Identification of Potential Biomarkers for Ovarian Cancer by Urinary Metabolomic Profiling

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Supporting Information

ABSTRACT: To evaluate the application of urinary metabolomics on discovering potential biomarkers for epithelial ovarian cancer (EOC), urine samples from 40 preoperative EOC patients, 62 benign ovarian tumor (BOT) patients, and 54 healthy controls were collected and analyzed with ultraperformance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-QTOF/MS). Good separations were obtained for EOC vs BOT, EOC vs healthy controls analyzed by partial least-squares discriminant analysis, or principal component analysis. Twenty-two ascertained metabolomic biomarkers were found to be disturbed in several metabolic pathways among EOC patients, including nucleotide metabolism (pseudouridine, N4-acetylcytidine), histidine metabolism (L-histidine, imidazol-5-yl-pyruvate), tryptophan metabolism (3-indolelactic acid), and mucin metabolism (3′-sialyllactose and 3-sialyl-N-acetyllactosamine). In addition, the concentrations of some urinary metabolites of 18 postoperative EOC patients among the 40 EOC patients changed significantly compared with those of their preoperative condition, and four of them suggested recovery tendency toward normal level after surgical operation, including N4-acetylcytidine, pseudouridine, urate-3-ribonucleoside, and succinic acid. These metabolites would be highly postulated to be associated with EOC. In conclusion, our study demonstrated that urinary metabolomics analysis by UPLC-QTOF/MS, performed in a minimally noninvasive and convenient manner, possessed great potential in biomarker discovery for EOC.

KEYWORDS: metabolomics, epithelial ovarian cancer, urine, biomarker, mass spectrometry

INTRODUCTION

Epithelial ovarian cancer (EOC), the most common form of malignant ovarian tumor, is the leading cause of death among gynecological cancers.1 Almost 90% of EOC patients were diagnosed at an advanced stage (III/IV), with a poor 5-year survival rate of less than 30%.2 The cancer antigen 125 (CA125) and transvaginal ultrasound are two major techniques used to diagnose EOC currently; however, the identification of EOC was unsatisfied by them in clinical practice. The increased level of CA125 is not present in all EOC women, and it could also occur in women with other cancers (e.g., pancreatic, breast, and lung) as well as benign diseases (e.g., uterine fibroids and benign ovarian tumors (BOT)).3 The diagnostic accuracy of transvaginal ultrasound has been reported to be better than that of CA125, while transvaginal ultrasound is invasive and expensive, and its accuracy mainly depends on the subjective experience of the examiners.4

Metabolomics, the global quantitative assessment of endogenous small molecule metabolites within a biological system,5 has been successfully utilized in cancer biomarker discovery using specimens of biofluids or tissues,6 such as breast cancer,7 colon cancer,8 oral cancer,9 and prostate cancer.10 Recently, metabolomic analyses for EOC were conducted with mass spectrometry (MS) or nuclear magnetic resonance (NMR), which revealed significantly perturbed metabolic expression in EOC patients compared with healthy women.2,11−16 Among them, two studies concentrated on urinary metabolic signatures of EOC patients. Urinary metabolites are the downstream products of cellular processes, and urinary metabolomic analysis could provide complementary information to metabolomic analysis of serum and tissue. In addition, urinary test is noninvasive, inexpensive,
and convenient in clinical practice. All these advantages of urinary metabolomics make it applicable in biomarker discovery. Slupsky et al. found differences of urinary metabolic profiling between 50 female EOC and 72 female volunteers analyzed by NMR.

Woo et al. reported different metabolite patterns in urine between 9 patients with ovarian cancer and 22 normal controls analyzed by LC-MS. However, both studies employed healthy women as their controls and one of them was limited by small sample size. In addition, the results reported in the two studies were diverse, which would provide us evidence that different metabolites related to EOC would be detected from different research designs. Therefore, further research revealing differences in urinary metabolic profiling between EOC patients and other than normal controls is needed, which would provide more information for the complete elucidation of the underlying metabolic mechanisms of EOC.

In this study, we performed a urinary metabolomic analysis on ultraperformance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-QTOF/MS) to discriminate 40 preoperative EOC patients from 62 women with BOT or 54 healthy controls (HC) and tried to reveal the differential metabolites between these groups. In addition, we investigated the potential of urinary metabolomics in evaluating the metabolic changes between pre- and postoperation in 18 EOC patients.

## MATERIALS AND METHODS

### Urine Collection

Urine samples of 40 EOC patients preoperation and 18 patients postoperation on the seventh day, 62 BOT patients, and 54 healthy controls were collected from Department of Gynecology of Harbin Medical University Tumor Hospital (Harbin, China) between September 2009 and November 2010. All participants signed informed consent forms under local research ethics committee approval. Each EOC patient was diagnosed with different histopathological features and staged according to the International Federation of Gynecology and Obstetrics (FIGO) staging system for ovarian cancer: stage I, 7 patients; stage II, 5 patients; stage III, 20 patients; stage IV, 2 patients; undocumented, 6 patients. The demographic and clinical chemistry characteristics of enrolled subjects are shown in Table 1. All urine samples were collected in the morning before breakfast, centrifuged at 3000 rpm for 15 min to remove impurities, and then frozen immediately and stored at −80 °C until analysis.

### Sample Preparation and Pretreatment

Prior to sample preparation and UPLC-QTOF/MS analysis, all the urine samples were randomized along with 18 pooled quality control (QC) urine samples. The pooled QC samples were prepared by mixing equal amounts of urine samples from three EOC patients and three healthy controls. All urine samples were thawed in a 4 °C water bath for 20–30 min and then centrifuged at 14000g for 10 min. An aliquot of 150 μL of supernatant was transferred to a 2 mL centrifuge tube, and then 450 μL of water was added to the tube to make the dilution ratio of urine water be 1:3. The total 600 μL solutions were vortexed for 2 min, placed into the sampling vial, and stored at 4 °C pending UPLC-QTOF/MS analysis.

### UPLC-QTOF/MS Analysis of Urine Samples

A 2 μL aliquot of the pretreated sample was injected into a HSS T3 2.1 mm × 100 mm × 1.8 μm column (Waters, Milford, USA) held at 35 °C using an Acquity ultraperformance liquid chromatography system (Waters, Milford, USA). The UPLC mobile phase consisted of water with 0.1% formic acid (solution A), and acetonitrile with 0.1% formic acid (solution B). The column was eluted with a linear gradient of 2–20% B for 0–6 min, 20–35% B for 6–7 min, 35–70% B for 7–8 min, 70–98% B for 8–10 min, and kept 98% B for 10–11.5 min. The gradient was then changed to 70% B for 11.5–12 min, 20% B for 12–13 min, and finally to 2% B for 13–15 min. The flow rate was 0.35 mL/min, and all of the samples were maintained at 4 °C during the analysis.
Mass spectrometry was performed using a Waters Micromass Q-TOF (Waters, Manchester, U.K.) equipped with an electrospray ionization source operating in negative-ion mode (ESI−). The source temperature was set at 110 °C with a cone gas flow rate of 50 L/h. Meanwhile, the desolvation gas temperature was 300 °C with a desolvation gas flow rate of 650 L/h. The capillary voltage and the cone voltage were set to 2.8 kV and 35 V, respectively. Centroid data were collected from 50 to 1000 m/z with a scan time of 0.4 s, an interscan delay of 0.1 s, and a lock spray frequency of 10 s.

Data Preprocessing and Annotation

The raw UPLC-QTOF/MS ESI data were first transformed to NetCDF files by Databridge (Waters, Manchester, U.K.), and then the files were imported to the xcms package in the R platform for preprocessing, including nonlinear retention time (RT) alignment, matched filtration, peak detection, and peak matching.18 Full width at half-maximum (fwhm) was set to 10, and the retention time window was set to 10 (bw = 10), while the values of other parameters were default.19 The preprocessing results by xcms offered a three-dimensional matrix containing arbitrarily assigned peak indices of RT and mass-to-charge ratio (m/z) pairs, sample names, and ion-intensity information. The R package CAMERA was used for annotation of isotope peaks, adducts, and fragments in the processed peak data by xcms.20 Normalization to total peak intensities for each sample was done before statistical analysis.

Statistical Analysis

Both the unsupervised method (principal component analysis, PCA) and the supervised method (partial least-squares discriminant analysis, PLS-DA) were employed to reveal the global metabolic changes of EOC vs BOT, EOC vs HC, and pre-vs postoperative EOC patients using SIMCA-p 11.5 (Umetrics AB, Umea, Sweden), and the corresponding variable importance in the projection (VIP values) was calculated in the PLS-DA model as well.25 A validation plot was used to assess the validity of the PLS-DA model by comparing the goodness of fit (R2 and Q2) of the PLS-DA models with the goodness of fit of 100 Y-permutated models.26 Meanwhile, the nonparametric Kruskal–Wallis rank sum test was performed to determine the significance of each metabolite, and the relevant false discovery rates (FDR) based on the p-values were estimated in the context of multiple testing.27 A potential metabolic biomarker was selected when the value of its VIP was more than 1 and FDR was less than 0.05.

Statistical analysis was performed on the R platform,28 with the exception that PCA and PLS-DA were carried out on SIMCA-p.

RESULTS

The typical UPLC-QTOF/MS chromatograms are shown in Figure 1. The final data table contained 353 variables (chromatographic peaks).

Metabolic Profiles of EOC, BOT, and Healthy Controls

Although the PCA scores plots only showed a tendency of difference in metabolic profiling for EOC vs HC and EOC vs BOT (Figures S1 (A and B) in the Supporting Information), the PLS-DA score plots revealed a clear separation for EOC vs HC (Figure 2A) and EOC vs BOT (Figure 2C). The PLS-DA model for EOC vs HC contained four factors with the performance of R2X = 0.347, R2Ycum = 0.817, and Q2cum = 0.376. And the validation plot presented in Figure 2B strongly indicated the validity of the PLS-DA model, since the Q2 regression line in blue had a negative intercept and all permuted R2-values in green on the left were lower than the original point of the R2-value on the right. The PLS-DA model for EOC vs BOT contained four latent variables with the performance of R2X = 0.332, R2Ycum = 0.817, and Q2cum = 0.776, and Q2cum = 0.301, and the validation plot supported the validity of the PLS-DA model (Figure 2D). For employing metabolomic profiles to discriminate BOT patients from healthy controls, either PCA or PLS-DA showed overlaid results between

![Figure 1. Typical UPLC-QTOF/MS chromatograms of urine samples for a healthy woman, a benign ovarian tumor (BOT) patient, and an epithelial ovarian cancer (EOC) patient in the preoperative and postoperative situation.](image-url)
these two groups (Figures S1 (D) and S2 in the Supporting Information, respectively).

**Metabolic Changes between Pre- and Postoperative EOC Patients**

Both the PCA scores plot (Figure S1 (C) in the Supporting Information) and the PLS-DA scores plot (Figure 3A) demonstrated excellent separation between pre- and postoperative EOC patients (for PLS-DA: 1 component, with R2X = 0.258, R2Ycum = 0.787, Q2cum = 0.612). The validation plot supported the validity of this PLS-DA model (Figure 3B).

**Discovery and Identification of Metabolic Biomarkers**

A total of 42 urine metabolites were selected as potential biomarkers to classify EOC vs HC (34 metabolites) or EOC vs BOT (23 metabolites), and 13 of them were statistically significant.
between pre- and postoperative EOC patients (Table S1 in the Supporting Information). Among them, 22 metabolites were identified through MS/MS (tandem mass spectroscopy) experiments and online databases: 19 metabolites for EOC vs HC and 8 metabolites for EOC vs BOT (Table 2). The average %RSDs of these 22 metabolites were 83.7, 77.2, 74.8, and 93.6 for the preoperative EOC patients, BOT women, health controls, and postoperative EOC patients, respectively, which suggested a consistent intragroup variation among the four groups. The %RSD of these 22 metabolites were 83.7, 77.2, 74.8, and 93.6 for the preoperative EOC patients, BOT women, health controls, and postoperative EOC patients, respectively, which suggested a consistent intragroup variation among the four groups.

Detailed information about metabolites identification was shown in Table S2 and Figure S3 in the Supporting Information, and five of them, including t-histidine, N-acetylglutamine, succinic acid, taurine, and 3-sialyl-N-acetyllactosamine were additionally verified by external reference standards. The MS/MS spectra of these five biomarkers in our urine sample matched well with those of the reference standards, respectively (the identification of 3-sialyl-N-acetyllactosamine was shown in Figure S5 in the Supporting Information). In addition, the concentrations of nine metabolites (imidazol-5-yl-pyruvate, N-acetyllactosamine, pseudouridine, succinic acid, (S)-reticuline, N-acetylneuraminic acid, 3-sialyl-N-acetyllactosamine, β-nicotinamide mononucleotide, and 3'-sialylactose) were found to be significantly different in EOC patients who were in different FIGO stages (Figure S6 in the Supporting Information), and the classification between EOC patients in early stages (I and II) and those in advanced stages (III and IV) was detected in our study, with the value of the area of the receiver operating characteristic curve (AUC) obtained by these nine differentiated metabolites of 0.828. Furthermore, the possible matching pathways for these 22 identified biomarkers were searched in online pathway databases of KEGG (http://www.genome.jp/kegg/) and SMPDB (http://www.smpdb.ca/), which mainly involved nucleotide metabolism (pseudouridine, N4-acetylcytidine), histidine metabolism (t-histidine, imidazol-5-yl-pyruvate), tryptophan metabolism (3-indolelactic acid), and mucin metabolism (3'-sialyllactose and 3-sialyl-N-acetyllactosamine).

### DISCUSSION

Our study suggested that the urinary metabolomics analyzed by UPLC-QTOF/MS could be used to discriminate EOC from BOT/HC, and the 22 ascertained urinary metabolic biomarkers were highly possible to be associated with EOC. In addition, the metabolomic changes were found between pre- and postoperative EOC patients, and some metabolites showed recovery tendency toward the normal level.

In line with previous results of metabolic analysis from serum, urine, or tissue samples,39,40 our findings demonstrated good separations between EOC and BOT/HC as well. Meanwhile, we found that the ascertained urinary biomarkers could improve the diagnostic accuracy of CA125, the most commonly used plasma biomarker in the clinic currently,3,4,30 especially for differentiating between EOC and BOT (the values of AUC of combination of eight metabolites and CA125: 0.870; 8 metabolites: 0.728). Our study further confirmed that urinary metabolomics could be used to differentiate EOC patients from healthy controls or BOT patients through urine samples analyzed by UPLC-QTOF/MS.

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**Table 2. Urinary Metabolic Biomarkers for EOC vs Healthy Controls, EOC vs BOT, or Pre- vs Postoperative EOC Patients**

<table>
<thead>
<tr>
<th>marker</th>
<th>identity</th>
<th>EOC vs healthy control</th>
<th>EOC vs BOT</th>
<th>pre- vs postoperative</th>
</tr>
</thead>
<tbody>
<tr>
<td>v80</td>
<td>imidazol-5-yl-pyruvate</td>
<td>1.26</td>
<td>0.00727</td>
<td>1.3</td>
</tr>
<tr>
<td>v26</td>
<td>t-histidine</td>
<td>1.58</td>
<td>0.00394</td>
<td>−0.89</td>
</tr>
<tr>
<td>v56</td>
<td>N-acetyllactosamine 9-phosphate</td>
<td>1.55</td>
<td>0.00101</td>
<td>1.06</td>
</tr>
<tr>
<td>v160</td>
<td>N4-acetylcystidine</td>
<td>1.24</td>
<td>0.0116</td>
<td>0.37</td>
</tr>
<tr>
<td>v159</td>
<td>urate-3-ribonucleoside</td>
<td>1.57</td>
<td>0.00019</td>
<td>1.09</td>
</tr>
<tr>
<td>v78</td>
<td>pseudouridine</td>
<td>1.31</td>
<td>0.00727</td>
<td>9.09</td>
</tr>
<tr>
<td>v117</td>
<td>3-dehydroquinic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>v272</td>
<td>3-indolelactic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>v73</td>
<td>Na-acetyl-l-glutamine</td>
<td>1.56</td>
<td>0.00852</td>
<td>−1.16</td>
</tr>
<tr>
<td>v63</td>
<td>succinic acid</td>
<td>1.31</td>
<td>0.00912</td>
<td>0.22</td>
</tr>
<tr>
<td>v345</td>
<td>LPA(P:16:0/0:0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>v197</td>
<td>(S)-reticuline</td>
<td>1.46</td>
<td>0.00217</td>
<td>2.12</td>
</tr>
<tr>
<td>v67</td>
<td>taurine</td>
<td>1</td>
<td>0.00135</td>
<td>0.87</td>
</tr>
<tr>
<td>v342</td>
<td>prasterone sulfate</td>
<td>1.33</td>
<td>0.00316</td>
<td>−16.36</td>
</tr>
<tr>
<td>v45</td>
<td>N-acetyllactosaminic acid</td>
<td>1.55</td>
<td>0.000196</td>
<td>2.52</td>
</tr>
<tr>
<td>v57</td>
<td>3-sialyl-N-acetyllactosamine</td>
<td>1.62</td>
<td>9.03 × 10^{-5}</td>
<td>1.97</td>
</tr>
<tr>
<td>v74</td>
<td>β-nicotinamide mononucleotide</td>
<td>1.08</td>
<td>0.00466</td>
<td>0.44</td>
</tr>
<tr>
<td>v59</td>
<td>prollylhdroxyproline</td>
<td>1.94</td>
<td>2.38 × 10^{-6}</td>
<td>4.17</td>
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<tr>
<td>v110</td>
<td>selenocystathionine</td>
<td>1.24</td>
<td>0.0121</td>
<td>0.63</td>
</tr>
<tr>
<td>v44</td>
<td>3'-sialyllactose</td>
<td>1.52</td>
<td>4.3 × 10^{-5}</td>
<td>2.36</td>
</tr>
<tr>
<td>v49</td>
<td>N-acetylgalactosamine 4-sulfate</td>
<td>1.23</td>
<td>0.000981</td>
<td>1.46</td>
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<tr>
<td>v348</td>
<td>glycodeoxycholate</td>
<td>1.18</td>
<td>0.0121</td>
<td>−2.15</td>
</tr>
</tbody>
</table>

**Abbreviations:** EOC, epithelial ovarian cancer; BOT, benign ovarian tumor. **Variable importance in the projection (VIP)** was obtained from PLS-DA with a threshold of 1.0. **The p-value** was calculated from the nonparametric Kruskal–Wallis rank sum test. **Fold change (FC)** was calculated from the arithmetic mean values of each group; FC with a positive value indicates that the concentration of a certain metabolite is up-regulated in preoperative EOC compared to healthy controls, BOT, or postoperative EOC. **Relative standard deviation (%RSD)** in the levels of marker metabolites in the 18 quality control samples.
Among the 22 ascertained metabolic biomarkers, five of them (3-indolelactic acid, N-acetylglutamine, succinic acid, taurine, prasterone sulfate) have been previously reported,15,31–35 in which significantly disturbed concentrations of these five biomarkers in EOC patients compared to normal controls or BOT patients were not completely consistent with our results. However, only one of these studies employed urine metabolomics analyzed by NMR in which succinic acid was reported, and others employed metabolic profiles from serum or tissue samples. In addition, the eight urinary metabolic biomarkers identified to discriminate EOC from BOT in the current study were quite different from the six biomarkers in our recent study of differentiating 80 EOC from 90 BOT women by plasma metabolomics,29 which suggested that urinary metabolic analysis might provide complementary information to plasma metabolomic analysis for the complete elucidation of the underlying metabolic mechanisms of EOC. Notably, the possible related pathways of the ascertained urinary metabolic biomarkers were further investigated in the current study.

Urinary concentrations of some nucleosides, N4-acetylcytidine, pseudouridine, and urate-3-ribonucleoside, were increased in EOC patients compared to healthy controls. These elevated nucleosides should result primarily from the degradation of a large amount of synthesized RNA,36,37 as most of the modified nucleosides coming from the degradation of tRNA were excreted in urine.38 Our results suggested enhanced nucleotide metabolism in EOC patients. Meanwhile, the urinary L-histidine in EOC patients was down-regulated relative to that in healthy controls, which was consistent with the results of urinary metabolomic study on colorectal cancer.33 Imidazol-5-yl-pyruvate, one metabolic product of L-histidine, was up-regulated relative to that in controls, which was consistent with the results of urinary metabolic study on colorectal cancer.33 Imidazol-5-yl-pyruvate, one metabolic product of L-histidine, was up-regulated relative to that in healthy controls, which further indicated the disturbance of the L-histidine metabolic pathway in EOC patients. It has been suggested that the biosynthesis of histidine is inherently linked to the pathways of nucleotide formation through 5-phosphoribosyl 1-pyrophosphate (PRPP).39 In addition, N-acetylglutamine, a liquid-stable source of glutamine,40,41 was found down-regulated in EOC patients, and it is clear that glutamine is engaged in the PRPP-mediated nucleotide metabolism.36 The abnormal L-histidine metabolism and nucleotide metabolism found in our study might indicate the turbulence of the PRPP-mediated histidine-nucleotide superpathway in EOC patients (shown in Figure 4).

Figure 4. Disturbance of the PRPP-medicated histidine-nucleotide superpathway in ovarian cancer patients.

Elevated urinary concentration of 3-indolelactic acid was observed in EOC patients relative to BOT patients, which suggested the disturbed metabolic pathway of tryptophan in EOC patients, since 3-indolelactic acid is a direct degradation product of L-tryptophan.32 Two oligosaccharides, 3′-sialyllactose and 3-sialyl-N-acetyllactosamine, were found to be up-regulated in EOC patients as compared with those in both BOT patients and healthy controls. Oligosaccharides are known to make up a large fraction of the mass of mucins,43 and mucins have been suggested to be associated with the pathogenesis of cancer adenocarcinomas.44 It is well-known that elevated serum mucins of CA125 (MUC16) and MUC1 can be found in EOC patients.35 The high urinary concentrations of 3′-sialyllactose and 3-sialyl-N-acetyllactosamine in our study would provide complementary information to serum test of mucins.

Urinary concentration of succinic acid in EOC patients was observed to be higher than that in healthy controls. Studies have suggested that accumulated succinic acid can contribute to prolyl hydroxylase (PHD) inhibition and HIF-α hydroxylation, which in turn induces expression of genes that facilitates angiogenesis, metastasis, and metabolism of aggressive tumors.45 β-Nicotinamide mononucleotide, which is involved in the biosynthesis of nicotinamide adenine dinucleotide (NAD+),46 was found elevated in the urine of EOC patients compared to their healthy controls. NAD+, carrying electrons from one reaction to another, plays a critical role in energy production.47 Therefore, the increase of β-nicotinamide mononucleotide, resulting in the elevation of NAD+, would meet large amounts of energy consumption of cancer cells growth. In addition, taurine was significantly up-regulated in EOC patients compared to that in healthy controls, which might indicate the disturbance of taurine metabolism but need to be validated in further studies.

Among the differential metabolites of EOC patients before and after surgical operation, four of them (N4-acetylcystidyne, pseudouridine, urate-3-ribonucleoside, and succinic acid) showed recovery tendency toward normal level, while others (N-acetylglutamine, 3-indolelactic acid, 3′-sialyllactose, and 3-sialyl-N-acetyllactosamine) showed completely opposite tendency to recovery. Our results demonstrated metabolic changes occurred between pre- and postoperative EOC patients, but these changes could be attributed to nutritional supplementation, increased oxidative stress, or surgical curative effect.33 The metabolites showing recovery tendency, which are most likely due to the surgical curative effect, would be most highly postulated to be associated with EOC. However, these metabolic changes were based on small number of EOC patients and deserve further investigation in large cohorts in the future.

Although age and menopausal status were imbalanced between EOC and controls in this study, the potential 22 biomarkers were still statistically significant after being adjusted for age and menopause in the logistic regression models (data not shown). In addition, the classification between EOC patients in early stages (I and II) and those in advanced stages (III and IV) was detected in our study; however, the classification of EOC patients who were in different FIGO stages (I, II, III, and IV) was not detected, which might be limited by the relatively small sample size. Further investigations consisting of larger sample sizes are needed to validate our findings.

The findings in our study showed the valuable potential of urinary metabolomics to discover biomarkers in discriminating EOC patients from BOT patients or healthy controls. The urinary biomarkers, up- or down-regulated in EOC patients compared to those in BOT patients or healthy controls, would suggest the disturbed metabolic pathways in EOC patients, including metabolisms of nucleotide, histidine, tryptophan, and mucin. Our results also indicated that the urinary metabolomics
could provide evidence on ascertaining the metabolites which may relate to EOC, when they show recovery tendency after surgical operation. In conclusion, the urinary metabolomics possessed great potential in biomarker discovery and investigation of the underlying metabolic mechanisms for EOC.

■ ASSOCIATED CONTENT

2 Supporting Information

Strategy of metabolite identification; list of 42 selected biomarkers (Table S1) and detailed identification information of 22 ascertained metabolites (Table S2); PCA score plots for EOC vs healthy controls (Figure S1 (A)), EOC vs BOT (Figure S1 (B)), BOT vs healthy controls (Figure S1 (C)), and BOT vs healthy controls (Figure S1 (D)); PLS-DA score plot and validation plot for BOT vs healthy controls (Figure S2); typical process of identifying potential biomarkers (Figure S3 and S5); PCA scores plot comprising EOC, postoperative EOC, BOT, and validation plot for BOT vs healthy controls (Figure S2); typical process of identifying potential biomarkers (Figure S3 and S5); PCA scores plot comprising EOC, postoperative EOC, BOT, healthy controls, and quality controls (Figure S4); and metabolite profiles of potential biomarkers of epithelial ovarian cancer (EOC) patients with different FIGO stages (Figure S6). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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