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Primary Culture of Cancer Cells Derived from Human Lung Tumors

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Lung Cancer,
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Primary Culture

Abstract: Primary cell culture is an important research platform for studying the biochemistry and molecular biology relevant to individual cancer types and understanding cancer cell physiology. Therefore, in this study, it was aimed to perform primary cell culture from tissues of non-small cell lung cancer (NSCLC) patients with different clinical course in our own laboratory conditions. Thus, it will throw light on future studies on tumor biology and pathogenesis in NSCLC, the most common subtype of lung cancer, which is the most malignant type of cancer. **Materials and Methods:** Patients diagnosed with NSCLC who required surgical intervention as a result of pulmonary function tests and thorax computed tomography and gave written consent were included in the study. After the pathologically confirmed tumor tissue was removed by surgical resection, the cells obtained by mechanical and enzymatic digestion with collagenase/hyaluronidase were cultured in Dulbecco's Modified Eagle Medium (DMEM) under sterile conditions. **Results:** In this study, cancer cells were successfully isolated and cultured from the tumor tissues of 3 patients diagnosed with NSCLC, whose tumor diameters were 2*1.5*1.2, 8*7*6, 4*3.7*3, without metastasis and aged between 43-70 years. **Conclusions:** This study presents a successful primary cell culture protocol that includes important steps for obtaining patient-derived tumor cells in lung cancer based on information in the literature and personal experience on the subject. Primary cell culture is an *in vitro* model that is thought to contribute to the development of personalized treatments in future studies for the effective treatment of lung cancer by bridging the gap between the laboratory and the clinical decision process of the physician.

İnsan Akciğer Tümörlerinden Elde Edilen Kanser Hücrelerinin Primer Kültürü

Anahtar Kelimeler
Akciğer Kanseri
Küçük Hücreli Dışı Akciğer Kanseri
Primer Hücre Kültürü

Özet: Primer hucre kulturu bireysel kanser turleri ile ilişkili biyokimya ve moleküler biyolojiyi inceleme ve kanser hücresi fizyolojisini anlamak için kullanılan önemli bir araştırma platformudur. Bu nedenle bu çalışmada en ölümcülkanser türü olan akciğer kanserinin en sık görülen alt tipi olan küçük hücreli dışı akciğer kanserinde (KHDAK) tümör biyolojisi ve patogenezi ile ilgili ileride yapılacak olan çalışmalara ışık tutması için farklı klinik seyir gösteren KHDAK hastalarına ait dokulardan kendi laboratuvar koşullarımızda primer hücre kültürü yapılması amaçlanmıştır. **Gereç ve Yöntem:** KHDAK tanısı alan solunum fonksiyontestleri ve toraks bilgisayarlı tomografisi cerrahi müdahaleye uygun olan ve yazılı olarak onay veren hastalar çalışmaya dahil edilmiştir. Patolojik olarak doğrulanan tümör dokusu cerrahi rezeksiyon ile çıkarıldıktan sonra steril şartlarda mekanik ve kollajenaz/hyaluronidaz ile enzimatik parçalanmasıyla elde edilen hücreler Dulbecco's Modified Eagle Medium (DMEM) içerisinde kültüre edilmiştir. **Bulgular:** Bu çalışmada tümör çapları 2*1.5*1.2, 8*7*6, 4*3.7*3 olan, metastazı olmayan ve yaşları 43-70 arasında değişen KHDAK tanısı alan 3 hastanın tümör dokularından kanser hücreleri başarılı bir şekilde izole edilip kültüre edilmiştir. **Sonuç:** Bu çalışma, literatürdeki bilgilere ve konuyla ilgili kişisel deneyimlere dayanarak akciğer kanserinde hasta kaynaklı tümör hücrelerinin elde edilebilmesi için önemli adımları içeren başarılı bir primer hücre kültürü protokolü sunmaktadır. Primer hücre kültürü laboratuvar ile hekimin klinik karar süreci arasında köprü oluşturarak akciğer kanserinin etkili bir şekilde tedavi edilebilmesi için ileride yapılacak çalışmalarla kişiselleştirilmiş tedavilerin geliştirilmesine katkı sağlayacağı düşünülen *in vitro* bir modeldir.

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1. Introduction

Among all cancers, lung cancer is the cause of cancer-related deaths worldwide, with a rate of 18.4% [1]. Although there are many subtypes of lung cancer, non-small cell lung cancer (NSCLC) [2]. Nevertheless NSCLC is one of the leading causes of cancer deaths worldwide [3]. Despite significant progress in many areas, such as cancer initiation, progression, metastasis, and tumor microenvironment, clinical success in long-term studies of lung cancer is still limited. The most important reason for this situation is the heterogeneous nature of cancer, resulting in multiple disease manifestations. The different variations between patients (intertumoral heterogeneity), the primary tumor mass of the patient, and the large diversity of cell populations in individual metastases (intratumoral heterogeneity) have a great impact on patient have a great impact on patient prognosisand treatment options [4].

In vivo animal models and *in vitro* studies, which are widely used in cancer research, are of extraordinary importance for elucidating the molecular mechanisms underlying cancer development and for developing new treatment strategies. However, *in vivo* models are quite expensive in terms of infrastructure and maintenance and cannot be organized as easily as *in vitro* models [5]. In this context, immortalized cancer cell lines have been an accessible, easy-to-use biological model for studying cancer biology and potential anticancer drug efficacy for many years. However, there are genetic and epigenetic differences between cell lines and cancer cells derived from patient tissues. In this context, this appears to be a parameter that makes it difficult to assess how much of the original tumor biology is reflected in established cell line models that are maintained *in vitro* for long periods of time. Therefore, cell lines do not adequately represent the diversity, heterogeneity, and drug-resistant tumors that occur in patients [6,7]. On the other hand, *in vivo* animal models may not be able to predict clinical outcomes because they are not fully adequate to show cancerogenesis with intertumoral heterogeneity and basic aspects such as invasion and metastasis that involved in cancer development. Moreover previous studies, have shown that the conversion rate of results from animal model studies to clinical trials is less than 8% [8]. Cell cultures prepared from patient tissues are particularly important for studies aimed at better preserving the molecular characteristics of patient tumors and supporting clinical decision-making and counseling as a better alternative to studying cancer biology and drug sensitivity [9,10]. Therefore, primary culture plays a critical role in better mimicking patient-specific tumor behavior, detecting differences between patients and, developing personalized treatments.

The aim of this study was to generate a primary cell culture model under our laboratory conditions from tissues of NSCLC patients with different clinical courses, and it is expected to contribute to future studies on the biology and pathogenesis of lung cancer in this model.

2. Material and Method

2.1. The selection and determination of patients

Patients with NSCLC who presented to the Hospital of Erciyes University Faculty of Medicine, Department of Thoracic Surgery and whose pulmonary function tests and thoracic computed tomography were suitable for surgical intervention were included in the study. This work programme is in accordance with the Declaration of Helsinki. This research was supported by TUBITAK 1001 project number 215S849. Patients included in the study were informed of the purpose of the study and asked to read the “Voluntary Informed Consent Form”, afterwhich verbal and written consents were obtained from the patients.

2.2. Pathological confirmation of the tumor

After the tumor margins were determined by a pathologist at Erciyes University Pathology, hematoxylin-eosin (H&E) and immunostaining were performed for diagnosis and accurate subtyping, and it was finally determined to be NSCLC.

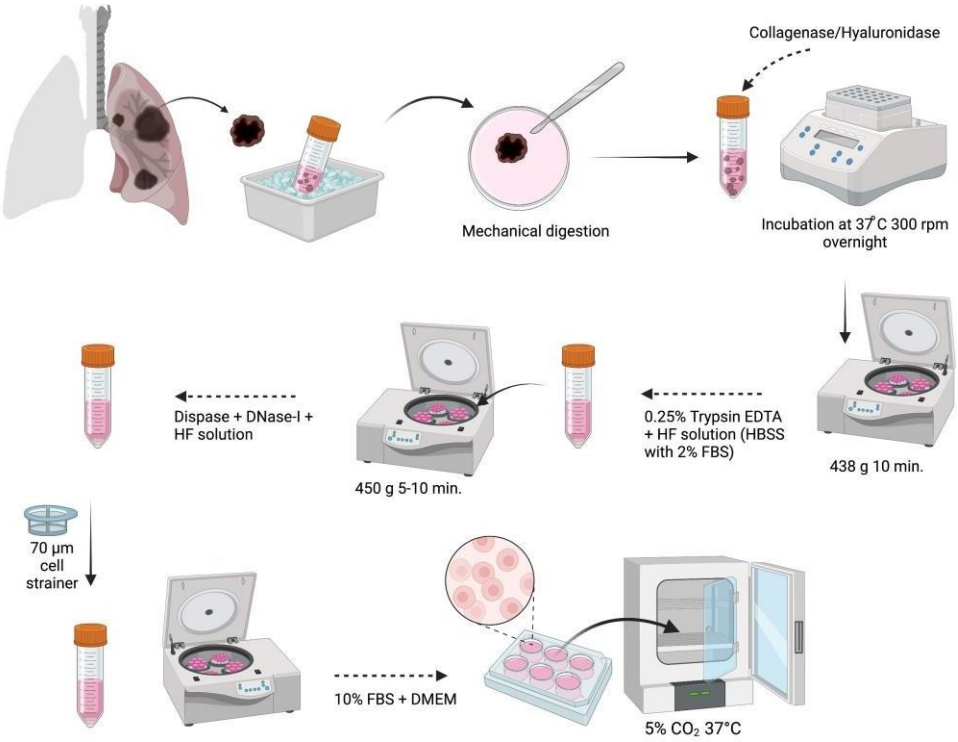

Figure 1. Schematic representation of the experimental procedure in the study. Tumor tissues from patients diagnosed with NSCLC were transferred from Erciyes University Pathology Department to Erciyes University Genome and Stem Cell Center in a cold environment. After mechanical lysis of the transferred tumor tissues, enzymatic lysis with collagenase/hyaluronidase was performed. The obtained cells were cultured in a 37°C incubator with 5% CO_2.

2.3. Transport procedure of tumor tissues

According to the required procedures in the Pathology Department of Erciyes University, 0.5-1 cm^3 of tumor tissue was transported to the Genome and Stem Cell Center under cold and sterile conditions in a Dulbecco's Modified Eagle Medium (DMEM) (STEM CELL, #5630) containing 10% Fetal Bovine Serum (FBS) (Thermo Fisher, #10270106), 1% Penicillin-Streptomycin (Pen-Strep) (Thermo Fisher, #15070063), 1% Amphotericin (Thermo Fisher, #15290026), and 1% L-Glutamine (STEMCELL, #7100). This provided minimal conditions for performing primary cell culture studies.

2.4. Obtaining a single-cell suspension for primary cell culture

2.4.1. Mechanical digestion

Tissues transferred to the Genome and Stem Cell Center were transferred to a Petri dish under sterile conditions, and non-tumor tissues such as vascular fragments, necrotic structures, and tar were removed with a scalpel and forceps. After washing with Hanks Balanced Salt Solution (HBSS) (STEM CELL, #37150) containing 1% Pen- strep, mechanical digestion was performed.

2.4.2. Enzymatic digestion

Tissues cut into small pieces for enzymatic lysis were transferred to a centrifuge tube and incubated overnight with the enzyme Collagenase/Hyaluronidase (STEM CELL, #7912) at 37°C in a shaking incubator at 300 rpm. It was then centrifuged at 438 g for 10 minutes to remove the supernatant from the suspended cells. To obtain a single-cell suspension, the following procedures were carried out:
First, 0.25% Trypsin-EDTA (Thermo Fisher, 25200056) was added, followed by HBSS (HF solution) containing 2% FBS and centrifuged at 450 g for 5-10 minutes, then 2 ml Dispase (STEM CELL, 7913) and 1mg/1mL DNase- I (STEM CELL, 7900) were added and allowed to stand for 3-5 minutes. Then 10 mL of cold HF solution (HBSS with 2% FBS) was added, filtered, and centrifuged again, then cultured in a 37°C incubator with 5% CO_2 and inoculated with DMEM with 5% FBS.

2.5. Cryopreservation of cells

To preserve and stock the cells obtained, cells were frozen with Dimethyl Sulfoxide (DMSO) (AppliChem, A3672,0100) in cryogenic vials and stored at -80°C/Liquid Nitrogen tank. After formation of the cell pellet, cells were resuspended with medium containing 5% FPS. After calculating the cell number, 10% DMSO was added to the cell suspension, transferred to the cryogenic vials, brought to -20°C and then stored at -80°C.

3. Results

Clinical outcomes of patients with Non-Small Cell Lung Cancer

The tumor diameter, metastasis status, and adjuvant treatment status of NSCLC patients used within the scope of this project are given in Table 1. According to information obtained from the Erciyes University Department of Thoracic Surgery, no metastases were detected in the patients, and only Patient 2 was reported to have received chemotherapy.

Table 1. Clinical outputs of patients with Non-Small Cell Lung Cancer

Patient Numbers	Age	Tumor Diameter	Metastasis	Adjuvan Treatment
Patient 1	64	2*1.5*1.2	-	-
Patient 2	43	8*7*6	-	Chemotherapy
Patient 3	70	4*3.7*3	-	-

Immunostaining of Non-Small Cell Lung Cancer Patients

The immunostaining of patient tissues diagnosed with NSCLC in the Department of Pathology, Erciyes University, is shown in Table 2. H&E staining for diagnosis and subtyping of tumor cells from patients with non-small cell lung cancer is shown in Figure 2, and immunohistochemical staining is shown in Figure 3.

Table 2. The immunostaining of patient tissues diagnosed with Non-Small Cell Lung Cancer

Patient Numbers	**Immunostaining**
Patient 1	Napsin A+, TTF-1+, CK7+, Musin-
Patient 2	TTF-1 +, ß-catenin +, Synaptophysin +, Chromogranin-, Ostrogen-, Glypican 3 –
Patient 3	P40-, P63-, TTF-1+, Napsin A-, CD56-, Synaptophysin-, Chromogranin-, Musin +

*TTF-1: Thyroid transcription factor 1; CK7: Cytokeratin 7

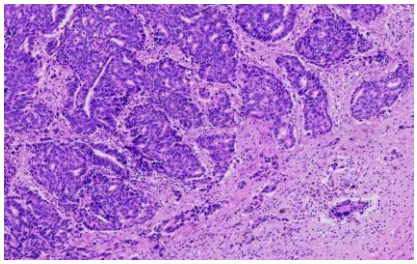

Figure 2. H&E staining of primary tumor cells from a patient with Non-Small Cell Lung cancer. Groups of malignant epithelial cells with marked atypia forming adenoid structures and occasionally solid islands were observed in the fibrotic stroma (x200).

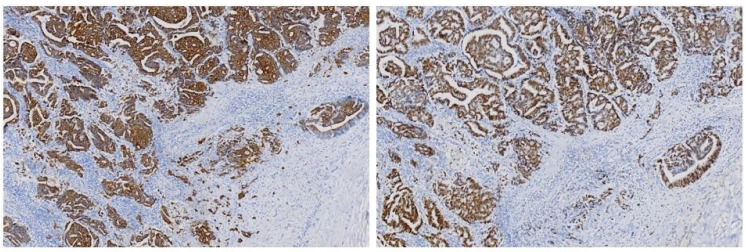

Figure 3. Immunohistochemical staining of tumor cells in the primary tumor in a patient with non-small cell lung cancer. CK7 and TTF1 immunostaining model in a patient with non-small cell lung cancer. NSCLC tumor cells are positive for CK7 and TTF1 (x200).

Retrieval and mechanical fragmentation of tumor tissue from Non-Small Cell Lung Cancer patients

Figure 4 shows images of the tissue removed by surgical resection and submitted to the Department of Pathology. The resection was performed in the Thoracic Surgery Department of the Hospital of the Faculty of Medicine of Erciyes University. Subsequently, cancer diagnosis and subtyping of the tissues brought to the Pathology Department was performed by the pathologist. Confirmed samples were forwarded to the Genome and Stem Cell Centre. Figure 5 shows the representation of the mechanical digestion process.

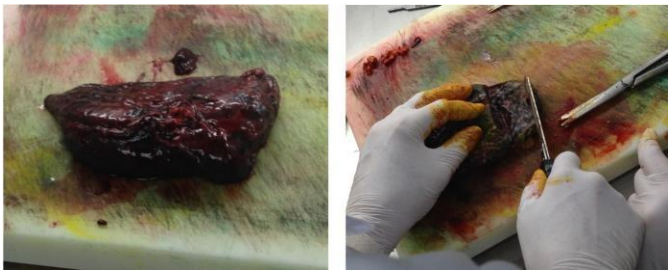

Figure 4. Tissue images removed by surgical resection and submitted to the Department of Pathology. Resection was performed at the Hospital of Erciyes University Faculty, Thoracic Surgery. Cancer diagnosis and subtyping of the tissues submitted to the Department of Pathology was performed by a pathologist.

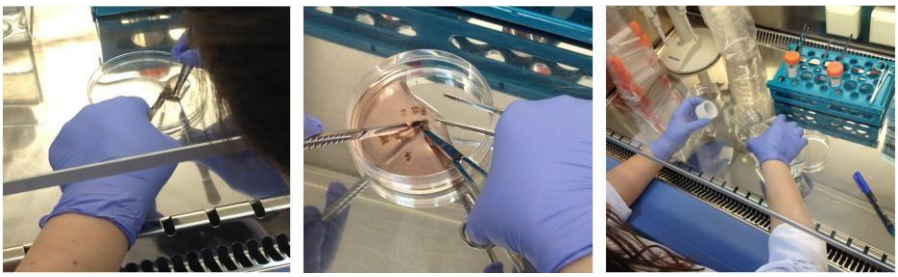

Figure 5. Images of tissues transferred from the Department of Pathology to the Genome and Stem Cell Center. Mechanical disruption is demonstrated by removal of blood vessels and necrotic parts from the transferred tissues.

Primary cell culture images of Non-Small Cell Lung Cancer patients

Figure 6 shows images of primary cell culture of all patients at day 1, day 5, and day 10.

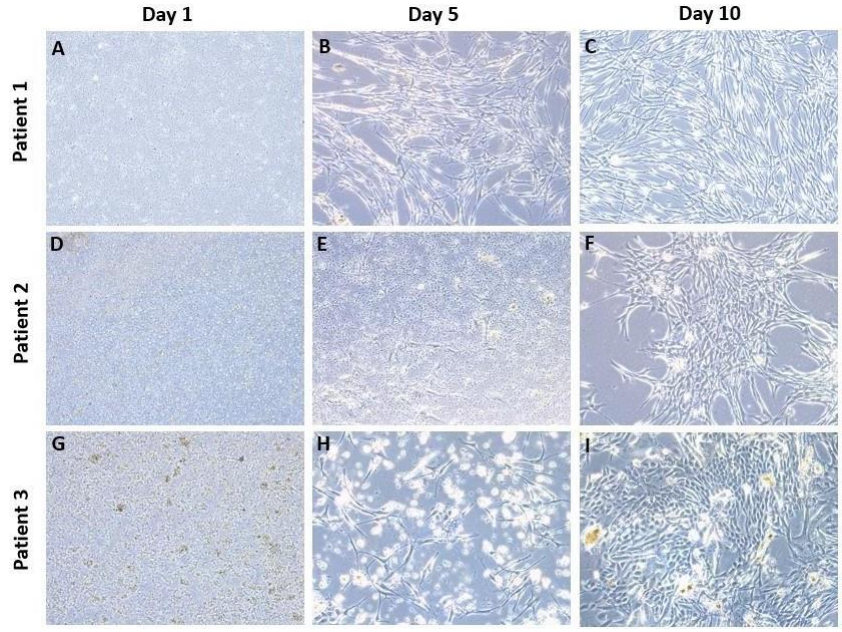

Figure 6. Images of primary cell cultures, from all patients. (A-C) Patient 1, 1st, 5th, and 10th days in passage 0, (D-F) Patient 2, 1st, 5th, and 10th days in passage 0, (G-I) Patient 3, 1st, 5th, and 10th days in passage 0 were recorded with a 10X objective under a Leica inverted light microscope.

4. Discussion and Conclusion

Despite advances in early cancer detection and multimodality therapy, the vast majority of lung cancer patients are locally advanced or metastatic at the time of diagnosis, suggesting that lung cancer cells are very aggressive. The mechanisms underlying the invasiveness and metastasis of the lung cancer are not fully understood[11]. To elucidate this mechanism, it is very important to perform a successful primary cell culture and consider the specific characteristics of the tumor in each patient. Therefore, a pilot study was performed with 3 patients, in which primary cell cultures were established from the tissues of patients with NSCLC, the most common subtype of lung cancer.

Isolation and cultuvation of solid tumor cells in vitro that resemble the microenvironment of the patient-specific tumor has always been considered challenging and requires special techniques in this context. Although there are several methods for primary cell culture, each method has its advantages and disadvantages [6]. Although primary cell culture is considered the best method for studying tumor tissues, it is difficult to control due to the heterogeneity of cellular, genetic, and epigenetic composition among patients and the high risk of contamination [12,13]. For this reason, the risk of contamination is controlled with antibiotics and antifungals added to the transport medium used for tissue transfer to successfully harvest cancer cells. In addition, it should be emphasized that tumor tissue must be cleared of necrosis or fibrous lesions as soon as possible to obtain parenchymal tumor cells in primary culture [14]. Therefore, as shown in Figure 5, the tar, blood vessels, and necrotic parts were excised and removed from the incoming tumor tissue, and then mechanical fragmentation was performed (Figure 5).

Lung cancer tissue with epithelial phenotype is rich in stromal cells, and fibroblast contamination must be removed for successful lung cancer cell culture [15,16]. Cleavage of cancer cells by collagenase has been shown to be less harmful to epithelial cells than lysis by other enzymes [17]. In this study, collagenase/hyaluronidase was used to isolate primary lung cancer cells (Figure 1). It has been shown that overnight incubation with collagenase/hyaluronidase can successfully obtain and culture cancer cells without affecting the adhesion or proliferation ability of the isolated cells (Figure 6). The choice of culture medium for primary culture of lung cancer cells is a key factor. Media such as DMEM, RPMI-1640, HITES, and ACL-4 can be used for adenocarcinoma culture [14,18]. In the study of Ho et al, the enzyme collagenase was used to isolate cancer cells from NSCLC patients and the cells were successfully cultured in DMEM medium [19]. In view of these studies, we used DMEM with penicillin and streptomycin as culture medium in this study. Another important factor to be considered in primary culture is the use of extracellular matrix (ECM). ECM proteins such as collagen and fibronectin are used in many studies because they allow cells to adhere better to the culture dish [15]. However, ECM proteins can also cause cell differentiation [20]. For this reason, our study demonstrated that isolated cancer cells can be cultured without the use of ECM proteins.

While no adherent cells were observed on the first day after primary culture, they were observed to adhere and proliferate in the following days. With increasing culture time, the number of adherent cells increased. Cells from patient 1 were observed to adhere and proliferate earlier compared to other patients. Cells that appeared spindle-shaped or polygonal gradually aggregated into clumps and filled the culture dish. After an average of 5 days, the cells grew rapidly, and by day 10, cells from patients 1 and 3 were 70-80% confluent. This shows us that the culture times required for attachment of cancer cells from different patients are different (Figure 6).

This suggests that each patient has a specific phenotype of cell behavior for their tumor tissue. Therefore, the creation of an *in vitro* model using patient tissue can help clinicals perform individual drug screening for personalized cancer treatment, test potential drug candidates, and thus determine the clinician decision for predictive oncology, based on the fact that each patient's response to therapy is unique [21-23].

Primary cell culture is an important research platform to explore the biological characteristics of patient-specific lung cancer cells. For this reason, it is considered a method that can bridge, the gap between the laboratory and the clinician's clinical decision-making process. Consequently, the successful implementation and developmentof primary cell culture method is expected to contribute the understanding of tumor biology and future studies of personalized treatments.

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References

1. de Koning, H. J., van der Aalst, C. M., de Jong, P. A., Scholten, E. T., Nackaerts, K., Heuvelmans, M. A., Lammers, J. J., Weenink, C., Yousaf-Khan, U., Horeweg, N., van 't Westeinde, S., Prokop, M., Mali, W. P., Mohamed Hoesein, F. A. A., van Ooijen, P. M. A., Aerts, J. G. J. V., den Bakker, M. A., Thunnissen, E., Verschakelen, J., Vliegenthart, R., … Oudkerk, M. 2020. Reduced Lung-Cancer Mortality with Volume CT Screening in a Randomized Trial. The New England Journal of Medicine, *382*(6), 503–513.
2. Nooreldeen, R., & Bach, H. 2021. Current and Future Development in Lung Cancer Diagnosis. International Journal of Molecular Sciences, *22*(16), 8661.
3. Reck, M., Heigener, D. F., Mok, T., Soria, J. C., & Rabe, K. F. 2013. Management of Non-Small-Cell Lung Cancer: Recent Developments. Lancet, 382(9893):709-19.
4. Dagogo-Jack, I., & Shaw, A. T. 2018. Tumour Heterogeneity and Resistance to Cancer Therapies. Nature reviews. Clinical Oncology, *15*(2), 81–94.
5. Kohl, C., Aung, T., Haerteis, S., Ignatov, A., Ortmann, O., & Papathemelis, T. 2022. The 3D in vivo Chorioallantoic Membrane Model and Its Role in Breast Cancer Research. Journal of Cancer Research and Clinical Oncology, *148*(5), 1033–1043.
6. Mitra, A., Mishra, L., & Li, S. 2013. Technologies for Deriving Primary Tumor Cells for Use in Personalized Cancer Therapy. Trends in Biotechnology, *31*(6), 347–354.
7. Gazdar, A. F., Girard, L., Lockwood, W. W., Lam, W. L., & Minna, J. D. 2010. Lung Cancer Cell Lines as Tools for Biomedical Discovery and Research. Journal of the National Cancer Institute, *102*(17), 1310–1321.
8. Mak, I. W., Evaniew, N., & Ghert, M. 2014. Lost in Translation: Animal Models and Clinical Trials in Cancer Treatment. American Journal of Translational Research, *6*(2), 114–118.
9. Huo, K. G., D'Arcangelo, E., & Tsao, M. S. 2020. Patient-Derived Cell Line, Xenograft and Organoid Models in Lung Cancer Therapy. Translational Lung Cancer Research, *9*(5), 2214–2232.
10. Naipal, K. A., Verkaik, N. S., Sánchez, H., van Deurzen, C. H., den Bakker, M. A., Hoeijmakers, J. H., Kanaar, R., Vreeswijk, M. P., Jager, A., & van Gent, D. C. 2016. Tumor Slice Culture System to Assess Drug Response of Primary Breast Cancer. BMC Cancer, *16*, 78.
11. Hu, W., Liu, Y., Zhou, W., Si, L., & Ren, L. 2014. CXCL16 And CXCR6 Are Coexpressed in Human Lung Cancer in vivo and Mediate The İnvasion of Lung Cancer Cell Lines in vitro. PloS One, *9*(6), e99056.
12. Neve, R. M., Chin, K., Fridlyand, J., Yeh, J., Baehner, F. L., Fevr, T., Clark, L., Bayani, N., Coppe, J. P., Tong, F., Speed, T., Spellman, P. T., DeVries, S., Lapuk, A., Wang, N. J., Kuo, W. L., Stilwell, J. L., Pinkel, D., Albertson,

D. G., Waldman, F. M., … Gray, J. W. 2006. A Collection of Breast Cancer Cell Lines For The Study of Functionally Distinct Cancer Subtypes. Cancer Cell, *10*(6), 515–527.

13. Stacey G. N. 2011. Cell Culture Contamination. Methods in Molecular Biology *(Clifton, N.J.)*, *731*, 79–91.
14. Si, L. L., Lv, L., Zhou, W. H., & Hu, W. D. 2015. Establishment and Identification of Human Primary Lung Cancer Cell Culture in vitro. International Journal of Clinical and Experimental Pathology, *8*(6), 6540–6546.
15. Xi, K. X., Wen, Y. S., Zhu, C. M., Yu, X. Y., Qin, R. Q., Zhang, X. W., Lin, Y. B., Rong, T. H., Wang, W. D., Chen, Y. Q., & Zhang, L. J. 2017. Tumor-Stroma Ratio (TSR) İn Non-Small Cell Lung Cancer (NSCLC) Patients After Lung Resection is a Prognostic Factor for Survival. Journal of Thoracic Disease, *9*(10), 4017–4026.
16. Nazareth, M. R., Broderick, L., Simpson-Abelson, M. R., Kelleher, R. J., Jr, Yokota, S. J., & Bankert, R. B. 2007. Characterization of Human Lung Tumor-Associated Fibroblasts and Their Ability to Modulate The Activation of Tumor-Associated T Cells. Journal of Immunology *(Baltimore, Md.: 1950)*, *178*(9), 5552–5562.
17. Valente, M. J., Henrique, R., Costa, V. L., Jerónimo, C., Carvalho, F., Bastos, M. L., de Pinho, P. G., & Carvalho, M. 2011. A Rapid and Simple Procedure for The Establishment of Human Normal and Cancer Renal Primary Cell Cultures from Surgical Specimens. PloS One, *6*(5), e19337.
18. Saforo, D., Omer, L., Smolenkov, A., Barve, A., Casson, L., Boyd, N., Clark, G., Siskind, L., & Beverly, L. 2019. Primary Lung Cancer Samples Cultured Under Microenvironment-Mimetic Conditions Enrich for Mesenchymal Stem-Like Cells that Promote Metastasis. Scientific Reports, *9*(1), 4177.
19. Morgan, M. M., Johnson, B. P., Livingston, M. K., Schuler, L. A., Alarid, E. T., Sung, K. E., & Beebe, D. J. 2016. Personalized in vitro Cancer Models to Predict Therapeutic Response: Challenges and a Framework for Improvement. Pharmacology & Therapeutics, *165*, 79–92.
20. Ho, M. M., Ng, A. V., Lam, S., & Hung, J. Y. 2007. Side Population in Human Lung Cancer Cell Lines and Tumors is Enriched with Stem-Like Cancer Cells. Cancer Research, *67*(10), 4827–4833.
21. Järveläinen, H., Sainio, A., Koulu, M., Wight, T. N., & Penttinen, R. 2009. Extracellular Matrix Molecules: Potential Targets in Pharmacotherapy. Pharmacological Reviews, *61*(2), 198–223.
22. Cree, I. A., Glaysher, S., & Harvey, A. L. 2010. Efficacy of Anti-Cancer Agents in Cell Lines Versus Human Primary Tumour Tissue. Current Opinion in Pharmacology, *10*(4), 375–379.
23. Esparza-López, J., Martínez-Aguilar, J. F., & Ibarra-Sánchez, M. J. 2019. Derıvıng Prımary Cancer Cell Cultures for Personalızed Therapy. Revista de investigacion clinica; organo del Hospital de Enfermedades de la Nutricion, *71*(6), 369–380.