SPHINGOLIPID METABOLISM IN LUNG DISEASES

Ph.D thesis

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1. Introduction

Sphingolipids are bioactive lipids that play key roles not only as structural membrane components but also as mediators of inflammation, apoptosis, and cell survival. The concept of the "sphingolipid rheostat" describes the balance between ceramides, which induce growth arrest and apoptosis, and sphingosine-1-phosphate (S1P), which promotes proliferation, angiogenesis, and survival. Disturbances in this balance contribute to the pathogenesis of multiple diseases including cancer, metabolic and vascular disorders.

In cancer, dysregulated sphingolipid metabolism critically influences tumor progression and therapy response. Ceramide accumulation induces apoptosis, autophagy, and growth arrest. Conversely, S1P promotes tumor survival, invasion, chemoresistance, and shapes the tumor microenvironment by enhancing angiogenesis and immune evasion. Targeting sphingolipid pathways has emerged as a promising therapeutic approach, with both ceramide-based strategies and S1P-directed interventions under investigation.

Obstructive sleep apnea (OSA), characterized by recurrent airway collapse and intermittent hypoxia, is associated with cardiovascular, metabolic and neurocognitive comorbidities.

Intermittent hypoxia promotes systemic inflammation, oxidative stress, sympathetic activation, and endothelial dysfunction. Sphingolipids, as key regulators of inflammation, metabolism and vascular function, are likely to play a role in OSA-related pathologies. Proinflammatory cytokines are known to stimulate sphingolipid synthesis. Ceramides contribute to insulin resistance and obesity-related inflammation, while S1P influences endothelial activation and atherosclerosis. Although systematic studies are lacking, overlapping mechanisms suggest sphingolipid metabolism as a potential mediator in OSA comorbidities and a possible target for future research.

This doctoral thesis aims to investigate the involvement of the sphingolipid rheostat in non-small cell lung cancer (NSCLC) and OSA, focusing on S1P and anti-ceramide antibody as potential non-invasive biomarkers.

2. Objectives

The main objectives are as follows:

1. To investigate the potential of S1P and anti-ceramide antibody as biomarkers in unresectable and advanced stage NSCLC:
    - via assessing circulating levels of S1P in NSCLC patients
    - via assessing circulating levels of anti-ceramide antibody in NSCLC patients
    - via assessing anti-ceramide antibody levels in bronchial washing fluid (BWF) of NSCLC patients
    - via correlating survival data with S1P and anti-ceramide antibody levels
2. To investigate the possible role of S1P and anti-ceramide antibody in OSA:
    - via assessing circulating levels of S1P in OSA patients
    - via assessing circulating levels of anti-ceramide antibody in OSA patients
    - via correlating clinical variables with anti-ceramide antibody levels

3. Methods

3.1. Study design

Two parallel case-control studies were conducted to evaluate sphingolipid metabolism in OSA and NSCLC. Patients were recruited from the Department of Pulmonology, Semmelweis University. The primary endpoint in both studies was to compare levels of S1P and anti-ceramide antibody between the control and patient groups. In the NSCLC study, as a secondary endpoint, we analyzed survival data in relation to these biomarkers.

Both studies were approved by the institutional ethics committees, and informed consent was obtained from all participants.

3.2. Study populations

In the NSCLC study, 32 patients with stage III–IV unresectable NSCLC and 34 healthy controls were included for plasma biomarker measurements. In addition, BWF samples were collected from 20 NSCLC patients and 3 controls. NSCLC patients were diagnosed by histology or cytology, with staging based on UICC TNM 8th edition. Exclusion criteria were infections in the last 2 months and history of any malignancy in the control groups.

In the OSA study 68 subjects with suspected OSA were recruited from the Sleep Unit. Exclusion criteria included uncontrolled chronic disease, history of malignancy in the last 10 years, or recent infection. All subjects underwent attended polysomnography (n=41) or cardiorespiratory polygraphy (n=27), scored according to AASM guidelines. OSA was defined as apnea–hypopnea index (AHI) $\geq$5/h. OSA was confirmed in 31 subjects.

In both studies, clinical and demographic data were collected, and venous blood samples were analyzed for S1P and anti-ceramide antibody levels. In the NSCLC study, BWF samples were analyzed for anti-ceramide antibodies.

3.3. Sample processing and biomarker measurement

Venous blood and BWF samples were processed within 2 hours and stored at –80 °C. Commercial ELISA kits (MyBioSource, USA) were used to measure S1P and anti-ceramide antibodies in plasma (both studies) and in BWF (NSCLC). Assays were performed in duplicates according to the manufacturer's instructions and mean concentrations were used as inputs for analysis.

3.4. Statistical analysis

Statistical analyses were conducted using R (v 4.1.3) and JASP (v 0.14.1). The sample size was calculated to detect a minimum effect size of 0.70 for differences in anti-ceramide antibody or S1P levels between groups, with 80% power at $\alpha=0.05$. P values of <.05 were considered significant. Data are expressed as median [range] or mean ± SD. All plots were generated using *ggplot2*.

Data normality was assessed using the Shapiro-Wilk test. In the NSCLC study, both anti-ceramide antibody and S1P levels were normally distributed, while in the OSA study, distributions were non-parametric.

In the NSCLC study, biomarker levels were compared with linear regression adjusted for age, COPD status, and BMI. Chi-squared tests were used for categorical variables, and t-tests were used to compare COPD and non-COPD subgroups. Survival analysis was performed using the log-rank test, and Kaplan-Meier plots were generated. Survival differences based on biomarker levels were also assessed. ROC analysis (*rocit* package) was used to determine an optimal cutoff for anti-ceramide antibody levels, while the median value was used for S1P cutoff.

In the OSA study, Mann-Whitney U-tests and chi-squared tests were used to compare biomarker levels and clinical variables between OSA patients and controls. A non-parametric ANCOVA adjusted for age, gender, and BMI was used to assess biomarker differences. Correlations with clinical parameters were tested by Spearman's and logistic regression analysis.

4. Results

4.1. NSCLC study

32 stage III–IV NSCLC patients and 34 controls were enrolled. Patients were older, more frequently smokers, and had higher prevalence of COPD, hypertension, and elevated CRP (all $p<0.05$). Most tumors were adenocarcinomas (71.9%); KRAS mutations were detected in 61%, and PDL1 positivity in 72%.

Plasma S1P levels were markedly higher in NSCLC patients compared to controls (3771 ± 762 vs. 367 ± 249 ng/ml, $p<0.001$), independent of age, BMI, COPD, KRAS or PDL1 status.

Anti-ceramide antibody concentrations were also elevated in NSCLC both in plasma (279 ± 19 vs. 179 ± 18 ng/ml, $p=0.007$) and BWF samples (155 ± 28 vs. 106 ± 10 ng/ml, $p<0.001$). However, regarding BWF measurements data is limited due to small control numbers.

Median overall survival (OS) in NSCLC was 13.4 months. Patients with high anti-ceramide antibody levels showed a tendency toward longer OS (24.3 vs. 8.6 months, $p=0.098$). In contrast, S1P levels did not correlate with OS ($p=0.17$).

4.2. OSA study

68 subjects underwent sleep studies; OSA was diagnosed in 31 cases. Patients with OSA were older, had higher BMI, hypertension, triglycerides, CRP, AHI and ODI, lower HDL-C, and lower minimum oxygen saturation (all $p<0.05$).

Plasma anti-ceramide antibody levels were significantly elevated in OSA patients (892 [4–1494] vs. 210 [36–1725] ng/ml, $p<0.001$), independent of age, sex, and BMI. Antibody levels correlated positively with BMI, CRP, AHI, ODI and TST90%, and negatively with $MinSatO_2$. All OSA severity subgroups (mild, moderate, severe) had higher antibody levels compared to controls, but no differences were observed between severity groups.

Plasma S1P levels were also significantly higher in OSA (1760 [99–1760] vs. 290 [42–1760] ng/ml, $p<0.001$). However, most OSA values exceeded the upper detection limit of the ELISA, precluding further subgroup or correlation analysis.

5. Conclusions

- Sphingolipid metabolism is altered in both NSCLC and OSA, two lung diseases associated with chronic inflammation.
- Plasma levels of S1P and anti-ceramide antibodies were significantly elevated in advanced NSCLC patients compared to controls.
- BWF levels of anti-ceramide antibodies were elevated in NSCLC, suggesting local origin in the TME
- High anti-ceramide antibody levels showed a tendency for longer overall survival in NSCLC, suggesting potential prognostic value.
- In OSA, both biomarkers were significantly elevated and anti-ceramide antibody levels correlated with BMI, CRP, AHI, and hypoxemia.
- Antibody levels were already increased in mild OSA, indicating early metabolic involvement, but did not differ across severity groups.

These findings highlight the broader role of sphingolipid metabolism in lung diseases and support sphingolipids as promising biomarkers and potential therapeutic targets.

6. Bibliography of the candidate's publications related to the thesis

Bűdi L, Hammer D, Varga R, Müller V, Tárnoki ÁD, Tárnoki DL, Mészáros M, Bikov A, Horváth P. Anti-ceramide antibody and sphingosine-1-phosphate as potential biomarkers of unresectable non-small cell lung cancer. Pathol Oncol Res. 2025 Jan 6;30:1611929. doi: 10.3389/pore.2024.1611929. PMID: 39835329; PMCID: PMC11742942.
IF: 2,3
Journal rank: Q2

Horváth P, Büdi L, Hammer D, Varga R, Losonczy G, Tárnoki ÁD, Tárnoki DL, Mészáros M, Bikov A. The link between the sphingolipid rheostat and obstructive sleep apnea. Sci Rep. 2023 May 11;13(1):7675. doi: 10.1038/s41598-023-34717-4. PMID: 37169814; PMCID: PMC10175248.
IF: 3,8
Journal rank: D1