İSTANBUL UNIVERSITY PRESS

RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ
Journal of Advanced Research in Health Sciences / Sağlık Bilimlerinde İleri Araştırmalar Dergisi 2023
DOI: 10.26650/JARHS2023-1221034

DETERMINATION OF METASTATIC CAPACITY IN PRIMARY LUNG CANCER CELLS: REFLECTION OF PATIENT PROFILE IN THE CLINIC USING *IN VITRO* METHODS

PRİMER AKCİĞER KANSER HÜCRELERİNDE METASTATİK KAPASİTENİN BELİRLENMESİ: KLİNİKTEKİ HASTA PROFİLİNİN *İN VİTRO* YÖNTEMLERLE YANSITILMASI

Seçil YILMAZ[1], Medine DOĞAN SARIKAYA[1], Elif YAŞAR[1], Burcu ŞEN BAĞCI[1], Özlem CANÖZ[2], Ömer ÖNAL[3]

[1] Erciyes University, Genome and Stem Cell Center, Kayseri, Turkiye
[2] Erciyes University, Faculty of Medicine, Department of Pathology, Kayseri, Turkiye
[3] Erciyes University, Faculty of Medicine, Department of Thoracic Surgery, Kayseri, Turkiye

ORCID ID: S.Y. 0000-0001-9381-828X; M.D.S. 0000-0003-0435-6066; E.Y. 0000-0001-5974-4176; B.Ş.B. 0000-0002-4526-5198; Ö.C. 0000-0002-0200-6970; Ö.Ö. 0000-0002-9971-7401

Citation/Atıf: Yilmaz S, Dogan Sarikaya M, Yasar E, Sen Burcu B, Canoz O, Onal O. determination of metastatic capacity in primary lung cancer cells: reflection of patient profile in the clinic using *in vitro* methods. Journal of Advanced Research in Health Sciences 2023;6(3):302-311. https://doi.org/10.26650/JARHS2023-1221034

ABSTRACT

Objective: There is a scarcity of *in vivo* models that accurately reflect tumor growth and metastasis in cancer research. Research using cell lines with increasing passage numbers may give misleading results because the tumor loses its characteristic feature. Primary culture is the best method to represent the cellular profile of cancer patients in the laboratory environment. Therefore, we worked with patient-derived primary culture. The most common subtype of the most diagnosed lung cancer worldwide is Non-Small Cell Lung Cancer (NSCLC). Therefore, we aimed to determine patient-specific metastatic capacities by comparatively examining the migration abilities of primary cancer cells of NSCLC patients on the same platform.

Materials and methods: The migration abilities of primary cancer cells of NSCLC patients were demonstrated through wound healing assays on cisplatin and non-cisplatin groups, and measurements were made with Image J software.

Results: In the results of the wound healing assays performed on the cancer cells of five patients, it was observed that there was correlation between the wound widths, wound areas, wound closure percentages, and metastasis in the groups with and without cisplatin in Patient 3, Patient 4, and Patient 5.

Conclusion: To reflect the profile of patients visiting the clinic using patient-derived primary tumor cells, the wound healing assay can be used as a tool to demonstrate tumor behaviors, such as the patients' responses to treatment and their metastasis-forming capacity. Detailed studies are needed in a larger population so that the physician can use in vitro tools in the decision support mechanism.

Keywords: Lung cancer, primary culture, migration, wound healing assay

ÖZ

Amaç: Kanser çalışmalarında tümöre dair büyüme ve metastaz durumunu birebir yansıtan *in vivo* modellerin azlığı söz konusudur. Hücre hatları kullanılan çalışmalarda ise pasaj sayıları ilerledikçe tümörün karakteristik özelliğini kaybetmesinden dolayı yanıltıcı sonuçlar verebilmektedir. Kanser hastalarının hücresel profilini laboratuvar ortamına taşıyabilmek için en iyi yöntem primer kültürdür. Bu nedenle çalışmamızda hasta kaynaklı primer kültür ile çalıştık. Dünya çapında en çok tanı konulan akciğer kanserinin en sık görülen alt tipi Küçük Hücreli-Dışı Akciğer Kanseri (KHDAK)'dir. Bu nedenle çalışmamızda KHDAK tanısı alan hastalardan elde edilen primer kanser hücrelerinin migrasyon yeteneklerinin tek bir platformda karşılaştırmalı olarak incelenerek hastaya özgü metastatik kapasitelerinin belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: KHDAK hastalarına ait primer kanser hücrelerinin migrasyon yetenekleri yara iyileştirmesi deneyiyle cisplatin içeren ve içermeyen grup üzerinde gösterilmiştir ve ölçümler Image J yazılımıyla yapılmıştır.

Bulgular: Beş hastanın kanser hücresine ait yapılan yara iyileşmesi deneyi sonuçlarında; Hasta 3, Hasta 4 ve Hasta 5'e ait cisplatin içeren ve içermeyen gruplarda yara genişlikleri, yara alanları ve yara bölgesini kapatma yüzdeleri ile metastaz arasında ilişki olduğu gözlendi.

Sonuç: Kliniğe başvuran hasta profilinin *in vitro* ortama en iyi şekilde yansıması için hasta kaynaklı primer tümör hücreleri kullanılarak hastaların tedaviye karşı vermiş oldukları yanıtların çeşitliliği ve metastaz oluşturma kapasiteleri gibi birçok tümör davranışının gösterilmesinde yara iyileşmesi deneyi bir araç olarak kullanılabilir. Hekimin karar destek mekanizmasında *in vitro* araç olarak kullanılabilmesi için daha geniş popülasyonda detaylı çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Akciğer Kanseri, primer kültür, migrasyon, yara iyileşmesi deneyi

Corresponding Author/Sorumlu Yazar: Seçil YILMAZ **E-mail:** siyilmaz@erciyes.edu.tr

Submitted/Başvuru: 19.12.2022 • **Revision Requested/Revizyon Talebi:** 07.02.2023 • **Last Revision Received/Son Revizyon:** 18.04.2023 • **Accepted/Kabul:** 28.04.2023 • **Published Online/Online Yayın:** 10.10.2023

INTRODUCTION

Lung cancer is one of the most common cancers in the world, with approximately 2 million new cases and 1.76 million deaths per year, and is the leading cause of cancer-related death (1). Lung cancer consists of molecular and histologically heterogeneous subtypes. Two of these are non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) (2). NSCLC accounts for 85% of all lung cancer diagnoses (3). Despite significant advances in treatment, NSCLC after surgical resection has a poor prognosis. Lung carcinomas are mostly in metastatic stage IV when diagnosed, and there are targeted organs for metastasis, such as the brain, bones, and adrenal glands (4,5). Lung carcinomas metastasize to lymphatic as well as blood vessels (6). When resected lung carcinomas are carefully evaluated, vascular invasion is often observed in low-stage tumors, which often leads to an increased incidence of recurrence and reduced patient survival (5). Therefore, identifying the molecular mechanisms underlying lung cancer progression may aid in the development of potential biomarkers and new therapeutic targets for malignancy (7).

For years, it has been suggested that collective cell migration plays important roles in the invasion and metastasis of malignant tumors (8). Collective cell migration is a fundamental process, a coordinated movement of grouped cells connected via cell-cell connections (9). Various in vitro techniques have been developed to study the dynamic process of collective cell migration. Among these techniques, the wound-healing assay is one of the most fundamental and commonly used methods to study collective cell migration because of its potential to visualize cells during migration (10). It is also commonly used in drug trials to test the effectiveness of potential therapeutic drugs and similar approaches (11,12).

In vivo and in vitro studies are frequently applied in cancer research. However, the development and preclinical testing of new cancer treatments is limited due to the scarcity of *in vivo* models that demonstrate tumor growth and metastatic progression (13). In addition, *in vivo* models are insufficient to demonstrate essential aspects of human malignancies such as invasion and metastasis; they are not predictive of clinical outcomes and are expensive, as well as time-consuming (14,15). However, *in vitro* models allow quantitative analysis and control of most experimental variables in the tumor microenvironment (16). In studies with cell lines, cell lines lose the characteristics of the primary tumor and may produce different results, since increasing passage numbers of cell lines can cause genotypic and phenotypic differences (17,18). Furthermore, understanding the genetic and epigenetic diversity of millions of patients from a small number of cell lines is difficult because there is wide variation in response to treatment between patients. This is the driving force behind personalized medicine and the development of methods for obtaining and culturing primary tumor cells from patients (19).

In addition to measuring the migration capacities of primary cells obtained from NSCLC patients, the aim of this study was to determine the treatment resistance profiles of patients by demonstrating the effect of cisplatin, one of the "first choice" drugs to treat lung cancer, on cell migration with the wound healing assay.

MATERIAL AND METHODS

The experiments carried out within the scope of this project are briefly schematized in Figure 1.

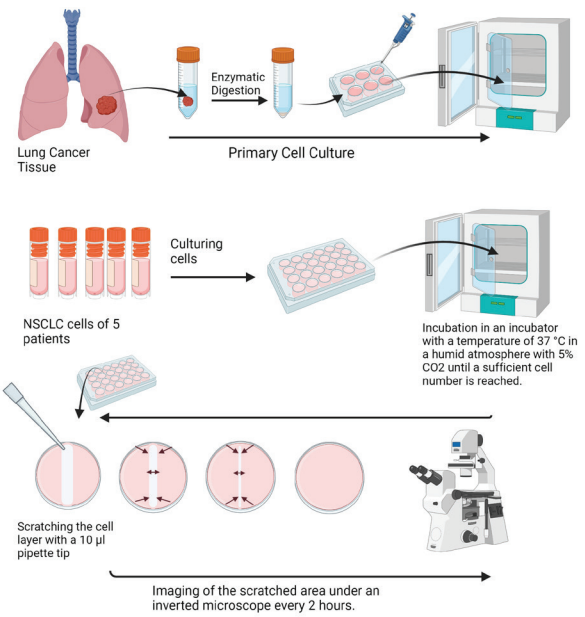

Figure 1: Summary of the experiments carried out within the scope of the project. Lung cancer cells isolated within the scope of the previous project and stocked at -80°C were re-cultured and incubated until sufficient cell number was reached. After reaching a sufficient number, a vertical scratch was created on the cell layer with a 10 µl pipette tip. Images of the scratched area were taken every 2 hours under an inverted microscope.

Collection of samples and primary cell culture

Patients who were diagnosed with definite NSCLC as a result of pathological examinations after surgical resection at Erciyes University Thoracic Surgery Department were included in the study. An informed consent form was signed from the patients before the samples were collected. The cells used in this study were obtained from the TUBITAK 1001 project "Genomic Profiling of Cancer Stem Cells in Lung Cancer Patients," numbered 215S849. This study was approved by the clinical research ethics committee of the Erciyes University Faculty of Medicine under decision number 2015/372, dated 26.08.2015, and was carried out according to the Declaration of Helsinki Principles (www.wma.net/e/policy/b3.htm). For primary culture of patient-derived lung cancer cells, cancer tissues were brought to the Genome and Stem Cell Center from Erciyes University Medical Pathology Department in a cold transport medium (Dulbecco's Modified Eagle medium (DMEM; Gibco, Grand Island, NY, USA, Cat. No. 41966029)) with 1% penicillin-streptomycin (Thermo Fisher Scientific Waltham, Massachusetts, USA, Cat. No. 15070063) within 30 minutes. Cells isolated from

the tumor tissue after mechanical and enzymatic digestion in the cabinet, with 1% penicillin-streptomycin (Thermo Fisher Scientific Waltham, Massachusetts, USA, Cat. No. 15070063), 1% Amphotericin (Thermo Fisher Scientific Waltham, Massachusetts, USA, Cat. No. 15290026) and 1% L-Glutamine (STEMCELL Technologies Inc., Vancouver, Canada, Cat. No. 7100) contained in the DMEM (Gibco, Grand Island, NY, USA, Cat. No. 41966029), were cultured at 37°C incubator with 5% CO_2 atmosphere.

Wound healing assay for determination of patient-specific migration ability 4-8

For the wound healing experiment, which was designed with 3 repetitive groups, cells with passage numbers in the range 4-8 were cultured in a 24-well plate with cell densities of $2x10^4$ cells/well. A total of $12x10^4$ NSCLC cells were used. When the cells were at 80% confluence in the 24-well plate, a wound was created by scratching using a sterile 10 µl micropipette tip, which created a cell-free gap. Images were recorded with the microscope every two hours for 24 hours to measure the wound size.

Drug preparation and administration in wound healing experiment

For the wound healing experiment, which was carried out in 3 repetitive drug groups 5 µM cisplatin and 3 repetitive control groups, the cells with passage numbers in the range 4-8 were cultured in a 24-well plate with $2x10^4$ cells/well. A total of $12x10^4$ NSCLC cells were used. 5 µM cisplatin was prepared, while the cells were expected to coat the 24-well plate.

Cisplatin preparation

cis-Diamineplatinum (II) dichloride (Sigma, Cat No: 479306) was prepared in accordance with the protocol for making stock solution from powder form. The protocol is explained below in brief:

-Stock cisplatin was prepared with 1 mg of cisplatin in 1 ml of medium.

-Since 5 µM cisplatin would be used within the scope of this study, 5 µl of stock cisplatin was taken and 995 µl of falcon with medium was taken. After pipetting to ensure heterogeneous distribution in the solution, the 5 µM cisplatin dose was kept at 4°C, ready to use. It was added to the culture dishes by pipetting before use.

When the cells covered the culture dishes, the wound was created by scratching the cell layer with a 10 µl pipette tip and was then washed with DPBS to eliminate the raised cells from the culture dishes. Then, 5 µM cisplatin was added to the drug group, while only the medium was added to the control group. Wound closure was observed every two hours with an inverted light microscope (DMi1, Leica, Germany). Images of the created wound were recorded using the Leica application package V4 software.

Analysis

The wound area was measured using Java's Image J software (http://rsb.info.nih.gov). The migration of cells towards wounds is expressed as percent wound closure. Wound closure percentages were calculated according to the formula % wound closure= [(At=0h- At=Δh)/At=0h] X 100% (20). The correlation of the migration in the patients' cells between the hours was analyzed by calculating their mean and standard deviation.

RESULTS

Clinical information of lung cancer patients

Tumor diameter, metastasis status, survival, and adjuvant treatment options for patient-derived NSCLC cells used in this study are given in Table 1. According to the information obtained from the Erciyes University Thoracic Surgery Department, it was seen that Patient 1 and Patient 3 had brain and lymph node metastases.

Wound healing assay of patient-derived lung cancer cells

Images of the wounds created with a 10 µl pipette tip in the wound healing assay are shown in Figure 2. When cells cultured in 24-well plates were scratched after covering the culture dish, it was considered the 0th hour of the wound healing assay. Cell images were recorded every two hours for 24 hours.

Table 1: Clinical information of patients with non-small cell lung cancer

Patient Numbers	Age	Tumor Diameter	Stage	Metastasis	Survival	Adjuvant treatment
Patient 1	52	6*4*4	III	Brain-Lymph node	Ex	-
Patient 2	58	1.2*0.8*0.*8	I	-	Living without disease	-
Patient 3	67	6*4*4	III	Brain-Lymph node	Living with disease	-
Patient 4	72	4*3*3	I	-	Ex	Chemotherapy
Patient 5	52	5.5*4.5*4	II	-	Living without disease	Chemotherapy

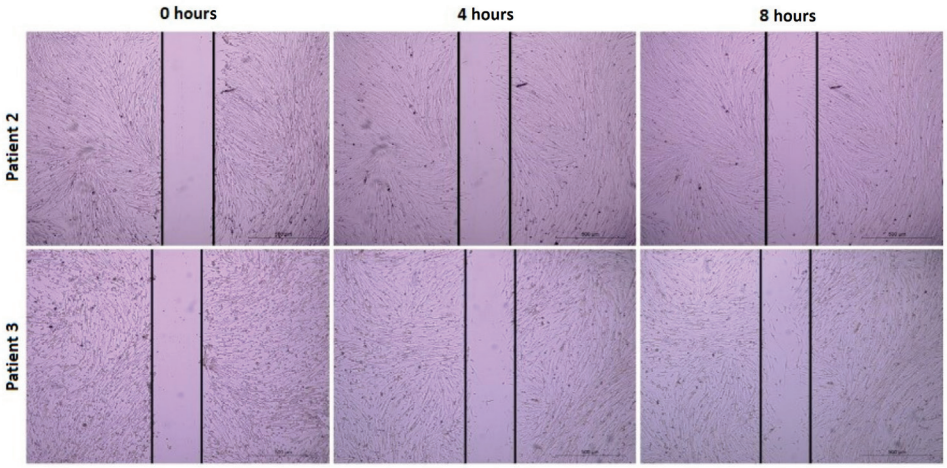

Figure 2: Wound healing assay images of cancer cells of Patient 2 and Patient 3. The wound area is indicated by lines. Images of the wound area at 0, 4, and 8 hours were recorded with a 4X objective. (Three repetitions)

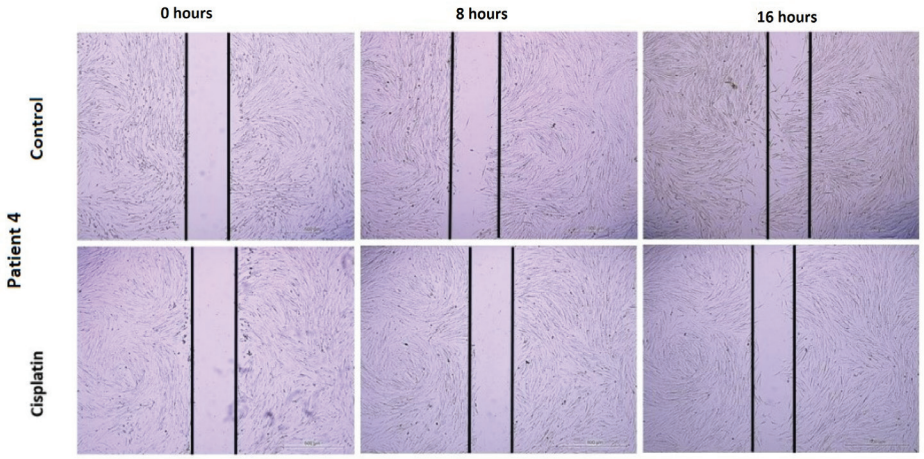

Figure 3: Wound healing assay images of Patient 4, which was performed by creating an experimental group containing 5 µM cisplatin and a control group without cisplatin in cancer cells of NSCLC patients. 0, 8 and 16 hours are shown. Cell images with a scratch area were recorded with a 4X objective. (Three repetitions)

Wound healing assay of patient-derived lung cancer cells (cisplatin)

Within the scope of this study, cell images of two different groups, the experimental group containing 5 µM cisplatin and the control group without cisplatin, were recorded every two hours for 24 hours. Likewise, images of wounds created with a 10 µl pipette tip are shown in Figure 5 for both groups. When cells cultured in 24-well culture dishes were scratched after covering the culture dish, it was considered the 0th hour of the wound healing experiment. Width (Figure 4A-B) and area measurements were made on the recorded cell images with the ImageJ program (Figure 4C-D).

Comparison of wound widths in patient-derived primary lung cancer cells

The findings of the comparison of wound widths of both groups with and without cisplatin are shown in Figure 5. In order to show the differences between the patients more clearly, the graph of each patient is shown separately.

Comparison of wound areas in patient-derived primary lung cancer cells

The findings of the comparison of the wound areas of both groups with and without cisplatin are shown in Figure 6.

Comparison of wound closure percentages in patient-originated primary lung cancer cells

Wound closure percentages were calculated according to the formula % wound closure= [(At=0h- At=Δh) /At=0h] X 100% in order to compare the clinical data and CSC rates of the patients according to the closure between the experimental and control groups (Figure 10).

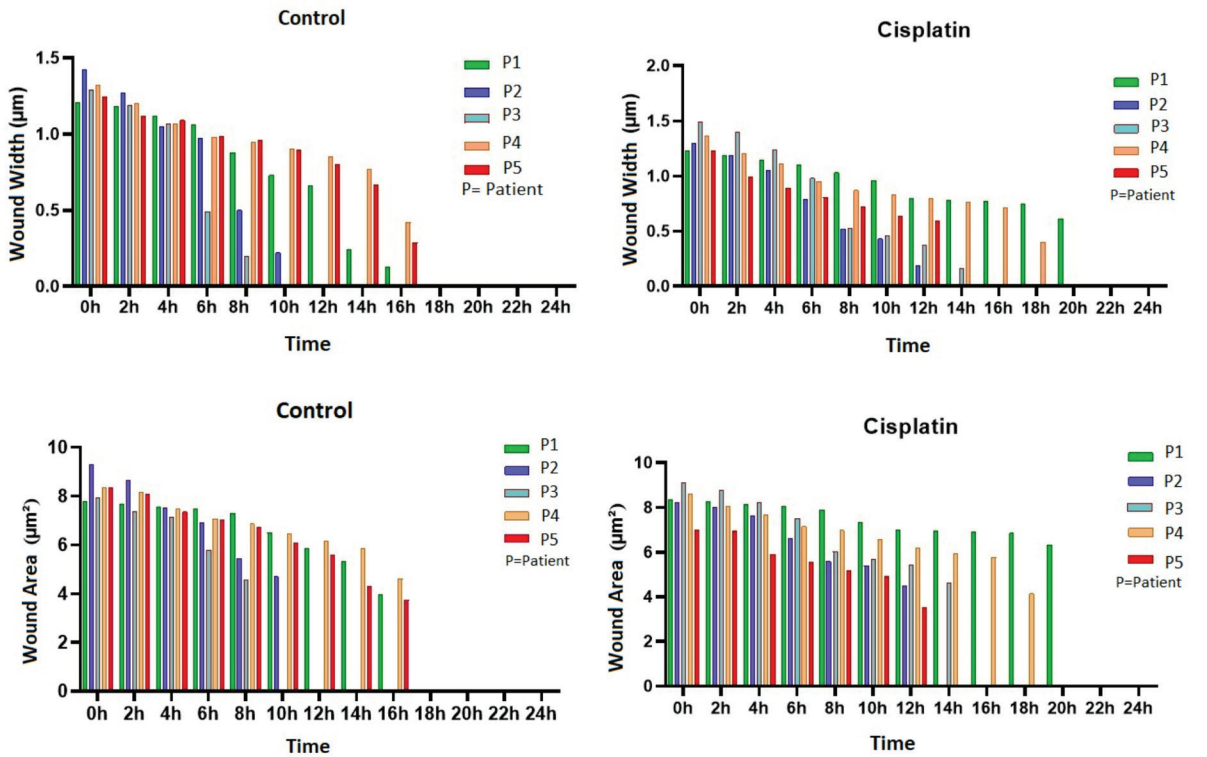

Figure 4: Measurements of the experimental and control groups obtained as a result of the 24-hour follow-up of the wound widths and wound areas created as a result of the wound healing assay of NSCLC patients. (A-B) Measurements of wound widths every two hours for 24 hours in the drug and control groups in the wound healing assay of NSCLC patients. (C-D) Measurements of wound areas every two hours for 24 hours as a result of the wound healing assay of NSCLC patients. All experiments were repeated three times.

DISCUSSION

NSCLC migration is the most prevalent subtype of lung cancer, with a five-year survival rate of roughly 15% (21). At the time of diagnosis, the majority of NSCLC patients are in the metastatic stage (22). 90% of all cancer-related deaths are thought to be caused by tumor metastases (23). Studies both *in vivo* and *in vitro* demonstrate that metastatic cancer cells spread one at a time (24). For a better understanding of metastasis, it is essential to comprehend the methods through which these tumor cells migration and penetrate (25). The molecular underpinnings of lung cancer and studies on metastasis must be further investigated because lung cancer is already metastatic when it is diagnosed. Therefore, our study compared the migration capabilities of patient-derived primary cells with different profiles in NSCLC, the most common subtype of lung cancer. Table 1 displays the patients' clinical profiles. It is seen that Patient 1 (P1) and Patient 3 (P3) have metastases, and Patient 4 (P4) and Patient 5 (P5) receive chemotherapy.

Although cell migration has an important role in cancer and metastasis, approaches to studying cell migration are very important in oncology, since cell migration is related to the effects of new therapeutic drugs and chemoattractants in the metastatic process. (26,11). Particularly, collective cell migration is a common type of migration seen during wound healing and metastasis of epithelial cancers (27,28). In addition, cell migration, which plays a role in re-epithelialization, is a mediator of angiogenesis and tumor invasion (29-31). Therefore, cell migration may also mediate cancer. Cell migration is an essential component of pathological and physiological processes encompassing all these (10). It is possible to monitor the metastasis potential of cancer cells with the wound healing assay, which is a common in vitro biological assay used to investigate cancer cell migration and to test drugs with therapeutic efficacy. In the study by Bahar et al. on four ovarian cancer cell lines, it was shown that the cell lines have different migration capacities (11). In the study by Pijuan and colleagues, the migration capacity of melanoma cells was demonstrated by a wound healing experiment (32). In another study by Jonkman and colleagues, the migratory capacity of breast cancer cells was demonstrated with a wound healing experiment in MCF7, a breast cancer cell line (33). In addition, in the study by Sullivan et al., it was shown that the wound area decreased three times faster in MDA-MB-231 cells compared to the less invasive MDA-MB-468 cells (34).

Wound healing assay is frequently used in the literature to measure the migration ability of cells. Although the wound healing assay is widely used, there are some limitations to the traditional wound healing experiment. First, the scratch rate

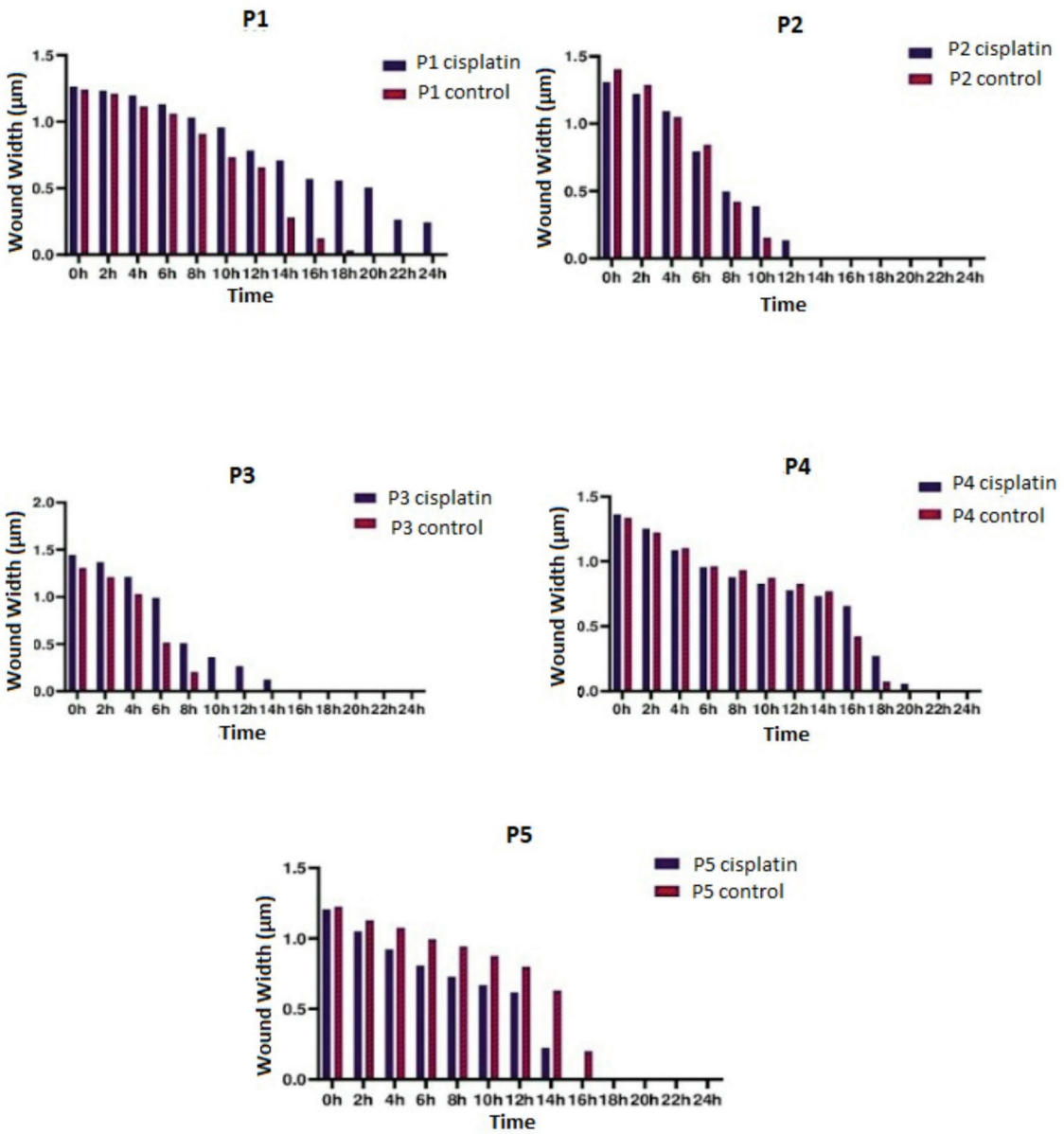

Figure 5: Comparison of wound widths in cisplatin and control groups of NSCLC patients (Three repetitions)

and shape of the injured area may vary between different experiments. Second, scraping involves mechanical damage to cells and cellular contents may be released into the environment. The extent of cell damage is difficult to control and can complicate the process of cell migration (10). These limitations can make comparisons between experiments difficult. For this reason, the optimization of the experiment was performed and the images of the cells were taken at intervals of two hours, as the experiments were repeated in triplicate. Two-way verification was provided by calculating the area as well as the wound width. The Image J program was used to avoid manual errors in the measurements. As a result of measuring the wound width according to a fixed area determined, at the 10th hour of the cancer cells of P3 and the 12th hour of the cancer cells of P2 the cancer cells completely covered the wound width it was observed that the cancer cells of P1, P4, and P5 completely covered the wound width at the 18th hour (Figure 4A). Consistent with these data, cancer cells were detected in the entire wound area belonging to P3 at the 10th hour and cancer cells of P2 at 12th hour. It was observed that the cancer cells of P1, P4, and P5 completely closed at 18 hours (Figure 4B). Although they closed the wound area at the same time, it was observed that the cancer cells with the slowest rate of closing the wound area belonged to P4, followed by P5 and P1, respectively. It was observed that the cancer cells that closed the wound area the fastest belonged to P3. These results show that cells from different patients exhibit different profiles. Images of the wound healing experiment in the cancer cells of P2

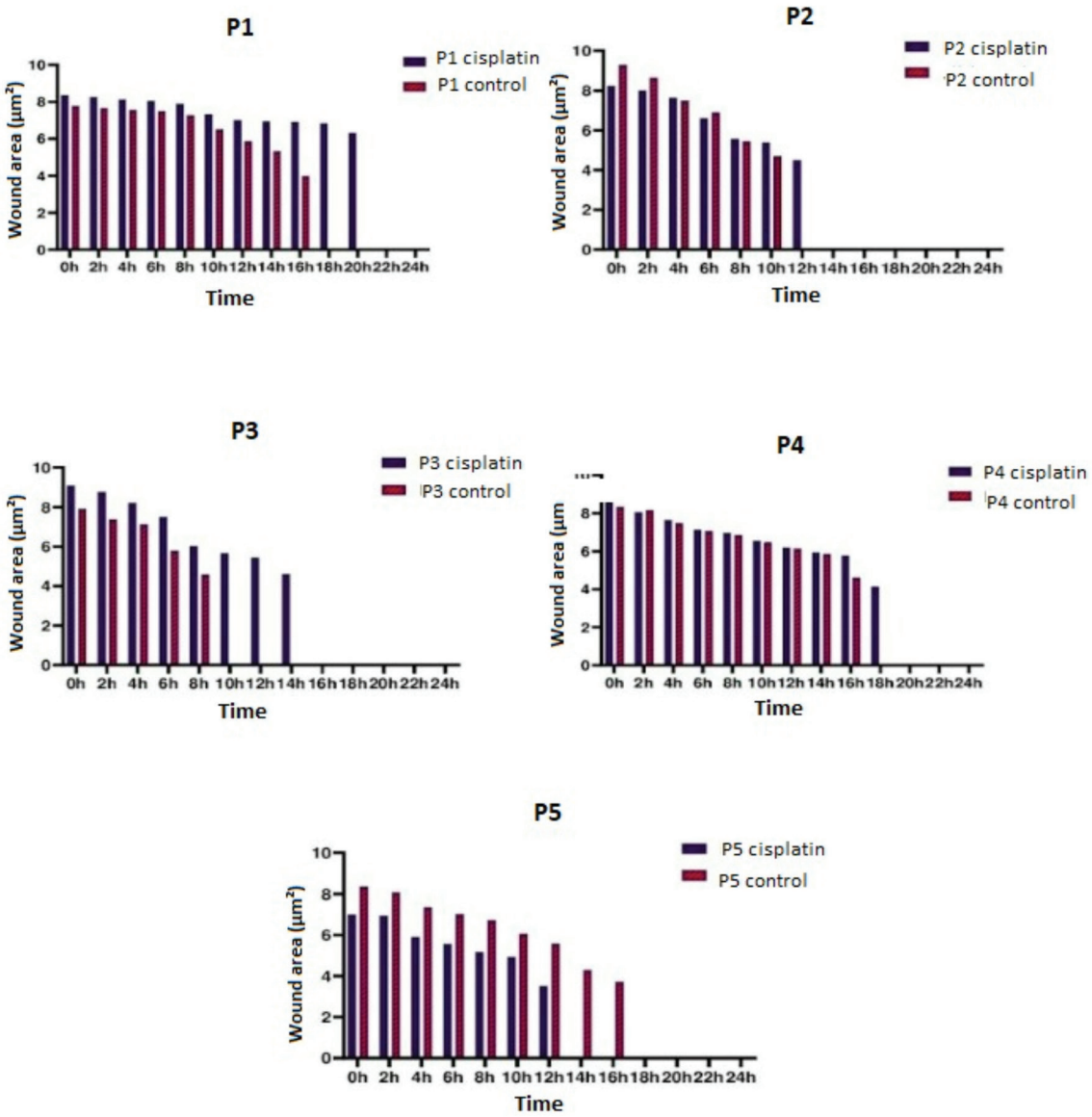

Figure 6: Comparison of the wound area in the cisplatin and control groups of NSCLC patients (Three repetitions)

and P3 (both NSCLC patients) are given in Figure 2. When we look at the images of the wound area at 0 hours, 4 hours, and 8 hours in patients whose wound area is indicated by lines, it is seen that P3's cells migrated to the injured area more quickly compared to P2. In these images of both patients, it is seen that the migration abilities of the cancer cells of the two patients are different. Considering the clinical information of the patient in Table 1, which confirms this finding, it is seen that P3 has brain-lymph node metastasis. It can be said that this migration ability of P3's cells is related to metastasis.

Adjuvant cisplatin-based chemotherapy is the standard of care in patients with completely resected NSCLC stage II-III and in patients with stage I NSCLC (35). Cisplatin interacts with DNA double strands by formation of interstrand and intrastrand adducts, and thereby induces apoptosis in cancer cells through the interference with DNA replication and gene transcription. Similar to other chemotherapeutic agents, the effect of cisplatin is commonly limited by the resistance of cancer cells. Cisplatin resistance can be intrinsic or acquired (36). Manguinhas and colleagues investigated cancer cell migration and invasion with the chemotherapeutic agent cisplatin and the cytotoxicity E3330 using the NSCLC cell line H1975 with a wound healing assay used to assess collective cell migration, maintenance of functional cell-cell junctions, and movement of cells across a horizontal surface (37). To investigate the effect of cisplatin on human lung cancer epithelial H460 cells migration, Maiuthed and colleagues performed wound healing assays (38). In this

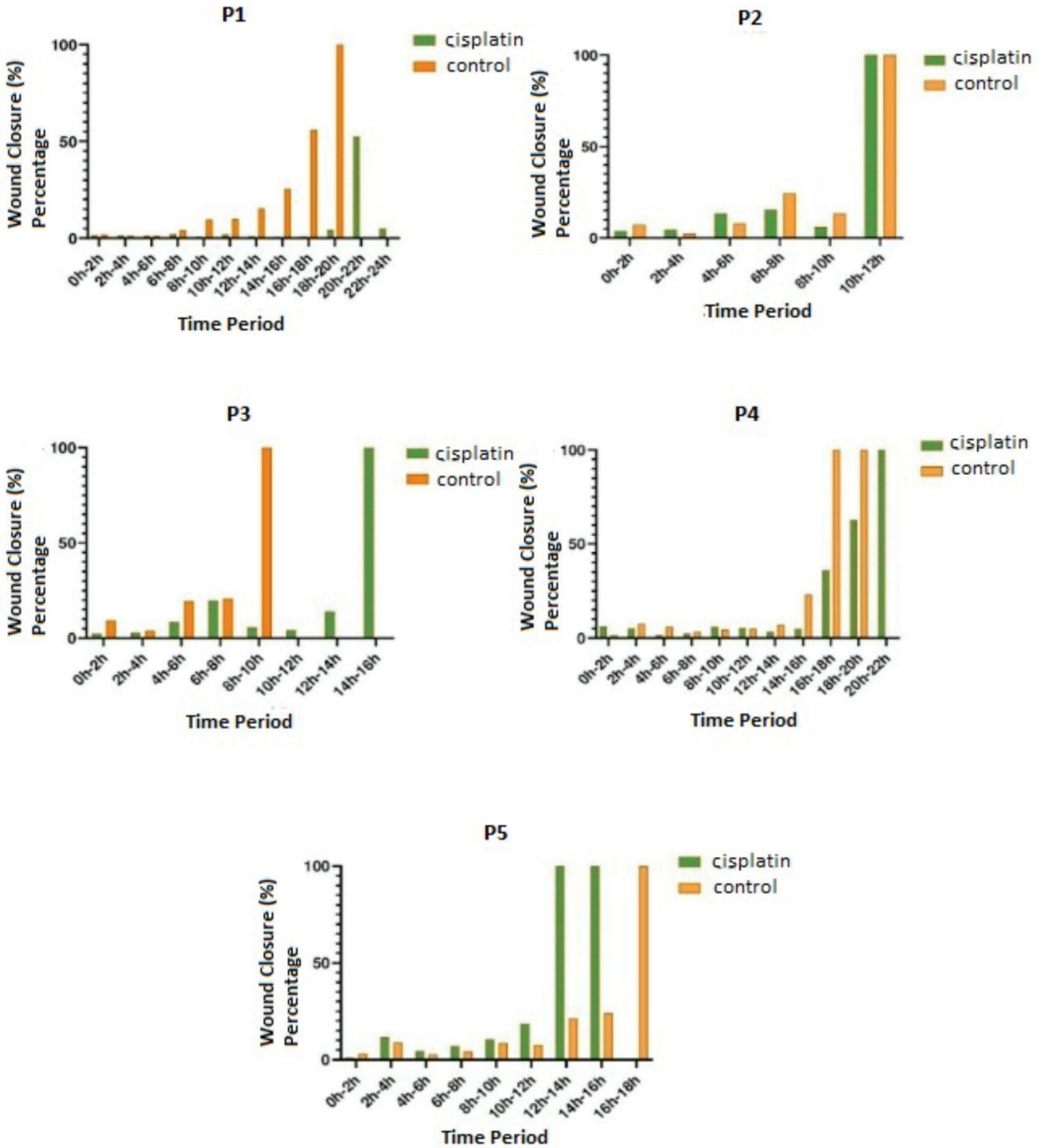

Figure 7: Comparison of the percentage of wound closure in the cisplatin and control groups of NSCLC patients (Three repetitions)

project, cisplatin, as the most widely used drug in NSCLC patients, was chosen for migration profiling of patient-derived cells in cell migration, since it blocks cell division. As a result of the experiments, the cell images of both the control and drug groups of P4, one of the cisplatin-administered groups, are given in Figure 3. It was observed that the wound areas were completely closed as follows. According to a fixed region determined in the groups, the wound width was determined by the cancer cells of P2 and P5 at the 12th hour, the cancer cells of P3 at the 16th hour, the cancer cells of P4 at the 20th hour, and the cancer cells of P1 at the 20th hour. (Figure 4B). Consistent with these data, in the cisplatin administered groups, it was observed that the wound area was completely closed at the 12th hour in the cancer cells of P2 and P5, at the 16th hour in the cancer cells of P3, at the 20th hour in the cancer cells of P4, and at the 22nd hour in the cancer cells of P1 (Figure 4D). When the closure rate was compared among the patients, it was observed that although P2 and P5 closed the wound area at the same time, the cancer cells with the fastest rate of closing the wound area belonged to P2. P3, P4, and P1 followed the rate of closure of the wound area by the cancer cells from high to low (Figure 4). The fact that both wound widths and wound areas, which were formed from triplicate groups according to the patient, supported each other, and that similar quantitative values showed the standardization of the study, provided support for the differences between

patients with more data (Figure 5,6). When the graph obtained is matched with the patients' clinical information, the absence of metastases in P2 and P5 can be interpreted as reduced aggressiveness and being more affected by the chemotherapeutic agent cisplatin. The closure of P1 in the cisplatin-containing group at the 22nd hour was examined. This patient's clinical data includes metastasis information, which does not reflect the aggressiveness and invasiveness of P1's cancer cells.

Wound closure percentages were calculated according to the formula in the literature in Figure 7 (20). When these graphs are examined, (i) as expected, the control group of Patient 1 was found to close earlier than the cisplatin group. It is found in the clinical data that this patient has metastasis. Although there is metastasis, delayed closure of the wound area compared to other patients may also be the result of heterogeneity between patients. (ii) P2 does not seem to have a history of metastasis or chemotherapy. In the wound healing experiment created from the cancer cells of this patient, it was observed that both the control group and the drug group containing cisplatin were closed very quickly at the 12th hour. When compared with the clinical picture of other patients, there was no correlation between metastasis and closure rate. (iii) It was observed that the wound area created in P3 closed faster than the cisplatin-containing drug group, as expected in the control group. Considering that cancer cells belonging to P3 are the fastest closure group compared to other patients and they have metastases in their clinical information, there may be a relationship between the ability to migrate and metastasize. (iv) In the wound healing experiment of P4, who was known to have received chemotherapy and had no metastases, it was observed that the control group had closed before, as expected. Compared to the closure time of the wound created in other patients, P4 closed later and the slower migration ability compared to other patients may be associated with metastasis. (v) The clinical picture of P5 shows that the patient received chemotherapy and has no metastases. In the areas where wound damage was created, closure occurred in both groups at close hours. In another study, it was shown that cisplatin-resistant cancer cells migrated more than non-resistant cells in a wound healing assay (39). The reason for this may be that cancer cells develop resistance to chemotherapy and therefore close at similar times between the drug group and the control group. In addition, considering that the control group closed later than other patients, slow migration abilities compared to other patients may be associated with metastasis.

Wound healing assay can be used as a tool in demonstrating the patient-specific migration ability of patients applying to the clinic *in vitro*, measuring differences in response to treatment and differences in drug resistance between patients. Detailed studies should be carried out by analyzing more patients and different parameters in order to be used as support in clinical decisions, such as the treatment to be given by the physician in the clinic according to the patient profile.

The authors' contributions to the study were equal and they did not receive any support for the study. The authors do not declare any conflict of interest.

Acknowledgements: We would like to thank Nilhan Mutlu for her help in this study, and the Erciyes University BAP unit and TUBITAK for their financial support.

Ethics Committee Approval: This study was approved by Erciyes University Faculty of Medicine Clinical Research Ethics Committee (Date: 26.08.2015 No: 2015/372).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- S.Y.; Data Acquisition- S.Y., Ö.Ö., Ö.C., M.D.S., B.Ş.B., E.Y.; Data Analysis/Interpretation- S.Y., Ö.D.S., E.Y.; Drafting Manuscript- S.Y., Ö.D.S., E.Y.; Critical Revision of Manuscript- S.Y., Ö.Ö., Ö.C., M.D.S., B.Ş.B., E.Y.; Final Approval and Accountability- S.Y., Ö.Ö., Ö.C.; Material and Technical Support- S.Y., Ö.Ö., Ö.C., M.D.S., B.Ş.B., E.Y.; Supervision- S.Y., Ö.Ö.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The study was supported by the Erciyes University Scientific Research Projects Unit (Project Number: THD_2021_11187) and the Scientific and Technological Research Council of Turkey (TÜBİTAK) 1001 project (Project Number: 215S849).

REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin 2022;72(1):7-33.
2. Howlader N, Forjaz G, Mooradian MJ, Meza R, Kong CY, Cronin KA, et al. The effect of advances in lung-cancer treatment on population mortality. N Engl J Med 2020;383(7):640-9.
3. Cengiz H, Demirci A, Varım C, Mandel NM, Turna Z. Küçük hücreli dışı akciğer kanserinde neoadjuvan ve adjuvan tedavi sonuçları. Sakarya Tıp Dergisi 2020;10(3):450-8.
4. Riihimäki M, Hemminki A, Fallah M, Thomsen H, Sundquist K, Sundquist J, et al. Metastatic sites and survival in lung cancer. Lung Cancer 2014;86(1):78-84.
5. Popper HH. Progression and metastasis of lung cancer. Cancer Metastasis Rev 2016;35(1):75-91.
6. Achen MG, Stacker SA. Molecular control of lymphatic metastasis. Ann N Y Acad Sci 2008;1131:225-34.
7. Wu Q, Zhang B, Li B, Cao X, Chen X, Xue Q. PTBP3 promotes migration of non-small cell lung cancer through regulating E-cadherin in EMT signaling pathway. Cancer Cell Int 2020;20:172.
8. Deisboeck TS, Couzin ID. Collective behavior in cancer cell populations. Bioessays 2009;31(2):190-7.
9. Friedl P, Gilmour D. Collective cell migration in morphogenesis, regeneration and cancer. Nat Rev Mol Cell Biol 2009;10(7):445-57.
10. Riahi R, Yang Y, Zhang DD, Wong PK. Advances in wound-healing assays for probing collective cell migration. J Lab Autom 2012;17(1):59-65.
11. Bahar E, Yoon H. Modeling and predicting the cell migration

properties from scratch wound healing assay on cisplatin-resistant ovarian cancer cell lines using artificial neural network. Healthcare (Basel) 2021;9(7):911.

12. Kauanova S, Urazbayev A, Vorobjev I. The Frequent sampling of wound scratch assay reveals the "opportunity" window for quantitative evaluation of cell motility-impeding drugs. Front Cell Dev Biol 2021;9:640972.
13. DeRose YS, Wang G, Lin YC, Bernard PS, Buys SS, Ebbert MT, et al. Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. Nat Med 2011;17(11):1514-20.
14. Kondo J, Inoue M. Application of cancer organoid model for drug screening and personalized therapy. Cells 2019;8(5):470.
15. Hait WN. Anticancer drug development: the grand challenges. Nat Rev Drug Discov 2010;9(4):253-4.
16. Katt ME, Placone AL, Wong AD, Xu ZS, Searson PC. In vitro tumor models: Advantages, disadvantages, variables, and selecting the right platform. Front Bioeng Biotechnol 2016;4:12.
17. Kaur G, Dufour JM. Cell lines: Valuable tools or useless artifacts. Spermatogenesis 2012;2(1):1-5.
18. Fallahi P, Ferrari SM, Elia G, Ragusa F, Patrizio A, Paparo SR, et al. Primary cell cultures for the personalized therapy in aggressive thyroid cancer of follicular origin. Semin Cancer Biol 2022;79:203-16.
19. Mitra A, Mishra L, Li S. Technologies for deriving primary tumor cells for use in personalized cancer therapy. Trends Biotechnol 2013;31(6):347-54.
20. Grada A, Otero-Vinas M, Prieto-Castrillo F, Obagi Z, Falanga V. Research techniques made aimple: Analysis of collective cell migration using the wound healing assay. J Invest Dermatol 2017;137(2):e11-6.
21. Fangjun L, Zhijia Y. Tumor suppressive roles of eugenol in human lung cancer cells. Thorac Cancer 2018;9(1):25-9.
22. Schettino C, Bareschino MA, Rossi A, Maione P, Sacco PC, Colantuoni G, et al. Targeting angiogenesis for treatment of NSCLC brain metastases. Curr Cancer Drug Targets 2012;12(3):289-99.
23. Spano D, Heck C, De Antonellis P, Christofori G, Zollo M. Molecular networks that regulate cancer metastasis. Semin Cancer Biol 2012;22(3):234-49.
24. Fares J, Fares MY, Khachfe HH, Salhab HA, Fares Y. Molecular principles of metastasis: a hallmark of cancer revisited. Signal Transduct Target Ther 2020;5(1):28.
25. Bravo-Cordero JJ, Hodgson L, Condeelis J. Directed cell invasion and migration during metastasis. Curr Opin Cell Biol 2012;24(2):277-83.
26. Simpson KJ, Selfors LM, Bui J, Reynolds A, Leake D, Khvorova A, et al. Identification of genes that regulate epithelial cell migration using an siRNA screening approach. Nat Cell Biol 2008;10(9):1027-38.
27. Friedl P, Wolf K. Tumour-cell invasion and migration: Diversity and escape mechanisms. Nat Rev Cancer 2003;3(5):362-74.
28. Wang X, Decker CC, Zechner L et al. In vitro wound healing of tumor cells: inhibition of cell migration by selected cytotoxic alkaloids. BMC Pharmacol Toxicol 2019;20:4.
29. Tonnesen MG, Feng X, Clark RA. Angiogenesis in wound healing. J Invest Derm Symp Proc 2000;5(1):40-6.
30. Small JV, Geiger B, Kaverina I, Bershadsky A. How do microtubules guide migrating cells? Nat Rev Mol Cell Bio 2002;3(12):957-64.
31. Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G, Parsons JT, Horwitz AR. Cell migration: integrating signals from front to back. Science. 2003;302(5651):1704–9.
32. Pijuan J, Barceló C, Moreno DF, Maiques O, Sisó P, Marti RM, et al. In vitro cell migration, invasion, and adhesion assays: From Cell Imaging to Data Analysis. Front Cell Dev Biol 2019;7:107.
33. Jonkman JE, Cathcart JA, Xu F, Bartolini ME, Amon JE, Stevens KM et al. An introduction to the wound healing assay using live-cell microscopy. Cell Adh Migr 2014;8(5):440-51.
34. Sullivan R, Holden T, Tremberger G, Jr J, Cheung E, Branch C, et al. Fractal dimension of breast cancer cell migration in a wound healing assay. Int J Biomed Biol Engine 2008; 2(8):186-91.
35. Pirker R. Adjuvant chemotherapy of non-small cell lung cancer. Tanaffos 2012;11(1):12-7.
36. Han XJ, Yang ZJ, Jiang LP, Wei YF, Liao MF, Qian Y, Li Y, Huang X, Wang JB, Xin HB, Wan YY. Mitochondrial dynamics regulates hypoxia-induced migration and antineoplastic activity of cisplatin in breast cancer cells. Int J Oncol 2015;46(2):691-700.
37. Manguinhas R, Fernandes AS, Costa JG, Saraiva N, Camões SP, Gil N, et al. Impact of the APE1 redox function inhibitor E3330 in non-small cell lung cancer cells exposed to cisplatin: increased cytotoxicity and impairment of cell migration and invasion. Antioxidants 2020;9(6): 550.
38. Maiuthed A, Chanvorachote P. Cisplatin at sub-toxic levels mediates integrin switch in lung cancer cells. Anticancer Res 2014;34(12):7111-7.
39. Wang H, Zhang G, Zhang H, Zhang F, Zhou B, Ning F, et al. Acquisition of epithelial-mesenchymal transition phenotype and cancer stem cell-like properties in cisplatin-resistant lung cancer cells through AKT/β-catenin/Snail signaling pathway. Eur J Pharmacol 2014;723:156-66.