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ORIGINAL ARTICLE

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# PRACTICAL EXPERIENCE OF A LUNG CANCER PRIMARY CELL CULTURE COLLECTION CREATION AT THE NATIONAL MEDICAL RESEARCH CENTRE FOR ONCOLOGY

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## ABSTRACT

**Purpose of the study.** Testing of new chemotherapeutic agents in translational and biology medicine needs studies on immortalized cell lines. However, such models do not always have the biological properties of a tumor *in situ*, in contrast to primary cell cultures. Primary cultures of lung cancer cells have biological, morphological and molecular characteristics close or identical to tumor cells *in vivo*. Obtaining collections of primary lung cancer cell lines is an important task in creating various models for preclinical studies.

**Materials and methods.** The materials are represented by postoperative tumor samples obtained from 25 patients with newly diagnosed lung cancer without prior treatment. The following methods were used to obtain primary cultures: enzymatic dissociation in Hanks' solution with the addition of 300 units/ml collagenase I (Thermo Fisher Scientific, USA), enzymatic dissociation using the Brain Tumor Dissociation Kit (Miltenyi Biotec, Germany) and 150 units/ml. ml of collagenase I, as well as the method of explants. The following methods were used to remove fibroblasts: the use of the FibrOut™ system (CHI Scientific, USA), magnetic separation of fibroblasts using Anti-Fibroblast MicroBeads (Miltenyi Biotec, Germany), and cold trypsinization.

**Results.** We have obtained 15 primary lung cancer cell cultures that have passed the zero order passage. In this work, the method of enzymatic dissociation turned out to be the most effective. Incubation of lung tumor samples with collagenase for 1 hour preserves the viability and adhesiveness of the cells. The explant method did not show its effectiveness for long-term cultivation, there was no migration of tumor cells to plastic. Magnetic separation, as a method of removing stromal components of fibroblasts, showed the greatest efficiency, while maintaining the viability of tumor cells.

**Conclusion.** The obtained primary cell cultures of lung cancer can be used for many tasks of experimental oncology: studies of the biological characteristics of lung cancer, development of preclinical models for the studies on new chemotherapeutic drugs.

**Keywords:**
primary cell culture, lung cancer, mechanical dissociation, enzymatic dissociation, collagenase, explant method, fibroblast removal

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ОРИГИНАЛЬНАЯ СТАТЬЯ

# ОПЫТ СОЗДАНИЯ КОЛЛЕКЦИИ КЛЕТОЧНЫХ КУЛЬТУР НЕМЕЛКОКЛЕТОЧНОГО РАКА ЛЕГКОГО В ФГБУ «НМИЦ ОНКОЛОГИИ» МИНЗДРАВА РОССИИ

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## РЕЗЮМЕ

**Цель исследования.** Тестирование новых химиотерапевтических агентов в трансляционной и биомедицине нуждается в исследованиях на иммортализованных клеточных линиях. Однако такие модели не всегда обладают биологическими свойствами опухоли *in situ*, в отличие от первичных культур клеток. Первичные культуры клеток рака легкого обладают близкими или идентичными опухолевым клеткам *in vivo* биологическими, гистологическими и молекулярными характеристиками. Получение коллекций первичных клеточных линий рака легкого является важной задачей в создании различных моделей для доклинических исследований.

**Материалы и методы.** В работе использовали послеоперационные образцы опухоли, полученные от 25 пациентов с впервые выявленным раком лёгкого без предварительного лечения. Для получения первичных культур использовали следующие методы: ферментативной диссоциации в растворе Хэнкса с добавлением 300 ед./мл коллагеназы I (Thermo Fisher Scientific, США), ферментативной диссоциации с использованием набора Brain Tumor Dissotiation Kit (Miltenyi Biotec, Германия) и 150 ед./мл коллагеназы I, а также метод эксплантатов. Для удаления фибробластов использовали следующие методы: применение системы FibrOut™ (CHI Scientific, США), магнитная сепарация фибробластов с использованием Anti-Fibroblast MicroBeads (Miltenyi Biotec, Германия) и метод холодной трипсинизации.

**Результаты.** Получено 15 первичных клеточных культур рака легкого, прошедших нулевой пассаж. В данной работе наиболее эффективным оказался метод ферментативной диссоциации. Инкубирование образцов опухоли легкого с коллагеназой в течение 1 ч. сохраняет жизнеспособность и адгезивную способность клеток. Метод эксплантатов не показал своей эффективности в целях долгосрочного культивирования, отсутствовала миграция клеток опухоли на пластик. Магнитная сепарация, как метод удаления стромальных компонентов фибробластов, показала наибольшую эффективность, сохраняя жизнеспособность клеток опухоли.

**Заключение.** Полученные первичные клеточные культуры рака легкого могут быть использованы для многих задач экспериментальной онкологии: исследования биологических особенностей рака легкого, разработки доклинических моделей исследования новых химиотерапевтических препаратов.

**Ключевые слова:**
первичная клеточная культура, рак легких, механическая диссоциация, ферментативная диссоциация, коллагеназа, метод эксплантатов, удаление фибробластов

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## INTRODUCTION

Lung cancer is one of the most common oncological diseases with a high mortality rate in the Russian Federation as well as in the world. In Russia, the incidence of lung cancer is among the top three, while in men it ranks first and accounts for 16.5 % of the total number of all oncological pathologies [1]. Being a heterogeneous disease, lung cancer includes several subtypes that are important for the clinical and pathological course of the disease. Histologically, lung tumors are divided into two main histotypes: small cell carcinoma (SCC) and non-small cell lung cancer (NSCLC). Small cell lung cancer accounts for 15–20 % of primary lung tumors and is the most aggressive form of malignant neoplasms of this localization. Non-small cell lung cancer accounts for about 80 % of the total number of lung tumors and is divided into four histological subtypes: lung adenocarcinoma, squamous cell carcinoma, large cell lung cancer and bronchial carcinoid tumor [2]. Molecular genetic subtypes of NSCLC are determined by the presence or absence of mutations in the EGFR, KRAS, translocations in the ALK, ROS1 genes, which allows based on these data to make decisions about the appointment of targeted therapy, and PD-L1 protein expression to make the decision about immunotherapy [3]. Despite all the successes in the treatment of lung cancer achieved through the use of radiation, chemotherapy, and immunotherapy, the prognosis for patients remains disappointing: SCC is characterized by early metastasis and the overall five-year survival rate is about 5 %, NSCLC has better prognosis, however, given the fact that the disease is more often diagnosed at stage III–IV, the five-year survival rate remains low and is 15–19 % [4]. The search for new drugs includes screening libraries of antitumor compounds on primary cultures and permanent cancer cell lines [5]. Primary culture consists of tumor cells placed in the culture medium after mechanical or enzymatic disaggregation or as a result of migration from explants. The primary cell culture that has passed the first passage, i.e. passed the first replanting after the cells isolated from the tumor sample have attached to the culture plastic, is called a cell line. A permanent cell line is a transformed *in vitro* cell line that has overcome the so–called Hayflick limit – the limit number of divisions determined by the length of telomeres [6]. Permanent lung cancer cell lines were first obtained in the 80s of the twentieth century, 25 years after the creation of the first cervical cancer cell line (HeLa). After the development of serum-free media, for example, ACL4 and HITES media, the introduction of lung cancer cell lines into culture reached its peak. Currently, more than 200 permanent cell lines of this nosology are known [7; 8].

The use of permanent cell lines makes it possible to evaluate the direct cytotoxic and cytostatic effects, the organ-specific toxicity of the tested chemical compounds and preparations, to determine their mechanism of action, as well as target proteins and genes [9]. However, permanent cell culture *in vitro* after prolonged subcultivation cannot demonstrate the heterogeneity of the original tumor *in vivo*, which occurs due to the presence in the cellular composition of the primary tumor of several subclones genotypically different from each other, which is characteristic, in particular, for non-small cell lung cancer. The heterogeneity of lung cancer is characterized by differences in the rate of cell growth, their karyotype, the presence of cell surface receptors, enzyme production, gene expression [10], sensitivity to various cytostatics [11]. It is known that differences in the biological characteristics of tumor cells underlie the metastatic progression of the primary tumor, its acquisition of resistance to targeted and chemotherapy, the occurrence of relapses. Neglecting tumor heterogeneity at the early stages of preclinical studies is one of the reasons for the failure of clinical trials of new antitumor drugs, which leads to large economic costs and slowing progress in this area [3; 12].

Keeping this in mind, a more adequate source for the creation of cellular models of tumor growth are primary cell cultures, in which the heterogeneity of the tumor is reproduced at the level of its histopathological, molecular and genetic features. The creation of collections of primary lung cancer cultures is a common practice in various scientific institutions around the world. Such collections serve to reproduce the population features of the disease [13], to study the response of NSCLC to chemotherapy [14] and to study various aspects of oncogenesis [15; 16].

There are many methods for obtaining primary cell cultures, which researchers are constantly optimizing depending on the characteristics of the tumor material and the goals facing the study. A key step in the process of creating a cell culture

is to obtain, during enzymatic or mechanical tissue disaggregation, a pool of viable tumor cells, with a composition close to the heterogeneous cellular composition of the original tumor. For tumors with a dense structure, which include lung cancer, the method of enzymatic dissociation is preferred, while the composition and viability of the resulting cells directly depend on the reagents used and fermentation conditions [17]. One of the most popular components of mixtures for enzymatic dissociation of dense tumor tissue is collagenase I, which has a high specificity with respect to collagen fibers, the main protein of the extracellular matrix, leaving intact the proteins of cell membranes, which is important for preserving the viability and biological characteristics of isolated cells [18]. Collagenase I is often used in the production of primary lung cancer cell lines, both in pure form [19] and in a mixture with other enzymes (trypsin) [13]. As an alternative to tissue dissociation, the explant method is used, which is based on the migration of intact cells to culture plastic from small fragments of the tumor. The advantage of this approach is the high survival rate of cells with the most complete preservation of the heterogeneity of their composition, however, the effectiveness of obtaining a cell line is limited by the mobility of tumor cells [6; 14]. Obtaining primary cultures is a rather complex process due to the small number of initial tumor cells, as well as the partial loss of cell viability after tumor resection and the use of methods disaggregation of the material [17].

Regardless of the method of obtaining the primary culture of lung cancer, as well as other malignant neoplasms with a pronounced fibrous component, contamination by stroma cells is a big problem, of which the most numerous are fibroblasts, which *in vitro* quickly switch to division and thereby are able to suppress tumor cells. There are a number of ways to combat fibroblasts in culture, which can be divided into three groups: 1) the use of cytostatics specific for fibroblasts; 2) using the properties of differential adhesion of primary culture cells and 3) cell sorting. Each of these methods has its own advantages and disadvantages [20].

**The purpose of the study** was to evaluate the effectiveness of various methods of isolating tumor cells when creating a collection of cell cultures of non-small cell lung cancer.

## MATERIALS AND METHODS

In the period from March to September 2021, tumor material from 25 patients with non–small cell lung cancer (lung adenocarcinoma) was selected for research, of which 20 samples were obtained from the primary focus and 5 samples from brain metastasis.

The patients were treated in the Department of thoracic surgery and in the Department of Neuro-Oncology at the National Medical Research Centre for Oncology in 2021. The histological diagnosis was confirmed in the pathology and anatomical department of National Medical Research Centre for Oncology. The patients were aware of their participation in the scientific study and signed an informed consent to the collection of biological material. The study was approved by the local ethical committee National Medical Research Centre for Oncology Protocol No. 6/1 of February 10, 2020.

Samples from the operating room were transferred in Hanks solution (HBSS, Gibco, USA) with the addition of 1 % penicillin-streptomycin (Biolot, Russia) at a temperature of +4–8 °C to the Laboratory of Cellular Technologies National Medical Research Centre for Oncology in an interval of no more than 20 minutes after removal of the tumor drug. To work out the technique of obtaining viable cells, we selected several variants of enzymatic dissociation protocols, as well as the explant method. In 15 cases, we used the procedure of enzymatic dissociation using pure collagenase I in the case of the primary focus ($n$ = 10) and using a combination of collagenase I and enzymes for dissociation of brain tissue in the case of samples obtained from metastases ($n$ = 5). In 10 cases, the explant method was used to obtain a cell culture from the primary focus. A schematic representation of the protocols and methods used in the work is shown in Figure 1. In all cases, a standard culture medium was used for growing primary cell lines, which is a DMEM medium (Gibco, USA) with the addition of 10 % FBS (HyClone, USA), 1 % insulin-transferrin-sodium selenite (Biolot, Russia), 10 ng/ml FGF-2 (Miltenyi Biotec, Germany), 10 ng/ml EGF (Miltenyi Biotec, Germany), 1 % NEAA (Gibco, USA).

Visual examination of cell morphology and photofixation were performed using an inverted Axio Vert microscope. A1 (Carl Zeiss Microscopy, Germany).

**Protocol No. 1.** *Enzymatic dissociation in a solution of Hanks and collagenase I (Thermo Fisher Scientific, USA).* The protocol used corresponds to the usual practice of enzymatic treatment of lung tumor tissue [19]. The tumor fragments were placed in a Petri dish (d = 35 mm) (Eppendorf, Germany) with Hanks solution (with $Ca^{2+}$ and $Mg^{2+}$ ions) (Gibco, USA) and the samples were fragmented with a surgical blade to a size of 1–2 $mm^3$, after which 300 units/ml of collagenase I were extracted (Thermo Fisher Scientific, USA) and incubated for 1 hour in a thermostat at 37 °C and 5 % $CO_2$. At the end of cultivation, the sample was resuspended by passing through a plastic tip several times, resulting in a homogeneous cell suspension. Next, 3 ml of standard culture medium was introduced, centrifuged for 5 min at 300g and the supernatant was decanted. 5 ml of DMEM nutrient medium (Gibco, USA) was added to the cell sediment and resuspended, after which the suspension of tumor cells was passed through a sterile nylon filter (d = 70 nm) (Becton Dickinson, USA). Cell viability was calculated and determined in the Goryaev chamber with a 0.4 % solution of trypan blue (Biolot, Russia). The samples, depending on the number of cells, were passed into vials with a culture surface area of 25 $cm^2$ or 75 $cm^2$ (Thermo Fisher Scientific NUNCtm EasYFlasktm, Denmark) in a nutrient medium for primary cell lines of the Primary Cancer Culture System (PromoCell®, Germany) and placed in a $CO_2$ incubator for further cultivation at 37 °C. and 5 % $CO_2$. On the 3rd day of cultivation, 2 ml of fresh nutrient medium Primary Cancer Culture System was added. Further, the replacement of the nutrient medium was carried out once every three days. After a month of cultivation, the nutrient medium for the primary cell lines was replaced with a standard culture medium.

**Protocol No. 2.** *Enzymatic dissociation with the combined use of Brain Tumor Dissociation Kit (Miltenyi Biotec, Germany) and 150 units/ml of collagenase I (Thermo Fisher Scientific, USA).* The samples were crushed with scalpels to a size of 1–2 $mm^3$. For enzymatic dissociation, the Brain Tumor Dissociation Kit (Miltenyi Biotec, Germany) was used according to the manufacturer's instructions. Cultured for 2 hours in a $CO_2$ incubator (Binder, Germany) at 37 °C and 5 % $CO_2$ on a mechanical stirrer, programming the device according to the instructions for the kit. Further, 150 units/ml of collagenase I were added to the suspension of enzymes and tumor fragments and incubated for another 18 hours at +4…+8 °C. After the incubation time, the sample was washed, filtered, and cells were counted and passed, as indicated in Protocol 1.

**Protocol No. 3.** *Explants method.* The tumor material was fragmented with a scalpel up to 2 mm in a 35 mm diameter Petri dish (Eppendorf, Germany) with 5 ml of culture medium for the cultivation of primary cell lines of the Primary Cancer Culture System (PromoCell®, Germany), after which the obtained explants were cultured in an incubator at 37 °C, 5 % $CO_2$. After a few weeks of cultivation, the non-adhesive explants were transferred from a Petri dish, crushed by pipetting and then washed, filtered, counted and passed cells, as indicated in protocol 1.

Removal of fibroblasts from the primary culture was carried out by several methods widely used in the practice of obtaining cell lines from solid tumors [20].

**Method No. 1.** *Removal of fibroblasts using the FibrOut™ system (CHI Scientific, USA).* The FibrOut™ system was introduced into vials with primary lung cancer cell cultures at the rate of 1 ml of the system to 500 ml of culture medium. Further, the cells were cultured for 3–5 days until the fibroblasts were completely detached, after which the medium was replaced with a standard culture medium.

**Method No. 2.** *Magnetic separation of cells using a set of Anti-Fibroblast MicroBeads (Miltenyi Biotec, Germany).* At the first stage, primary cultures were removed using a trypsin-versin solution (1:1) (Biolot, Russia) at a temperature of 37 °C, then fibroblasts from the cell suspension were separated on a magnetic column according to the manufacturer's instructions. The negative fraction depleted by fibroblasts was passaged in a standard culture medium and placed in a $CO_2$ incubator for further cultivation at 37 °C and 5 % $CO_2$.

**Method No. 3.** *Cold trypsinization.* The spent nutrient medium was decanted from culture vials with primary cultures, after which a cooled trypsin-versene solution (1:1) was introduced (Biolot, Russia) (+4…+8 °C) and incubated for 3 minutes at room temperature, observing the process of detaching cells from the bottom of the vial with a microscope. The fibroblasts separated from the bottom of the vial were carefully washed with a DMEM medium containing 5 % FBS and removed from the vial. Then a standard

culture medium was introduced into the vial with the remaining cells and placed in a $CO_2$ incubator for further cultivation at 37 °C and 5 % $CO_2$.

## RESEARCH RESULTS

In all cases, with enzymatic dissociation of the material, we were able to obtain a suspension with a proportion of viable cells of about 90 %. Moreover, the viability was not reduced even in samples obtained from metastases, despite their prolonged incubation at a reduced temperature (+4 … +8 °C). On the 2nd day after exposure to enzymes, a small number of attached cells were observed, while tumor cells formed clusters, and fibroblasts were observed in the form of single spindle-shaped cells (Fig. 2A). As the cultivation time increased, the number of adhered cells grew, fibroblasts occupied the entire surface

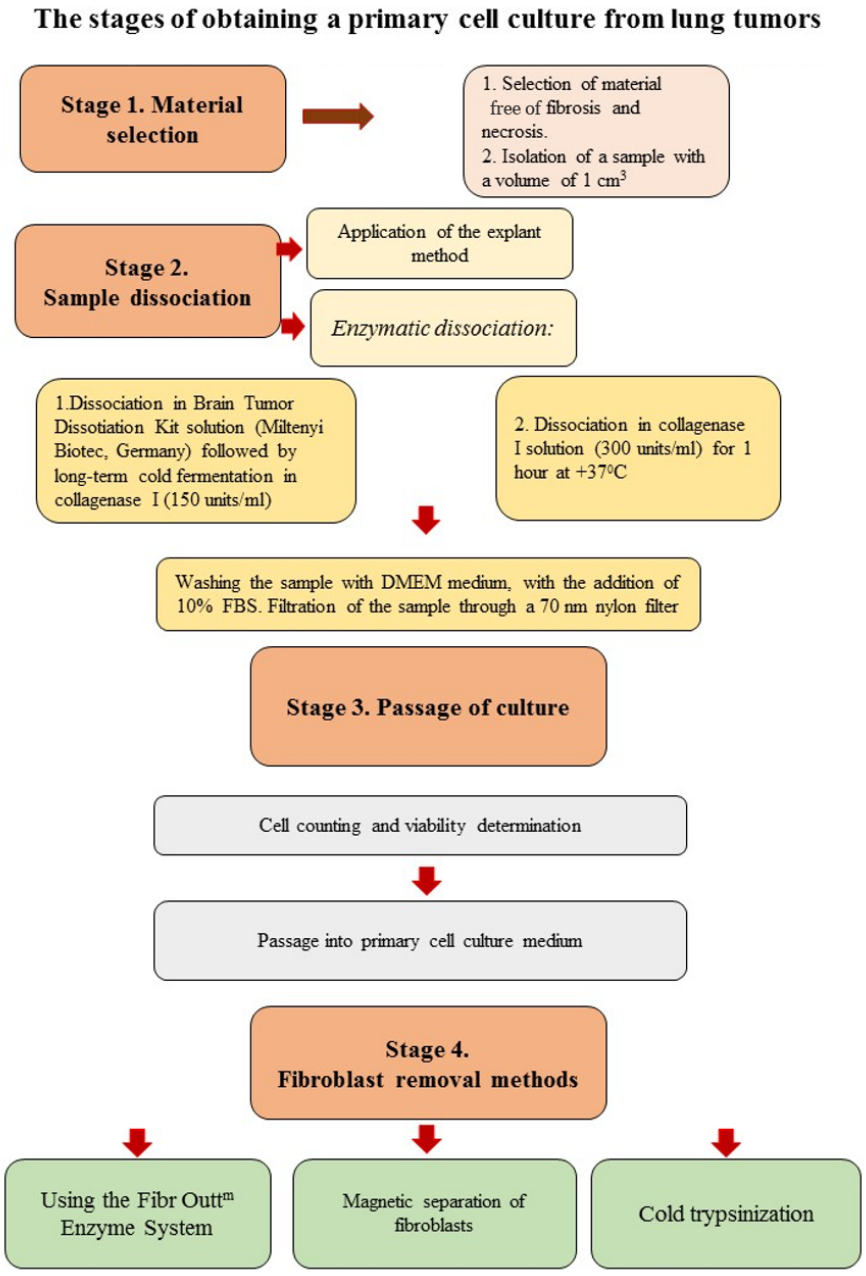


Fig. 1. Diagram that shows the stages of obtaining primary lung cancer cell lines.

of the vial, and tumor cells formed single clusters of rounded shape (Fig. 2B). The first replacement of the nutrient medium was carried out for 5 days. cultivation, then every 3 days. Elimination of erythrocytes and cellular detritus from the nutrient medium occurred during decanting of the waste medium.

Explants were attached to the bottom of the vial for 2 days. In the explant samples, after attachment to the surface of the Petri dish, only a small number of migrated cells were observed, which also showed no signs of proliferation even after several weeks of cultivation. Taking advantage of the fact that the fragments of tumor tissue became loose after a long stay in the nutrient medium, the remnants of explants were subjected to mechanical dissociation. The resulting cell sediment was passed through a 70 nm nylon filter and passed into culture vials with a surface area of 25 $cm^2$. The proportion of viable cells with this method of obtaining primary lung cancer culture was 70–80 %. After 2 days, the cells attached to the surface of the vial, and after 7 days. Their proliferation was observed after cultivation (Fig. 2C).

Working with samples of lung cancer metastases to the brain, contamination was often noted with cells morphologically similar to glial cells, having a fusiform shape and translucent processes forming a network (Fig. 2D). Unlike fibroblasts, these cells did not proliferate, their pool was depleted after each passage of primary cell lines. With a large number of glial cells, an additional mechanical cleaning of the sample was carried out using a scrapper under control in an inverted microscope in a laminar box.

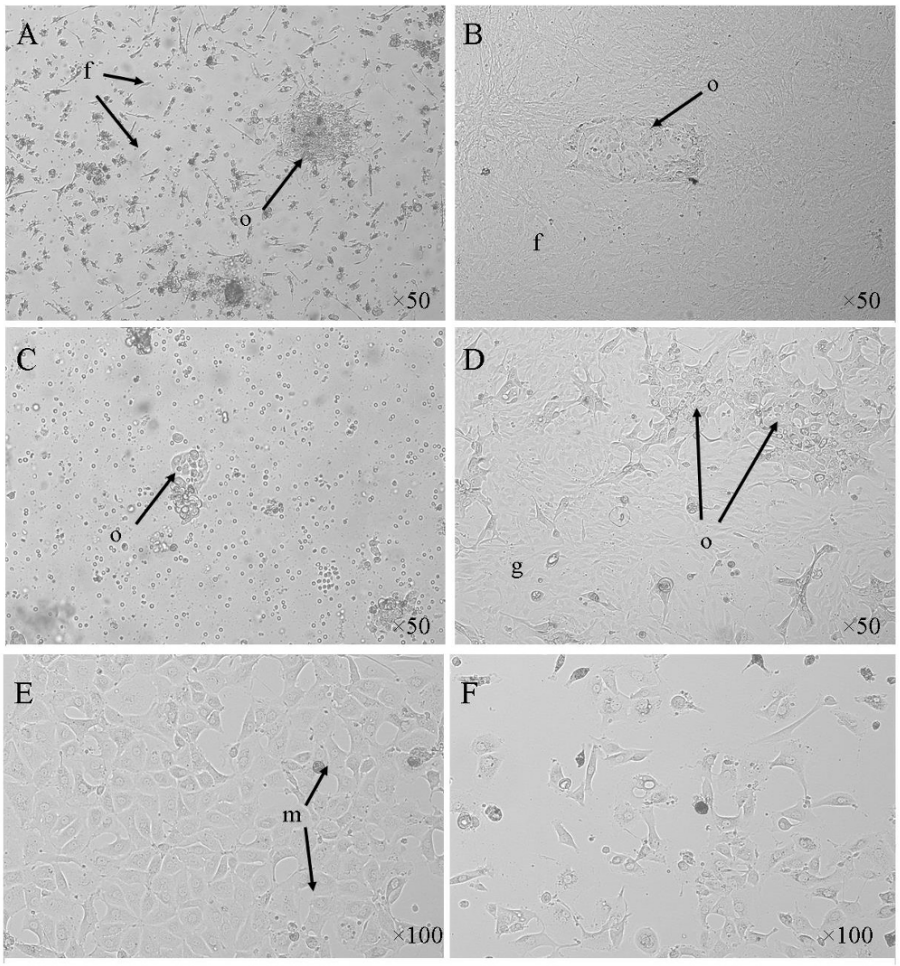


Fig. 2. View of primary lung cancer cell lines. A – cells after enzymatic dissociation on the 2nd day of cultivation. B – cell proliferation after 7 days of cultivation. C – proliferation of cells from the explant after disaggregation. D – primary lung cancer culture obtained from brain metastasis. E is a proliferating primary culture of epithelial morphology that has passed the zero passage. F – degradation of the cell line: vacuolization of tumor cells. Designations: *o* – tumor cells, *f* – fibroblasts, *g* – glial cells, *m* – mitoses.

In all cultures obtained from samples of locally advanced lung cancer, contamination with fibroblasts was observed (Fig. 2B), for the elimination of which several purification methods were used. The FibrOut™ system was used in 7 samples. In all cases, in addition to the detachment of fibroblasts, loss of adhesion and death of tumor cells were also observed, which may have been due to their low confluence (no more than 20 %) on the surface of the culture vial. Using systems for targeted elimination of fibroblasts, it is necessary to take into account their possible toxic effect on tumor cells and strictly maintaining the concentration, select safe dosages and incubation time. Magnetic separation of fibroblasts using Anti-Fibroblast MicroBeads was used in 7 cases. After magnetic separation, viable tumor cells were preserved, which quickly attached to the surface of the vial and then actively proliferated. However, the removal of fibroblasts was incomplete, and after a while their growth was observed again, which required repeated separations. When using cold trypsinization (6 samples), the least complete removal of fibroblasts from the surface of the vial was observed, since the exposure time with trypsin was strictly limited due to the high probability of loss of tumor cells. To maintain a low fibroblast content, it was necessary to repeat the cold trypsinization procedure, which was eventually carried out more often than the procedure of magnetic separation of fibroblasts. Comparative characteristics of methods for removing fibroblasts from primary lung cancer culture are presented in Table 1.

According to the results of the study, 25 primary lung cancer cell cultures were obtained, of which only 15 passed the zero passage and were cryopreserved at passages 1, 2 and 4. The cells of the successful lines were spread out in a monolayer (confluence 100 %) along the bottom of the vials, had a predominantly polygonal shape corresponding to epithelial morphology, single mitoses were observed (Fig. 2E). In the remaining lines, gradual degradation of cells was observed for 21 days: vacuolization and detachment from the adhesive surface (Fig. 2F).

## DISCUSSION

The process of creating primary cell cultures from lung tumors is accompanied by some difficulties, compared with other types of epithelial tissues, and the probability of successful release of primary lung cancer cell cultures is no more than 40 % [21], which corresponds to our experience. Other authors note the multinucleation of cells and vacuolization of the cytoplasm [22] gradual degradation of tumor cells during long-term cultivation [19].

In this work, we used the methods of enzymatic dissociation with collagenase I as the most effective for obtaining primary cell lines [19]. Enzyme cocktails are actively used by researchers to produce spheroids and organoids of lung cancer that have similar properties and genetic mutations as the original tumor [22]. Collagenase I gradually cleaves collagen fibrils, while new sections of the fiber become available for interaction with the enzyme. Degradation of collagen and extracellular matrix leads to the release of cells from tumor tissue [23]. In our work, we also confirmed that incubating a lung cancer sample with collagenase for 1 hour does not inhibit other cells, preserves their viability and adhesive and proliferative ability.

**Table 1. Comparison of the fibroblasts extraction methods from primary lung cancer cells culture**

| | FibrOut™ | Anti-Fibroblast MicroBeads | Cold trypsinization |
|---|---|---|---|
| Reduction of the proportion of fibroblasts, % | 100 % | 80 % | 40 % |
| Effect on the viability of tumor cells | It has a toxic effect on tumor cells. It is necessary to select the optimal cultivation time | Shows no effect on the viability of tumor cells | Shows no effect on the viability of tumor cells |
| The optimal frequency of the procedure to achieve the effect | Cultivation in conjunction with the FibrOut™ system for 3–5 days. A repeat of the procedure is not required | For complete separation of tumor cells and fibroblasts, it is necessary to carry out 2–3 magnetic separation procedures | For complete elimination of fibroblasts, 3–4 procedures should be performed |

Explants are an effective model for drug testing for many nosologies of solid cancer [24]. In particular, in the work of Karekla et al. explants of non-small cell lung cancer were used as an ex vivo model to evaluate the response to cisplatin therapy [14]. In our work, long-term cultivation was assumed, during which, as we believed, tumor cells would begin to exit the explant, which was observed in our practice earlier when working with glial tumors [25] and prostate cancer, when the migration of tumor cells to plastic occurred within a week [26]. However, in the present study, in all cases, prolonged cultivation did not lead to the release of a sufficient number of tumor cells from the explant to the culture plastic. What biological features of lung cancer underlie the low mobility of tumor cells in primary culture have yet to be established.

An important aspect of working with tumor material obtained from lung tissue is the removal of fibroblasts, which are present in large numbers in this type of tissue. It is known that fibroblasts are a source of a number of signaling influences regulating the proliferation and mobility of tumor cells both in situ [27] and in culture [28] Despite the fact that fibroblasts can support the growth of tumor cells in culture [29], their excessive proliferation leads to a restriction of the proliferation of tumor cells, since fibroblasts divide faster *in vitro* and gradually displace tumor cells from the vial. In this regard, the tactics of regular "thinning" of fibroblasts using the methods of cold trypsinization and magnetic cell sorting, which we implemented in our work, is the most successful for samples with a small number of tumor cells, since it allows us to preserve the signaling functions of fibroblasts while restraining their excessive reproduction. However, at later stages, when the tumor cells have reached the cell density necessary for successful cultivation, as well as in samples with the number of tumor cells initially sufficient to create the necessary contact interaction that supports their viability and proliferation, it may be recommended to switch to more radical methods of fibroblast removal, for example, the use of commercial FibrOut™ (CHI) systems Scientific, USA) or Genetecin™ (Thermo Fisher Scientific, USA), designed to target the suppression of this type of cells.

Finally, the decisive factor determining the success of the cultivation of primary cell lines is the choice of nutrient medium. In our work, we did not set the goal of selecting the optimal environment for the introduction of lung cancer cells into the culture, but used ready-made commercial solutions and the experience of other authors. At the first passage, we used the Primary Cancer Culture System (PromoCell®, Germany) environment for primary cell lines with the manufacturer's additives. The medium used by us at a later stage of cultivation was prepared on the basis of DMEM nutrient medium, one of the most common and widely used media for the cultivation of various tumor cells, including those with a high proliferation rate [30]. The medium also included fetal bovine serum (10 %), a mixture of insulin-transferrin-sodium selenite (1 %), 10 ng/ml FGF-2, 10 ng/ml EGF and a solution of essential amino acids (1 %). Serum, essential amino acids and the addition of insulin-transferrin-selenite are essential components for maintaining the vital activity of cells undergoing adaptation to *in vitro* conditions [31; 32].

The presence of growth factors, such as fibroblast growth factor and epidermal growth factor, is necessary for selective stimulation of the division of malignant tumor cells, the addition of these factors to the culture medium is due to their small concentrations in serum and is a commonly used technique to increase the efficiency of obtaining primary lung cancer cell lines. At the same time, the concentration of growth factors in the cultivation medium in various studies ranges from 10 ng/ml [16] to 20 ng/ml [32].

## CONCLUSION

The result of the work that has been carried out were 15 primary lung cancer cell lines obtained. The most effective method of obtaining a primary lung cancer culture was enzymatic dissociation with collagenase I, followed by removal of fibroblasts using magnetic separation. The obtained cell lines can be used to solve a wide range of research tasks in the field of experimental oncology. Primary lung cancer cell cultures, unlike immortalized cell lines undergoing genetic aberrations with an increase in the number of passages, have biological characteristics of tumor cells similar or identical to those *in vivo*, which makes them ideal models for preclinical studies of new drugs, research of biological features of lung cancer, creation of xenographic models, etc.

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