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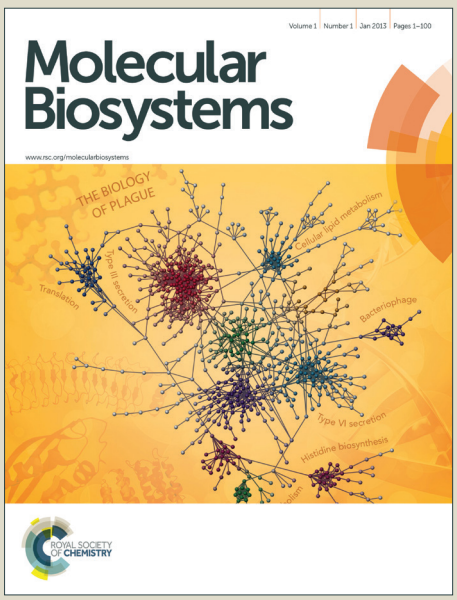

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Metabolomics Approach for Predicting Response to Neoadjuvant Chemotherapy in Cervical Cancer Patients

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Abstract

Cervical cancer is a clinical and pathological heterogeneity disease, which makes different type of treatments and leads to a variety of outcomes. In the clinical practice, only some patients could benefit from chemotherapy treatment. Identifying patients who will be response to chemotherapy could increase their survival time, which has important implications to personalized treatment and outcomes, while identifying non-responders may reduce the likelihood for these patients to receive the ineffective treatment and thereby enable them to receive other potentially effective treatments. Plasma metabolite profiling was performed in this study to identify the potential biomarkers that could predict the response to neoadjuvant chemotherapy (NACT) for cervical cancer patients. Metabolic profiles of plasma from 38 cervical cancer patients with complete, partial and non-response to NACT were studies using a combination of liquid chromatography coupled with mass spectrometry (LC/MS) and multivariate analysis methods. L-valine and L-Tryptophan were finally identified and verified for the potential biomarkers. A prediction model constructed with L-valine and L-tryptophan correctly identified approximately 80% of patients who are non-response to chemotherapy and 87% of patients who were pathologically complete response to chemotherapy, and has an excellent discriminant performance with AUC of 0.9407. These results show promise for larger studies that could produce more personalized treatment protocols for cervical cancer patients.

KEYWORDS: Cervical carcinoma; biomarker; Neoadjuvant chemotherapy; Radical hysterectomy; Tumor Response

Introduction

Cervical cancer is characterized by a clinical and pathological heterogeneity disease, which makes different type of treatments and leads to a variety of outcomes. It is the most prevalent female malignancy in some developing countries[1, 2]. In developing countries, most patients are diagnosed at the locally advanced stage[3] and the majority

of cases are squamous cell carcinoma (SCC). Currently, the strategy for such patients involves the use of neoadjuvant chemotherapy (NACT) prior to either radical surgery or radiotherapy[4, 5], which was currently considered to be a novel and promising treatment approach for locally advanced cervical cancer, especially in patients with FIGO stage IB2-IIB[6].Several studies have reported that for inpatients at similar FIGO stages, preoperative NACT may facilitate subsequent radiation, simplify the surgical procedures and promote the possible transformation of inoperable tumors[7-10]. Previous studies, however, have suggested that approximately 30% of the patients with SCC are nonresponsive to chemotherapy regimens[11, 12]. Identifying patients who will be response to chemotherapy could increase their survival time, which has important implications to individual treatment and outcomes, while identifying non responders may reduce the likelihood for these patients to receive the ineffective treatment and thereby enable them to receive other potentially effective treatments. An ability to predict response to NACT has important implication in developing personalized treatment protocols, improving survival rates and reducing unnecessary exposure of patients to toxic drugs.

Currently, MRI is the standard technique used for the response evaluation and is used to compare the pretreatment and preoperative tumor size. The MRI findings are then used by oncologists to further determine the treatment protocol for cervical cancer patients [11, 13, 14]. Previous studies have indicated that the evaluation of tumor volume after chemoradiation therapy using MRI increases the risk of false-positive results, which leads to increased post-treatment morbidity following multimodality therapy, unnecessary expense and accelerated tumor growth [15]. It is reported that the diffusion-weighted magnetic resonance imaging has been used for assessing the response of locally advanced cervical cancer to NACT [16]. However, this technique is more expensive than conventional MRI and, thus, cannot be widely applied in assessment procedures, especially in developing countries. Especially, Vives et al (1995) demonstrated that MRI could not be a precise evaluation for chemotherapy response [17].

In the recent years, genomics have been performed to find the useful molecular to

predict the pathological complete response (pCR) or partial response (PR) to chemotherapy in cervical cancer patients. Chung et al (2006) reported that XRCC1 R399Q polymorphism is associated with response to platinum-based neoadjuvant chemotherapy in bulky cervical cancer[18]. Kim et al (2008) reported that XRCC1 Arginine194Tryptophan and GGH-401Cytosine/Thymine polymorphisms are associated with response to platinum-based neoadjuvant chemotherapy in cervical cancer[19]. These two studies focused on investigating the association between single nucleotide polymorphisms (SNPs) of genes and the response to platinum-based NACT in cervical cancer. However, both studies just focus on the association rather than the prediction performance in the response to chemotherapy in cervical cancer. Although Scambia et al (1996) reported that pretreatment serum levels of SCC, together with CA 15.3 assay, may be a useful tool in the determination of response to chemotherapy, these two biomarkers showed a lower sensitivity the prediction of cervical carcinoma[20].

Metabolomics involves the quantitative detection of multiple small molecule metabolites in biological systems[21]. A major advantage in the application of metabolomics comes from an improved ability to detect thousands of metabolites in parallel, which could help us to monitor the dynamic picture for disease progression [21]. In the past few years, ultra-performance liquid chromatography coupled to time-of-flight mass spectrometry (LC-MS), an information-rich analytical technique, has become one of the most advanced and useful tool. In comparison with other biomarker approach, metabolomics might provide more insight into oncogensis. Importantly, plasma tests based on metabolic profiles are relatively cheap, rapid and automated. Although metabolomics has been widely used in the molecule discovery for the early diagnosis, disease detection, targeted therapy and drug response[21-25], so far few studies were performed in the biomarker discovery for predicting response to chemotherapy in cervical cancer patients.

In this prospective study, plasma metabolite profiling was performed in this study to identify the potential biomarkers that could predict the response to NACT for cervical cancer patients. Metabolic profiles of plasma from cervical cancer patients

with complete, partial and non-response to NACT were studies using a combination of liquid chromatography coupled with mass spectrometry and multivariate analysis methods. The predictive performance was evaluated in terms of sensitivity, specificity and accuracy based on random forest and leave one out cross validation [26-28]. These results showed promise for larger studies that would provide more personalized treatment protocols for cervical cancer patients.

2. Materials and Methods

Patients were enrolled in this study if they satisfied the following inclusion criteria 1) diagnosis of stage IB2-IIB SCC according to the FIGO classification; 2) SCC histologic subtype; and 3) no prior hysterectomy, pelvic radiotherapy, systemic chemotherapy and medical contraindications to chemotherapy; 4) no other metabolic diseases, like diabetes mellitus; 5) no inflammatory conditions for patients. The exclusion criteria included 1) the inability to undergo MRI examinations; 2) missing MRI or SCC-ag levels data at the initial visit; 3) drop-out during the study period; 4) protocol violation. All patients provided written informed consent in accordance with institutional guidelines, and the study was approved by the institutional review board of the affiliated Tumor Hospital of Harbin Medical University.

Neoadjuvant Chemotherapy Regimen

Eligible patients received NACT in the form of a regimen that consisted of three cycles of paclitaxel and carboplatin. Once every 3 weeks, patients received paclitaxel at 150 mg/m^2 intravenously (IV) over 3 hours at the 1st day in addition to carboplatin (area under the serum concentration-time curve=5) IV over 30 minutes with simultaneous monitoring of blood pressure, ECG and blood oxygen saturation. The doses and schedules of the drug administration were modified according to drug toxicity evaluation before each course.

Magnetic Resonance Imaging

All patients underwent MRI scans at the initial visit, and if they received NACT treatment, they would undergo an additional MRI scan on the 21st day upon completion of chemotherapy. All examinations were performed using a 1.5-T NVi/CVi magnetic resonance machine (GE, Waukesha, USA) with a standard phased array torso coil. The MR protocol for the localization of the tumor and the measurement of its dimensions is described below.

A sagittal T1- and T2-weighted fast spin echo acquisition was obtained with a 24×24cm field of view (FOV), an echo time (TE) of 97·6 cm, and a repetition time (TR) of 1600 ms. Slices that were 4 mm thick were acquired with a 1-mm gap between the slices using a 256×256 matrix. The scan ranged from the iliac crests to the pubic symphysis. Based on the sagittal view, axial slices were obtained through the tumor using a T2-weighted fast recovery fast spin echo sequence with an FOV of 32×32 cm, a TE/TR of 83.6/4520 ms, 3-mm slices (no gap) and a 256×256 matrix.

Once the tumor was fully visualized, diffusion-weighted images (DWI) were acquired in straight axial and sagittal planes (planned on the T2-weighted sagittal scan) and centered through the middle of the tumor using an epi-based diffusion tensor imaging sequence. The epi-based sequence was limited to straight axial slices. All slices were acquired with a 40×40-cm FOV, a 2-mm slice thickness, a TR/TE of 6800 ms/70 ms, and a 160×256 matrix. Data were acquired with a *b* value of 1000. The ADC value was obtained from diffusion tensor images on each slice.

Image Analysis

The images were evaluated by two experienced radiologists who were informed about the patients' clinical data during the evaluation but unaware of the results of other imaging studies. The response based on imaging was determined using and T1- and T2-weighted images in the sagittal and axial planes, and the clinical response was determined from the measurement of the largest diameter of the target lesion. When more than one measurable lesion was present at baseline, the sum of the largest

diameters was calculated at baseline and at the subsequent evaluation. If there was a discrepancy between the two readings, a third experienced radiologist served as an independent and final arbiter.

Response Evaluation

The response evaluation was based on the response evaluation criteria for solid tumors (RECST, version 1.1)[13] and was classified as follows: pathologically complete response (pCR) was defined as a complete disappearance of all lesions; partial response (PR) was defined as at least a 30% decrease in the sum of the largest diameter (LD) of the targeted lesions; stable disease (SD) was defined as neither shrinkage that qualified as PR nor sufficient increase that qualified as progressive disease (PD); and PD was defined as at least a 20% increase in the sum of the LD of the target lesions. For statistical analysis, the overall response was defined as CR plus PR.

Pathological Assessment

All specimens removed during surgical procedures were submitted for pathological analysis. A consistent protocol for the assessment of the specimens was performed by the Department of Pathology, which included macroscopic measurement of the lesion size and microscopic determination of the boundary of the lesion based on frozen tissue. The largest diameter for the lesion was considered to be the SCC pathological result.

Sample collection

The patients who were initially diagnosed as cervical cancer were enrolled from the Department of Gynecology of Harbin Medical University Tumor Hospital (Harbin, China) between September 2009 and March 2011. All patients signed informed consent forms. After fasting and being avoided alcohol and medication for 12 h, each participant was collected 5 ml of whole blood before cytoreductive surgery,

radiotherapy or chemotherapy via venipuncture. Then the blood was transferred into the evacuated blood collection tubes that contained anticoagulant sodium dihydrogen phosphate (EDTA-K3). After separation by centrifugation within 1 h, the plasma was separated into five 400-μl portions and stored at −80 °C until the time of the assay.

Sample preparation and pretreatment

In the laboratory, the plasma samples were thawed and refrozen in a 4 °C water bath. A 300 μl aliquot was injected into a vial and vortexed for 30 s, and then the mixed solution was extracted with 1500 μl of methanol and vortexed for 2 min. After being stored for 10 min at 20 °C, the solutions were centrifuged at 14,000 g for 10 min at 4 °C. An aliquot of supernatant was transferred to a sampling vial to nitrogen-dry at 37 °C. The residue was derivatized using a 2-step procedure. First, 300 μl of acetonitrile/water (3:1) was added to the vial, which was kept at 30 °C for 10 min and then vortexed for 60 s. The supernatant was then placed into the sampling vial, pending UPLC-QTOF/MS analysis.

Chromatography

A2 μl aliquot of the pre-treated sample was injected into a 100 mm×2.1 mm, 1.7 μm BEH C18 column (Waters, Milford, MA) held at 35 °C using an Acquity ultra-performance liquid chromatogra- phy system (Waters). Samples from 5 BOT and 5 EOC patients were alternately run. A gradient consisting of 2 solutions, A (water with 0.1% formic acid) and B (acetonitrile with 0.1% formic acid), was run. The column was eluted with a linear gradient of 2–20% B for 0–1.5 min, 20–70% B for 1.5–6 min, and 70–98% B for 6–10 min and held for 2 min. The gradient then returned to 40% B for 12–14 min and finally to 2% B for 16–18 min. The flow rate was 0.35 ml/min, and all of the samples were maintained at 4 °C during the analysis [23]

Mass spectrometry

Mass spectrometry was performed using a Waters Micromass Q-TOF (Waters,

Manchester, U.K.) equipped with an electrospray ionization source operating in positive-ion mode (ESI+). The source temperature was set at 100 ℃ with a cone gas flow rate of 25 l/h. Meanwhile, the desolvation gas temperature was 300 ℃ with a desolvation gas flow rate of 650 l/h. In the case of positive-ion modes, the capillary voltage was set to 2.8 kV, and the cone voltage was set to 35 V. Centroid data were collected from 50 to 1000 m/z with a scan time of 0.4 s, an inter-scan delay of 0.1 s, and a lock spray frequency of 10 s. In the tandem mass spectrometry (MS/MS) experiments, argon was employed as the collision gas and collision energy was altered between 10 and 30 eV.

Data preprocessing and annotation

The data preprocessing and annotation were similar to our previous work[29]. The raw UPLC-QTOF/MS ESI+ data were first transformed to NetCDF files by Databridge (Waters), and then the files were imported to xcms package in R platform for preprocessing, including nonlinear retention time alignment, matched filtration, peak detection, and peak matching. 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.

The preprocessing through xcms offered a 3-dimensional matrix containing retention time (RT)-mass-to-charge ratio (m/z) pairs, sample names (observations), and ion-intensity information (variables). Then for the processed peak data by xcms, the R package CAMERA was used for annotation of isotope peaks, adducts and fragments in the peak lists[30, 31]. Normalization to total peak intensities for each sample was done before statistical analysis.

Statistical analysis

Categorical data are described as frequency counts and their percentages. Comparisons between the clinical pathological characteristics such as FIGO staging, lymph node metastasis and differentiation in responsive and non-responsive patients

were performed using *Pearson*'s chi-square test. The partial least-squares discriminant analysis (PLS-DA) was employed to present the discrimination performance of metabolites between responders and nonresponders, and variable importance in the project (VIP) was calculated as well[32]. Univariate nonparametric Kruskal–Wallis rank sum test and multivariate analysis were used to select the potential biomarkers that were used for predicting the chemotherapy response. Potential metabolic biomarkers were selected with the criteria of VIP≥1 and $p<0.05$[25, 29]. Random forest (RF) [33]prediction model were constructed with the potential biomarkers. The predictive performance for the potential biomarkers were evaluated by sensitivity, specificity, accuracy and the area under the receiver operating characteristic (ROC) based on . the leave-one-out cross-validation using R package. All statistical procedures were performed using the R platform.

3. Results

Demographic and Clinical Characteristics

Between September 2009 and March 2011, 38 eligible previously untreated patients who met the inclusion and exclusion criteria were enrolled in this prospective study, of whom 15 patients were diagnosed as pCR, 14 patients were PR, while the remaining 9 patients were non-responsive to chemotherapy based on the pathological examination. They underwent NACT followed by radical surgery. The demographic and clinical characteristics of the prospective cohort were shown in Table 1. The baseline characteristics were comparable in each group.

Plasma metabolomic profiles

In order to visualize the classification performance of metabolic profiling, the PLS-DA score plot was depicted (Figure 1). Figure 1 revealed a clear separation trend between pCR and SD, and the PR group lies in the between pCR and SD, while no clear separation was found between pCR and PR.

Discovery and identification of metabolic biomarkers

According to the identity check by raw data and the annotation of peaks by CAMERA, 562 peaks (variables) were identified. We employed the Wilcoxon Kruskal–Wallis test and multivariate analysis approach to identify a set of metabolites that had the highest independent ability to predict response before chemotherapy treatment. The potential biomarkers were selected with the criteria of VIP≥1 and p<0.05. Four biomarkers were identified and two of them (L-valine and L-tryptophan) were verified by external reference standard. In addition, the boxplots for the four potential biomarkers were given in Figure 2. From Figure 2, it is seen that concentrations of L-valine, L-tryptophan and prasterone sulfate had the consistently decreased trend from pCR to SD, while Cer(d18:0/12:0) concentration was increased in PR and decreased in SD compared to pCR.

Biomarkers for the prediction of response to chemotherapy

Since the two biomarkers, L-valine and L-tryptophan, were finally selected as biomarkers candidates for predicting the chemotherapy response and verified by external reference standard. L-valine provided an AUC of 0.7333 in the discrimination between pCR and SD and AUC of 0.7183 between pCR combined with PR (pCR+ PR) and SD. L-tryptophan provided an AUC of 0.9185 in the discrimination between pCR and SD and AUC of 0.8175 between pCR+ PR and SD. The error rate from RF regression modeling with these two biomarkers was 16.67%. The combination of these two biomarkers had the sensitivity of 0.87 and specificity of 0.8, with an AUC of 0.9407 in the chemotherapy response prediction.

Metabolic disturbance associated with chemotherapy response

We mapped all the differential metabolites into KEGG database and found that these metabolites were involved in the pathway of amino acid metabolism, tryptophan metabolism and lipid metabolism. These results suggested metabolic disturbance

might be associated with cervical cancer.

4. Discussion

In this study, we present the metabolomics approach for predicting the chemotherapy response for cervical cancer patients before NACT. By applying ultra-performance liquid chromatography coupled to time-of-flight mass spectrometry, a powerful technique that has high sensitivity and specificity, two amino acids, one sphingolipids and one steroids are shown to be highly correlated with pCR. Although there are quite heterogeneous in the clinical and histopathological characteristics of patients in each response group, the plasma samples still can group them into distinct cluster. In order to evaluate the impact of clinical pathological parameters of cervical cancer on the predictive performance of four biomarkers, we perform multivariate analysis to assess whether these biomarkers are the independent predictors for chemotherapy response with these biomarkers and clinicalpathological parameters and found that these biomarkers are still the independent predictors of chemotherapy response.

The prediction model constructed with the selected model has high sensitivity and specificity. Since chemotherapy response prediction for cancer remains challenging around the world, this promising metabolomics approach might open a new view for patients to select the promising treatment or even truly ‘personalized treatment’ in the clinical practice. Importantly, as seen from the multivariate regression analysis between chemotherapy responses and predictors consisting of biomarkers and clinicalpathological parameters (data not shown), the selected biomarkers are strongly associated with chemotherapy response. The altered metabolic pathways associated with the selected biomarkers are strongly connected with chemotherapy response. However, studies with large-scale cohort are needed to substantiate these finding and identify metabolic link with pathologically different subtypes, not limited to the squamous cervical cancer.

The prediction performance of the two biomarkers detected by LC-MS analysis was excellent and showed an AUC of 0.9407 in the discrimination between pCR from

SD. In addition, the metabolites detected by LC-MS provides a number of additional information about better insight into the cellular metabolism and provides a more robust model that could effectively predict chemotherapy response when validated in a larger cohort of patients. Two metabolites from LC-MS distinguish the three cervical cancer patients groups, pCR, PR and SD with good performance. The excellent classification performance through plasma metabolites suggests metabolomics approach might be particularly noteworthy when the prediction of response to NACT in cervical cancer patients remains challenging around the world. We found that PR samples were dispersing between pCR and SD and some of them are predicted as pCR while others cluster with SD patients.

The metabolic processes in the cancer patients are different to normal subjects [22, 29, 34]. The alternation of the amino acid metabolism might be a consequence of metabolic dysfunction. Tryptophan, an essential amino acid, is catabolized in the local microenvironment of tumors, immune-privileged sites or inflammation sites [35]. In the cancer cells, immune cells create an environment that suppress antigen-specific T-cell responses by tryptophan depletion [36]. The tryptophan catabolism is induced by inflammatory mediators, which might be an endogenous mechanism that restrict excessive immune responses, thereby presenting immunopathology. Previous study demonstrate that suppression of anti-tumor immune responses in lesions by tryptophan catabolism promotes tumor growth [37]. The change and variation in the amino acids profiles might be attributable to accelerated gluconeogenesis and the increase in the synthesis of proteins in the liver [38]. The concentration of L-valine, one of the amino acid, was increase in the responders might be a consequence of the destruction of cellular proteins of the tumor and the decrease in their synthesis.

The response evaluation in this study is not limited to chemotherapy in SCC, but can be extended to radiotherapy in such cancers and all malignant tumors that originate from squamous tissue such as lung squamous cell carcinoma and head and neck squamous cell carcinoma; however, further large-scale cohort studies on these cancers should be performed.

Conflicts of interests

None declared.

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REFERENCES

1. K. Hemminki, X. Li and P. Mutanen, European journal of epidemiology, 2001, 17, 323-328.
2. A. P. Vizcaino, V. Moreno, F. X. Bosch, N. Munoz, X. M. Barros-Dios, J. Borras and D. M. Parkin, International journal of cancer. Journal international du cancer, 2000, 86, 429-435.
3. I. T. Katz and A. A. Wright, The New England journal of medicine, 2006, 354, 1110.
4. P. G. Rose, B. N. Bundy, E. B. Watkins, J. T. Thigpen, G. Deppe, M. A. Maiman, D. L. Clarke-Pearson and S. Insalaco, The New England journal of medicine, 1999, 340, 1144-1153.
5. P. B. Panici, G. Scambia, G. Baiocchi, S. Greggi, G. Ragusa, A. Gallo, M. Conte, F. Battaglia, G. Laurelli, C. Rabitti and et al., Cancer, 1991, 67, 372-379.
6. A. Duenas-Gonzalez, L. Cetina, I. Mariscal and J. de la Garza, Cancer treatment reviews, 2003, 29, 389-399.
7. T. Iwasaka, K. Fukuda, K. Hara, M. Yokoyama, Y. Nakao, M. Uchiyama and H. Sugimori, Gynecologic oncology, 1998, 70, 236-240.
8. S. W. deSouza NM, Rustin G, Mahon MM, Jones B, Dina R, McIndoe GA., Br J Cancer., 2004.
9. P. Benedetti-Panici, F. Maneschi, G. Cutillo, S. Greggi, M. G. Salerno, M. Amoroso, G. Scambia and S. Mancuso, Cancer, 1996, 78, 2359-2365.
10. M. Yin, F. Zhao, G. Lou, H. Zhang, M. Sun, C. Li, Y. Hou, X. Li, F. Meng and X. Chen, International journal of gynecological cancer : official journal of the International Gynecological Cancer Society, 2011, 21, 92-99.
11. E. Micheletti, B. La Face, E. Bianchi, E. Cagna, P. Apostoli, G. Ruggeri, R. Verardi and E. Sartori, American journal of clinical oncology, 1997, 20, 613-620.
12. D. C. Park, J. H. Kim, Y. O. Lew, D. H. Kim and S. E. Namkoong, Gynecologic oncology, 2004, 92, 59-63.
13. E. A. Eisenhauer, P. Therasse, J. Bogaerts, L. H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe and J. Verweij, European journal of cancer, 2009, 45, 228-247.
14. L. P. Peters WA 3rd, Barrett RJ 2nd, Stock RJ, Monk BJ, Berek JS, Souhami L, Grigsby P, Gordon W Jr, Alberts DS., J Clin Oncol, 2000, 18, 8.
15. E. Vincens, C. Balleyguier, A. Rey, C. Uzan, E. Zareski, S. Gouy, P. Pautier, P. Duvillard, C.

Haie-Meder and P. Morice, Cancer, 2008, 113, 2158-2165.
16. C. Fu, D. Bian, F. Liu, X. Feng, W. Du and X. Wang, International journal of gynecological cancer : official journal of the International Gynecological Cancer Society, 2012, 22, 1037-1043.
17. A. Vives, C. Castelo-Branco, X. Iglesias, X. Tomas, H. Palombo, J. Pahisa, J. A. Lejarcegui, A. Salvador and L. Puig-Tintore, Acta obstetricia et gynecologica Scandinavica, 1995, 74, 467-471.
18. H. H. Chung, M. K. Kim, J. W. Kim, N. H. Park, Y. S. Song, S. B. Kang and H. P. Lee, Gynecologic oncology, 2006, 103, 1031-1037.
19. K. Kim, S. B. Kang, H. H. Chung, J. W. Kim, N. H. Park and Y. S. Song, Gynecologic oncology, 2008, 111, 509-515.
20. G. Scambia, P. Benedetti, E. Foti, G. Ferrandina, F. P. Leone, M. Marciano and S. Mancuso, European journal of cancer, 1996, 32A, 259-263.
21. G. A. Gowda, S. Zhang, H. Gu, V. Asiago, N. Shanaiah and D. Raftery, Expert review of molecular diagnostics, 2008, 8, 617-633.
22. T. Zhang, X. Wu, C. Ke, M. Yin, Z. Li, L. Fan, W. Zhang, H. Zhang, F. Zhao, X. Zhou, G. Lou and K. Li, Journal of proteome research, 2013, 12, 505-512.
23. W. X. Zhang T, Yin M, Fan L, Zhang H, Zhao F, Zhang W, Ke C, Zhang G, Hou Y, Zhou X, Lou G, Li K., Clin Chim Acta, 2012, 413, 861-868.
24. L. Tenori, C. Oakman, W. M. Claudino, P. Bernini, S. Cappadona, S. Nepi, L. Biganzoli, M. C. Arbushites, C. Luchinat, I. Bertini and A. Di Leo, Molecular oncology, 2012, 6, 437-444.
25. S. Wei, L. Liu, J. Zhang, J. Bowers, G. A. Gowda, H. Seeger, T. Fehm, H. J. Neubauer, U. Vogel, S. E. Clare and D. Raftery, Molecular oncology, 2013, 7, 297-307.
26. A. M. K. Michael Haenlein, Understanding statistics, 2004, 3, 283-297.
27. W. R. atthew Barker, Journal of chemometrics, 2003, 17, 166-173.
28. P. H. Garthwaitea, Journal of the American Statistical Association, 1994, 89, 122-127.
29. T. Zhang, X. Wu, M. Yin, L. Fan, H. Zhang, F. Zhao, W. Zhang, C. Ke, G. Zhang, Y. Hou, X. Zhou, G. Lou and K. Li, Clin Chim Acta, 2012, 413, 861-868.
30. M. Katajamaa and M. Oresic, Journal of chromatography. A, 2007, 1158, 318-328.
31. R. A. van den Berg, H. C. Hoefsloot, J. A. Westerhuis, A. K. Smilde and M. J. van der Werf, BMC genomics, 2006, 7, 142.
32. A. L. Boulesteix and K. Strimmer, Briefings in bioinformatics, 2007, 8, 32-44.
33. L. Breiman., Machine learning, 2001, 45, 5-32.
34. G. X. Xie, T. L. Chen, Y. P. Qiu, P. Shi, X. J. Zheng, M. M. Su, A. H. Zhao, Z. T. Zhou and W. Jia, Metabolomics : Official journal of the Metabolomic Society, 2011, 8, 220-231.
35. A.L. Mellor and D.H. Munn, Nature Reviews Immunology, 2008, 8, 74-80.
36. A.J. Muller, M.D. Sharma, P.R.Chandler, J.B. Duhadaway, M.E. Everhart, B.A. Johnson 3rd, D.J. Kahler, J. Pihkala, A.P. Soler, D.H. Munn, G.C. Prendergast, A.L .Mellor, proceddings of the National Academy of Sciences of the United States of America, 2008, 105, 17073-17078.
37. G. C. Prendergast, M.F. Chang, L. Mandik-Navak, R. Metz and A. J .Muller, Current medicinal chemistry, 2011, 18, 2257-2262.
38. K. Lundholm, A. C. Bylund, J. Holm and T. Scherstén, European journal of cancer, 1976, 12, 465-473.

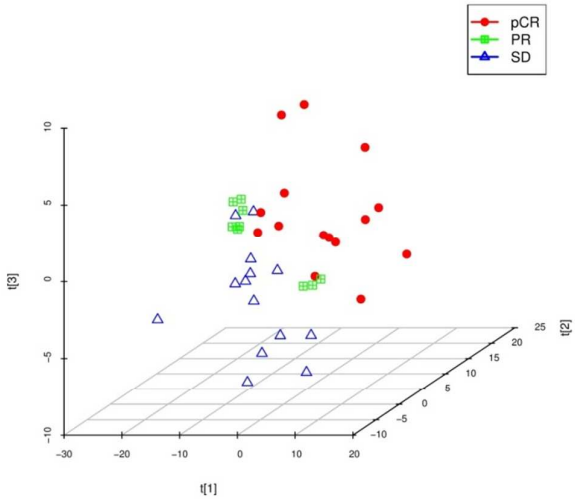

Figure 1 The PLS-DA score plot for pCR, PR and SD patients.

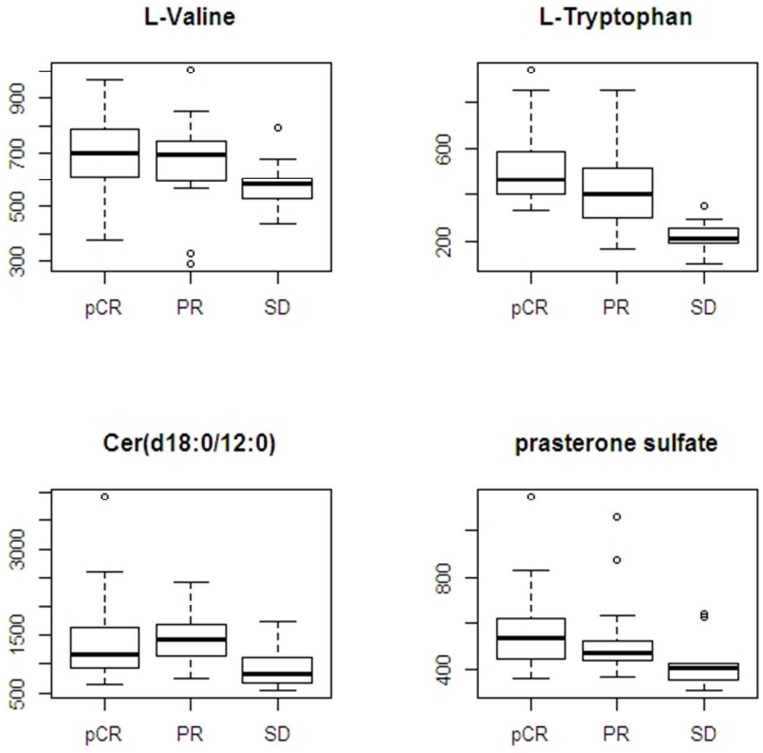

Figure 2 Individual metabolite box plots for the different groups of patients: (a) L-Valine; (b) L-Tryptophan; (c) Cer(d18:0/12:0); (d) prasterone surfate

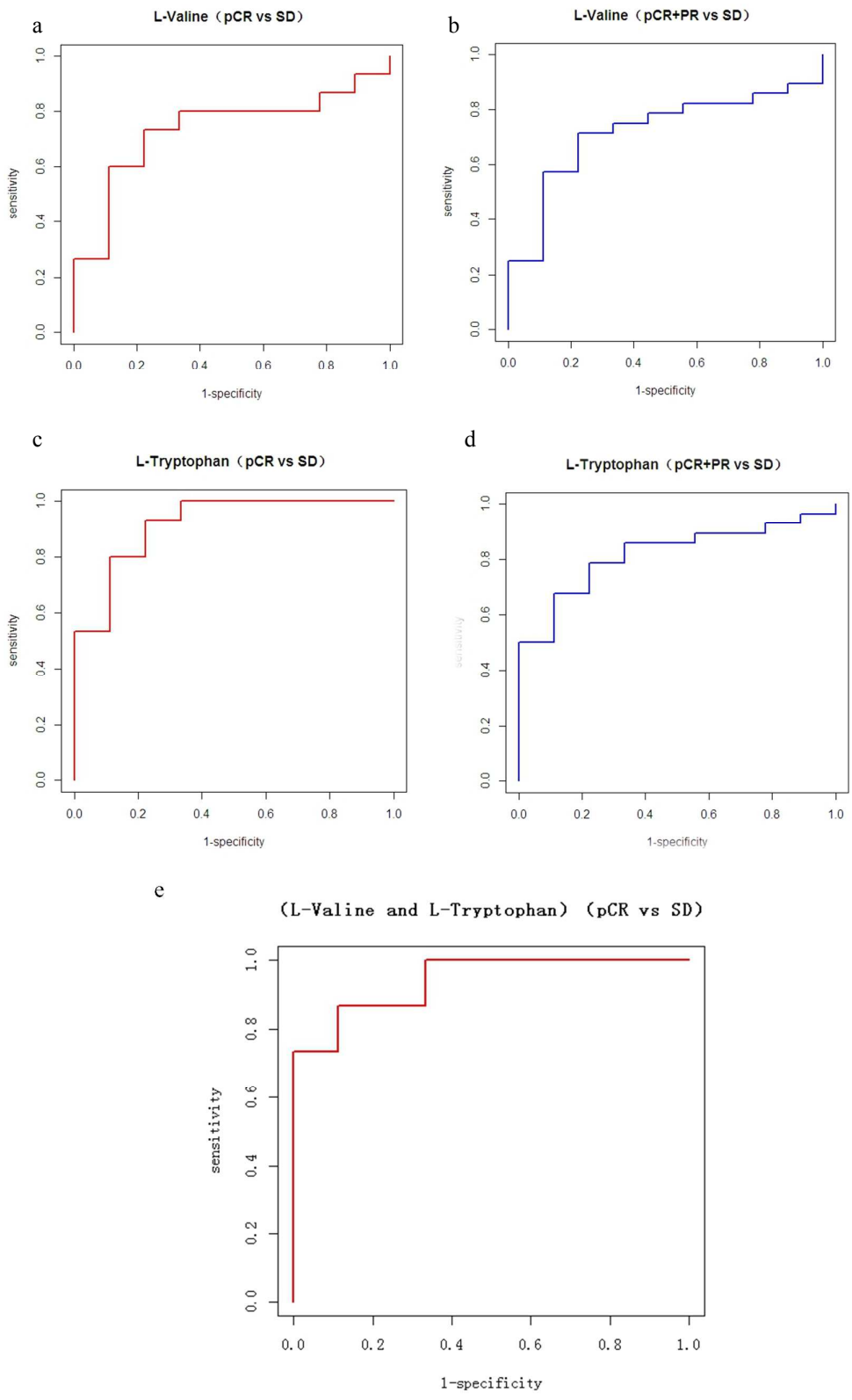

Figure 3 The prediction results for RF model based on L-valine and L-tryptophan.(a)

ROC curve for pCR vs SD using cross-validation based on L-valine (AUC=0.7333); (b) ROC curve for pCR together with PR vs SD using cross-validation based on L-valine (AUC=0.7183)；(c) ROC curve for pCR vs SD using cross-validation based on L-tryptophan (AUC=0.9185)；(d) ROC curve for pCR together with PR vs SD using cross-validation based on L-tryptophan (AUC=0.8175)；(e)The combination of L-valine and L-tryptophan provide an AUC of 0.9407.

Table 1 Demographics and Clinical Characteristics of Cervical cancer Patients in this Prospective Cohort

Characteristics		pCR(n=15)	PR(n=14)	SD(n=9)	*p* value
Age(year)	Mean(Std)	50.66(10.99)	50.02(7.45)	46.44(9.80)	0.7390
	Min,Max	31.72,66.15	36.30,59.45	27.39,56.82	
	Median	54.43	50.02	48.57	
Menses	Non-menopause	7(46.67%)	4(28.57%)	1(11.11%)	0.2476
	menopause	8(53.33%)	10(71.43%)	8(88.89%)	
SCC	Mean(Std)	2.76(3.41)	3.48(5.34)	3.67(2.49)	0.3760
	Min,Max	0.40,14.20	0.50,21.60	0.80,7.80	
	Median	2.20	2.25	4.10	
FIGO Stage	IB2	1(6.67%)	1(7.14%)	2(22.22%)	0.4378
	IIA	8(53.33%)	4(28.57%)	4(44.44%)	
	IIB	6(40.00%)	9(64.29%)	3(33.33%)	
Lymph node metastasis	Negative	15(100.00%)	11(78.57%)	9(100.00%)	0.0531
	Positive	0(0.00%)	3(21.43%)	0(0.00%)	
Differentiation	Well	3(20.00%)	2(14.29%)	1(11.11%)	0.9151
	Moderate	8(53.33%)	8(57.14%)	4(44.44%)	
	Poor	4(26.67%)	4(28.57%)	4(44.44%)	

The values in the parenthese represented the percentage frequency;

Table 2 The differential metabolites in the prediction of chemotherapy response

Measured	RT(min)	Delta Mass(ppm)	identity	p-value (pCR vs SD)	p-value (pCR+PR vs PR)
118.0862	0.71	0	L-Valine	0.0456	0.0413
205.0990	2.32	9	L-Tryptophan	0.0148	0.0261
484.4689	9.31	7	Cer(d18:0/12:0)	0.0287	0.0434
369.1753	8.45	6	prasterone sulfate	0.0315	0.0461