Frontiers in Life Sciences and Related Technologies 5(3) (2024) 188-194

Contents lists available at Dergipark

Frontiers in Life Sciences and Related Technologies

Journal homepage: http://dergipark.org.tr/en/pub/flsrt

Research article

Determination of biomarker candidates with proteomics approach in small cell lung cancer: NCI-H209 cell line

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Abstract

Proteins, the primary building blocks of the cell membrane, play crucial roles in communication between cells as well as interactions with the extracellular matrix. They make for an excellent resource for disease identification due to their potential as biomarkers. In order to perform the study, HEL-299 (CCL-137™) and NCI-H209 lung cells were incubated at 37°C in a chamber that contained 5% CO_2. Trypsinization was used to transfer the cells into Eppendorf tubes. Proteomics analyses were carried out using LC-QTOF equipment, and the corresponding procedures of denaturation, alkynylation, trypsinization, and purification were carried out by adding the required chemicals. The Searchquie and PeptideShacker software interfaces were used to assess the analysis findings. Proteins that differ across groups are displayed by classifying them based on their roles as cellular components, molecular activities, and biological processes. Proteomics data showed that the lung cancer cell line NCI-H209 lacked 14 proteins that were present in the healthy lung cell HEL-299. These are the proteins ANK3, PIK3R2, INPP5F, HSF1, VIM, NFAM1, SHROOM3, ETV4, RNF31, LMNA, BRD8, PRTN3, TERT, SMAD9. There were discovered to be 5 distinct proteins in the lung cancer group compared to healthy lung HEL-299 cells. These proteins are AHSG, NCOA6, VCP, DNAJC19, NCL. Given the heterogeneity of lung cancer, a thorough and in-depth investigation of lung cancer proteome profiling is necessary for effective target treatment. The examination of proteins as prospective lung cancer biomarker candidates shows that it will make up a viable source for clinical investigations. These proteins differ in the direction in this study. Potential clinical applications of the biomarkers identified in this study, such as early diagnosis, monitoring treatment response, and determining disease prognosis, may contribute to the development of personalized medicine approaches.

Keywords: *Biomarker; cancer; proteomics; small cell; lung cancer*

1. Introduction

Lung cancer was a relatively uncommon disease in the 1800s, but it is now one of the most common cancers, with an estimated 2.21 million new cases and 1.80 million deaths per year (Nadaf et al., 2022). Lung cancer accounts for more deaths in recent years than breast, colon, and prostate cancer combined. In India, the mortality rate from lung cancer has increased in women in the last ten years, in contrast to men, whose rate has started to decline more than 20 years ago (Ghosh et al., 2023).

The lack of early diagnostic techniques, along with smoking, environmental pollution, and genetic predisposition, is a major factor contributing to the rise in lung cancer incidence. Large populations are particularly challenging to screen for diseases in because of the general lack of knowledge on the benefits of routine blood work and physical examinations. The development of methods such as low dose computed tomography (LDCT), liquid biopsies, and advanced bronchoscopy techniques has become increasingly important in the biological early diagnosis of lung cancer (Rusch et al., 2020; Wang et al., 2021). The fact that lung cancer's overall five-year survival rate is just 14% suggests that the disease's individual

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https://doi.org/10.51753/flsrt.1511261 Author contributions
Received 05 July 2024; Accepted 05 October 2024
Available online 30 December 2024

death rates have not yet dropped considerably. As a result, it's critical to establish procedures for tracking the disease's progression and early diagnosis. Validated biomarkers are seen to be the most significant of these approaches, so finding novel, targeted biomarkers for lung cancer is necessary.

Proteomic studies have been extensively used in the investigation of many diseases, particularly in cancer research, and are now acknowledged as a potent tool for large-scale assessment of protein expression (Matsuda et al., 2020; Zhang et al., 2021; Chen et al., 2022; Lee et al., 2023).

A key component of contemporary proteomic research is the identification and confirmation of biomarkers, which enable early cancer diagnosis, treatment, monitoring, and prognosis. Since mRNA levels do not always fully reflect the number of molecules or proteins involved in biological processes, the use of protein-based biomarkers in cancer diagnosis and treatment has become increasingly important (Meyer et al., 2020; Zhang et al., 2022; Atasever, 2024).

Post-translational modifications (such as phosphorylation, glycosylation, and methylation) are more valuable and instructive than transcriptomics or genomics, as well as revealing the functions of tumors and their interactions with the tumor microenvironment. Proteomic analyses have been used to study malignancy in many types of cancer, including gastric, lung, colorectal, breast, prostate, and pancreatic cancer. There are still a lot of pre-, analytical, and post-analytical issues with MS techniques, despite their many benefits and insights into cancer biology (Liu et al., 2022; Zhao et al., 2023; Wang et al., 2023; Gao et al., 2024).

When applied to fresh tissue or blood samples, certain mass spectrometry techniques like MALDI/MS do not always allow for the direct identification of proteins. Bioinformatics biostatistical artifacts are the primary cause of post-analysis constraints. Because blood serum is easily accessible from patients through routine blood chemistry, it is most frequently employed in biomarker investigations. Many circulating protein fragments originating from the tumor microenvironment and biomarkers detected in biopsied cancer tissue are examples of potential biomarkers that are predominantly found in blood (Schumacher et al., 2021; Kalluri and LeBleu, 2022; Duffy et al., 2023).

The effects of meclofenamic acid on the KHAK cell line DMS114 were examined in the study carried out by Yanar et al. (2023). The findings of the study demonstrated that the administration of meclofenamic acid altered cellular energy metabolism. Glycolysis was inhibited, although oxidative phosphorylation and mitochondrial activity rose. Thus, the proteomics results changed. Proteomic analysis of lavage fluids from SCLC patients revealed 460 BALF proteins. In addition, CNDP2 and RNPEP were identified as potential subtype markers for ASCL1 and NEUROD (Vu et al., 2023). A further study carried out in 2023 to enhance the therapy of lung cancer revealed 73 microRNAs (miRNAs). Ten miRNAs were shown to be tumor suppressors in lung cancer and four miRNAs to be oncogenic based on patient survival data and tumors compared to normal lung tissues. DGKE and WDR47 were found to be significant in lung cancer among these microRNAs (Ye et al., 2023). 33 distinct proteins were found in the study of Gasparri et al. (2023) on the profiling of serum proteomics of early-stage lung cancer. ACTR3B, CD59, PRKCA, and ARSA are among these proteins. PRKCA and ARSA were highlighted as being crucial. The thorough proteogenomic characterization of small cell lung cancer (SCLC) has been established by Liu et al. (2024) study. This work has demonstrated the cancer biology resulting from genetic aberrations using integrated multi-omics analysis, emphasizing the carcinogenic functions of RB1 deletion, FAT1 mutation, and chromosomal 5q loss. HMGB3 and CASP10 are two prognostic biomarkers that have been found.

Moreover, it has been documented that HMGB3 overexpression stimulates SCLC cell migration by transcriptionally regulating genes linked to cell attachment. Four distinct subtypes have been identified using multi-omics clustering, each with unique treatment limitations. 67 of the 1162 circulating proteins that were examined in the 2023 study were linked to an increased risk of lung cancer. Reproducible correlations between lung cancer diagnosis risk and 36 proteins were found (Ahamed et al., 2024).

The 36 proteins were examined for their known mechanistic roles, and it was discovered that they performed a variety of molecular fuertnctions. These include chemokines, various growth factors, tumor necrosis factor receptors, and cytokines. The only protein that has been found to have a negative correlation with lung cancer is SCF. SCF may control cell survival, proliferation, and hematopoiesis, according to certain theories. In 2023, the proteomic profiles of plasma exosomes from patients diagnosed with metastatic lung cancer were examined using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The samples comprised five healthy controls (HC), twenty-six solitary brain metastases (BM), sixteen solitary liver metastases (LM), and twenty-five non-metastatic locally advanced (LA) lung cancer cases. Lung cancer LM and BM showed deregulation of 143 and 120 exosome-based proteins, respectively. Additionally, it was found that SELL and MUC5B could be used as diagnostic markers of BM, while APOH, CD81, and CCT5 could be used to diagnose LM in LC patients (Li et al., 2023). In a study aiming to find invasive biomarkers as plasma proteins in the early diagnosis of lung cancer, 2941 proteins were analyzed in 131 cases, 237 controls, and numerous plasma samples taken one to 10 years anterior to diagnosis. Samples obtained 1-3 years before the diagnosis revealed that 240 proteins had been significantly changed in cases; samples taken 1-5 years before the diagnosis revealed 150 more proteins, 117 of which were significantly altered (Davies et al., 2023).

Currently, while there are no widely approved serum biomarkers for the early detection of lung cancer, ongoing research is focused on enhancing early and targeted identification as well as disease surveillance. Changes in protein glycosylation are ubiquitous indicators of malignant transformation and tumor advancement in human cancer, manifesting in every cell. Target protein research is therefore crucial to the treatment of cancer and the advancement of the disease. Characterization and biological function of proteins depend heavily on extensive and methodical protein analysis (Koch et al., 2020). Recent research has demonstrated that protein alterations in cancerous cells are significant during different phases of the tumor's development (Pérez-Rodríguez et al., 2021). Proteomic data sets are necessary to comprehend the biological significance of the distinct protein networks of healthy and malignant cells, to clarify their molecular interactions, to determine the functional architectures of proteins, and to determine how these relationships relate to diseases. To ascertain the function of tumor-associated chemicals as putative biomarkers in the NCI-H209 small cell lung cancer cell line, a proteomics investigation was carried out using the LC-QTOF/MS instrument (Chen et al., 2024).

2. Materials and methods

2.1. Cell proliferation

The study used the NCI-H209 cell line and HEL-299 (CCL-137TM) cell line. The Safe Fast Elite (EN 12469 2000) flow chamber was used to cultivate the cell line. Bovine serum-containing media (MULTICELL (FBS-HI-IIA)) was used for cell passage and culture, together with medium, 1% L-glutamine (MULTICELL (609-065-E2), and 1% penicillin-streptomycin [MULTICELL (450-201-Z2)]. The medium was kept at 37 °C and 5% CO2 in sterile incubators (PANASONIC). Up until the fifth passage, cultured cell lines were repeated; at that point, stocks were prepared for utilization. DMSO (MERCK 67-68-15) was placed in medium-containing cryotubes and kept at a temperature of -150°C (PANASONIC). All experiments began at the fifth passage of the cell lines and ended at the fifteenth passage. In this study, cells were standardized for proteomic analysis with three independent replicates on samples obtained from cell lines and grown under the same conditions. This process was designed to increase the reliability of the analyses and aimed to achieve statistical significance of the obtained data.

2.2. Determination of glycoproteins by proteomics study (LC-QTOF/MS)

First, urea solution (i.e., DTT) was added to the proliferating cells, and incubated at room temperature for a few hours to denature them. To prevent the formation of disulfide bonds, 1.5 µL of 200 mM iodoacetamide (IAA) was added, and the mixture was left to sit at room temperature in the dark for an hour. To reduce the urea content, 20 µL of 50 mM ABC/2 mM $CaCl_2$ was added to 100 µL of the reaction mixture. To separate the glycans, 2 µL of the PNGaseF enzyme was added, and the samples were incubated for 3-4 hours at 37ºC. The proteins were broken down into small pieces by trypsinization, which was followed by an overnight (16-18 hours) incubation at 37ºC for digestion. (An example would be to add 12.5 µL of 0.2 µg/µL trypsin solution to 100 mg of total protein to create a 1:40 ratio. After incubation, 10% TFA was added to stop the reaction. Purification was carried out in the SEP-PAK C18 solid-phase extraction system. Proteomics analysis was performed with three independent replicates from each cell line. The cartridge column was conditioned with methanol and 20 mM ammonium acetate solution. The elution was carried out with 20%-40%-75% acetonitrile: water (v:v), fractions were collected and evaporated under nitrogen and solubilized with 1% TFA. When the obtained data were evaluated on a repeat and fraction basis, it was aimed to obtain statistically significant results. Proteomics analysis was performed with Liquid Chromatography-Time-of-Flight Mass Spectrometry (AB-Sciex 4600 QTOF). The parameters of the method are presented in Table 1. The samples were injected into the LC-QTOF/MS system and the fragmentation ions were characterized by analyzing with high efficiency. There are many different interfaces available for proteomics analysis. However, in this study, SearchGUI (Compomics, 2024) was chosen as a search interface because it integrates various search tools (Fan, et al., 2024) into a single platform, rather than functioning as a database. By setting the search parameters, data was made available for search using various definition software techniques for the examination and interpretation of the collected data. SearchGui is an interface that allows the comparison of results using multiple search engines simultaneously and provides more reliable results. It offers advanced features for the integration and analysis of the obtained data. Precursor and product mass tolerance is applied at 10 ppm and carbamidomethylation of C (Cystine) is a fixed modification and oxidation of M (methionine) is a variable modification. In this study, a 1% False Discovery Rate (FDR) was applied to the SearchGui interface to increase the reliability of the proteins obtained by controlling the rate of false positive results, reduce the number of false positives, reveal proteins with real biological meaning more clearly and increase the statistical power of the analysis. For the processing and interpretation of search results; the PeptideShaker interface was used which contains post-translational modification (PTM) and peptide sequence matching (PSM) in the verification process. In addition, the PeptideShaker applies a comprehensive validation process to increase the reliability of the peptide and protein results obtained, reduces the false positive rate, helps to reveal results with real biological meaning, and allows researchers to easily visualize and understand the results thanks to its user-friendly interface (Compomics, 2024). In addition, the obtained analysis results allowed the comparison of the expression levels of certain proteins and contributed to the emergence of biologically significant findings.

Table1
Proteomics method and LC-QTOF parameters.

High-performance liquid chromatography parameters for proteomics method	
System	Agilent 1260 (Autosampler, Binary pump, Column Oven, Solvent Cabin)
Column	300SB-C18 column (Agilent Technologies) with 50 mm×1.0 mm, 3.5 µm particle size
Mobile phase (A)	Formic acid in water (0.1 %)
Mobile phase (B)	Formic acid in acetonitrile (0.1%)
Autosampler temperature	4°C
Flow rate Gradient:0 min. %5 B, 8.0 min. %5 B, 40 min. %40 B, 41 min. %80 B, 55 min %80 B, 56 min. %5 B, 75 min. %5 B	0.12 mL·min^{-1}
Column temperature	35°C
Injection volume	5 µl
Total run time	75 min
High resolution mass spectrometry parameters for proteomics method	
System	AB-Sciex 4600 QTOF
Ionization mode	Positive electrospray ionization
Ion source gas (1)	25 l\min
Ion source gas (2)	40 l\min
Curtain gas	30 l\min
Temperature	350°C
Ion spray voltage	5500 Volt
Declustering potential	120 Volt
Collision energy	35
Ms Method	300-2000 mass range
MsMs	IDA experiment, 50-1750 mass range

3. Results and discussion

The discovery of various tumor markers and other proteins associated with cancer through genomic and clinical studies has made the development of appropriate proteomic analyses of interest. It is of great importance to evaluate proteomic approaches for early diagnosis and treatment of cancer cells.

Table 2
Small cell lung cancer primary cell culture proteome: cancer-related proteins.

Group	Description	Chromosome	Gene Name	MW [kDa]	Molecular Function
Control	Ankyrin-3	10	ANK3	480,113	structural component of the cytoskeleton, cadherin binding, cytoskeletal protein binding
Control	Phosphatidylinositol 3-kinase regulatory subunit beta	19	PIK3R2	81,495	phosphotyrosine residue binding, protein heterodimerization, phosphatidylinositol 3-kinase regulatory activity, 1-phosphatidylinositol-3-kinase activity, 1-phosphatidylinositol-3-kinase regulatory activity, and protein binding—both protein phosphatase and protein binding are examples of these activities.
Control	Phosphatidylinositide phosphatase SAC2 (Fragment)	10	INPP5F	20,622	The enzymes that catalyze the breakdown of inositol monophosphate include phosphatase, phosphatidylinositol phosphate 4-phosphatase, phosphatidylinositol phosphate 5-phosphatase, protein binding, phosphatitol monophosphate 3-phosphatase, and phosphatidylinositol-4-phosphate phosphatase.
Control	Heat shock factor protein 1	8	HSF1	53,560	chromatin binding, sequence-specific single-stranded DNA binding, transcription repressor activity, protein binding, and STAT family protein binding.
Control	Vimentin	10	VIM	49,623	Double-stranded RNA binding, scaffold protein binding, keratin filament binding, protein domain-specific binding, structural molecule activity, and protein C-terminal binding are examples of structural cytoskeleton components.
Control	NFAT activation molecule 1	22	NFAM1	17,849	transmembrane signaling receptor activity
Control	Protein Shroom3	4	SHROOM3	207,922	actin filament binding
Control	ETS translocation variant 4	17	ETV4	48,120	Sequence-specific DNA binding, DNA-binding transcription activator activity and protein binding are all associated with the cis-regulatory region of RNA polymerase II.
Control	RBR-type E3 ubiquitin transferase (Fragment)	14	RNF31	102,332	transferase activity, protein binding, ubiquitin-protein transferase activity, and ubiquitin-protein ligase binding.
Control	Prelamin-A/C	1	LMNA	14,212	Similar protein binding and structural molecule action.
Control	Bromodomain-containing protein 8 (Fragment)	5	BRD8	31,591	transcription factor activity that binds DNA, nuclear receptor activity.
Control	Myeloblastin	19	PRTN3	27,789	protein binding, signal receptor binding, serine-type endopeptidase activity, and peptidase activity.
Control	Telomerase reverse transcriptase	5	TERT	126,916	protein nucleotidyltransferase activity, telomeric DNA binding, and protein C- and N-terminal binding.
Control	Mothers against decapentaplegic homolog 9	13	SMAD9	16,519	transcription factor activity specific to DNA binding, metal ion binding, protein binding, and RNA polymerase II specific.
Cancer	Alpha-2-HS-glycoprotein	3	AHSG	39,387	kinase inhibitor activity and cysteine type endopeptidase inhibitor activity.
Cancer	Nuclear receptor coactivator 6	20	NCOA6	219,008	nuclear receptor coactivator activity, transcription coactivator activity, retinoid X receptor binding, enzyme binding, chromatin binding, thyroid hormone receptor binding, protein binding.
Cancer	Vesicle-fusing ATPase	9	VCP	50,962	Protein-containing complex binding, ubiquitin-specific protease binding, hydrolase activity, RNA binding, and ubiquitin-like protein ligase binding are some of the binding mechanisms associated with the BAT3 complex.
Cancer	Mitochondrial import inner membrane translocase subunit TIM14	3	DNAJC19	5,333	protein binding
Cancer	Nucleolin	2	NCL	68,552	mRNA 5'-UTR binding, protein C-terminal binding, nucleic acid binding, telomeric DNA binding, DNA topoisomerase binding, protein, RNA, and identical protein binding.

Therefore, it was decided to examine protein differences by propagating HEL-299 (CCL-137™) and NCI-H209 lung cells under appropriate conditions and performing a proteomic analysis with the LC-QTOF/MS approach. After exporting the reports to Excel, an in-built tool was used to extract the percentage of each GO term in the Genome Ontology (GO) dataset for the cellular component of each protein. Unclassified proteins were validated by comparing with entries in Swiss-Prot, Human Protein Reference Database, and Bioinformatics Harvester to search for annotations for biological components.

The overlap between the proteins found in each cell line and between each cell line's three replicates was assessed using a method that was developed internally. Additionally, the Plasma Proteome Database was searched for all extracellular and membrane-bound proteins. According to the proteomics results, it was determined that 14 proteins found in the healthy lung cell HEL-299 were not found in the lung cancer cell line NCI-H209. These are ANK3, PIK3R2, INPP5F, HSF1, VIM, NFAM1, SHROOM3, ETV4, RNF31, LMNA, BRD8, PRTN3, TERT, SMAD9 proteins. In the lung cancer group, a total of 5 proteins were found to differ from those in healthy HEL-299 lung cells. These proteins are AHSG, NCOA6, VCP, DNAJC19, NCL. The results obtained are presented in Table 2.

Complex protein mixtures have traditionally been separated and compared using the proteomics approach as the main method. Nevertheless, there are significant variances in this method due to sample processing, protein loading, and gel staining (Kulasingam and Diamandis, 2007). Another disadvantage of 2DE in terms of proteomics is the inability to adequately recover proteins from the gel for MS. For this reason, techniques like multidimensional LC have been looked for as ways to enhance or replace 2DE (Sardana et al., 2008). High throughput protein identification is achieved using multidimensional LC-MS/MS analysis, in contrast to the relatively slow and laborious 2DE-MS/MS techniques. Through the analysis of complex protein combinations seen in biological fluids, tissues, or cell cultures, this technique has been utilized to find cancer biomarkers (Cho et al., 2007; Shaw et al., 2007; Li et al., 2023). The LC-QTOF/MS method was employed in this work to detect protein variations in two lung cell lines. Because lung cancer is a diverse disease, the secretomes of cell lines from various backgrounds were examined to better characterize the proteome of the disease and increase the possibility of finding biomarkers for its pathology (Gonzalez et al., 2021; Kawasaki et al., 2024).

According to the proteomics results, it was determined that 14 proteins found in the healthy lung cell HEL-299 were not found in the lung cancer cell line NCI-H209. These are ANK3, PIK3R2, INPP5F, HSF1, VIM, NFAM1, SHROOM3, ETV4, RNF31, LMNA, BRD8, PRTN3, TERT, SMAD9 proteins. In the lung cancer group, a total of 5 proteins were determined to be different from healthy lung HEL-299 cells. These proteins are AHSG, NCOA6, VCP, DNAJC19, NCL.

The AHSG protein exhibits distinct expression patterns in lung, colorectal, and breast cancers. Five potential biomarkers found in serum samples from lung cancer patients were analyzed using boxplots, and the median levels of the control group were compared to those in the small cell, adenocarcinoma, and squamous cell carcinoma. The AHSG levels of 55 lung cancer patients were found to be higher after the serum samples were analyzed (Petrik et al., 2008; Qiu et al., 2008).

It has been demonstrated that the 59 kDa glycoprotein AHSG, primarily produced in the liver, mediates growth signals in breast carcinoma cells (Ren et al., 2020). In contrast to the control group, the differential expression of AHSG was shown to be significant only in breast cancer in this study., and its abundance levels were also reduced. Petrik et al. (2008) used SELDI-TOF to find that in glioblastoma patients, AHSG is a peak that becomes less noticeable as tumor grade rises. Thus, in a separate cohort of 72 glioblastoma patients, AHSG was later verified as a survival marker in glioblastoma using ELISA.

It was found that across all cancer types examined, C3, which has a high AUC value, was elevated. Increased levels of this APP have been linked to cancer in the past, and C3 is a crucial part of the complement system in this study (Fang et al., 2020; Xing et al., 2023). Using gel electrophoresis, it was previously discovered that the serum of patients with pancreatic adenocarcinoma had higher levels of C3 than the sera of healthy individuals (Costantini et al., 2021).

The TCGA-LUAD database shows that AHSG expression is substantially greater in cancer tissues than in normal tissues. Additionally, a pan-cancer analysis identified aberrant AHSG expression in many tumor types. The expression of AHSG may be a separate predictor of overall survival in lung adenocarcinoma, according to a later study. According to survival analysis, patients in the low-expression group in the TCGA-LUAD database fared better overall than those in the high-expression group. The AHSG gene is involved in many physiological and pathological pathways in lung cancer cell lines. Xiong et al. discovered in their 2023 study that patients with lung cancer may use AHSG as a predictive indicator for OS. They also underlined the possibility that it is a therapeutic target and could sustain the biological behavior of lung adenocarcinoma (Zhou et al., 2021).

NCOA6 is mainly found in the colon of human fetal trophoblasts and also in endometrial transplants. Following NCOA6 knockdown, HTR-8/SVneo cell invasion and migration were markedly diminished, and transcriptional suppression led to a decrease in MMP9 secretion. Additionally, it was discovered that NCOA6 co-activates NF-κB-mediated MMP9 transcription. Furthermore, it was discovered that patients with early-onset sPE have altered NCOA6 expression in their placentas. The study's findings indicate that NCOA6 plays a crucial role in cytotrophoblast invasion and migration, possibly through its activation of NF-κB-mediated MMP9 transcription. Additionally, it has been discovered that NCOA6 downregulation may have a role in the pathophysiology of early-onset sPE (Wu et al., 2020).

Valosin-containing protein (VCP)/p97, a molecular chaperone of AAA ATPase, regulates critical cellular functions and protein processing. Furthermore, a recent study showed a relationship between the prognosis and course of non-small cell lung cancer (NSCLC) and the expression levels of VCP. Additionally, the precise function of VCP in the development and course of NSCLC was identified in this study. According to the study, VCP has also been shown to directly influence the levels of the proteins NFκB and p53, which may be a way to control the proliferation and advancement of tumor cells. The investigation further assessed the therapeutic potential of VCP inhibition and found that EerI therapy greatly inhibited the growth of NSCLC tumors in vitro and in xenograft mouse (athymic-naked) models. Thus, focusing on VCP in NSCLC might offer a fresh approach to raising p53 and NFκB levels, enhancing tumor development, and enhancing tumorigenesis—all of which could result in better clinical results (Costantini et al., 2021). Multiple driver oncogenes exist for NSCLC. Disease-associated protein DNAJC19 is a crucial part of the mitochondrial membrane translocation mechanism. The function of DNAJC19 in NSCLC cell proliferation and metastasis is described here. Immunohistochemistry (IHC) was used to investigate DNAJC19 expression in clinical samples of non-small cell lung cancer in this study. NCI-H1299 or A549 lung cancer cells were subjected to overexpression or knockdown experiments using lentiviral vectors. After their functionalities were evaluated, mouse xenograft and metastatic tumor models were created using DNAJC19-knockdown A549

cells. RNA-seq data, western blotting, PCR and IHC-based evaluations were performed. As a result, the study determined that DNAJC19 expression was higher in tumors than in non-cancerous tissues. A survival analysis revealed a correlation between lower DNAJC19 levels and higher progression-free survival. DNAJC19 was significantly decreased in terms of proliferation, survival, migration, and invasion by shRNA-mediated knockdown. Additionally, RNA-seq research revealed that the PI3K/AKT signaling pathway was involved in molecular events when shDNAJC19 was treated in A549 cells. By controlling PI3K/AKT signaling, DNAJC19 was discovered to significantly enhance NSCLC cell proliferation and lung metastasis, offering a potential therapeutic target for the treatment of NSCLC patients (Zhou et al., 2021).

Proteomics studies performed with LC-QTOF/MS analysis have some limitations. First of all, in proteomics analysis with LC-QTOF/MS, there may be difficulties in detecting low-abundance proteins, which may cause important biological information to be missed (Bache et al., 2018; Kim et al., 2020). In particular, proteins with post-translational modifications (e.g., phosphorylated and glycosylated proteins) may not be adequately represented in these analyses (Guan et al., 2019). In addition, chemicals used during proteomics analysis may have an effect on the integrity of the samples; the potential of some reactors or chemicals to disrupt protein structure may threaten the accuracy of the analysis results (Shin et al., 2021). Therefore, it is important to consider these limitations in future studies and discuss the potential effects in detail.

Based on the findings, this study fills a significant gap in the current literature and provides an innovative approach to proteomic profiling of small cell lung cancer. The applied methodology provides new insights into cancer biology by analyzing proteins that differ between both HEL-299 and NCI-H209 cell lines in detail. Furthermore, easy-to-use software interfaces and comprehensive analysis processes allow researchers to reliably evaluate the results. This innovative methodology provides a solid foundation for the discovery of potential biomarkers for future clinical studies and contributes to the development of personalized medicine approaches.

4. Conclusion

Although this study demonstrates that there is now a great deal more understanding of the molecular causes of cancer, there are still gaps in the knowledge of the disease's mechanisms and the creation of practical early detection and treatment plans. The most important element influencing a patient's ability to get successful therapy is an understanding of the pathophysiology of the illness in its later stages. Currently, it is standard procedure to use molecular techniques to diagnose biological samples based on many markers. Changes in these markers' concentrations can be detected to facilitate early diagnosis, prognosis prediction, monitoring of therapy response, and disease screening of high-risk people. According to the findings in this study, tiny cells may be biomarkers for lung cancer that can be detected early on. With the support of further studies, the use of target protein markers in the clinic can be made widespread.

Ethical approval: The development, acquisition, authentication, cryopreservation, and transfer of cell lines between laboratories were followed according to the guidelines published in the British Journal of Cancer, 2014.

Conflict of interest: The authors declare that they have no conflict of interests.

Informed consent: The authors declare that this manuscript did not involve human or animal participants and informed consent was not collected.

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Cite as: Turker, N. P., & Celik, S. (2024). Determination of biomarker candidates with proteomics approach in small cell lung cancer: NCI-H209 cell line. *Front Life Sci RT, 5*(3), 188-194.