Metabolome Analysis Reveals a Diversity of Cancer Tissues in Advanced Epithelial Ovarian Cancer

Kosuke Yoshida

Nagoya University Graduate School of Medicine Faculty of Medicine: Nagoya Daigaku Daigakuin
Igakuken Kenkyuka Igakubu

Nobuhisa Yoshikawa (✉ n-yoshikawa@med.nagoya-u.ac.jp)

Nagoya University Graduate School of Medicine Faculty of Medicine: Nagoya Daigaku Daigakuin
Igakuken Kenkyuka Igakubu <https://orcid.org/0000-0001-8662-9421>

Kazuhisa Kitami

Nagoya University Graduate School of Medicine Faculty of Medicine: Nagoya Daigaku Daigakuin
Igakuken Kenkyuka Igakubu

Satoshi Tamauchi

Nagoya University Graduate School of Medicine Faculty of Medicine: Nagoya Daigaku Daigakuin
Igakuken Kenkyuka Igakubu

Yoshiki Ikeda

Nagoya University Graduate School of Medicine Faculty of Medicine: Nagoya Daigaku Daigakuin
Igakuken Kenkyuka Igakubu

Akira Yokoi

Nagoya University Graduate School of Medicine Faculty of Medicine: Nagoya Daigaku Daigakuin
Igakuken Kenkyuka Igakubu

Kimihiro Nishino

Nagoya University Graduate School of Medicine Faculty of Medicine: Nagoya Daigaku Daigakuin
Igakuken Kenkyuka Igakubu

Kaoru Niimi

Nagoya University Graduate School of Medicine Faculty of Medicine: Nagoya Daigaku Daigakuin
Igakuken Kenkyuka Igakubu

Hiroaki Kajiyama

Nagoya University Graduate School of Medicine Faculty of Medicine: Nagoya Daigaku Daigakuin
Igakuken Kenkyuka Igakubu

Research Article

Keywords: Metabolome, Epithelial ovarian cancer, Omental metastasis

Posted Date: April 7th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-361726/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Epithelial ovarian cancer remains one of the leading causes of cancer deaths among women worldwide, and advanced epithelial ovarian cancer frequently metastasizes to the omentum. Characteristics of the metastatic cancer may be different from that of primary ovarian cancer reflecting the unique omental microenvironment. This study investigated metabolomic differences in epithelial ovarian cancers.

Methods: Patients with advanced epithelial ovarian cancer are eligible for this study. There were five patients who underwent surgery and resected primary ovarian and omental metastatic cancers at Nagoya University. Metabolome analysis was performed using these paired cancer and metastatic cancer tissues through a facility service (C-SCOPE) at Human Metabolome Technologies, Inc. The concentrations of 116 compounds were measured by CE-TOFMS and CE-QqQMS, and 30 metabolic parameters were calculated. For statistical analyses, Welch's *t*-test was used for comparisons between two independent groups.

Results: Metabolite profiles were different from each other reflecting a diversity in the cancer tissues. Of the measured compounds, urea was the only metabolite that was significantly decreased in the omental metastatic cancers compared with the primary cancers ($p = 0.031$). Moreover, in omental metastatic cancers, the pentose phosphate pathway was more dominant than glycolysis. Furthermore, lactic acids in omental metastatic cancers were markedly decreased compared with primary cancers in some cases. With regard to histological characteristics, the total amount of amino acids, especially the percentage of glutamine, were significantly rich in serous carcinomas than in non-serous carcinomas ($p = 0.004$ and $p = 0.001$, respectively). Moreover, the reduced form of glutathione and polyamines were also abundant in serous carcinomas than in non-serous carcinomas ($p = 0.025$ and 0.048 , respectively).

Conclusions: The metabolite profiles were different depending on the location of the tumor and the histological subtype. Metabolome analysis may be a useful tool for identifying cancer diagnostic and prognostic markers.

Background

Epithelial ovarian cancer (EOC) remains one of the leading causes of cancer deaths among women worldwide with an estimated 184,799 deaths in 2018 [1]. There are four major histological subtypes of EOC: high-grade serous ovarian carcinoma (HGSOC), clear cell carcinoma (CCC), endometrioid carcinoma (EC), and mucinous carcinoma [2]. The clinical and molecular biological features are different for each histological subtype [3, 4]. EOC typically spreads to the peritoneal cavity and frequently metastasizes to the omentum, which is characterized by an adipose-rich environment [3, 5, 6]. There is growing evidence that the tumor microenvironment plays a critical role in cancer progression [7, 8]. Adipocytes in the omentum promote cancer growth and omental metastasis by producing cytokines and chemokines [8, 9]. Moreover, upregulation of fatty acid binding protein 4 (FABP4) in the metastatic lesions of EOC was reported [10]. Therefore, as EOC progresses to the omentum, the molecular biological characteristics of

the cancer cells may change, thus reflecting the unique microenvironment. Therefore, understanding the differences based on the tumor site and histology is essential for elucidating EOC pathology.

Metabolome analysis represents a robust method to identify and quantitate small molecules related to cellular metabolic activity, such as sugars, amino acids, and other organic molecules [11]. Since cancer cell-specific metabolism known as the Warburg effect was discovered, energy metabolism in cancer tissue is considered important [12-15]. The expressed level of transcript and protein of a gene is regarded as a surrogate for predicting gene function. In addition, a variety of microRNAs exert their oncogenic and tumor-suppressive functions by regulating the expression of target genes [16]. However, in the case of enzymatic reactions that are part of a metabolic pathway, the level of expression and function are not necessarily correlated [17, 18]. Metabolome analysis is suitable for evaluating the functional differences in the cancer microenvironment because it enables one to analyze the metabolite changes reflecting enzyme function. Thus, in addition to next-generation sequencing and proteomics, metabolome analysis may be used for the identification of biomarkers and metabolic pathways altered in cancer [11, 18]. Several studies have indicated that serum and tissue metabolites may be potential diagnostic and prognostic biomarkers for EOC [19-22]. However, metabolomic differences between primary cancer (PC) and omental metastatic cancer (OMC) remain undefined and will be beneficial for understanding the spatial diversity of the tumor microenvironment. In this study, we investigated the metabolic changes in PC and OMC and the metabolic differences associated with the histological subtypes of EOC.

Methods

Patients and samples

Archival fresh frozen tissue samples of EOC were collected from the Nagoya University Hospital (Aichi, Japan). There were five patients who underwent PC and OMC tissue resection simultaneously from 2018. Patient characteristics are provided in Table 1. Cases 1 and 2 were histologically HGSOE, cases 3 and 4 were CCC, and case 5 was EC. Case 3 underwent interval debulking surgery after eight courses of carboplatin and paclitaxel combination chemotherapy, whereas the remaining four patients underwent primary debulking surgery. The study protocol was approved by the Ethics Committee of our institution (approval No. 2017-0497). We obtained written informed consent from each patient.

Immediately following resection, cancer tissues were macroscopically sectioned into small pieces and placed into 1.5 mL tubes. They were immediately frozen in liquid nitrogen and stored at -80°C . Approximately 40–50 mg of tissues were used for metabolome analysis.

Metabolite measurements

Metabolome analysis was performed using a facility service (C-SCOPE) at Human Metabolome Technologies, Inc. (Yamagata, Japan). Briefly, the tissues were homogenized in a 50% acetonitrile aqueous solution. The samples were then centrifuged at $2,300 \times g$ at 4°C for 5 min and the upper aqueous layer was centrifugally filtered through a 5 kDa cutoff filter at $9,100 \times g$ for 120 min at 4°C . The

filtrates were resuspended in 50 µl of Milli-Q water and metabolome analysis was done using CE-TOFMS and CE-QqQMS. The data was analyzed by MasterHands ver.2.17.1.11 (Keio University, Tsuruoka, Japan) and MassHunter Quantitative Analysis B.06.00 (Agilent Technologies, Santa Clara, CA), respectively, and the peak area of each metabolite was calculated. The peak area was then normalized with an internal standard, and the concentrations of metabolites were calculated using standard curves. As a result, 116 compounds were measured and 30 metabolic parameters were calculated.

Data analysis and statistical analysis

RStudio (RStudio, Boston, MA) and R software (ver. 3.5.0) were used for the analysis. For heatmap and principal component analyses, values below the detection sensitivity were designated as zero, and metabolites in which the concentration was zero were excluded from all samples. The data was converted to base 10 logarithms and z-scores. The heatmap.2 function of the gplots package (ver. 3.0.1) was used to generate a heatmap. The distance was calculated as “1–Spearman correlation coefficient” and the “ward.D2” clustering method was used. To calculate and visualize the principal component analysis, the prcomp and plot3d functions of the rgl package (ver. 0.99.16) were used. For statistical analyses, Welch’s *t*-test was used for comparisons between two independent groups. P-values less than 0.05 was considered statistically significant.

Results

We performed metabolome analysis using 10 fresh-frozen EOC tissues from 5 patients and 116 compounds were measured (Supplementary 1). A heatmap analysis revealed that metabolites from the paired PC and OMC tissues were similar and metabolites in HGSOC (cases 1 and 2) were quite different than those in non-HGSOC (cases 3–5, Fig. 1A). However, no significant differences were observed in the metabolite profiles between the PC and OMC groups. Likewise, the principal component analysis revealed a diversity in the cancer tissues based on histological subtypes and individual differences (Fig. 1B).

The urea cycle

We investigated the detailed differences in multiple metabolic pathways between the PC and OMC groups. The concentrations of most of the compounds were not significantly different between the two groups (Supplementary 1); however, urea concentration in the OMC group was significantly decreased compared with the PC group ($p = 0.031$). Notably, it decreased by approximately half in cases 1, 4, and 5 (Fig. 2A). The other compounds in the urea cycle were subsequently evaluated. The concentration of ornithine in the OMC group was also decreased in all cases as both urea and ornithine are derived from arginine (Fig. 2B). In addition to arginase, nitric oxide synthase (NOS) is a metabolic enzyme of arginine, and the citrulline/ornithine ratio is considered to be an index of their enzyme activity (Fig. 2C) [23]. The citrulline/ornithine ratio was elevated in all cases in the OMC group, although citrulline alone was not significantly altered (Fig. 2B, 2D). Therefore, the activity of NOS was increased in the OMC group, but there was no significant difference ($p = 0.352$, Supplementary 2). With regard to the other compound, no significant differences were observed (Fig. 2B).

The glycolysis and pentose phosphate pathway (PPP)

Next, we focused on glycometabolism. The glucose-6-phosphate (G6P)/ribose-5-phosphate (R5P) ratio was decreased in the OMC group than in the paired PC group, but there was no significant difference ($p = 0.204$, Fig. 3A and Supplementary 2). G6P was somewhat decreased in the OMC group, especially in case 3, whereas R5P was increased in the OMC group, particularly in case 5 (Fig. 3B). This suggested that the PPP was dominant in the OMC group. However, NADP⁺ and NADPH were almost identical in all of the samples (Supplementary 1).

Pyruvic acid is produced as a product of glycolysis and metabolized to lactic acid and acetyl-CoA. In cases 1, 3, and 5, lactic acid was remarkably decreased in the OMC group (Fig. 3B and Supplementary 1). Moreover, in all cases except case 4, the lactate/pyruvate ratio was decreased in the OMC group than in the paired PC group (Fig. 3C). However, due to individual differences, there was no statistically significant difference ($p = 0.248$). This suggested that the oxygen environment in the OMC group is different than that in PC group.

Amino acid composition

In addition to the PC and OMC groups, we evaluated metabolite differences by histological subtype. Comparing HGSOC (cases 1 and 2) with non-HGSOC (cases 3–5), HGSOC contained significantly more amino acids ($p = 0.004$, Fig. 4A). In all samples, glutamic acid was the major component, whereas glycine and alanine were also abundant (Fig. 4B). Notably, the proportion of glutamine was significantly higher in the HGSOC than in non-HGSOC samples ($p = 0.001$, Fig. 4B). As previously described, HGSOC samples contained more total amino acids, and thus, the absolute concentration of glutamine was also significantly higher (the HGSOC vs. non-HGSOC, $p = 0.004$). Therefore, glutathione metabolism-related amino acids were enriched in HGSOC than in non-HGSOC. (Fig. 4C). There are two forms of glutathione; a reduced form (GSH) and an oxidized disulfide form (GSSG). GSH is converted into GSSG during oxidative stress [24]. The HGSOC contained significantly more GSH than the non-HGSOC ($p = 0.025$), whereas no differences in GSSG were observed (Fig. 4D).

Ornithine, a metabolite of the urea cycle, is metabolized to polyamines including putrescine, spermidine, and spermine (Fig. 4E). The sum of these three polyamine forms was significantly higher in the HGSOC than in the non-HGSOC samples ($p = 0.048$, Fig. 4F).

Discussion

Metabolism represents the set of life-sustaining chemical reactions in cells and is essential for cancer progression. We evaluated metabolite profiles in advanced EOC tissues using metabolome analysis and analyzed alteration in energy metabolism.

Urea concentration was significantly decreased in the OMC group than in the PC group and alterations of the urea cycle were observed. The urea cycle is an essential pathway for the disposal of ammonia, and

enzymes of this cycle in non-hepatic cells are regulated by a variety of pro- and anti-inflammatory cytokines [25]. Thus, the relationship between the alteration of the urea cycle and cancer progression has been presented [26]. Our results indicated that the citrulline/ornithine ratio was increased in OMC and considered to reflect decreased arginase activity and increased NOS activity. As a result, NO was also considered to be increased. The function of NO is somewhat ambivalent, but NO primarily promotes cancer-related events, such as angiogenesis, apoptosis, and cell cycle [27]. Unfortunately, in this analysis, NO was not a target compound and further studies are needed to evaluate urea cycle dysfunction and NO during EOC progression.

Next, we focused on glycometabolism that is essential for cell survival and unique to cancer tissues according to Warburg [12-15]. In addition to glycolysis, the PPP is also a major pathway for glucose catabolism and plays an important role in cancer cell survival and growth [15, 28-30]. The PPP is classified into two biochemical branches known as the oxidative and non-oxidative PPP. In the former, the chemical reaction is irreversible and two molecules of NADPH are generated from one molecule of glucose, whereas, in the latter phase, the five-carbon sugar, R5P, is generated from a six-carbon glucose molecule. However, this phase is reversible (Fig. 3B). Therefore, depending on which phase of the PPP is activated, the production of NADPH and R5P is regulated [28-30]. Our results indicate that the OMC exhibited a low G6P/R5P ratio. Therefore, the PPP was dominant in the OMC and the demand for R5P and NADPH was increased. However, NADPH concentrations were similar in all of the samples. The consumption of NADPH may have been increased in the OMC due to oxidative stress. Otherwise, the OMC cells may produce more R5P than NADPH by activating the non-oxidative phase of the PPP. In addition, the proportion of cancer stem cells (CSCs) exhibiting enhanced PPP metabolism may be increased in the OMC tissues [28, 31]. Hence, the significance of the PPP in cancer progression should be reconsidered. Next, we evaluated glycolysis as the Warburg effect predicts that cancer cells use glycolysis even under aerobic conditions [12, 15]. We discovered that the lactate/pyruvate ratio was decreased in the OMC. This suggests that the oxygen environment is different at metastatic sites and cancer cells may change energy metabolism process based on their environment. Alteration of glycolysis between PC and OMC was also previously reported, although the detailed metabolic changes were not consistent with our results [32]. Therefore, the heterogeneity of cancer cells and individual differences may be significant and further larger-scale studies are needed.

We demonstrated that HGSOCs contained significantly more glutamine and other amino acids than non-serous carcinomas. Glutamine was the prominent amino acid and it has been reported that proliferating cells require glutamine since the 1950s [15, 33]. In addition, highly proliferative cells produce high levels of reactive oxygen species (ROS) [34]. To maintain the intracellular redox balance, NADPH and GSH play an important role and elevated GSH levels are observed in various tumor types [24, 35]. We observed high glutamine and GSH levels in HGSOC, which may reflect a high proliferative potential. Moreover, GSH is known to mediate resistance to platinum analogs through several mechanisms including increased DNA repair and the inhibition of apoptosis [35, 36]. Thus, GSH represents a potent therapeutic target and several inhibitors have been evaluated [35, 36]. Based on our results, the efficacy of GSH-targeted therapy should be separately evaluated for various histological subtypes.

Finally, we observed differences in polyamine concentrations among the histological subtypes. Polyamines are involved in many fundamental processes of cell growth and survival and polyamine metabolism is associated with cancer-driving pathways including the PTEN–PI3K–mTOR complex 1, WNT signaling, and RAS pathways [37]. In addition, a previous study reported that cycline E1-driven EOC was associated with activated polyamine synthesis and decreased cancer immunity [38]. Therefore, several polyamine-targeted drugs might exhibit efficacy in EOC, especially EGSOC [37].

This metabolome-based study has several limitations. First, detailed molecular biological changes such as genetic mutations, mRNA and protein expression, and enzyme activities were not evaluated. Second, lipidomics was not done, although it is an interesting approach because the omentum is characterized by an adipose-rich microenvironment. However, lipid metabolites were not measurable with of C-SCOPE. Therefore, these metabolomic changes related to those molecules are worth evaluating in the future.

Conclusion

Metabolome analysis revealed different metabolite profiles between PC and OMC, particularly in the urea cycle, glycolysis, and pentose phosphate pathways. Moreover, HGSOC contained more amino acids and GSH than non-serous carcinomas. These differences may reflect the diversity of biological processes in cancer cells. Metabolome analysis is expected to be a powerful tool for cancer biology.

List Of Abbreviations

EOC, Epithelial ovarian cancer; HGSOC, high-grade serous ovarian carcinoma; CCC, clear cell carcinoma; EC, endometrioid carcinoma; PC, primary cancer; OMC, omental metastatic cancer; NOS, nitric oxide synthase; PPP, pentose phosphate pathway; G6P, glucose-6-phosphate; R5P, ribose-5-phosphate; GSH, reduced glutathione; GSSG, oxidized glutathione; ROS, reactive oxygen species

Declarations

Ethics approval and consent to participate: The study protocol was approved by the Ethics Committee of our institution (approval No. 2017-0497). We obtained written informed consent from each patient.

Consent for publication: Not applicable

Availability of data and materials: All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests: The authors declare that they have no competing interests.

Funding: This study was supported by a Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research: Grant Number 19K18693.

Authors' contributions: KY and KK participated in the experiments, analyzed the data, and wrote the manuscript. NY designed the study and revised the manuscript. ST and AY interpreted the data. YI, KM, and KN collected cancer tissues. HK revised the manuscript and supervised the project. All authors read and approved the final manuscript.

Acknowledgements: This study was supported by the Program for Promoting the Enhancement of Research Universities as young researcher units for the advancement of new and undeveloped fields at Nagoya University. We thank Enago (<https://www.enago.jp/>) for English editing of this manuscript.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A: **Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.** *CA: a cancer journal for clinicians* 2018, **68**(6):394-424.
2. Nagase S, Ohta T, Takahashi F, Enomoto T: **Annual report of the committee on gynecologic oncology, the Japan Society of Obstetrics and Gynecology: Annual patients report for 2015 and annual treatment report for 2010.** *The journal of obstetrics and gynaecology research* 2019, **45**(2):289-298.
3. Cannistra SA: **Cancer of the ovary.** *The New England journal of medicine* 2004, **351**(24):2519-2529.
4. Jayson GC, Kohn EC, Kitchener HC, Ledermann JA: **Ovarian cancer.** *Lancet (London, England)* 2014, **384**(9951):1376-1388.
5. Lengyel E: **Ovarian cancer development and metastasis.** *The American journal of pathology* 2010, **177**(3):1053-1064.
6. Motohara T, Masuda K, Morotti M, Zheng Y, El-Sahhar S, Chong KY, Wietek N, Alsaadi A, Karaminejadranjbar M, Hu Z *et al*: **An evolving story of the metastatic voyage of ovarian cancer cells: cellular and molecular orchestration of the adipose-rich metastatic microenvironment.** *Oncogene* 2019, **38**(16):2885-2898.
7. Luo Z, Wang Q, Lau WB, Lau B, Xu L, Zhao L, Yang H, Feng M, Xuan Y, Yang Y *et al*: **Tumor microenvironment: The culprit for ovarian cancer metastasis?** *Cancer letters* 2016, **377**(2):174-182.
8. Rodriguez GM, Galpin KJC, McCloskey CW, Vanderhyden BC: **The Tumor Microenvironment of Epithelial Ovarian Cancer and Its Influence on Response to Immunotherapy.** *Cancers* 2018, **10**(8).
9. Thibault B, Castells M, Delord JP, Couderc B: **Ovarian cancer microenvironment: implications for cancer dissemination and chemoresistance acquisition.** *Cancer metastasis reviews* 2014, **33**(1):17-39.
10. Nieman KM, Kenny HA, Penicka CV, Ladanyi A, Buell-Gutbrod R, Zillhardt MR, Romero IL, Carey MS, Mills GB, Hotamisligil GS *et al*: **Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth.** *Nature medicine* 2011, **17**(11):1498-1503.
11. Beger RD: **A review of applications of metabolomics in cancer.** *Metabolites* 2013, **3**(3):552-574.
12. Warburg O: **On the origin of cancer cells.** *Science (New York, NY)* 1956, **123**(3191):309-314.

13. Lunt SY, Vander Heiden MG: **Aerobic glycolysis: meeting the metabolic requirements of cell proliferation.** *Annual review of cell and developmental biology* 2011, **27**:441-464.
14. Vander Heiden MG, Cantley LC, Thompson CB: **Understanding the Warburg effect: the metabolic requirements of cell proliferation.** *Science (New York, NY)* 2009, **324**(5930):1029-1033.
15. Counihan JL, Grossman EA, Nomura DK: **Cancer Metabolism: Current Understanding and Therapies.** *Chemical reviews* 2018, **118**(14):6893-6923.
16. Yoshida K, Yokoi A, Kato T, Ochiya T, Yamamoto Y: **The clinical impact of intra- and extracellular miRNAs in ovarian cancer.** *Cancer science* 2020, **111**(10):3435-3444.
17. Ter Kuile BH, Westerhoff HV: **Transcriptome meets metabolome: hierarchical and metabolic regulation of the glycolytic pathway.** *FEBS letters* 2001, **500**(3):169-171.
18. Griffin JL, Shockcor JP: **Metabolic profiles of cancer cells.** *Nature reviews Cancer* 2004, **4**(7):551-561.
19. Plewa S, Horala A, Derezinski P, Klupczynska A, Nowak-Markwitz E, Matysiak J, Kokot ZJ: **Usefulness of Amino Acid Profiling in Ovarian Cancer Screening with Special Emphasis on Their Role in Cancerogenesis.** *International journal of molecular sciences* 2017, **18**(12).
20. Bachmayr-Heyda A, Aust S, Auer K, Meier SM, Schmetterer KG, Dekan S, Gerner C, Pils D: **Integrative Systemic and Local Metabolomics with Impact on Survival in High-Grade Serous Ovarian Cancer.** *Clinical cancer research : an official journal of the American Association for Cancer Research* 2017, **23**(8):2081-2092.
21. Hilvo M, de Santiago I, Gopalacharyulu P, Schmitt WD, Budczies J, Kuhberg M, Dietel M, Aittokallio T, Markowitz F, Denkert C *et al*: **Accumulated Metabolites of Hydroxybutyric Acid Serve as Diagnostic and Prognostic Biomarkers of Ovarian High-Grade Serous Carcinomas.** *Cancer research* 2016, **76**(4):796-804.
22. Zhang F, Zhang Y, Ke C, Li A, Wang W, Yang K, Liu H, Xie H, Deng K, Zhao W *et al*: **Predicting ovarian cancer recurrence by plasma metabolic profiles before and after surgery.** *Metabolomics : Official journal of the Metabolomic Society* 2018, **14**(5):65.
23. Jung C, Gonon AT, Sjoquist PO, Lundberg JO, Pernow J: **Arginase inhibition mediates cardioprotection during ischaemia-reperfusion.** *Cardiovascular research* 2010, **85**(1):147-154.
24. Bansal A, Simon MC: **Glutathione metabolism in cancer progression and treatment resistance.** *The Journal of cell biology* 2018, **217**(7):2291-2298.
25. Morris SM, Jr.: **Regulation of enzymes of the urea cycle and arginine metabolism.** *Annual review of nutrition* 2002, **22**:87-105.
26. Keshet R, Szlosarek P, Carracedo A, Erez A: **Rewiring urea cycle metabolism in cancer to support anabolism.** *Nature reviews Cancer* 2018, **18**(10):634-645.
27. Choudhari SK, Chaudhary M, Bagde S, Gadbail AR, Joshi V: **Nitric oxide and cancer: a review.** *World journal of surgical oncology* 2013, **11**:118.
28. Ghanbari Movahed Z, Rastegari-Pouyani M, Mohammadi MH, Mansouri K: **Cancer cells change their glucose metabolism to overcome increased ROS: One step from cancer cell to cancer stem cell?**

29. Patra KC, Hay N: **The pentose phosphate pathway and cancer.** *Trends in biochemical sciences* 2014, **39**(8):347-354.
30. Jiang P, Du W, Wu M: **Regulation of the pentose phosphate pathway in cancer.** *Protein & cell* 2014, **5**(8):592-602.
31. Debeb BG, Lacerda L, Larson R, Wolfe AR, Krishnamurthy S, Reuben JM, Ueno NT, Gilcrease M, Woodward WA: **Histone deacetylase inhibitor-induced cancer stem cells exhibit high pentose phosphate pathway metabolism.** *Oncotarget* 2016, **7**(19):28329-28339.
32. Fong MY, McDunn J, Kakar SS: **Identification of metabolites in the normal ovary and their transformation in primary and metastatic ovarian cancer.** *PloS one* 2011, **6**(5):e19963.
33. Eagle H, Oyama VI, Levy M, Horton CL, Fleischman R: **The growth response of mammalian cells in tissue culture to L-glutamine and L-glutamic acid.** *The Journal of biological chemistry* 1956, **218**(2):607-616.
34. Lieu EL, Nguyen T, Rhyne S, Kim J: **Amino acids in cancer.** *Experimental & molecular medicine* 2020, **52**(1):15-30.
35. Traverso N, Ricciarelli R, Nitti M, Marengo B, Furfaro AL, Pronzato MA, Marinari UM, Domenicotti C: **Role of glutathione in cancer progression and chemoresistance.** *Oxidative medicine and cellular longevity* 2013, **2013**:972913.
36. Nunes SC, Serpa J: **Glutathione in Ovarian Cancer: A Double-Edged Sword.** *International journal of molecular sciences* 2018, **19**(7).
37. Casero RA, Jr., Murray Stewart T, Pegg AE: **Polyamine metabolism and cancer: treatments, challenges and opportunities.** *Nature reviews Cancer* 2018, **18**(11):681-695.
38. Guo T, Li B, Gu C, Chen X, Han M, Liu X, Xu C: **PGC-1alpha inhibits polyamine metabolism in Cyclin E1-driven ovarian cancer.** *Cancer medicine* 2019.

Tables

Table 1. Patients' characteristics

No.	Age	Stage	Histology	NAC
1	71	IIIC	HGSOC	No
2	57	IIIC	HGSOC	No
3	50	IIIC	CCC	Yes
4	67	IIIC	CCC	No
5	63	IVB	EC	No

HGSOC, high-grade serous ovarian carcinoma; CCC, clear cell carcinoma; EC, endometrioid carcinoma; NAC, neoadjuvant chemotherapy

Figures

Figure 1

Metabolome analysis for primary ovarian carcinoma (PC) and omental metastatic cancer (OMC) tissues. (A) Heatmap and (B) principal component analysis generated from the metabolome analysis. HGSOC, high-grade serous ovarian carcinoma; CCC, clear cell carcinoma; EC, endometrioid carcinoma.

Figure 2

Metabolomic profiles related to the urea cycle. (A) Changes in absolute urea concentration between primary ovarian carcinoma (PC) and omental metastatic carcinoma (OMC). (B) Metabolite changes related to the urea cycle. (C) Schema of two metabolic pathways of arginine. (D) Changes in the citrulline/ornithine ratio between primary and omental metastatic cancers. Error bars represent standard errors of the mean. N.D., not detected.

Figure 3

Metabolomic profiles related to the glycolysis and the pentose phosphate pathway. (A) Changes in the glucose-6-phosphate (G6P)/ribose-5-phosphate (R5P) ratio between primary ovarian carcinoma (PC) and omental metastatic carcinoma (OMC). (B) Metabolite changes related to the glycolysis and the PPP. (C) Changes in the lactate/pyruvate ratio between PC and OMC. Error bars represent standard errors of the mean. F6P, fructose 6-phosphate; F1,6P, fructose 1,6-bisphosphate; GAP, glyceraldehyde-3-phosphate; Ru5P, ribulose 5-phosphate; X5P, D-xylulose 5-phosphate; S7P, sedoheptulose 7-phosphate; E4P, erythrose 4-phosphate.

Figure 4

Metabolite differences based on histological subtype. (A) Absolute total amino acid concentration. (B) The proportions of amino acids. The top ten amino acids are shown. (C) Schema of glutathione metabolism. (D) Absolute glutathione (GSH/GSSG) concentration. (E) Schema of polyamine metabolism. (F) Absolute polyamine concentration. HGSOC, high-grade serous ovarian carcinoma; PC, primary ovarian carcinoma; OMC, omental metastatic carcinoma.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supple1.xlsx](#)
- [Supple2.xlsx](#)