Alterations in plasma lipid profile before and after surgical removal of soft tissue sarcoma

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Research Article

Keywords: Soft tissue sarcoma, Metabolomics, Lipid, Recurrence, Surgery

Posted Date: January 3rd, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3815397/v1
Abstract

Background

Soft tissue sarcoma (STS) is a relatively rare malignancy, accounting for about 1% of all adult cancers. It is known to have more than 70 subtypes. Its rarity, coupled with its various subtypes, makes early diagnosis challenging. The current standard treatment for STS is surgical removal. To aid in identifying prognosis and pathogenesis, we utilized an untargeted metabolomic approach to profile the altered endogenous metabolites in pre-operative and post-operative plasma samples of STS patients.

Methods

We collected pre-operative and post-operative plasma samples from 24 patients with STS who underwent surgical removal of masses. Plasma metabolic profiling was conducted using ultra-high performance liquid chromatography-quadrupole time-of-flight/mass spectrometry. Out of the 24 patients, 11 experienced recurrences after the operations. Multivariate analysis and permutation tests were conducted to identify putative altered metabolites. Univariate receiver operator characteristic analysis was performed to evaluate their predictive performance.

Results

Thirty-nine putative metabolites were identified based on the orthogonal projections to latent structures-discriminant analysis, with 34 of them showing statistical significance. These metabolites included phospholipids and acyl-carnitines, indicating changes in lipid metabolism. Specifically, phospholipids exhibited an increase in the post-operative samples, while acyl-carnitines showed a decrease. Notably, lysophosphatidylcholine (LPC) O-18:0 and LPC-O16:2 demonstrated predictive capabilities for STS recurrence, with area under the curve values of 0.748 and 0.797, respectively.

Conclusions

Our investigation revealed distinct alterations in the lipid profiles of plasma in STS patients after surgical resection of masses. We anticipate that these findings can contribute to the elucidation of the pathophysiology of STS and the development of further metabolic studies in this rare malignancy.

1. Introduction

Soft tissue sarcoma (STS) is a heterogeneous disease entity with approximately 70 subtypes, despite its prevalence being only 1% among adult malignancies [1]. STS originates from mesenchymal cells found in connective tissues such as muscles, blood vessels, neurons, cartilage, and adipose tissue. The scarcity
of cases and the absence of large-scale randomized controlled trials pose significant challenges in diagnosing and treating these rare malignancies.

The treatments for STS include local approaches such as surgery and radiation therapy. Currently, surgery is the standard curative treatment for localized STS, either alone or in combination with radiation therapy before and after the surgery [2]. The 5-year survival rate of STS is around 50%, but in cases of metastases, it rapidly decreases to around 10% [2, 3]. The prevalence of STS in young adults is relatively high compared to other epithelial cancers. Therefore, it is necessary to study the prognosis after surgery and understand the mechanisms of development and recurrence of STS to improve survival rates.

The Cancer Genome Atlas (TCGA) program and its accompanying studies have provided a wealth of valuable information about cancer. It is now believed that the malignancy of human bodies is primarily a result of oncogenic mutations, and there has been successful drugs targeting these mutations. However, STS cases exhibit a lower oncogenic mutational burden compared to other epithelial malignancies in the solid tumor categories [4, 5]. The lower mutational burden in STS is an unmet need in this era of TCGA and targeted therapies. Therefore, it is necessary to go beyond genomic alterations and explore the metabolic profiles to better understand the pathophysiology especially in STS. The tumor microenvironment (TME) has gained importance in the treatment of malignancies. The metabolic rewiring of tumors can be considered a part of the TME and a result of the interaction between tumor cells and the TME.

Metabolomics is the study of metabolites found in bio-fluids, tissues, and organisms. As metabolites are the byproducts of cellular processes, metabolomics provides a snapshot of the physiological state of an organism. Indeed, metabolites can be promising biomarkers associated with various diseases, especially cancers [6, 7]. In particular, untargeted metabolomics is advantageous as it allows for an unbiased analysis of metabolomes derived from various metabolic pathways. A previous study has reported physiological alterations in the serum of colorectal cancer (CRC) patients before and after surgery through untargeted and targeted metabolomics [8]. This type of research design, which compares pre-operative and post-operative profiling, can shed light on the process of tumorigenesis. Furthermore, metabolomics can provide insights into patient prognosis by evaluating metabolic profiles. A study has shown a prognostic nomogram that included metabolic profiles in gastric cancer patients [9].

However, only a limited number of studies have investigated the metabolites in pre-operative and post-operative samples, especially in rare malignancies such as STS. In this study, we examined the metabolome in plasma samples of STS patients before and after surgery using untargeted metabolomics based on ultra-high performance liquid chromatography-quadrupole time-of-flight/mass spectrometry (UHPLC-QTOF/MS). We aimed to gain valuable insights that could contribute to the early diagnosis of the disease or relapse, as well as to shed light on the pathophysiology of STS.

2. Materials and Methods
2.1 Reagents and Chemicals

High-performance liquid chromatography (HPLC) grade was used for analysis. Ultrapure distilled water and acetonitrile were purchased from J.T. Baker® (Phillipsburg, NJ, USA). Methanol was purchased from Merck (Darmstadt, Germany). Formic acid (LC-MS grade, > 98.0%) was purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Hexakis (2,2-difluoroethoxy) phosphazene for lock-mass was purchased from Apollo Scientific. Sodium formate solution (10 mM sodium hydroxide with 0.2% formic acid in isopropanol/water, 1:1 v/v) for internal calibration was purchased from Fluka-Honeywell (Charlotte, NC, USA).

2.2 Collection and preparation of samples

This was a prospective study that enrolled 36 patients who underwent surgical resection of STS from November 2018 to September 2021 at Pusan National University Hospital. Blood samples were collected from subjects before and after the operation. The collected blood samples had been centrifuged in 10 minutes each time and immediately kept frozen. Patients who did not meet the inclusion criteria were excluded, including cases where benign tumors were diagnosed based on histopathological findings ($n = 3$), cases where carcinoma was confirmed pathologically with surgical specimens ($n = 1$), cases where post-operative samples were not collected ($n = 6$), and cases where no tumor was visible during surgery ($n = 2$) (Fig. 1).

We grouped STSs based on chemosensitivity using a guideline and reported data [10, 11]. In 2016, the UK guidelines for STS provided information on the relative chemosensitivity of different STS subtypes. These guidelines categorized STS into five groups based on chemosensitivity: (1) chemotherapy integral to management, (2) chemosensitive, (3) moderately chemosensitive, (4) relatively chemo-insensitive, and (5) chemo-insensitive.

This prospective study was approved by the Institutional Review Board (IRB) of Pusan National University Hospital, with the requirement for written consent (IRB 1805-028-067). The study was performed in accordance with relevant guidelines and regulations. Plasma samples were prepared in 50 µL aliquots, and 100 µL of cold acetonitrile was added for protein precipitation. The samples were mixed thoroughly and centrifuged at 16,100 × $g$ for 15 minutes at 4°C. After drying the supernatant of 100 µL using a vacuum concentrator for 2.2 hours, 200 µL of 50% acetonitrile was added to the residuals.

2.3 Metabolomics analysis

The metabolomics analysis was performed using a UHPLC Dionex UltiMate 3000 series (Thermo Scientific, Dionex, Sunnyvale, CA, USA) with a Waters ACQUITY UPLC® BEH C18 column (100 mm × 2.10 mm, 1.7 µm, 130 Å; Waters, Milford, MA, USA) coupled to compact QTOF (Bruker Daltonics GmbH & Co. KG, Bremen, Germany). Separation was conducted at a flow rate of 300 µL/min using a mobile phase consisting of 0.1% formic acid in water (A) and acetonitrile (B). The gradient used was as follows: 1% B, 0.0–1.0 min; 1–65%, 0.5–3.0 min; 65–90%, 3.0–7.0 min; 90%, 7.0–35.0 min; 90–100%, 35.0–35.5 min; 100%, 35.0–41.5 min. The gradient then returned to the initial concentration (1% B) for 2 min before the
next sample. The auto-sampler and column temperature were maintained at 4°C and 40°C, respectively. The injection volume was 1.5 µL.

The mass spectrometer was operated in positive ionization mode for mass measurement, using the following parameters: mass scan range, full scan 50–1,000 mas`s-to-charge ratio (m/z); nebulizer gas pressure, 0.8 bar; capillary voltage, + 4,500 V; end plate offset, − 500 V; dry gas flow rate, 10.0 L/min; dry gas temperature, 200°C.

### 2.4 Putative identification of metabolites

The tandem mass (MS/MS) spectrum was compared to the libraries of MetaboScape 5.0 (Bruker Daltonics GmbH & Co. KG, Bremen, Germany), such as the Human Metabolome Database (HMDB) Metabolite Library, MetaboBASE Personal Library, and MS-DIAL LipidBlast (version 68). The annotation parameters were as follow: mass tolerance, 2.0–5.0 mDa; mSigma, 50–100. The mSigma is a measure of the goodness of fit between the measured and theoretical isotopic patterns.

### 2.5 Statistical analysis

ProfileAnalysis 2.1 (Bruker Daltonics, Billerica, MA, USA) was used to construct the feature table. The raw data was preprocessed by performing quantile normalization, log transformation, and pareto scaling. SIMCA 17.0.2 was employed for multivariate statistical analysis, such as principal component analysis (PCA) and orthogonal projections to latent structures-discriminant analysis (OPLS-DA). To verify the OPLS-DA results, a permutation test with 100 iterations was implemented. As the variable importance in projection (VIP) value represents the contribution of each feature, metabolites with high VIP values are more relevant for group separation [12]. A VIP value of 1.0 or higher was considered significant. A paired t-test was conducted to evaluate the differences in metabolites between the before and after surgery groups using SPSS 26.0 (IBM, Armonk, NY, USA).

To identify the metabolic signature contributing to group discrimination and evaluate the predictive performance of potential biomarkers in distinguishing recurrence of STSs, the univariate receiver operator characteristic (ROC) curve analysis was performed. For the ROC curve, the area under the curve (AUC) was calculated to assess the accuracy of the metabolites. A general guide was used to estimate the accuracy based on AUC values: 0.5–0.6, fail; 0.6–0.7, poor; 0.7–0.8, fair; 0.8–0.9, good; and 0.9–1.0, excellent [13]. MetaboAnalyst version 5.0 (https://www.metaboanalyst.ca) was used to perform the ROC curve analyses.

### 3. Results

#### 3.1 Patient characteristics

We conducted a study on 24 patients who had 10 different pathological subtypes of STS (Table 1). The median age of the patients, consisting of 11 females and 13 males, was 61 years (ranging from 42 to 76 years) at the time of diagnosis. The majority of the primary locations of the tumors were in the
extremities, except for one case of dedifferentiated liposarcoma in the retroperitoneum (Table 1). Among these 24 patients with STS, leiomyosarcoma (6 patients) was the most common subtype, followed by myofibrosarcoma (5 patients).

Thirteen out of 24 patients had relatively chemo-insensitive STSs, which included myxofibrosarcoma, dedifferentiated liposarcoma, well differentiated liposarcoma, undifferentiated pleomorphic sarcoma, and malignant peripheral nerve sheath tumor (MPNST). Moderately chemosensitive STS cases included leiomyosarcoma, angiosarcoma, and pleomorphic liposarcoma. Only 2 out of 24 patients with myxoid liposarcoma had chemosensitive STS. Pleomorphic leiomyosarcoma could not be evaluated due to very recently separate categorization from leiomyosarcoma and limited data on chemosensitivity. The median relapse-free survival (RFS) for these patients was 4 years.

### Table 1
Pathological characteristics of STS patients.

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Chemosensitivity</th>
<th>Anatomical location of primary lesion</th>
<th>Patients (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiosarcoma</td>
<td>Moderately chemosensitive</td>
<td>Hip</td>
<td>1</td>
</tr>
<tr>
<td>Dedifferentiated liposarcoma</td>
<td>Relatively chemo-insensitive</td>
<td>Calf, thigh, retroperitoneum</td>
<td>3</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>Moderately chemosensitive</td>
<td>Upper arm, hip, thigh (4)</td>
<td>6</td>
</tr>
<tr>
<td>MPNST</td>
<td>Relatively chemo-insensitive</td>
<td>Shoulder</td>
<td>1</td>
</tr>
<tr>
<td>Myxofibrosarcoma</td>
<td>Relatively chemo-insensitive</td>
<td>Upper arm, forearm, thigh (3)</td>
<td>5</td>
</tr>
<tr>
<td>Myxoid liposarcoma</td>
<td>Chemosensitive</td>
<td>Thigh</td>
<td>2</td>
</tr>
<tr>
<td>Pleomorphic leiomyosarcoma</td>
<td>NE</td>
<td>Thigh</td>
<td>1</td>
</tr>
<tr>
<td>Pleomorphic liposarcoma</td>
<td>Moderately chemosensitive</td>
<td>Thigh</td>
<td>1</td>
</tr>
<tr>
<td>Undifferentiated pleomorphic sarcoma</td>
<td>Relatively chemo-insensitive</td>
<td>Calf, hip, thigh</td>
<td>3</td>
</tr>
<tr>
<td>Well differentiated liposarcoma</td>
<td>Relatively chemo-insensitive</td>
<td>Hip</td>
<td>1</td>
</tr>
</tbody>
</table>

MPNST, malignant peripheral nerve sheath tumor; NE, not evaluable.

### 3.2 Metabolite profiles of sarcoma patients

The pre-operative and post-operative plasma samples were analyzed using untargeted metabolomics profiling. The quality control samples were clustered together in the PCA score plot, and there was a slight
separation observed between pre-operative and post-operative samples, although it was not very clear (Fig. 2). The OPLS-DA score plot clearly differentiated between pre-operative and post-operative STS samples, with an R2Y value of 0.971 and a Q2 value of 0.519 (Fig. 3A). In the permutation test to validate the OPLS-DA model, the y-intercept of the R2 and Q2 regression lines were 0.963 and -0.232, respectively (Fig. 3B).

3.3 Analysis of putatively identified metabolites

Based on the VIP value obtained from the OPLS-DA model, a total of 39 metabolites were screened (Table 2). Using either the paired t-test or Wilcoxon Rank-Sum test, it was found that 34 metabolites exhibited statistical significance. Among these, 9 metabolites showed downregulation, while the rest exhibited upregulation in postoperative STS patients. The trends of these metabolites are illustrated on a heatmap (Fig. 4).

Most of the putatively identified metabolites were lipids, specifically glycerophospholipids such as phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE), lysophosphatidylethanolamine (LPE), and lysophosphatidylserine (LPS), as well as fatty acids (FAs) and their derivatives.
Table 2
List of differential metabolites in plasma samples between pre-operative and post-operative STS patients.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>VIP</th>
<th>RT (min)</th>
<th>m/z</th>
<th>Formula</th>
<th>Trend</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Porphyrin metabolism; Bile secretion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilirubin</td>
<td>2.62</td>
<td>5.28</td>
<td>585.2713</td>
<td>C_{33}H_{36}N_{4}O_{6}</td>
<td>↓</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Fatty acid metabolism</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Palmitoyl threonine</td>
<td>1.00</td>
<td>5.98</td>
<td>358.2932</td>
<td>C_{20}H_{39}NO_{4}</td>
<td>↓</td>
<td>0.245</td>
</tr>
<tr>
<td>13Z-Docosenamide</td>
<td>1.34</td>
<td>12.96</td>
<td>338.3430</td>
<td>C_{22}H_{43}NO</td>
<td>↓</td>
<td>0.004</td>
</tr>
<tr>
<td>Nervonamide</td>
<td>1.20</td>
<td>15.89</td>
<td>366.3738</td>
<td>C_{24}H_{47}NO</td>
<td>↓</td>
<td>0.072</td>
</tr>
<tr>
<td>cis-4-Decenoylcarnitine</td>
<td>2.63</td>
<td>5.30</td>
<td>314.2328</td>
<td>C_{17}H_{31}NO_{4}</td>
<td>↓</td>
<td>0.002</td>
</tr>
<tr>
<td>cis-5-Dodecenoylcarnitine</td>
<td>3.04</td>
<td>5.64</td>
<td>342.2636</td>
<td>C_{19}H_{35}NO_{4}</td>
<td>↓</td>
<td>0.001</td>
</tr>
<tr>
<td>5Z,8Z-Tetradecadienoylcarnitine</td>
<td>2.63</td>
<td>5.79</td>
<td>368.2790</td>
<td>C_{21}H_{37}NO_{4}</td>
<td>↓</td>
<td>0.022</td>
</tr>
<tr>
<td>cis-5-Tetradecenoylcarnitine</td>
<td>3.19</td>
<td>6.04</td>
<td>370.2948</td>
<td>C_{21}H_{39}NO_{4}</td>
<td>↓</td>
<td>0.001</td>
</tr>
<tr>
<td>alpha-Linolenic acid</td>
<td>1.38</td>
<td>9.13</td>
<td>279.2312</td>
<td>C_{18}H_{30}O_{2}</td>
<td>↓</td>
<td>0.009</td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>2.39</td>
<td>9.38</td>
<td>329.2480</td>
<td>C_{22}H_{32}O_{2}</td>
<td>↓</td>
<td>0.014</td>
</tr>
<tr>
<td><strong>Glycerophospholipid metabolism</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPC 16:1</td>
<td>1.48</td>
<td>6.84</td>
<td>494.3263</td>
<td>C_{24}H_{48}NO_{7}P</td>
<td>↑</td>
<td>0.001</td>
</tr>
<tr>
<td>LPC 17:0</td>
<td>1.16</td>
<td>8.14</td>
<td>510.3557</td>
<td>C_{25}H_{52}NO_{7}P</td>
<td>↑</td>
<td>0.002</td>
</tr>
<tr>
<td>LPC 17:1</td>
<td>1.30</td>
<td>7.31</td>
<td>508.3422</td>
<td>C_{25}H_{50}NO_{7}P</td>
<td>↑</td>
<td>0.003</td>
</tr>
<tr>
<td>LPC 18:0</td>
<td>1.20</td>
<td>8.56</td>
<td>524.3714</td>
<td>C_{26}H_{54}NO_{7}P</td>
<td>↑</td>
<td>0.000</td>
</tr>
<tr>
<td>LPC 18:3</td>
<td>1.53</td>
<td>6.65</td>
<td>518.3248</td>
<td>C_{26}H_{48}NO_{7}P</td>
<td>↑</td>
<td>0.026</td>
</tr>
<tr>
<td>LPC 20:1</td>
<td>1.78</td>
<td>8.98</td>
<td>550.3888</td>
<td>C_{28}H_{56}NO_{7}P</td>
<td>↑</td>
<td>0.000</td>
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<tr>
<td>LPC 20:2</td>
<td>1.37</td>
<td>8.09</td>
<td>548.3735</td>
<td>C_{28}H_{54}NO_{7}P</td>
<td>↑</td>
<td>0.005</td>
</tr>
<tr>
<td>LPC 0-16:2</td>
<td>1.28</td>
<td>30.95</td>
<td>478.3303</td>
<td>C_{24}H_{48}NO_{6}P</td>
<td>↑</td>
<td>0.007</td>
</tr>
<tr>
<td>LPC 0-18:0</td>
<td>1.65</td>
<td>9.19</td>
<td>510.3930</td>
<td>C_{26}H_{56}NO_{6}P</td>
<td>↑</td>
<td>0.000</td>
</tr>
<tr>
<td>LPC 0-18:1</td>
<td>1.62</td>
<td>9.14</td>
<td>508.3753</td>
<td>C_{26}H_{54}NO_{6}P</td>
<td>↑</td>
<td>0.001</td>
</tr>
<tr>
<td>Metabolites</td>
<td>VIP</td>
<td>RT (min)</td>
<td>m/z</td>
<td>Formula</td>
<td>Trend</td>
<td>p-value</td>
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<tr>
<td>Porphyrin metabolism; Bile secretion</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPC P-18:0</td>
<td>1.18</td>
<td>8.13</td>
<td>508.3740</td>
<td>C_{26}H_{54}NO_{6}P</td>
<td>↑</td>
<td>0.002</td>
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<tr>
<td>LPE 18:2</td>
<td>1.01</td>
<td>7.10</td>
<td>478.2935</td>
<td>C_{23}H_{44}NO_{7}P</td>
<td>↑</td>
<td>0.011</td>
</tr>
<tr>
<td>LPE 22:5</td>
<td>1.19</td>
<td>7.25</td>
<td>528.3093</td>
<td>C_{27}H_{46}NO_{7}P</td>
<td>↑</td>
<td>0.068</td>
</tr>
<tr>
<td>LPE P-18:0</td>
<td>1.79</td>
<td>9.12</td>
<td>466.3305</td>
<td>C_{23}H_{48}NO_{6}P</td>
<td>↑</td>
<td>0.000</td>
</tr>
<tr>
<td>LPS O-18:0</td>
<td>1.68</td>
<td>6.80</td>
<td>512.3363</td>
<td>C_{24}H_{50}NO_{8}P</td>
<td>↑</td>
<td>0.001</td>
</tr>
<tr>
<td>PC 16:0/20:5</td>
<td>1.24</td>
<td>17.81</td>
<td>780.5541</td>
<td>C_{44}H_{78}NO_{8}P</td>
<td>↑</td>
<td>0.500</td>
</tr>
<tr>
<td>PC 18:0/20:4</td>
<td>1.86</td>
<td>25.36</td>
<td>810.6000</td>
<td>C_{46}H_{84}NO_{8}P</td>
<td>↑</td>
<td>0.000</td>
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<tr>
<td>PC 18:1/18:1</td>
<td>1.02</td>
<td>31.71</td>
<td>786.5999</td>
<td>C_{44}H_{84}NO_{8}P</td>
<td>↑</td>
<td>0.018</td>
</tr>
<tr>
<td>PC 18:2/18:3</td>
<td>1.21</td>
<td>15.43</td>
<td>780.5511</td>
<td>C_{44}H_{78}NO_{8}P</td>
<td>↑</td>
<td>0.069</td>
</tr>
<tr>
<td>PC 18:3/18:3</td>
<td>1.71</td>
<td>15.31</td>
<td>778.5419</td>
<td>C_{44}H_{76}NO_{8}P</td>
<td>↑</td>
<td>0.001</td>
</tr>
<tr>
<td>PC 18:4/18:2</td>
<td>1.11</td>
<td>18.75</td>
<td>778.5348</td>
<td>C_{44}H_{76}NO_{8}P</td>
<td>↑</td>
<td>0.039</td>
</tr>
<tr>
<td>PC 32:1</td>
<td>1.48</td>
<td>22.76</td>
<td>732.5541</td>
<td>C_{40}H_{78}NO_{8}P</td>
<td>↑</td>
<td>0.001</td>
</tr>
<tr>
<td>PC 34:3</td>
<td>1.87</td>
<td>19.57</td>
<td>756.5552</td>
<td>C_{42}H_{78}NO_{8}P</td>
<td>↑</td>
<td>0.003</td>
</tr>
<tr>
<td>PC 36:4</td>
<td>1.17</td>
<td>31.18</td>
<td>782.5684</td>
<td>C_{44}H_{80}NO_{8}P</td>
<td>↑</td>
<td>0.007</td>
</tr>
<tr>
<td>PC 38:6</td>
<td>1.14</td>
<td>25.01</td>
<td>806.5683</td>
<td>C_{46}H_{80}NO_{8}P</td>
<td>↑</td>
<td>0.003</td>
</tr>
<tr>
<td>PC 38:7</td>
<td>1.17</td>
<td>15.53</td>
<td>804.5562</td>
<td>C_{46}H_{78}NO_{8}P</td>
<td>↑</td>
<td>0.000</td>
</tr>
<tr>
<td>PC 40:7</td>
<td>1.11</td>
<td>19.64</td>
<td>832.5880</td>
<td>C_{48}H_{82}NO_{8}P</td>
<td>↑</td>
<td>0.000</td>
</tr>
<tr>
<td>PC 40:8</td>
<td>1.51</td>
<td>16.12</td>
<td>830.5694</td>
<td>C_{48}H_{80}NO_{8}P</td>
<td>↑</td>
<td>0.001</td>
</tr>
<tr>
<td>PE 36:4</td>
<td>1.45</td>
<td>22.07</td>
<td>740.5210</td>
<td>C_{41}H_{74}NO_{8}P</td>
<td>↑</td>
<td>0.014</td>
</tr>
</tbody>
</table>

A p-value below 0.05 was considered to indicate a significant difference.

VIP, variable importance in projection; RT, retention time; m/z, mass-to-charge ratio; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.
3.4 Analysis of receiver operating characteristics for potential biomarkers

After the operation, 11 out of 24 patients experienced a recurrence. To identify potential biomarkers that contribute to recurrence in STS patients, a univariate analysis was conducted. The pre-operative and post-operative plasma samples were classified into the recurrence and non-recurrence subgroups. Subsequently, metabolites showing significant differences between the recurrence and non-recurrence subgroups were selected. The levels of LPC O-18:0 and LPC O-16:2 in pre-operative plasma were significantly lower in patients who experienced the recurrence after the operation compared to those who did not, with \( p \)-values of 0.044 and 0.014, respectively (Fig. 5A and B).

To further assess the predictive potential of LPC O-18:0 and LPC O-16:0 for the recurrence of STSs, we conducted a univariate ROC analysis, which allowed us to obtain information about the sensitivity and specificity of these potential biomarkers. The AUC values for LPC O-18:0 and LPC O-16:2 were 0.748 and 0.797, respectively. The AUC values, with 95% confidence interval, are depicted in Fig. 5C and D. The corresponding sensitivity and specificity values are provided in Table 3.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>AUC value</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPC O-18:0</td>
<td>0.734 (0.510–0.951)</td>
<td>0.727</td>
<td>0.923</td>
</tr>
<tr>
<td>LPC O-16:2</td>
<td>0.797 (0.580–0.937)</td>
<td>0.727</td>
<td>0.615</td>
</tr>
</tbody>
</table>

The 95% confidence interval was calculated using 500 bootstrappings and is provided in parentheses.

LPC, lysophosphatidylcholine; AUC, area under the curve.

4. Discussion

In this study, we utilized UHPLC-QTOF/MS to perform untargeted metabolomics in the plasma of STS patients and found differences in the metabolic profiles between pre-operative and post-operative STS patients. The most significantly altered metabolic profiles in the post-operative plasma of STS patients were observed in phospholipids, particularly PC and LPC, which exhibited a considerable increase. PC is the most abundant phospholipid in mammalian cells, accounting for 40–50% of total cellular phospholipids [14]. It plays a crucial role in biological membranes and is involved in cell division, growth, and the synthesis of lipoproteins, which are responsible for lipid transport.

Tumors have a high ability to adapt their environment, allowing them to continue growing and survive even in unfavorable conditions. Malignant tumors exhibit a significant need for lipids to meet the energy
demands of processes related to cell growth, proliferation, invasion, and angiogenesis. This change in lipid metabolism is considered a hallmark of aggressive tumors [15, 16]. Ultimately, this reprogramming of lipid metabolism can also impact the lipid composition in the bloodstream. Numerous studies have been conducted on alterations in lipid metabolism in different types of solid tumors. For example, in the plasma of endometrial cancer patients, several PCs have been found to be significantly decreased compared to controls [17], and a decrease in plasma phospholipids has also been observed in lung cancer patients through untargeted metabolomics [18]. In CRC patients, a reduced plasma level of LPC has been reported, suggesting its potential as a biomarker for CRC [19]. The decreased levels of phospholipids in the plasma of patients with tumors may indicate that lipids are transferred from the bloodstream to the tumor due to the high lipid consumption by tumor cells. In addition, a study investigating gene alterations related to metabolic pathways across 32 different cancer types has suggested that the most pronounced genetic variations in sarcomas are associated with lipid metabolism [20]. The same study has also indicated that cancers with a higher frequency of genetic alterations in metabolic genes show shorter survival rates compared to those with fewer alterations. In our study, we found that the levels of PC and LPC in pre-operative STS patients were lower than those in post-operative patients. This suggests that STS cells present in the body consume excess amounts of lipids for their survival.

The high levels of plasma acyl-carnitines observed in pre-operative STS patients are presumed to be associated with increased energy supply caused by metabolic changes within tumor cells. Acyl-carnitines are conjugation of FAs with carnitine and serve as carriers transporting FAs to the mitochondrial matrix. This transportation facilitates fatty acid beta-oxidation (FAO) within cells, playing a crucial role in energy metabolism to sustain cell activity [21]. Acyl-carnitines have been implicated in various disease states, including insulin resistance [22], obesity [23], breast cancer [24], hepatocellular carcinoma [25], and nonalcoholic fatty liver disease (NAFLD) [26, 27]. In NAFLD patients, the serum levels of total acyl-carnitine increased gradually according to the progression of fibrosis, with even higher levels in hepatocellular carcinoma patients [27]. The high levels of acyl-carnitines in pre-operative samples in this study could indicate lipid metabolism reprogramming as well. These altered profiles suggest a potential shift in the utilization of lipid resources and may also reflect the interplay between the tumor cells and TME in energy metabolism.

FAO has been shown to be abnormally active in various tumors, which are closely related to the proliferation, metastasis, and resistance to chemotherapy of tumor cells [28]. In lipid metabolism, acyl-CoA synthetases (ACSL) convert long-chain fatty acids into fatty acyl-CoA esters. Subsequently, these esterified forms are further converted into acyl-carnitine by carnitine palmitoyltransferase 1 (CPT1). A recent study indicated that TGFβ1 treatment induces ACSL3 upregulation, promoting lipid metabolic reprogramming in CRC cells through the activation of the FAO pathway [29]. A British research team reported that ACSL3 and ACSL4 were highly expressed in STS cells. They found that the expression levels differed according to the subtype of STS, with the expression increasing in the order of liposarcoma, fibrosarcoma, leiomyosaroma, and rhabdomyosarcoma. Although the study was conducted with cell lines, we believe this difference could be associated with the chemosensitivity in vivo [30]. MPNST is
known as a relatively chemo-insensitive STS with poor prognosis. An American research team studied fatty acid synthase as a metabolic target in this STS and observed that MPNST cells accumulated lipid droplets, while the inhibition of FAO decreased oxygen consumption and reduced MPNST viability [31]. Among the three subtypes of CPT1, elevated levels of CPT1C mRNA have been reported in specific cancer types, particularly in STSs, with Ewing’s sarcoma and bone sarcoma following in rank [32]. This suggests a shift in lipid metabolic reprogramming towards the FAO pathway to meet energy demands during invasion of these malignancies.

There are some limitations in this study. The most disappointing factor was the small number of subjects due to the rarity of STS. However, we believe that this study is valuable considering the rarity of the disease. The second limitation is the timing of collecting post-operative samples. The intervals between the operation and post-operative sample collection ranged from 5 days to 45 days. The alterations of metabolic profiles might be attributable to the operation itself. It has been reported that lipid metabolism can be affected by circadian rhythm, and the feeding/fasting cycle affects the circadian system [33, 34]. However, only one patient had a sample acquired 5 days after the operation, and most samples were collected at least 7 days after the operation. We should have controlled the interval more homogenously, although 18 out of 24 patients offered their post-operative samples between 7 and 14 days after the surgeries.

We conducted survival analyses as well. The Kaplan-Meier curve showed distinct RFS lines based on the chemosensitivity and pre-operative chemotherapy. However, the number of patients was too small to yield statistically significant results. The observed \( p \)-values were around 0.15. In addition, we conducted \( t \)-tests and univariate ROC curve analyses to identify metabolic markers using the levels of 34 putative metabolites from pre-operative samples. Remarkably, 13Z-Docosenamide showed an AUC value exceeding 0.7 (0.707), indicating its potential significance as a metabolic marker. While the \( t \)-test did not indicate statistical significance, these findings suggest the need for further exploration and validation of its discriminatory power.

**Conclusion**

In conclusion, we characterize endogenous metabolite alterations in the pre-operative and post-operative plasma of STS patients. We observed a significant increase in the plasma levels of PC and LPC after the removal of the STS mass. This suggests that STS masses consume a high level of lipid as a source of energy or biomass. Our findings have potential to enhance the pathophysiological understanding of STS. Furthermore, a noteworthy discovery in this study was the identification of LPC O-18:0 and O-16:2 as potential biomarkers for predicting the prognosis of STS. However, further investigation is necessary to assess the clinical significance of LPC levels in larger cohorts. We recommend creating cohorts with groups based on the chemosensitivity of STS, taking into account the rarity of this disease and the results of previous studies, including this one.

**Abbreviations**
ACSL
acyl-CoA synthetase
AUC
area under the curve
CPT1
carnitine palmitoyltransferase 1
CRC
colorectal cancer
FAO
fatty acid beta-oxidation
HPLC
high-performance liquid chromatography
LPC
lysophosphatidylcholine
LPE
lysophosphatidylethanolamine
LPS
lysophosphatidylserine
MPNST
malignant peripheral nerve sheath tumor
MS/MS	
tandem mass
m/z
mass-to-charge ratio
NAFLD
nonalcoholic fatty liver disease
OPLS-DA
orthogonal projections to latent structures-discriminant analysis
PC
phosphatidylcholine
PCA
principal component analysis
PE
phosphatidylethanolamine
RFS
relapse-free survival
ROC
receiver operator characteristic
RT
retention time
Declarations

Availability of data and materials
The dataset generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval
This prospective study was approved by the Institutional Review Board (IRB) of Pusan National University Hospital, with the requirement for written consent (IRB 1805-028-067).

Competing interests
The authors declare no competing interests.

Funding
This work was supported by clinical research grant from Pusan National University Hospital in 2022.

Authors’ contributions
HJ Kim designed the study and collected the blood samples. SY Hwang always started centrifugation of every sample in 10 minutes each time HJ kim draw them from the patients and immediately kept them frozen. JI Kim enrolled and operated the patients. JH Lee and MR Gwon conducted the experiments and metabolomic analyses. HJ Kim, JH Lee, MR Gwon wrote the main manuscript text and prepared figures and tables. All authors reviewed the manuscript.

References


**Figures**

36 patients were enrolled

Surgery

Excluded
- Benign tumors according to histopathological findings ($n = 3$)
- Carcinoma according to histopathological findings ($n = 1$)
- No post-operative samples available ($n = 6$)
- No tumor observed during operation ($n = 2$)

24 patients were included in metabolomics study

**Figure 1**
Flowchart of the subject selection process.

Figure 2

The PCA score plot for pre-operative and post-operative STS samples.
Figure 3

The score and permutation test plot of the OPLS-DA. (A) OPLS-DA score plot. R2Y = 0.971, Q2 = 0.519. (B) Permutation test plot of the OPLS-DA model (n = 100).
Figure 4

The heatmap of 34 metabolites exhibiting significant alterations between pre-operative and post-operative STS patients.
Figure 5

Univariate analysis-based predictive potential of LPC O-18:0 and LPC O-16:2 in distinguishing between recurrence and non-recurrence STS patients at the pre-operative stage. (A, B) Box plots representing the levels of LPC O-18:0 and LPC O-16:2. (C, D) Univariate ROC analysis for LPC O-18:0 and LPC O-16:2, presenting the AUC and 95% confidence interval. *, \( p < 0.05 \); ns, not significant.