UPLC-MS based urine untargeted metabolomic analyses to differentiate bladder cancer from renal cell carcinoma

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Research article

Keywords: Metabolomics, UPLC-MS, Biomarkers, Bladder cancer, Renal cell carcinoma

Posted Date: July 31st, 2019

DOI: https://doi.org/10.21203/rs.2.12247/v1

Version of Record: A version of this preprint was published on December 5th, 2019. See the published version at https://doi.org/10.1186/s12885-019-6354-1.

Abstract

Purpose To discover biomarker panels that could distinguish cancers(BC & RCC) from healthy controls(HCs) and bladder cancers(BC) from renal cell carcinoma(RCC), regardless of whether with hematuria. Methods Totallys, 403 participants were enrolled in our study, with 146 BC patients(77 without hematuria and 69 with hematuria), 115 RCC patients (94 without hematuria and 21 with hematuria) and 142 gender- and age- matched HCs. Their midstream urine samples were collected and analysed by performing UPLC-MS. The statistical methods and pathway analyses were applied to discover potential biomarker panels and altered metabolic pathways. Results The panel of α-CEHC, β-cortolone, deoxyinosine, flunisolide, 11b,17a,21-trihydroxypreg-nenolone and glycerol tripropanoate could distinguish the cancers from the HCs(the AUC was 0.950) and the external validation also displayed a good predictive ability (the AUC was 0.867). The panel consisiting of 4-ethoxymethylphenol, prostaglandin F2b, thromboxane B3, hydroxybutyrylcarnitine, 3-hydroxyphloretin and N'-formylkynurenine could differentiate BC from RCC without hematuria. The AUC was 0.829 in the discovering group and 0.76 in the external validation. The metabolite panel comprising 1-hydroxy-2-oxopropyl tetrahydropterin, 1-acetoxy-2-hydroxy-16-heptadecyn-4-one, 1,2-dehydrosalsolinol and L-tyrosine could significantly discriminate BC from RCC with hematuria(AUC was 0.913). Pathway analyses revealed there existed alterd lipid and purine metabolism between cancers and HCs, together with disordered amino acid and purine metablism between BC and RCC with hematuria. Conclusions The UPLC-MS urine metabolomic analyses could not only differentiate the cancers from HCs but also discriminate the BC from RCC. Besides, pathway analyses could demonstrate the deeper metabolic mechanism of BC and RCC.

Background

Genitourinary cancers include cancers of bladder, kidney, prostate, and testicular. Other genitourinary cancers, such as adrenal, penile, ureteral, and urethral cancer, are relatively rare. Among them, bladder cancer (BC) and renal carcinoma (RC) are, respectively, the first two commonly occurring genitourinary cancers in China, and the second and third most common genitourinary cancers in Europe and North America[1]. Currently, cystoscopy and cytology are the standards for initial diagnosis and recurrence of BC, but limitations exist. Cystoscopy may fail to visualize certain areas within the bladder and may also fail to detect all cancers, particularly some cases of carcinoma in situ [2]. Cytology has high specificity and selectivity for high grade tumors but fails to provide strong predictive value for low grade tumors [3]. With regard to RC, computed tomography, magnetic resonance imaging, and positron emission tomography are commonly used imaging diagnostic techniques[4]. However, even with combined use of above three techniques, it remains difficult to detect early tumors because of their small size[5]. Therefore, developing novel and convenient techniques for detection of BC and RC with high sensitivity and specificity is urgently required.

Recently, metabonomic analyses are found to have increasing researches in the diagnosis of a number of pathologies[6–8] and elucidation of clinical pathogenesis of various diseases[9,10]. Metabonomics has

several major advantages, which include the ready availability and relative ease of analysis of biofluids, such as urine and plasma, as well as the derived metabolite profiles are sensitive to both environmental and genomic influences affecting the pathogenesis and progression of disease[11].

Urine is a particularly suited biofluid concerning bladder cancer and renal carcinoma, due to its intimate contact with the urinary system[12]. Therefore, urine metabolomics is a promising approach for BC and RC detection and marker discovery.

There are several researches on urine metabolomics analysis for bladder cancer biomarker discovery. In 2011, Huang et al. [13]found that a combined urinary biomarker composed of carnitine C9:1 and an unknown metabolite had high sensitivity and specificity in discriminating 27 BC patients from 32 healthy controls (HCs); In 2014, Jin et al. [14] applied LC/MS to profile urinary metabolites of 138 patients with BC and 121 control subjects. The study identified 12 putative markers that were involved in glycolysis and betaoxidation. Wittmann et al. [15] applied LC/MS to profile urinary metabolites of 66 BC and 266 non-BC subjects. They suggested that metabolites related to lipid metabolism may be potential BCa markers; In 2017, Zhou et al.[16] applied a urinary pseudotargeted method based on GC-MS for a BC metabolomics study. The study identified a combinatorial biomarker panel consisting of four differential metabolites was defined for BC and early-stage BC diagnosis.

Metabolomics has also been widely applied to research on renal carcinoma biomarker discovery. In 2011, Kim et al[17] used UHLC/MS and GC/MS platform to perform urine metabolomics against 29 kidney cancer patients and 33 control patients. The study identified 13 significant differentially expressed metabolites; In 2016, Monteiro et al.[12] analyzed urine metabolome of 42 RCC patients and 49 controls using NMR. A 32-metabolite/resonance signature, including 2-KG, N-methyl–2-pyridone–5-carboxamide (2-Py), bile acids, galactose, hypoxanthine, isoleucine, pyruvate, succinate etc., was successfully distinguishing RCC patients from controls in principal component analysis; In 2017, Falegan et al.[18] applied NMR and GC/MS platform to perform urine and serum metabolomics against 40 RCC patients and 13 benign patients. The results showed alterations in levels of glycolytic and tricarboxylic acid (TCA) cycle intermediates were detected in RCC relative to benign masses.

These studies have unveiled potential disease biomarkers in urine. However, most metabolic markers were discovered based on small pilot studies. The limited study cohort or lack of effective validation restricts further clinic applications of these biomarkers[19]. Moreover, to our knowledge, few studies have addressed the occurrence of false-positives with the approaches, e.g. the diagnosis of certain type of genitourinary cancer in patients with other genitourinary cancer or urologic disorder which present similar clinical symptoms[5]. For example, patients with BC usually present with hematuria, but hematuria can also be present in patients with other genitourinary cancers, hematuria can be a serious confounding variable. Therefore in our study, a urine metabolomics approach using Ultra-performance LC-MS (UPLC-MS) was carried out. A total of 403 urine samples, including 146 patients with BC (77 without hematuria and 69 with hematuria), 115 patients with RC (94 without hematuria and 21 with hematuria) and 142

gender- and age- matched healthy control were assessed. Multivariate statistical analysis and biomarker analysis were used to discover and externally validate the biomarker pannel. Previous studies have reported that the hematuria may greatly affect the outcomes of metabolic analyses. Therefore, BC patients without and with hematuria was distinguished from RC patients by biomarker panel from urine metabolomics that may used for differential diagnosis of BC and RC.

Methods

Sample Collection

This study was approved by the Institutional Review Board of the Institute of Basic Medical Sciences and Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, and all human subjects provided informed consent before participating in this study. Both the urine samples of patients and healthy controls were collected from Peking Union Medical College Hospital. Midstream urine was collected in the morning at 07:00 a.m –09:00 a.m after an overnight fasting to eliminate the disturbance of diet. Then all samples were immediately stored in –80 °C refrigerator and thawed on ice before analysis. A total of 403 urine samples, including bladder cancer (BC, n = 146), renal cell carcinoma (RCC, n = 115) and healthy controls(HCs, n = 142) were assessed.

Sample Preparation

For urine sample preparation, acetonitrile (200 ul) was added into each urine sample (200 ul), then the mixture was vortexed for 30 s and centrifuged at 14,000 × g for 10 min. The supernatant was dried under vacuum and then reconstituted with 200 µl of 2% acetonitrile. Urinary metabolites were further separated from larger molecules using 10 kDa molecular weight cut-off ultracentrifugation filters (Millipore Amicon Ultra, MA) before transferred to the autosamplers. The quality control (QC) sample was a pooled sample prepared by mixing aliquots of two hundred representative samples across different groups to be analyzed and therefore globally representative of the whole sample set. The QC samples were injected every ten samples throughout the analytical run to provide a set of data from which method stability and repeatability can be assessed.

UPLC-MS Analysis

Ultra-performance LC-MS analyses of urine samples were conducted using a Waters ACQUITY H-class LC system coupled with a LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific, MA. USA). Urinary metabolites were separated with a 17 min gradient on a Waters HSS C18 column (3.0× 100 mm, 1.7µm) at a flow rate of 0.5 ml/min. Mobile phase A was 0.1% formic acid in H_2O and mobile phase B was acetonitrile. The gradient was set as follows: 0–1 min, 2% solvent B; 1–3min, 2–15% solvent B; 3–6 min, 15–50% solvent B; 6–9 min, 50–95% solvent B; 9–9.1 min, 95–100% solvent B; 9.1–12 min, 100% solvent B; 12–12.1 min, 100–2% solvent B; 12–17 min, 2% solvent B. The column temperature was set as

50°C. Full MS acquisition scanned from 100 to 1000 m/z at a resolution of 60 K. Automatic gain control (AGC) target was 1×10^6 and maximum injection time (IT) was 100 ms. Subsequently differential metabolites identification was performed by UPLC targeted-MS/MS analyses of QC sample. It acquired at a resolution of 15 K with AGC target of 5×10^5, maximum IT of 50 ms, and isolation window of 3 m/z. Collision energy was optimized as 20, 40, 60 for each target with higher-energy collisional dissociation (HCD) fragmentation.

Data processing

Raw data files were processed by the Progenesis QI (Waters, Milford, MA, USA) software based on the published identification strategy [20,21]. The detailed workflow for QI data processing and metabolites identification was given in supplementary materials. Further data pre-processing including missing value estimation, Log transformation and Pareto scaling were carried out to make features more comparable using MetaAnalyst 4.0 (http://www.metaboanalyst.ca). Variables missed in 50% samples, were removed from further statistical analysis. Non-parametric tests (Wilcoxon rank-sum test) was used to evaluate the significance of variables. And adjusted P-value (FDR) cutoff was set as 0.05. Pattern recognition analysis (principal component analysis, PCA; orthogonal partial least squares discriminant analysis, OPLS-DA) was carried out using SIMCA 14.0 (Umetrics, Sweden) software. The selected differential variables must meet the following three conditions: 1) adjusted P-value <0.05; 2) Fold change between two groups >1.5; 3) VIP value obtained from OPLS-DA was above 1. Exploratory ROC analysis and external biomarker validation were carried out using “Biomarker discovery” module in MetaAnalyst 4.0 platform to evaluate the prediction results.

Quality Control

A strict quality control assessment is of great significance for the metabolomic analysis because some other factors like the sample collection, preparation or even the analytic procedures may also tremendously affect the outcomes. To eliminate the technical errors involved in our study, the samples were randomly distributed in the discovery or external validation group and our QC samples was also analysed to assess the stability. The injected QC samples in our study showed only a small variation ranging within 2 SD(Supplement Fig. 1A), conforming the stability and reproductivity of our data, as the tight clustering further demonstrated(Supplement Fig. 1B). The above analysis indicated that the analytic difference may arise from the internal metabolic variation within the samples rather than from the technical bias.

Results

Subjects

The workflow of this study was shown in Fig. 1. A total of 403 participants were enrolled in our study, with 146 BC patients(77 without hematuria and 69 with hematuria), 115 RC patients (94 without hematuria and 21 with hematuria) and 142 gender- and age- matched healthy controls. The baseline clinical information of all enrolled subjects is showed in Table 1. All the pathological diagnoses of the BC and RCC patients were confirmed after operation by more than two professional pathologists in our hospital. Since the control samples enrolled didn't have hematuria, the cancers (including BC&RCC) sampes without hematuria were explored to discover cancer biomarkers. First, a pilot differential analysis on urine metabolomics was performed to discriminate cancer patients from health subjects. Cancer biomarkers were discovered based on metabolic profiling analysis of 98 age- and gender- matched health subjects, 53 BC patients and 64 RCC patients. The potential biomarkers were further externally validated using an independent batch of cancer (24 BC patients and 30 RCC patients) and 44 control samples. Besides, a pilot differential analysis was performed to discriminate urinary metabolic profiling between BC and RCC. And the potential biomarkers were further externally validated using an independent batch of 24 BC patients and 30 RCC patients. Furthermore, to find promising biomarker panel that coud distinguish BC and RC with hematuria, the samples of 21 RCC patients and 69 BC patients with hematuria were diffenrentially analysed.

Untargeted metabolomics could distinguish Cancers(BC & RCC) from Healthy Controls

To find the biomarkers between the Cancers (BC & RCC) and healthy controls, an unsupervised PCA analysis was used to find the metabolic profiling differences. The results were shown in Supplement Fig. 2A. The score plot showed an significant difference between the two groups. Furthermore, in order to better show the difference between Cancer and Control groups, a supervised OPLS-DA model was lauched(Fig. 2A). Based on the value of important plot (VIP) value(VIP>1), a total of 37 statistically differential metabolic molecules were selected(Supplement Table 1a). According to Supplement Table 1a, a heatmap was launched to discover the metabolic disturbance(Fig. 2B), from which we could easily draw the conclusion that compared with the healthy controls, the lipid metabolism pathway was upregulated while the purine metabolism and acetaminophen metabolism were downregulated in Cancer group. To further explore the separating capacity of each metabolite, an ROC curve was applied to each molecure and the results were presented in Supplement Table 1b. As was depicted in the table, 8 metabolites show a good distingushing ability with the AUC above 0.8, along with 22 metabolites above 0.7. Furthermore, a mutlivariant ROC curve-based explorary analysis (http://www.metaboanalyst.ca/

faces/up-load/RocUpload View.xhtml) was used to discover the panel with the best predictive ability. As a result, a panel containing α-CEHC, β-cortolone, deoxyinosine, flunisolide, 11b,17a,21-trihydroxypregnenolone and glycerol tripropanoate was picked out. In our testing data, the AUC was 0.95 and 0.933 for 10-fold cross-validation(Fig. 2C). Our external vadidation data was used to test the predictive ability of the panel and the AUC was 0.867(Fig. 2D).

Untargeted metabolomics could distinguish BC from RCC without hematuria

To detect the differential metabolites between the BC and RC groups, the PCA was applied and the results were showed in Supplement Fig. 2B. The picture suggested an significantly differential ability. Then, a supervised OPLS-DA model was launched(Fig. 3A) and we selected a sum of 32 metabolites with the cutoff VIP value of 1(Supplement Table 2a). The ROC curve was used to evaluate the predictive precision later. Among the differential molecures, 3 metabolites showed potential diagnostic ability with an AUC above 0.7 and 26 metabolites with an AUC above 0.6(Supplement Table 2b). The mutlivariant ROC curve-based explorary analysis revealed that a metabolite panel including 4-ethoxymethylphenol, prostaglandin F2b, thromboxane B3, hydroxybutyrylcarnitine, 3-hydroxyphloretin and N'-formylkynurenine possessed the best predictive ability. The AUC under the discovering data was 0.829 and 0.784 for 10-fold cross-validation(Fig. 3B). Besides, the AUC of external vadilation was 0.76(Fig. 3C). The panel showed a good ability to distinguish 16 BC from 24 BC patients correctly and the rate was 24/30 for the RCC (Fig. 3D).

Untargeted metabolomics could distinguish BC from RCC with hematuria

Similarly, a PCA analysis was first applied to explore the difference between the BC and RC patients with hematuria and the results were shown in Supplement Fig. 2C. From the picture, we could clearly observe that there was an apparent sepration between the two subgroups. Then, the OPLS-DA model was structured (Fig. 4A). Based the VIP of OPLS-DA(VIP>1), 59 metabolic molecures in total were identified as significant differential metabolites between the two groups(Supplement Table 3a). From the metabolites, it's not diffcult to conclude that the metabolism concerning the nitrogen metabolism, D-glutamine and D-glutamate metabolism, purine metabolism, aspartate and glutamate metabolism were significantly altered between the two groups. Pathway power analysis revealed that the distinguishing metabolism could aid in the seperation(Fig. 4B). According to the ROC curve, 3 metabolites show good performance in seperating the BC from the RC groups with an AUC above 0.8 and other 33 metabolites showed AUC above 0.7(Supplement Table 3b). Further analysis indicated that a panel made up of 1-hydroxy–2-oxopropyl tetrahydropterin, 1-acetoxy–2-hydroxy–16-heptadecyn–4-one, 1,2-dehydrosalsolinol and L-tyrosine exibitated the best capacity to distinguish the independent subgroups. The AUC of the panel is 0.913 for the discovery group and 0.870 for 10-fold cross-validation(Fig. 4C).

Discussion

Through the high-throughput measurement of endogenous metabolites, metabolomics has showed enormous prospects in discovering diagnostic cancer biomarkers in the field of renal cell carcinoma and bladder cancer. However, to the best of our knowledge, although many cancer markers have been found in bladder cancer, most researches only focused on the differentiation between cancers and healthy subjects, thus ignoring the discrimination within the malignant tumors. As we know, our study is the first

one that explored the differential metabolites between BC and RCC, regardless of with or without hematuria. As a result, by comparing the BC, RCC and HCs, we found that: i) a panel made up of α-CEHC, β-cortolone, deoxyinosine, flunisolide, 11b,17a,21-trihydroxypreg-nenolone and glycerol tripropanoate could well distinguish the cancers (BC &RCC) from the healthy controls, which may provide significant information about the dysregulated metabolic pathways of malignant urinary tumors. ii) a panel consisiting of 4-ethoxymethylphenol, prostaglandin F2b, thromboxane B3, hydroxybutyrylcarnitine, 3-hydroxyphloretin and N'-formylkynurenine show a good ability to differentiate BC from RCC without hematuria. iii) Since previous studies have already indicated that hematuria may statistically affect the analytic outcomes of metabolomics, we also perform an exclusive experiment to certify the biomarker panel. As the result suggested, a panel comprising 1-hydroxy–2-oxopropyl tetrahydropterin, 1-acetoxy–2-hydroxy–16-heptadecyn–4-one, 1,2-dehydrosalsolinol and L-tyrosine could significantly discriminate BC from RCC among patients with hematuria.

The clustering heatmap between Cancers and Healthy controls suggested that the lipid metabolism was upregulated in Cancers, which was in accordance with the classical Warburg effect, demonstrating that cancer cells prefer to use glycolysis rather than aerobic oxidation even in the presence of oxygen[22]. As we know, the dysregulated lipid and phospholipid metabolism showed great significance in cel motality, cell invision and tumor metastasis, which may produce enormous tumor biomarkers[23]. In previous studies, the disturbance of lipid metabolism have been reported in various researches, including BC and RCC. By analysing the global lipidomic profiles of 165 bladder-derived tissues, Piyarathna etc. found that compared with benign tissues, the urothelial cancer of the bladder had higher levels of phospholipids and fatty acids, and reduced levels of triglycerides, which suggested that the reduced triglycerides may be used for producing energy, while the changed phospholipid may play an active role in membrane structure or signal transduction[24]. Bying performing comparative UPLC-MS of two isogenic human T24 bladder cancer cell lines, Young Lee et al. discovered that there was an statistically distinguished lipid species between cisplatin-sensitive and cisplatin-resistant cancer cells, suggesting that lipid-targeted new drug may improve the prognosis of cisplatin-resistant patients[25]. As for the RCC, an article reported that a lot of fatty acids were downregulated in nonmetastatic RCC tissue as a result of overly-active fatty acid oxidation. Besides, they also discovered that in metastatic RCC, the lipid metabolism was upregulated obviously, which may be related to the tumor progression[26]. In some other researches, metabolites of carnitine metabolism, which are responsible for the transportation of fatty acid into the mitochondria, have been found to be increased in high-grade tumor tissues, blood serum or urine[27–29], which may be a consequence of improved fatty acid β-oxidation to sustain higher rates of cell division and growth.

2,5,7,8-tetramethyl–2(2'-carboxyethyl)–6-hydroxychroman(also known as α-CEHC), is an end-product of α-tocopherol, one group of vitamin E generated through a set of enzymatic reaction[30]. As we know, vitamin E is a potent lipid-soluable antioxident and it could help strengthen the immune systems, inhibit cell proliferation and several inflammation pathways caused by infection or tumor progression[31,32]. In our study, the α-CEHC is upregulated compared with the healthy controls and the fold change is 4.38, which confirms an acceralated vitamin E metabolism. To the best of knowledge, our study is the first one that

discovered the upregulation of vitamin E metabolism in bladder caners, which may be caused by inflammation secondary to tumors. Concerning the renal cell carcinoma, Catchpole et al. observed that an increased level of α-tocopherol in RCC tumor tissues compared with the normal renal cortex tissue, consistent with the findings of Nikiforova etc [26,33]. Besides, analysing the 66 invasive ovarian carcinomas and 9 borderline tumor tissues by gas chromatography/time-of-flight mass spectrometry, Denkert etc. discovered that α-tocopherol 2 was elevated in cancers and the fold change of cancer vs borderline tumor was 2.5[34]. As all the vitamins in our bodies are gained through our digestation, we still can't rule out the possibility that the increased vitamin metabolism may be just a superfical phenomenon of an increased uptake of lipids, rather than caused by the cancers.

A disturbance of the purine metabolism has also been detected in our study not only in the panel of cancer vs healthy controls but also in the group of BC vs RCC. However, contrary to most previous studies, our researches showed that compared with the controls, deoxyinosine, one of the most common precusor of DNA was decreased in cancer groups. In 2007, Sahu ec al. enrolled 96 samples(including 72 urothelial carcinoma patients and 24 normal patients) and analysed their differential metabolites by performing UHPLC- MS/MS. As a result, both the purine and the puring metabolites were increased in urothelial cancer, suggesting the accelerated synthesis and degradation of nucleotides[35]. In a meta-analysis of 11 articles, the levels of guanine, cytosine, thymine, hypoxanthine, uracil and ribose were found eleveted in the urine of BC patients, indicating a higher level of nucleotide metabolism[36]. Concerning the RCC, few researches reported the differential metabolites of purine metabolism, making our study the first one to demonstrate the inner machanism. Compred with the BC, the purine metabolism of RCC was upregulated slightly, which suggested a higher nuclein metabolism. However, it's necessary to stess that the lowver purine level may be due to a much more obvious degradation together with a enhanced synthesis.

In our analysis, an perturbation of amino acid metabolism has also been revealed between the group of BC vs RCC with hematuria, namely alanine, aspartate, glutamate and D- glutamine metabolism, which suggested a distingushed protein metabolism between BC and RCC. Besides, the elevated prostaglandin F2b and thromboxane B3 occurred in BC, which are biologically active signaling components of the COX and LOX pathways. The COX and LOX pathways are closely associated with a function of inflammatory cell regulation, tumorigenesis, cell proliferation, and angiogenesis. Our results were supported by a previous metabolomic analysis of urothelial carcinoma[35], illustrating a hyperactive tumor metabolism and the consequent inflammtion.

There also exist some limitations in our study. Firstly, the sample scale in our study is relatively small and it's single-center, making it less convincible. Therefore, increasing the samples and enrolling more medical centers would be necessary in our further analysis. Secondly, our study focused on the discrimination of BC and RCC and revealed the deeper mechanism under the surface. However, due to the complete heterology of BC and RCC, it remains a question whether they are comparable. Beisdes, because of the epidemic difference between BC and RCC—the diagnostic age of BC is older than RCC, there is also a

possiblitiy that the metabolic disturbance between BC and RCC may be caused by the distinguished age between the two groups, rather than by the cancer. Last but not least, owning to the limitations of time and conditions, we merely used one method—metabolomics to predict the potential altered metabolism and thus we only focused on the small metabolites in urine. Therefore, a combination of proteomics, transcriptomics and genomics in the future could help us better understand the deeper mechanism in BC and RCC.

Conclusions

In conclusion, based on a highly sensitive metabolomics approach, we discovered three independent early diagnostic biomarker panels that could distinguish RCC, BC and Healthy Controls, which may significantly benefit the BC and RCC patients and thus improve their prognosis. Many altered metabolic pathways have been identified by comparative metabolomics including the lipid, vitamin E, purine, animo acid and eicosanoid metabolism.

Abbreviations

BC: bladder cancer; *RCC:* renal cell carcinoma; *HCs:* healthy controls; *AUC:* area under the curve; *UPLC-MS:* ultra-performance liquid chromatography mass spectrometry; *QC:* quality control; *α-CEHC:* 2,5,7,8-tetramethyl–2(2′-carboxyethyl)–6-hydroxychroman.

Declarations

Ethnical approval and consent to participate

This study was approved by the Institutional Review Board of the Institute of Basic Medical Sciences and Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, and all human subjects signed informed consent before participating in this study.

Consent to publish

Not applicable.

Availability of data and materials

All the necessary materials can be found in the text or supplementary materials. Due to the privacy policy, the confidential data materials could only be obtained with the permission of the corresponding authors.

Competing interests:

The authors declare that they have no conflict of interests.

Funding

This work was supported by National Basic Research Program of China (2014CBA02005).

Authors' contributions

ZW, XL, YZ and WS conceived and designed the study. XL, HS, ZG and GZ collected the clinical data and performed the experiments. ZW and XL drafted the first version of the manuscript. YZ and WS revised the manuscript toghther. All authors contributed to the interpretation of the results, edited and approved the final manuscript.

Acknowledgements

Not applicable.

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References

1.Issaq HJ, Nativ O, Waybright T, et al. Detection of bladder cancer in human urine by metabolomic profiling using high performance liquid chromatography/mass spectrometry. *The Journal of urology.* 2008;179(6):2422–2426.

2.van der Poel HG, Debruyne FM. Can biological markers replace cystoscopy? An update. *Current opinion in urology.* 2001;11(5):503–509.

3.Konety BR. Molecular markers in bladder cancer: a critical appraisal. *Urologic oncology.* 2006;24(4):326–337.

4.Siu KW, DeSouza LV, Scorilas A, et al. Differential protein expressions in renal cell carcinoma: new biomarker discovery by mass spectrometry. *J Proteome Res.* 2009;8(8):3797–3807.

5.Lin L, Huang Z, Gao Y, et al. LC-MS-based serum metabolic profiling for genitourinary cancer classification and cancer type-specific biomarker discovery. *Proteomics.* 2012;12(14):2238–2246.

6.Lewis GD, Wei R, Liu E, et al. Metabolite profiling of blood from individuals undergoing planned myocardial infarction reveals early markers of myocardial injury. *The Journal of clinical investigation.* 2008;118(10):3503–3512.

7.Spratlin JL, Serkova NJ, Eckhardt SG. Clinical applications of metabolomics in oncology: a review. *Clinical cancer research: an official journal of the American Association for Cancer Research.* 2009;15(2):431–440.

8.Sreekumar A, Poisson LM, Rajendiran TM, et al. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. *Nature.* 2009;457(7231):910–914.

9.Hirayama A, Kami K, Sugimoto M, et al. Quantitative metabolome profiling of colon and stomach cancer microenvironment by capillary electrophoresis time-of-flight mass spectrometry. *Cancer Res.* 2009;69(11):4918–4925.

10.Zhang A, Sun H, Yan G, Wang P, Wang X. Mass spectrometry-based metabolomics: applications to biomarker and metabolic pathway research. *Biomedical chromatography: BMC.* 2016;30(1):7–12.

11.Pasikanti KK, Esuvaranathan K, Ho PC, et al. Noninvasive urinary metabonomic diagnosis of human bladder cancer. *J Proteome Res.* 2010;9(6):2988–2995.

12.Monteiro MS, Barros AS, Pinto J, et al. Nuclear Magnetic Resonance metabolomics reveals an excretory metabolic signature of renal cell carcinoma. *Sci Rep.* 2016;6:37275.

13.Huang Z, Lin L, Gao Y, et al. Bladder cancer determination via two urinary metabolites: a biomarker pattern approach. *Molecular & cellular proteomics: MCP.* 2011;10(10):M111.007922.

14.Jin X, Yun SJ, Jeong P, Kim IY, Kim WJ, Park S. Diagnosis of bladder cancer and prediction of survival by urinary metabolomics. *Oncotarget.* 2014;5(6):1635–1645.

15.Wittmann BM, Stirdivant SM, Mitchell MW, et al. Bladder cancer biomarker discovery using global metabolomic profiling of urine. *PLoS One.* 2014;9(12):e115870.

16.Zhou Y, Song R, Ma C, et al. Discovery and validation of potential urinary biomarkers for bladder cancer diagnosis using a pseudotargeted GC-MS metabolomics method. *Oncotarget.* 2017;8(13):20719–

20728.

17.Kim K, Taylor SL, Ganti S, Guo L, Osier MV, Weiss RH. Urine metabolomic analysis identifies potential biomarkers and pathogenic pathways in kidney cancer. *OMICS.* 2011;15(5):293–303.

18.Falegan OS, Ball MW, Shaykhutdinov RA, et al. Urine and Serum Metabolomics Analyses May Distinguish between Stages of Renal Cell Carcinoma. *Metabolites.* 2017;7(1).

19.Luo P, Yin P, Hua R, et al. A Large-scale, multicenter serum metabolite biomarker identification study for the early detection of hepatocellular carcinoma. *Hepatology.* 2017.

20.Chen J, Zhao X, Fritsche J, et al. Practical approach for the identification and isomer elucidation of biomarkers detected in a metabonomic study for the discovery of individuals at risk for diabetes by integrating the chromatographic and mass spectrometric information. *Anal Chem.* 2008;80(4):1280–1289.

21.Zhang J, Yang W, Li S, et al. An intelligentized strategy for endogenous small molecules characterization and quality evaluation of earthworm from two geographic origins by ultra-high performance HILIC/QTOF MS(E) and Progenesis QI. *Analytical and bioanalytical chemistry.* 2016;408(14):3881–3890.

22.Armitage EG, Ciborowski M. Applications of Metabolomics in Cancer Studies. *Advances in experimental medicine and biology.* 2017;965:209–234.

23.Amara CS, Vantaku V, Lotan Y, Putluri N. Recent advances in the metabolomic study of bladder cancer. *Expert review of proteomics.* 2019;16(4):315–324.

24.Piyarathna DWB, Rajendiran TM, Putluri V, et al. Distinct Lipidomic Landscapes Associated with Clinical Stages of Urothelial Cancer of the Bladder. *European urology focus.* 2018;4(6):907–915.

25.Lee MY, Yeon A, Shahid M, et al. Reprogrammed lipid metabolism in bladder cancer with cisplatin resistance. *Oncotarget.* 2018;9(17):13231–13243.

26.Catchpole G, Platzer A, Weikert C, et al. Metabolic profiling reveals key metabolic features of renal cell carcinoma. *Journal of cellular and molecular medicine.* 2011;15(1):109–118.

27.Sato T, Kawasaki Y, Maekawa M, et al. Value of global metabolomics in association with diagnosis and clinicopathological factors of renal cell carcinoma. *International journal of cancer.* 2019.

28.Wettersten HI, Hakimi AA, Morin D, et al. Grade-Dependent Metabolic Reprogramming in Kidney Cancer Revealed by Combined Proteomics and Metabolomics Analysis. *Cancer research.* 2015;75(12):2541–2552.

29.Ganti S, Taylor SL, Kim K, et al. Urinary acylcarnitines are altered in human kidney cancer. *International journal of cancer.* 2012;130(12):2791–2800.

30.Mondul AM, Moore SC, Weinstein SJ, et al. Serum Metabolomic Response to Long-Term Supplementation with all-rac-alpha-Tocopheryl Acetate in a Randomized Controlled Trial. *Journal of nutrition and metabolism.* 2016;2016:6158436.

31.Al-Zalabani AH, Stewart KF, Wesselius A, Schols AM, Zeegers MP. Modifiable risk factors for the prevention of bladder cancer: a systematic review of meta-analyses. *European journal of epidemiology.* 2016;31(9):811–851.

32.Wang YY, Wang XL, Yu ZJ. Vitamin C and E intake and risk of bladder cancer: a meta-analysis of observational studies. *International journal of clinical and experimental medicine.* 2014;7(11):4154–4164.

33.Nikiforova NV, Kirpatovsky VI, Darenkov AF, Chumakov AM, Sevrukov EA, Darenkov SP. Liposoluble vitamins E and A in human renal cortex and renal cell carcinomas. *Nephron.* 1995;69(4):449–453.

34.Denkert C, Budczies J, Kind T, et al. Mass spectrometry-based metabolic profiling reveals different metabolite patterns in invasive ovarian carcinomas and ovarian borderline tumors. *Cancer research.* 2006;66(22):10795–10804.

35.Sahu D, Lotan Y, Wittmann B, Neri B, Hansel DE. Metabolomics analysis reveals distinct profiles of nonmuscle-invasive and muscle-invasive bladder cancer. *Cancer medicine.* 2017;6(9):2106–2120.

36.Cheng Y, Yang X, Deng X, et al. Metabolomics in bladder cancer: a systematic review. *International journal of clinical and experimental medicine.* 2015;8(7):11052–11063.

Figures

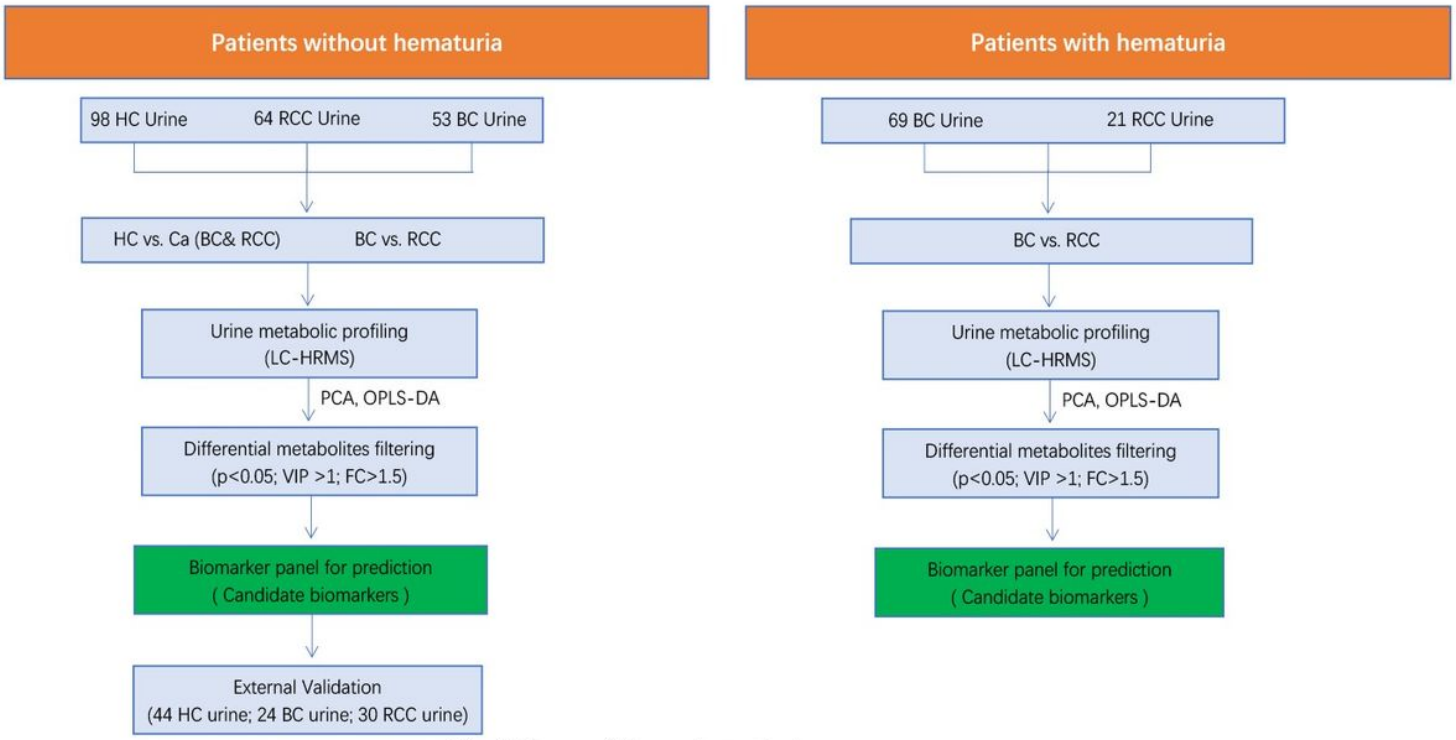

Fig.1 The workflow of our study.

Figure 1

The workflow of our study

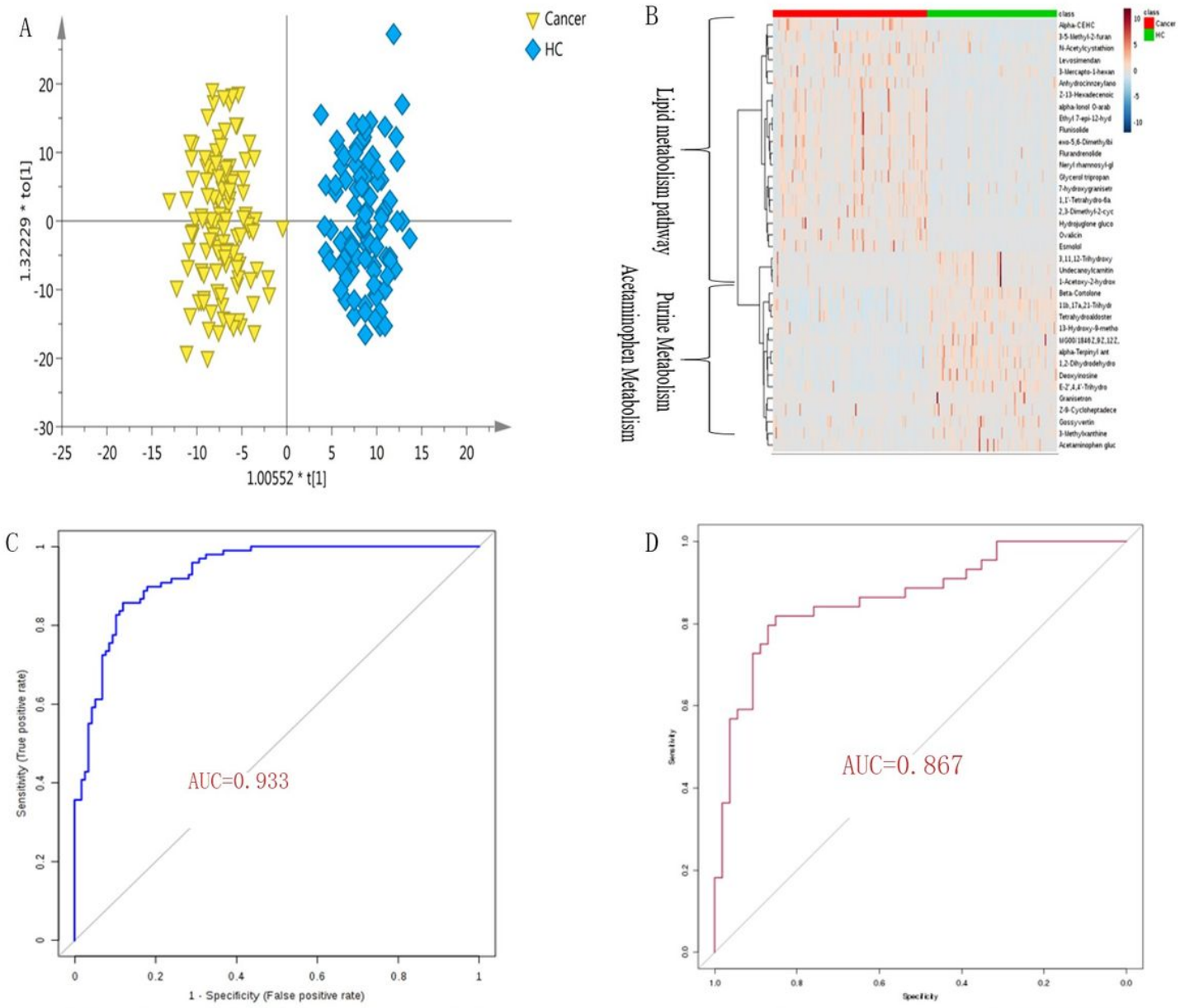

Fig. 2 (A) Plot of OPLS-DA.(B) Relative intensity. ROC curve with 10-fold cross validation(C) and external validation(D).

Figure 2

Analysis of metabolic profiling between cancers and controls. (A) Metabolic score plot of OPLS-DA.(B) Relative intensity between the cancers and controls. (C) ROC curve with 10-fold cross validation based on the biomarker panel. (D) ROC curve of external validation based on the biomarker panel

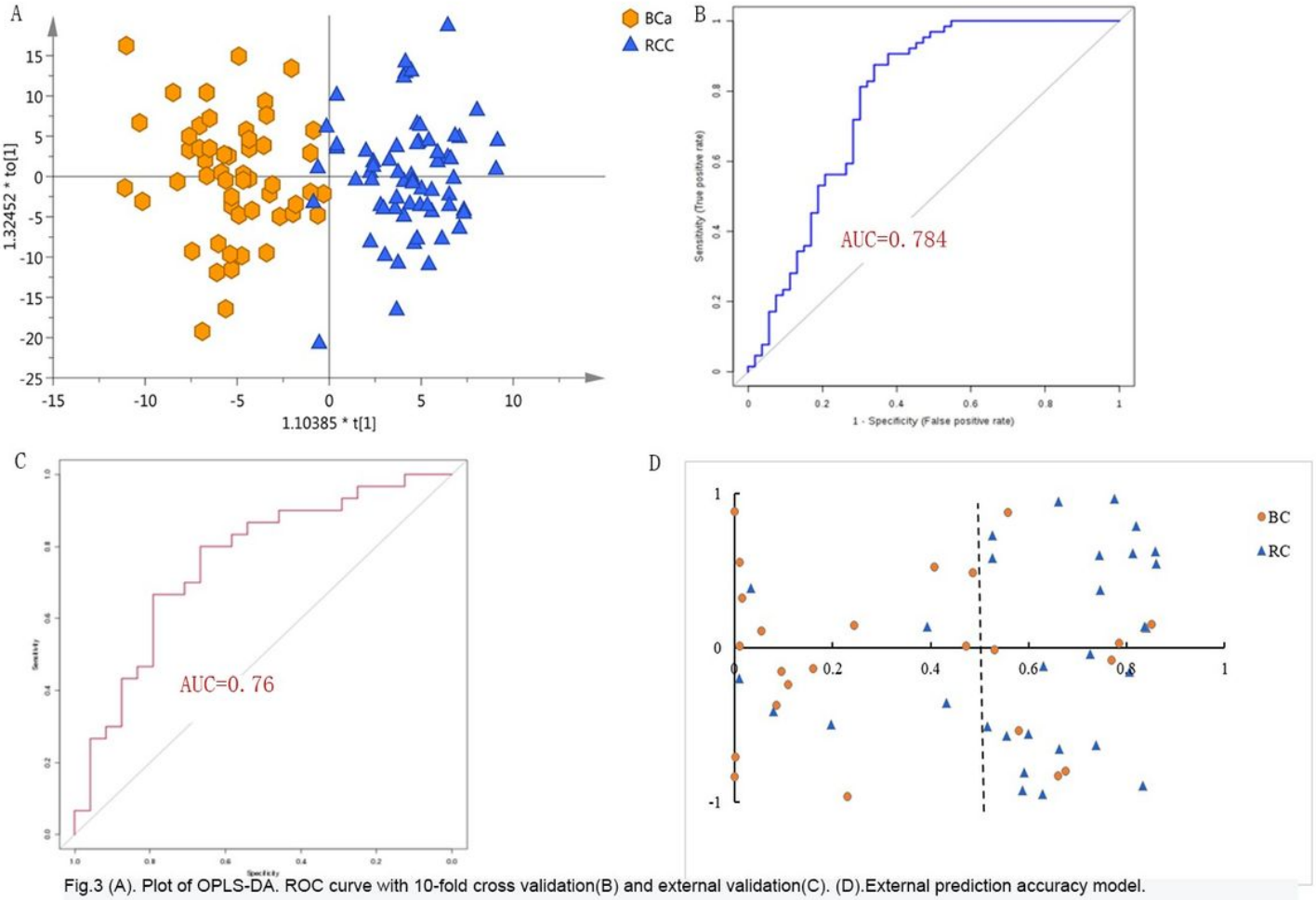

Fig.3 (A). Plot of OPLS-DA. ROC curve with 10-fold cross validation(B) and external validation(C). (D).External prediction accuracy model.

Figure 3

Analysis of metabolic analysis between BC and RCC without hemturia. (A). Metabolic score plot of OPLS-DA. (B). ROC curve with 10-fold cross validation based on the biomarker panel. (C). ROC curve of external validation based on the biomarker panel. (D). External prediction accuracy model based on the biomarker panel

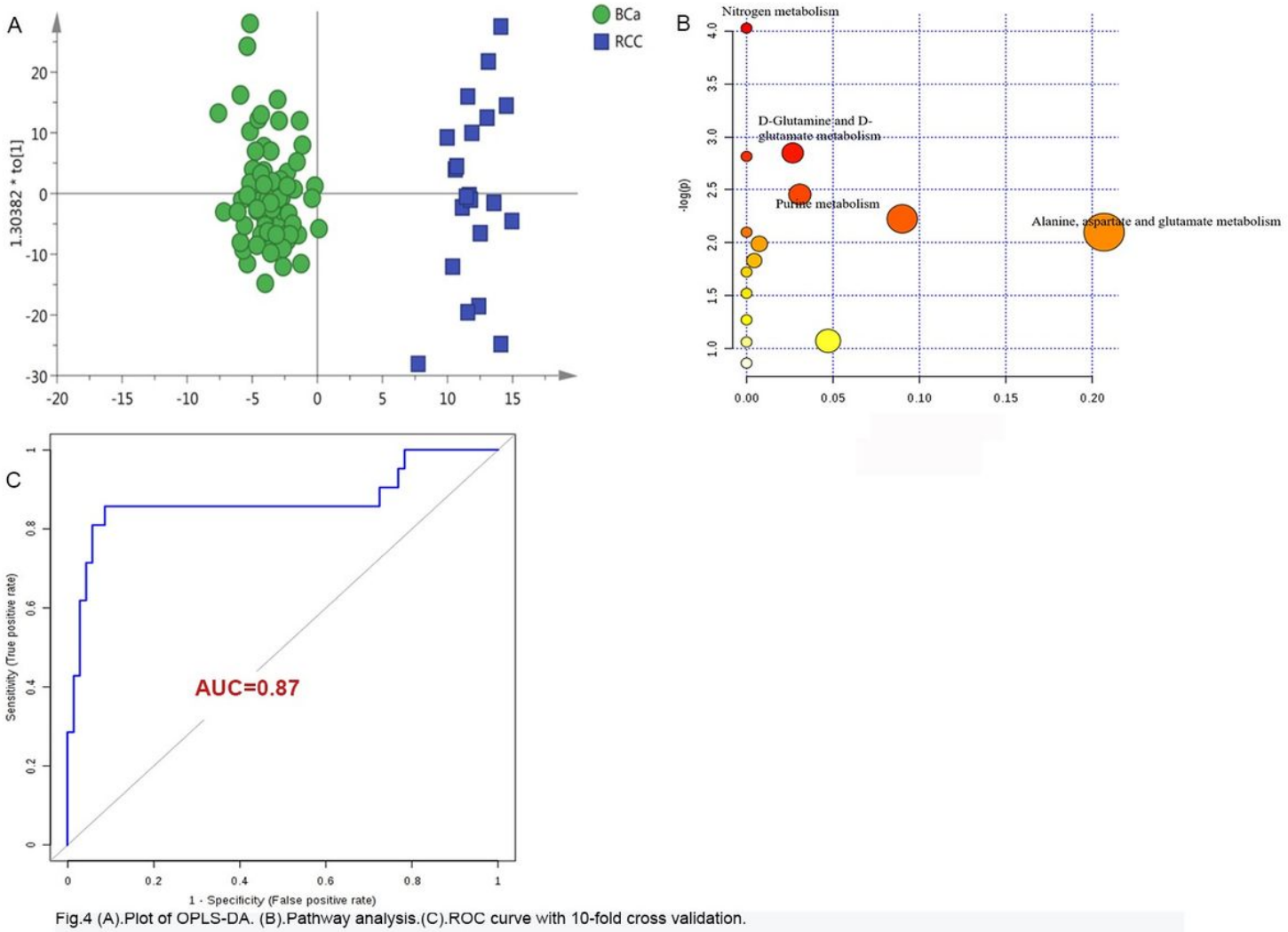

Fig.4 (A).Plot of OPLS-DA. (B).Pathway analysis.(C).ROC curve with 10-fold cross validation.

Figure 4

Analysis of metabolic anlysis between BC and RCC with hematuria. (A).Metabolic score plot of OPLS-DA. (B).Pathway analysis of the differential metabolites between the two subgroups. (C).ROC curve with 10-fold cross validation based on the metabolic biomarker panel.

Supplementary Files

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- supplement1.docx