС) та шкребків з оперованих пухлин бронхів, щоб з'ясувати природу росту РЛ. **Матеріали і методи.** Для вивчення росту пухлин у бронхах щодо слизової оболонки бронхів проведено цитологічне дослідження матеріалу, отриманого за допомогою ГБС та шкребків з поверхні оперованих пухлин бронхів, 31 пацієнта. Всі матеріали було верифіковано гістологічним методом згідно з Міжнародною гістологічною класифікацією пухлин. Було досліджено сквамозний рак у 19 (61 %) хворих, аденокарциному у 10 (32 %) та дрібноклітинний (недиференційований) рак у 2 (7 %) пацієнтів. **Результати.** У передопераційний період у матеріалі ГБС пухлинні клітини було виявлено лише у 1/3 пацієнтів. Для отримання оптимального матеріалу з екзофітних пухлин бронхів робили шкребки з усієї поверхні хірургічного матеріалу тих самих пацієнтів. У цитологічних препаратах отриманих шкребків підтверджено наявність ракових клітин більш ніж у 1/3 пацієнтів. У решті спостережень шкребки з поверхні пухлини містили лише клітини циліндричного епітелію. Під час вивчення матеріалів, отриманих шляхом ГБС та шкребків з поверхонь оперованих новоутворень, пухлинних клітин не виявлено у разі екзофітного росту у 8 (53 %) пацієнтів, у разі ендо-перибронхіального – в 1 (13 %), у разі перибронхіального росту – в 1 (17 %) хворого. **Висновки.** Цитологічні дослідження дали змогу стверджувати, що у 2/3 шкребків з поверхонь екзофітних пухлин бронхів були наявні лише клітини циліндричного епітелію. Імовірно, в таких випадках не відбувалось проростання пухлинами бронхіальної стінки. Проведені дослідження показали, що розвиток ракової пухлини починається під циліндричним епітелієм.

Ключові слова: цитологічні дослідження, гнучка бронхоскопія, шкребки з оперованих пухлин, ріст раку легені.

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