SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

A Comprehensive Approach to Lung Cancer: Oncogenic Signalling Mechanisms and Innovative Imaging Techniques in the Service of Personalized Diagnostics

by Attila Makai MD

Supervisor: Dr. Beáta Lontay

UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF MOLECULAR MEDICINE
DEBRECEN, 2026

A Comprehensive Approach to Lung Cancer: Oncogenic Signalling Mechanisms and Innovative Imaging Techniques in the Service of Personalized Diagnostics

By Attila Makai,
Doctor of Medicine

Supervisor: Dr. Beáta Lontay

Doctoral School of Molecular Medicine,
University of Debrecen

Head of the **Defense Committee**: Prof. Dr. László Csernoch, DSc

Reviewers: Dr. Zita Bognár, PhD
Dr. Péter Fülöp, PhD

Members of the Defense Committee:

Dr. Mónika Tünde Sztretye, PhD
Dr. László Urbán, PhD

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen
13:00, 26th of June 2026

Introduction

Current Challenges in the Diagnosis and Treatment of Lung Cancer

Epidemiology

Lung cancer is among the most common malignancies worldwide, accounting for approximately 12% of all new cancer cases (~2.09 million) in 2018. It was the second most frequent cancer in men after prostate cancer and in women after breast cancer. In Hungary in 2024, the incidence of bronchial cancer was 36.6 per 100,000 (3,506 new patients), while the prevalence of lung cancer was 203 per 100,000, corresponding to 19,486 registered patients. Five-year survival remains very low, averaging 19%, though it varies by histological type—23% for non-small cell lung cancer (NSCLC) and 6% for small cell lung cancer (SCLC). National mortality in 2023 was 83.9 per 100,000 (8,050 deaths), with particularly high incidence and mortality observed in the Northern Hungarian regions.

Screening

In Hungary, a significant proportion of lung cancer patients (35.1% in 2018) are diagnosed at stage IV, which is partly explained by the rapid growth of the tumor. For this reason, annual population-wide screening is currently not feasible, and (low dose CT) LDCT screening of high-risk groups has become the preferred approach. In Hungary, developments in this area are still ongoing within the framework of the HUNCHEST program.

Histological Types

The WHO 2015 histological classification is still used today, defining lung cancer subtypes based not only on morphology but also on molecular diagnostic findings. Lung cancer is divided into two major categories: SCLC, (15%) and NSCLC (85%). The most common NSCLC subtypes are

adenocarcinoma (ADC, 38.5%), squamous cell carcinoma (SCC, 20%), and large-cell arcinoma (3%).

ADC typically arises peripherally and exhibits glandular and papillary structures with mucin production, whereas SCC carcinoma is usually centrally located and characterized by keratin formation and intercellular junctions. Large-cell carcinoma is poorly differentiated and lacks keratinizing or glandular features. SCLC consists of small, oval-shaped cells with high mitotic activity and a strong tendency for rapid metastasis; it has the poorest prognosis among lung cancers.

Etiology

The primary known cause of lung cancer is smoking, accounting for approximately 85% of cases. Cigarette smoke contains over 4,000 compounds, of which at least 70 are carcinogenic, including polycyclic aromatic hydrocarbons that induce DNA damage and epigenetic alterations. Smoking and air pollution trigger oxidative stress and chronic inflammation through multiple inflammatory signaling pathways. Certain genetic factors, such as the Glutathione S-transferase Mu 1 (GSTM1) null genotype, increase the risk of lung cancer in smokers. Asbestos exposure also contributes to bronchial cancer development and exhibits a synergistic effect when combined with smoking, significantly raising the risk. Radon exposure plays a role as well, accounting for approximately 7% of bronchial cancer cases.

Genetic Alterations Involved in Pathogenesis

Carcinogenesis is a multistep process in which genetic changes transform normal cells into malignant ones. Intra-tumoral heterogeneity accelerates tumor evolution and the development of drug resistance. Common alterations include mutations in cell cycle regulatory genes (TP53, RB1), deletions (3p, 9p, p16), and c-MYC amplification, all of which promote increased cell proliferation, inhibition of apoptosis, and metastasis formation. In NSCLC, key “driver” and “druggable” mutations are primarily observed in

adenocarcinomas: KRAS (20–30%, p.G12C ~13%), EGFR (Caucasians 10–20%, Asians ≥50%, mainly exon 19 deletions and exon 21 L858R), BRAF (1.5–4.5%), ALK (~5%), ROS1 (1–2%), NTRK (0.2%), and RET (1–2%). HER2 mutations are rare but can also occur in NSCLC and are targetable with specific therapies.

Epigenetic Alterations Involved in Pathogenesis

During malignant transformation, numerous signaling pathways are altered, primarily regulated by post-translational modifications (PTMs) such as acetylation, methylation, phosphorylation, and ubiquitination. Among these, protein methylation has emerged as a key research focus, playing a crucial role on both histone and non-histone proteins in the control of transcription, RNA metabolism, and DNA damage repair. Phosphorylation is the most prominent regulatory PTM, coordinating enzyme and receptor function as well as cell division and proliferation; dysregulation of kinase activity is frequently observed in various tumors.

Histone proteins are fundamental structural components of chromatin, and modifications at their N- and C-termini significantly influence DNA condensation and gene expression. Activating modifications associated with chromatin relaxation—such as acetylation and certain demethylation events—enhance gene expression, whereas methylation and deacetylation generally induce repression. In lung tumors, increased H3K4 methylation has been reported, correlating with aberrant transcriptional programs; inhibition of the responsible SETD1A methyltransferase in xenograft models reduces tumor growth.

Protein arginine methyltransferases (PRMT1–9) catalyze arginine side-chain methylation. Among them, **PRMT5**, a type II enzyme, symmetrically dimethylates both histone and non-histone proteins. PRMT5's methyltransferase activity is mediated through a complex with MEP50 and regulates processes including transcriptional repression of the p53 gene and

various proliferative pathways. PRMT5 activity is finely tuned by multiple PTMs: certain phosphorylations (e.g., JAK2-dependent) inhibit it, whereas phosphorylation at Thr80 enhances its histone methylation activity. K63-linked ubiquitination, mediated by the TRAF6 E3 ubiquitin ligase, also has an activating effect.

A major negative regulator of PRMT5 is the serine/threonine-specific **myosin phosphatase (MP)**, composed of three subunits: the catalytic PP1c subunit, the MYPT1 (substrate targetting subunit 1), and a small regulatory subunit without any known function. MP exerts tumor-suppressive effects partly by dephosphorylating retinoblastoma protein and by removing activating phosphorylations on PRMT5, thereby reducing its methyltransferase activity. MP function is regulated by the phosphorylation state of MYPT1: Thr696 and Thr853 phosphorylation—mainly by ROK and other kinases—suppresses MP, whereas Ser695 phosphorylation (PKA/PKG-dependent) counteracts this inhibition.

MYPT1 phosphorylation is modulated not only by kinases but also by phosphatases. PP2A can remove inhibitory Thr696 phosphorylation, thereby enhancing MP activity. Similarly, the Mg^{2+}/Mn^{2+}-dependent phosphatase 1B (PPM1B) also dephosphorylates MYPT1 at Thr696, indirectly increasing MP activity. PPM1B regulates multiple signaling pathways, including MAPK, NF-κB, and TGF-β, and its stability is influenced by microRNAs and phosphorylation-driven degradation. Reduced PPM1B expression has been reported in several tumors and is associated with increased invasiveness and metastatic potential.

The **PPM1B/MP/PRMT5/histone pathway** exemplifies how phosphorylation and methylation events are interconnected in epigenetic regulation and tumorigenesis. Loss of PPM1B leads to persistent inhibitory phosphorylations on MYPT1, reducing MP activity. This results in sustained activating phosphorylations on PRMT5, promoting histone hypermethylation

and transcriptional programs that drive tumor progression. Thus, this multi-level regulatory axis represents a critical determinant of the epigenetic landscape in lung cancer and other malignancies.

Diagnostics

In the evaluation of cancer patients, the goal is to gather sufficient data according to current professional guidelines to establish the most optimal treatment algorithm, determined by a multidisciplinary oncology team. Essential information for decision-making includes the patient's performance status and functional condition, the tumor stage defined according to the TNM (tumor, node, metastasis) staging system, and the precise histological subtype. Accurate staging requires imaging studies such as CT, MRI, and PET-CT.

PET-CT combines positron emission tomography with CT, providing detailed anatomical and functional information simultaneously. The most commonly used tracer is 18F-FDG, which accumulates in regions of increased metabolism, often corresponding to malignant lesions, thus accelerating diagnostic accuracy. Virtual bronchoscopy is a CT-based, non-invasive method that provides an internal view of the airways, aiding in the precise localization of tumors and airway stenoses. Its advantages include non-invasive visualization and facilitation of biopsy planning, whereas its limitations are the inability to assess mucosal details and obtain direct tissue samples. The diagnostic yield of peripheral biopsies can be improved with virtual bronchoscopy, though success also depends on tumor size.

The combined use of PET-CT and virtual bronchoscopy further refines diagnostics: PET-CT identifies the most metabolically active lesions, while virtual bronchoscopy aids localization from a bronchological perspective. This optimizes biopsy strategy, improves the yield of bronchoscopic techniques, and provides complementary imaging information.

For histopathological confirmation, the least invasive bronchoscopic biopsy is performed first, followed if necessary by transthoracic needle biopsy or

surgical approaches. To minimize treatment risks, accurate assessment of cardiopulmonary status through pulmonary function tests and cardiology evaluation is essential. Although tumor markers alone are insufficient for diagnosis, they are useful for follow-up and planning therapy. In early-stage disease, assessment of PD-L1, EGFR, and ALK biomarkers is fundamental, while in more advanced stages, additional biomarker testing is often required to guide targeted therapies.

Treatment

Treatment of malignant tumors fundamentally relies on combinations of three modalities: surgery, radiotherapy, and systemic therapy. Lung cancers are commonly categorized based on stage into three groups: early-stage, locally advanced, and metastatic disease.

In early-stage NSCLC (I–IIIA), surgical intervention is the priority. The gold standard is lobectomy via VATS, with histopathological verification often performed intraoperatively in small tumors. To reduce recurrence risk, neoadjuvant chemotherapy and immune checkpoint inhibitors (ICI) may be used, while adjuvant therapy is indicated for T2b tumors or higher stages, and can be supplemented with targeted therapy if driver mutations are present. Radiotherapy is considered primarily when surgery is contraindicated, delivered as stereotactic body radiotherapy (SBRT) or post-operative radiotherapy (PORT).

For locally advanced, unresectable stage III disease, the standard of care is chemoradiotherapy, supplemented with consolidative ICI or targeted therapy. In metastatic NSCLC, treatment is primarily palliative, focusing on systemic therapy, palliative radiotherapy, or targeted therapy depending on biomarker status.

Objectives

Previous studies have demonstrated the role of the enzymes PRMT5, MP, and PPM1B in the development of various cancers. Their interactions define a finely tuned phosphorylation pathway, the imbalance of which contributes to malignant transformation. Although the interactions and regulatory mechanisms of these proteins have already been investigated in vitro in cervical cancer cells, their correlations in lung tumors and their different histological subtypes have not yet been described. In our work, we examined the abnormal function of the PPM1B/MP/PRMT5/histone pathway in the context of lung cancer.

Histological verification of thoracic tumors often encounters difficulties, as lesions are frequently hard to access. Therefore, the development of tools to support sampling remains highly important today. In the field of imaging, I participated in a study further developing functional virtual bronchoscopy.

The objectives of our work were as follows:

- To analyze the expression levels of elements in the PPM1B/MP/PRMT5/histone oncogenic signaling pathway.
- To examine the phosphorylations determining the activity of PPM1B/MP/PRMT5 enzymes and histone dimethylation.
- To assess the correlation between the expression and activity of the PPM1B/MP/PRMT5/histone oncogenic signaling pathway and patient overall survival using the Kaplan-Meier method.
- Another goal was to develop a dedicated functional virtual bronchoscopy (fVB) protocol that simultaneously enables the visualization of anatomical and functional information, providing a more detailed and structured depiction of the airways and leveraging the quality of high-definition CT images.

Materials and methods

Patients

Our study included 38 patients who underwent evaluation for suspected lung tumors and were recommended for surgical treatment by the multidisciplinary oncology team. Inclusion criteria required the presence of a lesion of at least 2 cm on chest CT. Preoperative histopathological diagnosis was not mandatory, as the exact histology was in several cases only established after tissue sampling. The size threshold was chosen to ensure that biopsy would not compromise pathological assessment; therefore, lesions smaller than this threshold were not sampled. Surgeries were performed at the Department of Thoracic Surgery, DEKK Surgical Surgical Department. Patient data were collected between December 11, 2019, and July 25, 2023, according to the ethical approval issued by the University of Debrecen (34199-L/2019/EKU).

Sampling and Histological Specimens

Tissue samples were obtained from surgically resected lobes or segments, including both tumor tissue and distant non-tumorous lung regions. Control samples were always collected outside the tumor infiltration zone, taking into account microscopic spread beyond the solid component (90th percentile: 12 mm; Grade 1: 13.0 mm, Grade 2: 9.7 mm, Grade 3: 4.4 mm). From both tumor and control sites, three types of samples were prepared: fresh-frozen tissue for Western blot analysis, tissue embedded in matrix and snap-frozen in liquid nitrogen in the operating room with subsequent storage at -70 °C, and tissue preserved in RNAlater and stored at -20 °C.

Protein extraction

Tissue samples (100 mg) were homogenized in RIPA buffer (50 mM Tris, pH 7.4, 0.2% sodium deoxycholate, 0.2% SDS, 1% Triton X-100, 1 mM EDTA) supplemented with protease and phosphatase inhibitors, 1 mM PMSF, and 1 µM microcystin-LR. The homogenates were processed on ice using a

TissueRuptor, followed by centrifugation at 3,000 g for 5 minutes at 4 °C, and the supernatant was collected. Protein concentration was determined using the Pierce BCA Protein Assay Kit (1:10 dilution, 540 nm ELISA reader, BSA standard), and data were analyzed by linear regression using GraphPad Prism software.

Western blot

Proteins were separated by size using SDS-PAGE (4–20% Criterion gel, Bio-Rad) and then transferred onto a nitrocellulose membrane (100 V, 90 min, Bio-Rad). Samples were diluted 1:5 with 6× SDS sample buffer, denatured at 100 °C for 5 min, and 30 µg of protein was loaded per gel. Membranes were blocked with 5% low-fat milk in PBST and incubated with primary antibody (90 min at room temperature or overnight at 4 °C), followed by treatment with HRP-conjugated secondary antibody. Detection was performed using WesternBright ECL substrate, and images were captured with the ChemiDoc Touch system, with GAPDH used as a loading control.

RNA Isolation

Total RNA was isolated from tumor and control lung tissues using TRIzol reagent (Life Technologies) according to a modified manufacturer's protocol. Samples were homogenized in 200 µl TRIzol using a TissueRuptor, followed by treatment with 200 µl chloroform and centrifugation at 13,000 rpm for 15 min at 4 °C. RNA was precipitated with 500 µl isopropanol and centrifuged at 13,000 rpm for 10 min. The pellet was washed with 75% ethanol and centrifuged at 13,000 rpm for 5 min. The final pellet was dissolved in 10 µl nuclease-free water, and RNA concentration was measured using a NanoDrop spectrophotometer.

Reverse Transcription and Quantitative PCR (RT-qPCR)

cDNA was synthesized from 1 µg of total RNA per sample using a Thermo Fisher kit at 37 °C for 120 min. Using the cDNA as a template, quantitative PCR was performed with 2× Xceed qPCR SYBR Green Master Mix (Institute

of Applied Biotechnologies) on a LightCycler 480 system (Roche) under the following cycling conditions: 95 °C for 3 min, followed by 50 cycles of 95 °C for 3 s, 60 °C for 30 s, and 72 °C for 90 s. Cp values were normalized to the geometric mean of the housekeeping genes GAPDH and Cyclophilin A.

Immunohistochemistry

The analyses were performed on formalin-fixed, paraffin-embedded sections from lung SCC and ADC samples, as well as on healthy lung tissues from the same patients. Paraffin was removed using xylene, followed by graded alcohol rehydration (100%, 96%, 70%). Antigen retrieval was carried out in heated citrate buffer (pH 6.0) under pressure, and nonspecific binding sites were blocked with 5% BSA for 1 hour. Sections were incubated with primary antibodies against MYPT1, PRMT5, and PPM1B (1:100 in BSA/Triton X-100/PBS) for 48 hours at 4 °C, followed by incubation with HRP-conjugated secondary antibody for 1 hour. Immunoreactivity was visualized using DAB substrate, counterstained with hematoxylin-eosin, and documented with a Leica microscope and MC120 HD camera.

Immunofluorescent Staining

Tumor and control lung tissues were fixed in 4% paraformaldehyde, and 40-60 µm sections were prepared using a vibratome. The sections were washed in PBS and TBS, then permeabilized and blocked with normal serum and Triton X-100. Next, the sections were incubated with primary antibodies (MYPT1, MYPT1^{pT853}, PRMT5, PRMT5^{pT80}) for 24 hours, followed by labeling with Alexa Fluor 488- and 546-conjugated secondary antibodies for 3 hours. Nuclei were stained with DAPI, and in confocal microscopy images, the intensity of overlapping (co-localized) pixels was optimized for better visibility.

Kaplan-Meier Survival Analysis

Overall survival (OS) and disease-free survival (DFS) were determined in months from patient enrollment until July 2024. Survival was plotted on

Kaplan-Meier curves according to histological subtype and the expression of molecular markers (MYPT1, PRMT5, H2A, PPM1B), as well as phosphorylation (MYPT1^{pT853}, PRMT5^{pT80}) and dimethylation (H2A) status. For each parameter, patients were categorized into "high" and "low" groups based on the cohort median. Survival differences were analyzed using the log-rank (Mantel-Cox) test, with $p < 0.05$ considered statistically significant.

Statistical Analysis

Densitometry of immunoblots was performed using ImageJ, and the data were plotted as bar graphs (mean ± SD, n = independent replicates) in GraphPad Prism 8. Phosphorylated proteins were normalized to total protein, and total protein was normalized to GAPDH. For comparisons between two groups, a t-test was used; for multiple groups, ANOVA followed by Tukey's test was applied. Outliers were identified using the Grubbs test, and $p < 0.05$ was considered statistically significant.

Functional Virtual Bronchoscopy: Methodological Settings

Patients who underwent PET/CT and hdCT examinations were selected. The hdCT images were acquired with a Siemens Emotion 16 scanner, and the ldCT and PET scans were performed using a Philips GEMINI TF TOF 64 scanner, with less than 1 year between scans; examination conditions were not standardized.

For virtual bronchoscopy, hdCT and ldCT images were registered using the elastix algorithm, while PET images were already registered to the ldCT. Lung-mask segmentation was performed with the M-SEGM algorithm, selecting voxels between -1000 and -400 HU to label bronchial airways and parenchyma. Airway segmentation was conducted using GeoS 2.2 or a custom region-growing method, allowing modeling of airways up to the origins of segmental bronchi.

The bronchial tree skeleton (centroids of the airways) enables virtual camera navigation and accurate bifurcation counting. A 3D surface model was

generated from the segmented voxels using the marching cubes algorithm, and FDG uptake was projected onto the surface model within the range of the transbronchial needle; affected vessels were also segmented.

Previously, registration quality was checked by a radiologist, which was subjective and time-consuming. The distances between lumen points on the hdCT and ldCT were reported in millimeters. Their correlation was examined under conditions of (non)matching parameters, the positive outcome of which made the quality of the registration objectively assessable.

Results and Conclusions

Clinical Data of the Selected Patients

In our study, we collected samples from 38 patients. The average age of participants was 65 years, with a predominance of males. With few exceptions, the patients were smokers. Their anamnesis clearly reflected the cases encountered in our daily practice, representing the routine challenges we face. The distribution of patient stages was clustered around T2 and N0, as this group was both ideal for surgical treatment and had tumors of sufficient size to allow sampling, making them the optimal target population for our study from a technical perspective. Regarding the histological types of the primary tumors, the studied population was quite heterogeneous, since preoperatively determined histology was not an exclusion criterion for patient enrollment. Further analyses were performed only on ADC and SCC, involving a total of 31 patients. The median overall survival (OS) was 44 months for patients with ADC and 20 months for patients with SCC.

PPM1B expression was significantly decreased in both ADC and SCC samples.

In previous studies, our research group identified PPM1B as a key regulator of the MP/PRMT5/histone signaling pathway, representing a novel mechanism in carcinogenesis in HeLa cells and cervical cancer.

Western blot analysis comparing relative PPM1B expression in ADC, SCC, and corresponding control samples showed significantly lower expression in tumor tissues compared to healthy lung tissue. Immunohistochemical analysis confirmed the same difference. However, RT-PCR analysis revealed no significant difference in mRNA expression between tumor and control samples.

To investigate the relationship between protein expression and survival, ADC and SCC patients were further divided into two groups based on high or low

PPM1B expression. Kaplan-Meier analysis showed that patients with lower PPM1B expression had significantly worse survival than those with higher expression. Overall, patients in the ADC group demonstrated generally better survival outcomes compared to SCC patients. In the SCC group, survival differences were less pronounced, suggesting that other molecular mechanisms may play a more prominent role in this group compared to ADC.

The expression of the MYPT1 regulatory subunit of myosin phosphatase was increased in both ADC and SCC tissues.

Previous studies have already revealed the tumor suppressor role of myosin phosphatase (MP), which functions by dephosphorylating the PRMT5 activating phospho-Thr80 site. This phenomenon was observed in hepatocellular carcinoma (HCC) and was investigated in lung adenocarcinoma (ADC) and squamous cell carcinoma (SCC) in the present study.

Western blot analysis showed that MYPT1 relative expression was higher in both SCC and ADC tumor samples compared to control tissues, differing from the observations in HCC. The difference between tumor and healthy lung tissue was even more pronounced at the mRNA level, as revealed by RT-PCR, suggesting strong transcriptional activity, possibly due to a compensatory mechanism. These results were further confirmed by IHC and immunofluorescence analyses.

The relationship between MYPT1 expression and survival was assessed using Kaplan-Meier analysis, dividing ADC and SCC patients into low and high expression groups. In both tumor types, higher MYPT1 expression was associated with a survival advantage compared to lower expression.

Phosphorylation of the inhibitory Thr853 residue of MYPT1 was increased in lung cancer tissue samples.

Increased phosphorylation of MYPT1 at the Thr853 residue leads to decreased activity of the MP holoenzyme, resulting in reduced dephosphorylation of the

PRMT5 Thr80 residue. Phosphorylation of PRMT5 at Thr80 is activating, so inhibition of MP increases PRMT5 enzymatic activity.

Western blot analysis showed that MYPT1^{pT853} phosphorylation was significantly higher in both tumor types compared to healthy lung tissue. Furthermore, when comparing ADC and SCC, the level of this inhibitory phosphorylation was significantly higher in SCC samples than in ADC. Immunofluorescent labeling revealed that MYPT1^{pT853} is also present in the nuclei of lung cancer tissues.

The relationship between MYPT1^{pT853} and patient survival was assessed using Kaplan-Meier analysis, as described previously, showing that lower phosphorylation levels were associated with better survival in both histological groups. According to our results, the impact of MYPT1 inhibitory phosphorylation on survival was greater than that of relative protein expression.

Elevated PRMT5 levels were detected in both ADC and SCC samples.

The relative expression of PRMT5 was also analyzed by Western blot. Our results showed that PRMT5 protein levels were significantly increased in both ADC and SCC samples compared to the corresponding control tissues. To confirm these findings, PRMT5 mRNA levels were measured by RT-qPCR. A significant increase was observed in ADC tissues, while no significant change was detected in SCC samples.

For further analysis, DAB immunohistochemistry was performed on ADC and SCC sections, showing stronger DAB staining in ADC tissues. The relationship between PRMT5 protein expression and overall survival was also assessed. Kaplan–Meier survival analysis revealed no statistically significant correlation between PRMT5 expression levels (high vs. low) and overall survival in either ADC or SCC lung cancer patients.

The level of activating PRMT5^{pT80} phosphorylation, and thus PRMT5 activity, was increased in both ADC and SCC samples.

The ratio of activating PRMT5 Thr80 phosphorylation relative to PRMT5 expression was also examined by Western blot. Measurements showed significantly higher phosphorylation levels in both SCC and ADC tumor tissues compared to their healthy controls, with SCC tumor samples exhibiting a significantly greater increase in phosphorylation than ADC samples. Immunofluorescent staining further confirmed the elevated PRMT5 Thr80 phosphorylation in tumor tissues, indicating pronounced nuclear localization and activating phosphorylation, which reflects its impact on PRMT5 gene expression.

The relationship of this PTM with survival was also assessed. Using the Kaplan-Meier method, patients were grouped as in previous analyses. Our results showed that higher phosphorylation levels were associated with a significant survival disadvantage in both tumor types, with this effect being much more pronounced in SCC compared to ADC. Thus, increased PRMT5 activity is associated with poorer survival outcomes.

Symmetric dimethylation of histone proteins (H2A and H4) is increased in both ADC and SCC.

We also examined the final component of the PPM1B/MP/PRMT5/H2A signaling pathway, assessing both H2A protein expression and its symmetric dimethylation (DM). While there was no significant difference in histone protein expression between healthy lung tissue and lung cancer samples, ADC samples showed significantly higher expression compared to SCC samples. Kaplan–Meier analysis revealed no significant correlation between H2A expression and survival in either histological type.

In contrast, symmetric dimethylation of H2A, which correlates with gene expression activity, was markedly increased in both SCC and ADC tumor samples compared to corresponding controls. Additionally, SCC tumors

exhibited significantly higher DM levels than ADC tumors, reflecting a functional consequence of the pathway's imbalance. Kaplan-Meier analysis dividing patients into low and high dimethylation groups showed that lower dimethylation levels were associated with better survival in both tumor types. In parallel, we analyzed H4 histone expression and symmetric dimethylation. Total H4 protein expression did not differ significantly between SCC, ADC, and control groups, similar to H2A. However, H4 symmetric dimethylation was significantly higher in both tumor groups compared to controls.

To directly examine the effect of histone symmetric dimethylation on gene expression, we analyzed changes in the expression levels of the well-characterized target gene, the tumor suppressor retinoblastoma protein (pRb), as a readout of the proto-oncogenic role of the pathway we investigated. Western blot analysis revealed that pRb protein levels were significantly decreased in tumor samples compared to their corresponding controls.

We also assessed the combined impact of PTMs on survival using an integrated Kaplan–Meier analysis. ADC patients with low PTM levels showed the most favorable survival, whereas SCC patients with high PTM levels exhibited the poorest survival.

Results of the Functional Virtual Bronchoscopy Development

The aim of the project was to improve the quality of functional virtual bronchoscopy (VB) through more accurate registration of ldCT and hdCT images. As a result, a reliable, functionally and morphologically based virtual bronchoscopy can be generated from existing PET and hdCT scans, independently of the institution, supporting the identification of the most suitable target areas for sampling (e.g., the most metabolically active tumor or lymph node regions).

A prerequisite for high-quality functional VB is correct ldCT–hdCT alignment, which requires a transformation matrix. Previous quality assessments performed by radiologists were subjective, so our goal was to

introduce an objective, numerical (dis)similarity metric. Among the voxel-based similarity parameters examined, only mutual information (MI) showed a strong correlation with the distance-based quality metric D. Using bootstrap-based confidence intervals, MI proved suitable for distinguishing acceptable from inadequate registrations; values falling into the "inadequate" range indicate that re-registration is necessary. The limitation of the method was the small number of patients.

New findings

1. By investigating specific components of the PPM1B/MYPT1/PRMT5/histone signaling pathway, we demonstrated that disruption of the balance of post-translational modifications contributes to the development and progression of lung adenocarcinoma and squamous cell carcinoma.
2. We showed that PPM1B expression was significantly decreased in both lung adenocarcinoma and squamous cell carcinoma.
3. We found that the relative expression of MYPT1 was increased in tumor samples.
4. In accordance with this, phosphorylation of the inhibitory side chain of MYPT1 at Thr853 was also elevated in lung cancer tissue samples.
5. The relative expression of PRMT5, the next component of the signaling pathway, was significantly increased in both examined histological types of lung cancer.
6. In parallel, the level of activating PRMT5 pT80 phosphorylation was also significantly higher in both lung cancer histological subtypes, indicating increased PRMT5 activity in tumor tissues.

7. We demonstrated that symmetric dimethylation of the known PRMT5 substrates, histone proteins H2A and H4, was significantly elevated in lung adenocarcinoma and squamous cell carcinoma samples, suggesting repression of gene transcription.
8. The gene-repressive effect of the alterations in the signaling pathway was confirmed by examining the well-known tumor suppressor retinoblastoma protein, whose expression was found to be reduced in tumor samples.
9. Based on a combined survival analysis of the post-translational modifications investigated, patients whose tumors exhibited lower levels of these modifications showed more favorable survival outcomes.
10. The novel functional virtual bronchoscopy method is based on automated fusion of high-resolution CT images with PET scans, providing significantly improved anatomical orientation. The method is applicable to CT examinations performed with different parameters and obtained at different institutions.

Summary

In summary, our work aimed to approach lung cancer in a comprehensive manner by examining a new oncogenic signaling pathway and an innovative imaging technique in the service of personalized diagnostics. In our study, we demonstrated that the PPM1B/MP/PRMT5/histone signaling pathway plays a role in the development and progression of lung cancer. Dysregulation of this pathway (the reduced expression of PPM1B, functional inactivation of MYPT1 despite its elevated expression, elevated PRMT5 activity, and increased methylation of histone H2A reveals new mechanisms of lung cancer development. The differences between the ADC and SCC subtypes further highlight the molecular heterogeneity of lung cancer, and we hope that our results may, in the future, contribute to the lung cancer treatment.

In addition to molecular mechanisms and potential diagnostic approaches, we also explored imaging possibilities. During the diagnostic process of lung cancer, patients usually undergo contrast enhanced CT and PET-CT examinations. The automated functional virtual bronchoscopy developed at our university combines the advantages of both PET and high-resolution CT while using retrospective scans, thereby avoiding additional radiation exposure. The use of functional information may enhance the success rate of tissue sampling, while vascular visualization could help to reduce the risk of complications.

List of Publications

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/644/2025.PL
Subject: PhD Publication List

Candidate: Attila Makai
Doctoral School: Doctoral School of Molecular Medicine

List of publications related to the dissertation

1. **Makai, A.**, Keller, I., Szalmás, A. F., Ungvári, Á., Horváth, D., Major, E., Enyedi, A., Takács, I., Lontay, B.: Decreased PPM1B Expression Drives PRMT5-Mediated Histone Modification in Lung Cancer Progression.
   Biomolecules. 15, 1-18, 2025.
   DOI: http://dx.doi.org/10.3390/biom15111581
   IF: 4.8 (2024)
2. Opposits, G., Nagy, M., Barta, Z., Aranyi, S. C., Szabó, D., **Makai, A.**, Varga, I., Galuska, L., Trón, L., Balkay, L., Emri, M.: Automated procedure assessing the accuracy of HRCT-PET registration applied in functional virtual bronchoscopy.
   EJNMMI Res. 11 (1), 1-13, 2021.
   DOI: http://dx.doi.org/10.1186/s13550-021-00810-w
   IF: 3.434

List of other publications

3. Lieber, A., **Makai, A.**, Orosz, Z., Kardos, T., Susil, J. I., Tornyi, I., Bittner, N.: The role of immunotherapy in early stage and metastatic NSCLC.
   Pathol. Oncol. Res. 30, 1-17, 2024.
   DOI: http://dx.doi.org/10.3389/pore.2024.1611713
   IF: 2.3

DEBRECENI EGYETEM Egyetemi és Nemzeti Könyvtár

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

4. Sebestyén, E., Major, N., Bodoki, L., **Makai, A.**, Balogh, I., Tóth, G., Orosz, Z., Árkosy, P., Vaskó, A., Hódosi, K., Szekanecz, Z., Szekanecz, É., Hungarian OncoRheumatology Network (HORN) initiative: Immune-related adverse events of anti-PD-1 immune checkpoint inhibitors: a single center experience.
Front Oncol. 13, 1-9, 2023.
DOI: http://dx.doi.org/10.3389/fonc.2023.1252215
IF: 3.5

Total IF of journals (all publications): 14,034
Total IF of journals (publications related to the dissertation): 8,234

The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

16 December, 2025

DEBRECENI EGYETEM
Egyetemi és Nemzeti Könyvtár