

Adenosine triphosphate-based tumor chemosensitivity assay may predict the clinical outcomes of gastric cancer patients receiving taxane-based post-operative adjuvant chemotherapy

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To the Editor: Gastric cancer is one of the most commonly diagnosed cancers in the world. For patients with a pathological tumor-node-metastasis stage of II or III, post-operative adjuvant chemotherapy is generally required to reduce recurrence risk by controlling residual tumor cells following curative resection.^[1] Although taxanes have recently shown promising activity in gastric cancer,^[2,3] non-responders may incur costs and experience adverse reactions without clinical benefit, and efforts to select effective regimens for individuals are very important. The application of *in vitro* chemosensitivity assays as predictive markers in personalized cancer treatment has been investigated in several studies.^[4] However, the heterogeneity of the gastric cancer response to taxane-based chemotherapy and the correlation between *in vitro* chemosensitivity and clinical outcomes remain unclear.

In this study, the chemosensitivity profiles of gastric cancer were evaluated in 638 patients using an adenosine triphosphate-based tumor chemosensitivity assay (ATP-TCA), which is a feasible method to study the chemosensitivity of individuals *in vitro* by measuring the intracellular adenosine triphosphate (ATP) levels in drug-exposed primary tumor cells.^[4,5] Furthermore, 59 patients who received standard gastrectomy and taxane-based post-operative chemotherapy regimens were followed up to obtain overall survival (OS) and disease-free survival (DFS) data, and Kaplan–Meier analysis was used to study the correlation between the ATP-TCA results and clinical outcomes. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 *Declaration of Helsinki* and its later amendments, and written informed consent was obtained from each patient. This study was approved by the Research Ethics Board of the First Affiliated Hospital

of Soochow University (No. 2020140). The flow chart of the study is shown in Supplementary Figure 1A, <http://links.lww.com/CM9/B87>. This retrospective study may provide more evidence for the application of ATP-TCA to guide personalized treatment for patients with gastric cancer.

The chemosensitivity assay was conducted for patients who received surgical treatment and with pathologically confirmed gastric cancer in the hospital from January 2014 to December 2018. The characteristics of the patients are shown in Supplementary Table 1, <http://links.lww.com/CM9/B87>. The gastric cancer specimens were treated with five chemotherapy regimens, namely, paclitaxel (PTX) + oxaliplatin (L-OHP), docetaxel (TXT) + L-OHP, 5-fluorouracil (5-FU) + L-OHP, 5-FU + irinotecan (SN-38), and 5-FU + etoposide (VP-16), and the response was assessed using ATP-TCA method. In brief, tumor specimens from every patient were collected in a sterile container with RPMI 1640 medium (Gibco, USA), and cells were immediately isolated under sterile conditions. Cell suspensions were seeded into 96-well plates with different chemotherapeutic agents at five different dilutions (12.5%, 25%, 50%, 100%, and 200%) of the plasma peak concentrations of the conventional clinical dose according to the manufacturers' protocols or Pharmacopeia [Supplementary Table 2, <http://links.lww.com/CM9/B87>]. Cells seeded without drug treatment or blank complete assay medium without cells were used as controls. The cells were incubated for 5 to 7 days and then lysed for ATP quantification by a luciferin-luciferase luminescence reaction using a microplate luminometer (Berthold Detection Systems, Dettenheim, Germany). The 90% and 50% inhibitory concentration (IC₉₀ and IC₅₀) values were calculated, and patients were divided into sensitive or resistant groups accordingly.^[5]

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Heterogeneity and multiple drug resistance were observed in 638 specimens [Supplementary Figure 1B, <http://links.lww.com/CM9/B87>]. The ATP-TCA results of 638 specimens tested in the study showed that the specimens were significantly more sensitive to PTX + L-OHP and TXT + L-OHP regimens than to the other regimens without taxanes. The sensitivity to the five chemotherapy regimens was ranked as follows: PTX + L-OHP (76.5%) > TXT + L-OHP (64.3%) > 5-FU + VP-16 (25.1%) > 5-FU + L-OHP (7.5%) > 5-FU + SN-38 (5.3%) [Supplementary Table 3, <http://links.lww.com/CM9/B87>]. Among the 638 specimens, 121 specimens (19.0%) were resistant to all five chemotherapy regimens, and 454 specimens (71.2%) were resistant to the three chemotherapy regimens except for PTX + L-OHP and TXT + L-OHP. In addition, the sensitivity to paclitaxel and docetaxel was found to be highly consistent in individual patients. A total of 539 specimens (84.5%) had consistent sensitivity to PTX + L-OHP and TXT + L-OHP. Of these specimens, 410 (64.3%) were sensitive to both PTX + L-OHP and TXT + L-OHP regimens, 129 (20.2%) were resistant to both regimens, and only 99 (15.5%) had inconsistent sensitivity between the two taxane-based regimens, which suggested cross-resistance to paclitaxel and docetaxel in patients with gastric cancer.

Furthermore, survival assessment was conducted for the patients who (1) underwent standard gastrectomy with D2 lymph node dissection; (2) had histologically confirmed stage II or stage III gastric cancer with no invasion of adjacent structures/organs or distant metastasis; and (3) received at least one cycle of taxane-based chemotherapy. Patients who underwent radiotherapy, targeted therapy, or pre-operative chemotherapy were excluded from the survival assessment. Patients eligible for survival assessment were divided into sensitive and resistant subgroups according to the ATP-TCA results after being divided into stage II or stage III groups based on histological results. The incidence of recurrence or death was obtained by means of follow-up with regular examination or by telephone or by means of the database of the Public Security Bureau System. OS and DFS were measured from the date of surgery until the date of death or disease progression. If no such event occurred, the date of the last follow-up was used as the end point of survival assessment (censored data).

Among the 638 patients with ATP-TCA results, 59 patients who underwent standard gastrectomy and received chemotherapy regimens of taxanes combined with fluoropyrimidines or oxaliplatin without radiotherapy or targeted therapy were eligible for the survival assessment. Among the 37 patients with stage III disease, 26 (70%) were sensitive to taxane-based regimens *in vitro*, and 11 (21%) were resistant. Of the 22 patients with stage II disease, 16 (73%) were sensitive and 6 (27%) were resistant to taxane-based regimens *in vitro*. The *in vitro* chemosensitivity of the patients included in the survival assessment to taxane-based regimens was consistent with that of the total 638 patients, which suggested that the patients who were followed up were well representative of

the population. There is no significant difference in the demographic characteristics of patients between the sensitive and resistant groups [Supplementary Table 4, <http://links.lww.com/CM9/B87>]. The clinical information of the patients is shown in Supplementary Table 5, <http://links.lww.com/CM9/B87>.

Based on the follow-up data until January 2020, the median follow-up period from the time of surgery was 37 months (4–72 months). Disease progression occurred in 14 patients (38%) with histological stage III disease and two patients (9%) with stage II disease, among whom ten patients (27%) with stage III disease and one patient (5%) with stage II disease died. The Kaplan–Meier analysis showed that compared with patients whose specimens were determined to be resistant to taxanes by ATP-TCA, patients who showed drug sensitivity had longer OS and DFS in both stage III ($P = 0.023$, $P = 0.179$) and stage II ($P = 0.121$, $P = 0.015$) groups [Supplementary Figure 1C, <http://links.lww.com/CM9/B87>]. Three-year survival data were obtained for 29 patients who received surgery before January 2017. For the patients with stage III disease, the 3-year OS and DFS rates were 72% (21/29) and 66% (19/29), respectively. For the patients with stage II disease, the 3-year OS and DFS rates were both 100% (9/9). The Kaplan–Meier curves for the 3-year survival of patients with stage III disease are shown in Supplementary Figure 1D, <http://links.lww.com/CM9/B87>, and significantly longer OS ($P = 0.003$) and DFS ($P = 0.016$) were observed in patients who were determined to be sensitive to taxanes by ATP-TCA. Since the survival rates of both groups were >50%, the median values for OS and DFS were not obtained in the study. These results showed that *in vitro* chemosensitivity was well correlated with the clinical outcomes of patients receiving taxane-based post-operative chemotherapy.

In conclusion, the response of gastric cancer to taxanes was heterogeneous, and the ATP-TCA results were well correlated with the clinical outcomes of gastric cancer patients who received taxane-based post-operative adjuvant chemotherapy. Therefore, this assay may aid in selecting patients who may benefit from taxane-based post-operative adjuvant chemotherapy and provide a prospective method to help with personalized medicine in addition to gene testing and biomarker detection. However, due to the limited cases included in the study, the conclusion can be further confirmed in large-scale multicenter studies.

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Conflicts of interest

None.

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