Significance of Platinum Distribution to Predicts Platinum Resistance in Ovarian Cancer After Platinum Treatment in Neoadjuvant Chemotherapy

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Abstract

Most advanced ovarian cancer patients experience recurrence and develop resistance to platinum-based agents. However, the diagnosis of platinum resistance based on platinum-free interval is not always accurate and timely. In this study, we employed laser ablation inductively coupled plasma mass spectrometry to visualize platinum distribution in the tissues at the time of interval debulking surgery following neoadjuvant chemotherapy. Twenty seven patients with advanced high grade serous ovarian cancer were enrolled. Two distinct patterns of platinum distribution were observed. Type A (n = 16): platinum accumulation at the adjacent stroma but little in the tumor; type B (n = 11): even distribution of platinum through tumor and adjacent stroma. Type A was significantly correlated with worse prognosis ($P = 0.031$). Patients classified in type A and treated with platinum-based adjuvant chemotherapy after operation were significantly shorter period of recurrence after last platinum-based chemotherapy ($P = 0.020$) and diagnosed with “platinum-resistant recurrence”. Treatment with non-platinum-based chemotherapy after operation could be effective for the patients who were classified in type A. Our data indicate that the platinum resistance can be predicted prior to recurrence with platinum distribution. Thus, we will be able to select more appropriate adjuvant chemotherapy, which may possibly lead to improve patient’s prognosis.

Introduction

Approximately 70% of ovarian cancer cases are diagnosed at an advanced stage with peritoneal dissemination¹. High grade serous ovarian carcinoma (HGSOC) is the most common subtypes. Primary debulking surgery followed by adjuvant chemotherapy is the standard treatment and neoadjuvant chemotherapy (NAC) followed by interval debulking surgery (IDS) is an alternative option (NAC-IDS)². Most cases of HGSOC are sensitive to initial platinum-based agents³. More than 70% of patients can achieve complete remission^{2,3}. However, nearly 80% of the patients experience recurrence and become resistant to platinum-based agents; this is related to poor long-term survival^{3,4}.

In clinical settings, the recurrence within 6 months after the last dose of platinum-based chemotherapy is referred to “platinum-resistant recurrence”. The interval between the date of the last platinum use and the date of relapse detection is also known as platinum-free interval (PFI)^{1,4}. Whether or not to use platinum-based agents at recurrence depends on PFI. The problem with this strategy is that it allows for the diagnosis of platinum resistance only at the time of tumor growth^{1,4,5}. Moreover, PFI is often inconsistent with true platinum resistance, that is because PFI does not always reflect molecular mechanisms^{5,6}. Novel biomarkers of platinum resistance are needed so that more appropriate drugs can be used earlier in adjuvant treatment, which could prolong survival and minimize adverse effects caused by platinum-based agents⁶.

The intracellular platinum concentration has been reported to be correlated with platinum sensitivity in lung, bladder, and ovarian cancer cell lines^{7–10}. Inductively coupled plasma mass spectrometry (ICP-MS)

can detect the platinum concentration in dissociated cells with nitric acid. Thus far, it is impossible to distinguish between platinum distributions in the tumor and stroma⁸. Recently, laser ablation ICP-MS (LA-ICP-MS) has been developed, that has a focused laser ablation system combined with ICP-MS¹¹ (Fig. 1). LA-ICP-MS is used to identify the distribution of trace elements in tissue and a few reports have shown platinum in cancer after using platinum-based agents^{12–16}. However, to our knowledge, no reports on the LA-ICP-MS analysis of platinum distribution to clarify drug resistance and prognosis have been published. The goal of this study is to identify the significance of platinum distribution using LA-ICP-MS to predict of platinum resistance and cancer prognosis at the time of NAC-IDS.

Results

Classification of NAC-IDS samples using LA-ICP-MS

First, primary debulking surgery specimens without usage of platinum-based agents were analyzed as a negative control (n=3). Created images using iQuant 2+ could be compared the H&E staining samples (**Fig. 2A**). The LA-ICP-MS revealed a high phosphorus and zinc content in tumor area, but platinum was not present in both the tumor and the stroma (**Fig. 2A, Table. 1**). Next, as positive control, clinically platinum-resistant recurrent ovarian cancer tissues were evaluated because of no response to platinum-based agents. In this case, platinum was not found in the tumor area and platinum was accumulated in the adjacent stroma (**Fig. 2B**).

Then, a total of 27 NAC-IDS specimens were classified into types A and B according to the distribution of platinum by LA-ICP-MS (**Fig. 3**). Representative images of each type are shown in **Fig. 4A**. Platinum counts in type A were significantly higher in the adjacent stroma compared to the tumor (**Fig. 4B, 4C**). The pattern of platinum distribution of type A was relatively the same of clinically platinum-resistant tumor as a positive control shown in Fig. 2B. By contrast, in type B, no apparent difference of platinum counts was observed between the tumor and the adjacent stroma (**Fig. 4B, 4C**).

The type of platinum accumulation at the adjacent stroma was related to platinum-resistant recurrence and poor prognosis

A total of 27 patients were enrolled to this study. **Table 2.** lists all these patient's background. The NAC cycles and duration from last NAC to IDS were 3–10 cycles and 23–53 days, respectively. In most cases, CA125 was decreased from diagnosis to surgery. Observational periods were 37-103 months.

Of the 27 cases, 16 (59.2%) were type A and the other 11 (40.7%) were type B. The patient's characteristics of both groups are presented in **Table 3**. No significant differences between two types were observed in age, NAC courses, duration from NAC to IDS, CA125 levels at diagnosis, and the rate of complete surgery. In terms of recurrence, no patients in type B experienced recurrence within 6 months from the last platinum-based drug. Conversely, 9 of the 16 (56.3%) type A patients experienced recurrence within 6 months (TFI < 6 moths). Among 16 patients, though 11 were treated with platinum-based agents in adjuvant chemotherapy, 4 were treated with non-platinum-based chemotherapy because

of clinical diagnosis of platinum resistance from pathological findings. 8 of 9 (88.9%) patients, whose PFI was within 6 months, were treated with the same platinum-based agents even in adjuvant chemotherapy. On the other hand, 3 of 4 patients in type A treated with non-platinum-based agents after IDS had TFI over 6 months. The Kaplan–Meier analysis did not reveal statistical difference in TFI between the type A and B patients (supplemental **Fig. 1A**, $P = 0.160$). However, the Kaplan–Meier analysis revealed significantly difference between the type A and B only in patients who were treated with platinum-based agents after operation (**Fig. 4D**, $P = 0.020$).

With respect to OS, all patients except 1 in type B lived over 3 years after diagnosis, whereas 8 of 16 patients in type A died within 3 years after diagnosis. As a result, type A had a significantly worse prognosis (**Fig. 4E**, $P = 0.031$). Comparing in type A, patients treated with non-platinum-based agents in adjuvant chemotherapy had tendency of longer PFI, though very small number (supplemental **Fig. 1B**, $P = 0.070$).

Lastly, we have analyzed the distribution of platinum in metastasis in type A patients. Accumulation of platinum in the adjacent stroma and little exist in tumor was also recognized in matched disseminated tumors, which were same distribution of primary tumor (**Fig. 5A, 5B** and **5C**, $n = 4$).

Discussion

In this study, we report the novel findings that LA-ICP-MS clearly revealed platinum distribution in NAC-IDS samples in ovarian cancer patients and that there are two characteristic patterns of platinum distribution, indicating platinum resistance and prognosis prior to recurrence. Platinum distribution using LA-ICP-MS can be a reasonable biomarker of platinum resistance at the time of NAC-IDS in patients with advanced ovarian cancer and useful information to select appropriate chemotherapy.

Platinum resistance is a major problem in ovarian cancer treatment^{1,3,5}. Currently, platinum resistance is defined based on PFI at the time of recurrence^{1,5,17}. This means that platinum resistance cannot be determined until recurrence. That's why platinum-based drugs are often used until disease (Supplementary **Fig. S2**). In this study, we analyzed NAC-IDS specimens using LA-ICP-MS and demonstrated that platinum could be detected in the cancer tissues and that the distribution of platinum was quite different between the tumor and the adjacent stroma. The pattern of platinum distribution was significantly associated with platinum resistance and long-term survival. According to these results, we speculated the possibility to predict the future acquisition of platinum-resistant recurrence at the time of IDS and to segregate a group of patients with a poor prognosis due to acquired platinum resistance.

LA-ICP-MS analysis revealed that type A patients showed a distinctive distribution of platinum, which accumulated in the adjacent stroma of the cancer tissues (**Fig. 4A**). Various studies on platinum resistance demonstrated that one of the most reliable mechanisms is platinum uptake reduction or enhanced extracellular efflux of platinum through transporters^{1,5,9,18,19}. However, previous studies have not been able to directly prove the distribution of platinum in cancer tissues. Considering that platinum

cannot enter into the tumor or is effluxed from the tumor leads to its accumulation at the tumor margins, the platinum distribution in type A with accumulation at the tumor margin is consistent with these mechanisms. To our best knowledge, this is the first study to identify the characteristic patterns of platinum distribution in cancer tissues. The platinum distribution by LA-ICP-MS can be a promising biomarker of platinum resistance in the treatment of ovarian cancer.

The TFI was significantly shorter in the type A than the type B, which is thought to be indicative of acquired platinum resistance in the patients treated with platinum-based agents in adjuvant chemotherapy (Fig. 4D, $P = 0.020$). In type A patients, the recurrence rate within 6 months, which is the current clinical definition of “platinum-resistant recurrence,” was 56.3% (9/16). 88.9% (8/9) of these were treated with the platinum-based agents which were same regimen even in adjuvant chemotherapy. This result may indicate the negative effect of same platinum-based agents and potential efficacies of non-platinum-based agents on TFI prolongation in type A patients, who have potentially acquired platinum resistance.

OS was also significantly worse in type A than in type B (Fig. 4E, $P = 0.031$). This reason can be because platinum-based drugs are often used for type A patients in the current clinical settings since no tool exists to determine platinum resistance at the time of IDS. Actually, 6 of 8 (75.0%) of the type A patients treated with the same preoperative platinum-based agents in postoperative treatment died within two years. The method of visualizing platinum distribution by LA-ICP-MS may serve as a better biomarker of platinum resistance and propose a better strategy for patients with acquired platinum resistance.

Platinum distribution in the peritoneal dissemination had the same pattern as that in the matched primary tumor (Fig. 5). The primary site (ovaries) is removed at the time of IDS, but the disseminated tumor sometimes remains. Over 70% of patients with advanced ovarian cancer experience recurrence in the abdomen, which is the most common cause of death in ovarian cancer patients. Therefore, even if the tumor is thought to be completely removed, most patients are likely to be treated with additional anticancer drugs after surgery^{1,5,19}. The results of LA-ICP-MS indicated that the pattern of platinum distribution in the disseminated lesions was the same as that of the primary tumor.

Analysis of NAC-IDS specimens using LA-ICP-MS can provide us with reliable information that could be used to determine postoperative ovarian cancer treatment. We suggest new ovarian cancer treatment strategy using LA-ICP-MS (Fig. 6). Using LA-ICP-MS analysis after platinum-based agents, we can segregate patients with poor prognosis in current clinical settings and choose appropriate anti-cancer agents. This strategy can improve their prognosis.

There are some limitations of this study. Firstly, only 27 patients from a single institution were included, and underlying biases in our analysis could not be controlled due to the retrospective study design. Prospective larger-scale studies are needed to verify whether treatment strategies that incorporate LA-ICP-MS prolong the prognosis. In this study, we have analyzed only the tissues of ovarian cancer, though platinum-based agents are widely used in various tumors. In addition, the area that can be analyzed by

LA-ICP-MS is limited. In the present study, analysis was performed using tissues merely from the border of the tumor and stroma.

In conclusion, the distribution of platinum was clearly visualized by LA-ICP-MS. The duration to subsequent recurrence was significantly shorter in the type A group, with lower concentration of platinum in the tumor than the adjacent stroma. LA-ICP-MS is a powerful tool to predict platinum resistance and long-term survival. In the future, analysis of NAC-IDS specimens using LA-ICP-MS can offer guidance toward more appropriate adjuvant anticancer treatment and can change the treatment strategy for patients with advanced ovarian cancer.

Methods

Ovarian cancer samples for LA-ICP-MS analysis

Our study was approved by the Ethics Committee at Nagoya University (No. 2017-0497). During January 2013 to December 2018, a total 308 patients who were suspected with ovarian malignancy and received operation at our institution were enrolled. Among of them, 171 patients were diagnosed with epithelial ovarian cancer. Twenty-seven patients with HGSOc in stage III or IV according to FIGO 2014 treated with NAC-IDS including platinum-based agents were analyzed in this study. Two patients were excluded due to outcome unknown and 4 were also excluded because of no remnant tumor cells. To analyze both primary and disseminated lesion, 4 patients whose tumor existed in both ovary and outside ovary were included (**Fig. 3**). The clinical information including age, NAC cycles, duration from last NAC to IDS, CA125 values at diagnosis and before IDS, interval from last chemotherapy to recurrence or death (defined as the treatment-free interval; TFI), and overall survival (OS), defined as the time from diagnosis to death, was retrospectively collected. In 4 cases, disseminated lesions in IDS were also included in the study. Tissue samples removed by debulking surgery without usage of platinum-based agents were used as a negative control, and we used clinically platinum-resistant recurrent samples as a positive control which were resistant to platinum-based agents after recurrence, then resected by surgery.

LA-ICP-MS

For analysis, we used a CETAC LSX-213 G2+ laser ablation system, which is equipped with frequency quintupled 213 nm Quantel Ultra Compact Q-Switched Nd: YAG MIL-SPEC Laser and coupled to an Agilent 7700X ICP-MS instrument (**Fig. 1**). The laser ablated the samples at a 20 μ m diameter spot on the surface. The detailed instrumental parameters are summarized in **Table 1**.

Sample preparation and classification of LA-ICP-MS images

The marginal area of the cancer tissue where the tumor and the adjacent stroma meet was analyzed. The analyzed samples were sliced into 4-6 μ m sections from formalin-fixed paraffin embedded blocks. The counts of ^{31}P , ^{195}Pt and ^{66}Zn were adjusted ^{13}C counts to make a per-specimen correction for the amount of laser-evaporated tissue. Localized images of these trace elements were generated in iQuant 2+²⁰. The

counts of ^{195}Pt and ^{31}P were performed as an average of 20 randomly selected spots from the tumor and the stromal sites.

The specimens analyzed by LA-ICP-MS were classified into two types, type A and type B, based on the distribution in the images and platinum counts, as follows: Type A refers to specimens with low platinum counts in the tumor but with the high platinum counts in the adjacent stroma, whereas type B refers to specimens with the comparable platinum counts both in the tumor and adjacent stroma. Type A was defined as the pattern that platinum counts ratio of stroma to tumor is over 2. Type B was the pattern that the ratio is 2 or less. We also compared platinum distribution pattern between primary tumors and matched disseminated tumors in type A patients.

Statistical analysis

Non-normally distributed data are reported as medians and minimum and maximal ranges. Categorical variables are presented numerically and as percentages. Non-normally distributed data were analyzed with non-parametric tests. A Kaplan-Meier survival analysis was used for the analyses of TFI and OS. The probability of significance was set at $P < 0.05$. All statistical analyses were performed using the IBM SPSS software package (version 27.0, IBM Corp., Armonk, NY, USA).

Abbreviations

Pt: platinum, LA-ICP-MS: laser ablation inductively coupled plasma mass spectrometry, HGSOC: high grade serous ovarian carcinoma, IDS: interval debulking surgery, NAC: neoadjuvant chemotherapy, PFI: platinum-free interval, TFI: treatment-free interval, OS: overall survival

Declarations

Authors' Contributions

Conception and design: K.U, N.Y, A.T, S.O, F.K, H.K

Development of methodology: K.U, N.Y, A.T, S.O, M.K

Analysis and interpretation of data (statistical analysis, biostatistics, computational analysis): K.U, N.Y, M.Y, K.K, S.I, K.M, M.S, Y.K

Writing, review, and/or revision of the manuscript: K.U, N.Y, A.T, S.O, M.Y, K.K, S.I, K.M, M.S, Y.K, S.T, Y.I, A.Y, M.K, F.K, H.K

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Conflicts of Interests:

The authors declare no potential competing interests.

Ethics approval and consent to participate

Our study was approved by the Ethics Committee at Nagoya University (No. 2017-0497). We have got written informed consents from all the participants. The study was performed in accordance with the Declaration of Helsinki.

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Tables

Table 1. Operational condition of LA-ICP-MS

Laser ablation system

Type	CETAC LSX-213 G2+
Wave length	213 nm
Pulse duration	< 5ns
Laser repetition rate	20 Hz
Laser spot size	20 µm
Laser energy	5.1 J/cm ² (25%)
Laser scan speed	20 µm/s
Laser beam geometry	circular
Helix He gas flow	0.225 flow rate
Innercup He gas flow	0.20 flow rate

Inductively coupled plasma mass spectrometry

Type	Agilent 7700X
Plasma power	1550W
Carrier Ar gas glow	1.07 flow rate
Collision cell gas	He
Collision cell gas flow	5.0 flow rate
Cone	Ni
Acquisition element	¹³ C, ³¹ P, ¹⁹⁵ Pt, ⁶⁶ Zn

Abbreviations: LA-ICP-MS, laser ablation inductively coupled plasma mass spectrometry; He, Helium; Ni, nickel; C, carbon; P, phosphorus; Pt, platinum; Zn, zinc

Table. 2 Patients background

Charateristics	Number or median	Percentage or range
Total patients	27	
Age at diagnosis	61	41-75
Clinical stage		
IIIC	18	66.7%
IVA	1	3.7%
IVB	8	29.6%
NAC cycle (times)	6	3-10
Duration from last NAC to IDS (days)	35	23-53
Complete surgery	18	66.7%
Adjuvant chemotherapy		
Platinum-based	21	77.8%
Non-platinum based	4	14.8%
No adjuvant therapy	2	7.4%
CA125 level (U/mL)	1,881	142-17,434
At diagnosis		
Before operation	26	8.2-587
Recurrence	24	88.9%
Death	19	70.3%

Abbreviations: NAC, neo-adjuvant chemotherapy; IDS, interval-debulking surgery

Table 3. Patients characteristics of both groups

	Type A (n = 16)	Type B (n = 11)	P-value
Age at diagnosis	61.5 (41-75)	60.0 (41-71)	0.394
NAC course (times)	5.5 (3-9)	6.0 (3-10)	0.121
Duration from NAC to IDS (days)	35.0 (23-50)	36.0 (24-53)	0.645
CA125 at daignosis (U/mL)	3,208 (161-17,434)	1,640 (142-6,220)	0.368
CA125 before operation (U/mL)	58.6 (8.2-587)	15.0 (11-34)	0.023
Complete surgery	10/16 (62.5%)	8/11 (72.7%)	0.692

Abbreviations: NAC, neo-adjuvant chemotherapy; IDS, interval-debulking surgery

Figures

Figure 1

Simple image of LA-ICP-MS. FFPE samples inserted into HelEx cell box are ablated by focused laser. The ablated samples are carried through the aerosol rapid introduction system (ARIS) to ICP-MS.

Figure 2

Phosphorus and platinum distribution in clinical samples using laser ablation ICP-MS (LA-ICP-MS). A, Representative images of LA-ICP-MS of high grade serous ovarian cancer in primary debulking surgery as negative control of platinum and phosphorus and zinc distribution in tumor. Scale bar; 200 μm . B, Images of phosphorus and platinum distribution in clinically platinum-resistant recurrent ovarian tumor. Platinum was not present in the tumor and accumulated in the borderline area of tumor and stroma. Scale bar; 200 μm .

Figure 3

Flowchart of patient inclusion.

Figure 4

Platinum distribution and survival in ovarian cancer patients. A, Representative images of type A and B. In type A, little platinum accumulation in the tumor but accumulation in stroma. In type B, platinum existed also in the tumor. Scale bar; 200 μ m. B, ¹⁹⁵Pt counts in tumor and stroma in both types. ¹⁹⁵Pt counts in tumor are in type A was significantly lower than that of stroma. C, Platinum counts in stroma in type A is more than twice higher than that of tumor. D, Kaplan-Meier analysis about Treatment-free interval in both groups in patients treated with platinum-based agents in adjuvant chemotherapy. Type A patients significantly earlier recurrence and most of them were diagnosed platinum-resistant recurrence ($P = 0.020$). E, Kaplan-Meier analysis about overall survival in both groups. Type A patients had shorter survival than type B ($P = 0.031$).

Figure 5

Platinum distribution of primary tumor and disseminated tumor. A, Representative images of abdominal dissemination tumor in patients in type A. Platinum was accumulated in marginal area in tumor and stroma and little existed in the tumor. Scale bar; 200 μm . B, Platinum distribution of matched primary tumor (ovary) of type A patients. Little platinum existed in the tumor compared with the stroma. Scale bar; 200 μm . C, Platinum counts in tumor and stroma in disseminated tumors (n=3).

Figure 6

New ovarian cancer treatment strategy using LA-ICP-MS. LA-ICP-MS reveal platinum resistant in NAC-IDS samples before recurrence. Since type A patients are worse prognosis when using the same platinum-based agents in adjuvant chemotherapy, we suggest for patients classified type A to change to non-platinum-based adjuvant chemotherapy to improve their disease progression and prognosis and to decrease side effects with inefficient platinum-based agents.

Supplementary Files

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