# CLPTM1L expression predicts recurrence of patients with intermediate- and high-risk stage IB-IIB cervical cancer undergoing radical hysterectomy followed by TP as adjuvant chemotherapy

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**Abstract.** According to the National Comprehensive Cancer Network clinical practice guidelines of cervical cancer, concurrent chemoradiotherapy or radiotherapy is suggested for patients who receive radical hysterectomy and have intermediate- and high-risk cervical cancer. However, adjuvant chemotherapy has been increasingly chosen given the adverse events associated with chemoradiotherapy or radiotherapy and the increase in evidence regarding the efficacy of adjuvant chemotherapy. Given that adjuvant chemotherapy is not a standard treatment at present, if recurrence after adjuvant chemotherapy could be predicted, it would assist the decision of gynecological oncologists selecting which adjuvant therapy (chemotherapy or radiation therapy) to use. Cleft lip and palate transmembrane protein 1-like protein (CLPTM1L; also known as cisplatin resistance-related protein 9) is associated with apoptotic mechanisms and is related to the proliferation of the tumor cells and resistance against chemotherapy. In the present study, the association between CLPTM1L expression and recurrence of intermediate- and high-risk stage IB-IIB cervical cancer in patients undergoing radical hysterectomy followed by treatment with cisplatin and paclitaxel (TP) as adjuvant chemotherapy was determined. Patients were divided into two groups: Recurrence group and no-recurrence group. CLPTM1L expression was examined using immunohistochemistry in paraffin-embedded sections using weighted scores. Regarding the characteristics of the patients, a histology of non-squamous cell carcinoma, lymph node metastasis and parametrium invasion were more common in the recurrence group compared with the non-recurrence group. In the recurrence group, CLPTM1L expression was significantly higher than that in the no-recurrence group. Next, patients were divided into low and high-expression groups based on the weighted score with a cut-off value of 6. In the high expression group, patients exhibited a higher rate of recurrence (37.5 vs. 5.1%) and had worse overall survival. Multivariate analysis revealed that high CLPTM1L expression was independently related to recurrence. In *in vitro* analysis, small interfering RNA-mediated knockdown of CLPTM1L enhanced the sensitivity of cervical cancer cells to cisplatin. In conclusion, the present study revealed that CLPTM1L expression may be a predictive biomarker of recurrence of intermediate- and high-risk stage IB-IIB cervical cancer in patients undergoing radical hysterectomy followed by TP as adjuvant chemotherapy.

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*Key words:* uterine cervical cancer, adjuvant chemotherapy, cisplatin, paclitaxel, cleft lip and palate transmembrane protein 1-like protein, cisplatin resistance-related protein 9

## Introduction

Cervical cancer is the fourth most common type of cancer in women globally accounting for 604,127 new cases and 341,831 new deaths in 2020 (1). Surgical treatment with radical hysterectomy and pelvic lymph node dissection is one of the options for the primary treatment of stage IB-IIB [2008 Federation of Gynecology and Obstetrics (FIGO) staging system] cervical cancer according to the National Comprehensive Cancer Network guidelines (2) and the Japan Society of Gynecologic Oncology guidelines (3). After surgical treatment, adjuvant therapy such as whole-pelvic irradiation or chemoradiation is recommended depending on risk factors for recurrence, which is evaluated using pathological factors of specimens from resected tissues (2,3). However, the combination of radical hysterectomy and whole-pelvic irradiation or chemoradiation is linked with an increased occurrence of complications such as lower-limb lymphedema, urinary disturbance, bowel obstruction, sexual dysfunction, and mental issues related to

those lasting issues (4-6). Chemotherapy has been reported as an effective post-surgical adjuvant therapy for patients with intermediate- and high-risk stage IB-IIB cervical cancer (7-9). For these reasons, chemotherapy has been increasingly chosen as post-surgical adjuvant therapy in clinical practice (10,11). As adjuvant chemotherapy is not a standard treatment yet, if the recurrence after adjuvant chemotherapy can be predicted, (that is patients who will not benefit from adjuvant chemotherapy), it would assist gynecological oncologists in deciding which adjuvant therapy (chemotherapy or radiation therapy) would be suitable.

Cleft lip and palate transmembrane protein 1-like protein [CLPTM1L, also known as cisplatin resistance-related protein 9 (CRR9)] has been identified as an overexpressed protein in human ovarian tumor cells resistant to cisplatin (12). CLPTM1L is a protein that is highly expressed in several types of tumor cells, including lung cancer, pancreatic cancer, prostate cancer, bladder cancer, glioma, melanoma, basal cell carcinoma, and cervical cancer (13-22). The expression of CLPTM1L is associated with the apoptosis mechanism and related to the growth of the tumor cells and resistance against chemotherapy (12,23).

In the present study, the relationship between CLPTM1L expression in patients with intermediate- and high-risk stage IB-IIB cervical cancer undergoing radical hysterectomy followed by treatment with cisplatin and paclitaxel (TP) as adjuvant chemotherapy and recurrence were determined. Additionally, the effect of knockdown of CLPTM1L on the sensitivity to chemotherapy agents using a cervical cancer cell line. The results showed that the expression of CLPTM1L in patients with intermediate- and high-risk stage IB-IIB cervical cancer undergoing radical hysterectomy followed by TP was associated with recurrence in those patients and suppression of expression of CLPTM1L on cervical cancer cell line can enhance the sensitivity to cisplatin.

## Materials and methods

*Patients*. The present study was a retrospective study in which a total of 91 patients were enrolled. The inclusion criteria were as follows: i) Patients diagnosed with FIGO 2008 stage IB-IIB cervical cancer; ii) patients who received radical hysterectomy at Osaka City University Hospital (Osaka, Japan) between January 1st, 2014 and December 31st, 2019; iii) patients who underwent TP chemotherapy [paclitaxel (135 mg/m$^2$) plus cisplatin (50 mg/m$^2$) every 3 weeks for 3-6 cycles) after surgery as adjuvant chemotherapy; and iv) the medical records were available to analyze. Information on the clinical factors such as FIGO stage, age, histology, pathological findings of the specimens from resected organs, laboratory data of blood samples, date of recurrence, and date of death was collected. Overall survival was defined as the duration between the date of primary surgery and death from any cause. Data of patients who were still alive were censored at the date when the patient's survival was last confirmed.

To investigate the relationship between CLPTM1L expression and cancer recurrence, the patients were divided into two groups; one in which cancer recurred within 2 years after primary surgery, and the other in which cancer did not recur within 2 years. Recurrence was evaluated by radiological assessments using computed tomography (CT) or Magnetic resonance imaging (MRI) after every three cycles of adjuvant chemotherapy, whilst a physical examination was performed after every cycle. After completion of adjuvant chemotherapy, a physical examination was conducted every 3 months and radiological assessments were conducted every year since the primary surgery was performed for 2 years. Radiological assessments were also conducted when physical examination detected any abnormal findings.

This study was performed at Osaka City University and Osaka Metropolitan University. All the patients enrolled in this study provided written informed consent for the treatment and the use of their samples in future research including the present study. The Institutional Review Board of Osaka City University Hospital approved this study (approval no. 2021-150).

*Immunohistochemical staining and scoring*. For immunohistochemistry, 4 $\mu$m sections from paraffin-embedded tissue blocks of cervical cancer which were dissected during the surgery were obtained. Following routine deparaffinization and dehydration, antigen retrieval was performed by immersing in Target Retrieval Solution, pH 9.0 (cat. no. S2367; Agilent Technologies, Inc.) and heating in an autoclave at 121˚C for 20 min. For the primary antibody, a rabbit monoclonal anti-CLPTM1L antibody (cat. no. HPA014791; MilliporeSigma; 1:400 dilution) was used and slides were incubated at 4˚C overnight. For the secondary antibody, a biotinylated goat immunoglobulin G antibody (cat. no. K0675; Agilent Technologies, Inc.) was used, and samples were incubated at room temperature for 10 min. To stabilize staining, the slides were immersed in DAB solution at room temperature for 10 min. Finally, the tissue sections were counterstained with hematoxylin at room temperature for 1 min.

CLPTM1L expression was quantified by weighted scoring, as described previously (24). This score is calculated by multiplying the score of the percentage of stained tumor cells by the score of staining intensity of each slide. The percentage stained was scored as follows: 0, <5%; 1, 5-25%; 2, 25-50%; 3, 50-75%; and 4, >75%. The intensity was scored as follows: 1, weak; 2, moderate; and 3, intense.

*Cell culture*. CaSki cells (cat. no. IFO50007; Japanese Collection of Research Biosources Cell Bank) were cultured in RPMI medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS and 1% penicillin. Cells were incubated in a humidified incubator at 37˚C supplied with 5% $CO_2$.

*CLPTM1L knockdown and cell survival assays*. For CLPTM1L siRNA transfections, Lipofectamine® RNAiMAX (Invitrogen; Thermo Fisher Science, Inc.) was used. CLPTM1L-specific siRNA (CLPTM1L siRNA; cat. no. 4392420; Invitrogen; Thermo Fisher Scientific, Inc.) or control siRNA (control siRNA-A; cat. no. 4390843; Invitrogen; Thermo Fisher Scientific, Inc.) were used. The sequence of the CLPTM1L siRNA was 5'-GAAUUUUUGUAGAUA CCAAtt; the sequence of the control siRNA was confidential according to the company's policy. CaSki cells were seeded at a density of 2x10$^3$ cells/well in 96-well tissue culture plates. Cells were incubated with medium containing CLPTM1L

Table I. Patient characteristics.

| Characteristics | Non-recurrence group | Recurrence group | P-value |
|---|---|---|---|
| No. of patients | 76 | 15 | |
| Median age, years (range) | 55 (27-78) | 55 (39-76) | 0.974[a] |
| FIGO stage, n | | | 0.163[b] |
| I | 46 | 6 | |
| II | 30 | 9 | |
| Histology, n | | | 0.013[b,c] |
| SCC | 64 | 8 | |
| Non-SCC | 12 | 7 | |
| Lymph node metastasis, n | | | 0.023[b,c] |
| Negative | 46 | 4 | |
| Positive | 30 | 11 | |
| Parametrium invasion, n | | | 0.011[b,c] |
| Negative | 58 | 6 | |
| Positive | 18 | 9 | |

[a]Mann-Whitney U test; [b]Fisher's exact test. [c]P<0.05. FIGO, Federation of Gynecology and Obstetrics; SCC, squamous cell carcinoma.

siRNA or control siRNA at 37˚C for 24 h, after which they were incubated with media containing 1.5, 3.1, 6.2, or 12.5 $\mu$M cisplatin at 37˚C for 48 h. Next, 10 $\mu$l Cell Counting Kit-8 solution (Dojindo Molecular Technologies, Inc.) was added per well, and cells were further incubated at 37˚C for 2 h, and subsequently, the absorbance at 450 nm was measured using a microplate reader (Corona Electric, Co., Ltd.).

*Reverse transcription-quantitative PCR (RT-qPCR).* After CLPTM1L siRNA transfection, RT-qPCR was used to confirm the knock down of CLPTM1L mRNA expression. TaqMan primer and probes for CLPTM1L (cat. no. Hs00363947_m1) and hypoxanthine phosphoribosyl-transferase 1 (cat. no. Hs02800695_ml; both from Thermo Fisher Scientific, Inc.) were used according to the manufacturer's protocol. Total RNA from CaSki cells was obtained using a RNeasy Mini Kit (Qiagen GmbH). Then total RNA (1 $\mu$g) was reverse transcribed into cDNA using a High-Capacity cDNA Reverse Transcription Kit according to the manufacturer's protocol (Thermo Fisher Scientific, Inc.). Finally, qPCR was performed using TaqMan Fast Universal PCR MasterMix (Thermo Fisher Scientific, Inc.). The following thermocycling conditions were used: Initial denaturation at 95˚C for 20 sec; followed by 40 cycles of 95˚C for 3 sec and 60˚C for 30 sec. To calculate the relative changes in gene expression, the $2^{-\Delta\Delta Cq}$ method was used (25).

*Statistical analysis.* GraphPad Prism version 9 (GraphPad Software, Inc.) was used to analyze the data. Data are presented as the median and the range. Significant differences between two groups were determined using a Fisher's exact test and a Mann-Whitney U-test. A receiver operating characteristic (ROC) curve was used to determine the cut-off value of Weighted scores of CLPTM1L expression. For prognostic analysis, the Kaplan-Meier method and log-rank tests were used. For risk factors for recurrence, which were detected in univariate analysis with Fisher's exact test, multivariate analysis was performed to detect independent risk factors using logistic regression analysis. P<0.05 was considered to indicate a statistically significant difference. Five replicates were performed for RT-qPCR, and ten replicates were performed for the cell survival assays.

## Results

*Patient characteristics.* There were 76 patients included in the no-recurrence group and 15 patients in the recurrence group. Regarding age and FIGO stage, there were no significant differences between the two groups. In the recurrence group, the proportion of cases of non-squamous cell carcinoma (non-SCC), positive lymph node metastasis, and positive parametrium invasion were significantly higher (P=0.013, P=0.023, and P=0.011, respectively; Table I). This suggests that non-SCC, positive lymph node metastasis, and positive parametrium invasion were risk factors for recurrence in the univariate analysis. Non-SCC histology includes five usual type endocervical adenocarcinomas and two large cell neuroendocrine carcinomas in the non-recurrence group and eight usual type endocervical adenocarcinomas, two endometrioid carcinomas, one clear cell carcinoma and one adenosquamous carcinoma in the recurrence group.

*CLPTM1L weighted score and cutoff value to predict recurrence.* The expression of CLPTM1L was compared between the two groups using immunohistochemical staining. CLPTM1L protein expression was observed in the cytoplasm of cervical cancer cells macroscopically which was identical to previous reports describing the localization of CLPTM1L in the mitochondria, endoplasmic reticulum and the plasma membrane (26-28). The representative images of weighted

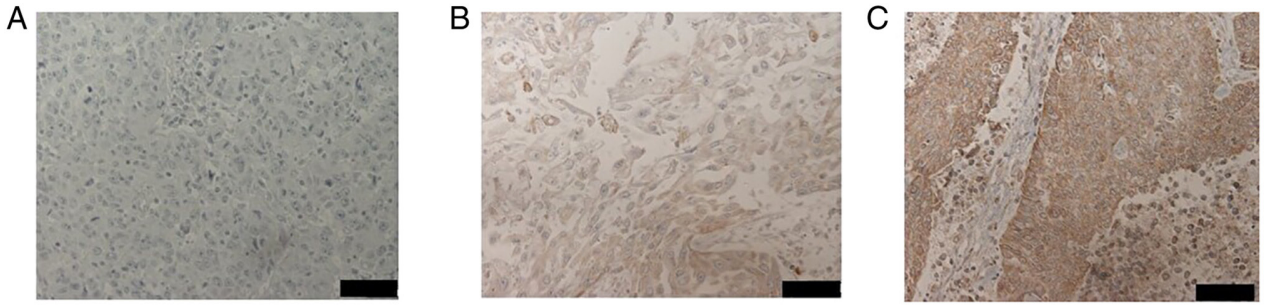


Figure 1. Immunohistochemical staining of cleft lip and palate transmembrane protein 1-like protein in uterine cervical squamous cell carcinoma specimens counterstained with hematoxylin. Scale bar, 50 $\mu$m. Representative image of a weighted score of (A) 0, (B) 6 and (C) 12.

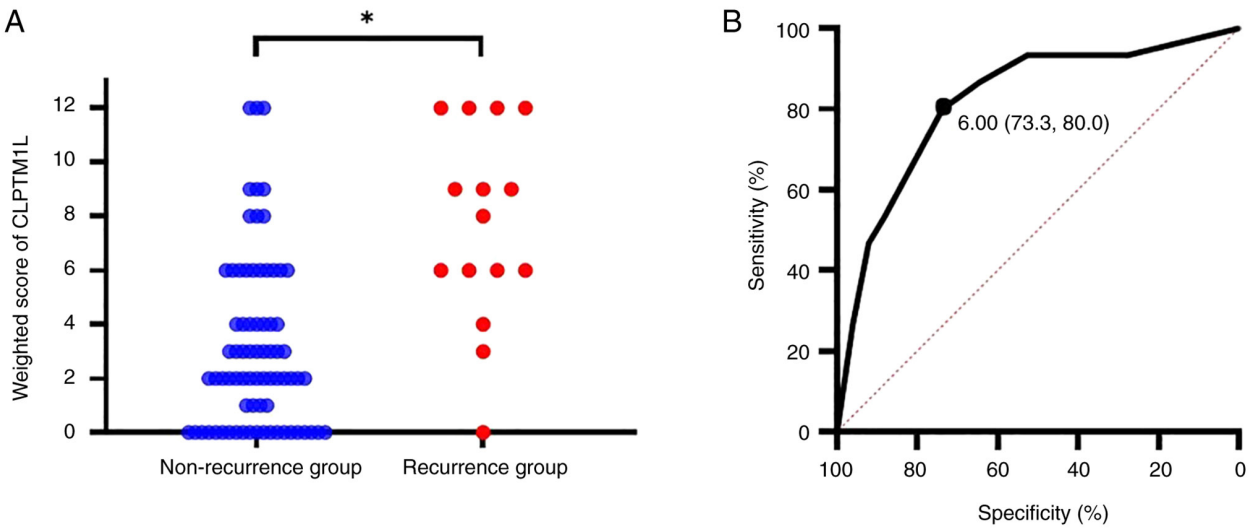


Figure 2. Comparison of the weighted score of CLPTM1L between the two groups and ROC curve to predict recurrence. (A) Comparison of the weighted score of CLPTM1L between the two groups revealed that the non-recurrence group had a significantly lower score compared with the recurrence group. (B) An ROC curve was generated using the weighted score of the two groups, and it showed that a cut-off value of 6 predicted recurrence with a sensitivity of 80.0% and specificity of 73.3% with an area under the curve value of 0.82 and a 95% confidence interval of 0.698-0.942. *P<0.05 (Mann-Whitney U-test). CLPTM1L, cleft lip and palate transmembrane protein 1-like protein; ROC, receiver operating characteristic.

scores 0, 6, and 12 are shown in Fig. 1. The expression of CLPTM1L which was evaluated using a weighted score was significantly higher in the recurrence group (P<0.05, Fig. 2A). To identify the optimal cut-off weighted score value of CLPTM1L to predict recurrence, ROC curves were drawn. They showed that a cut-off weighted score value of 6 predicted recurrence with a sensitivity of 73.3% and specificity of 80.0%, with an area under the curve of 0.82 and a 95% confidence interval of 0.698-0.942 (Fig. 2B).

*CLPTM1L expression for predicting recurrence and overall survival*. Based on the cut-off weighted score value of 6, patients were divided into two groups: low expression group (score ≤4; 59 patients) and high expression group (score ≥6; 32 patients). Regarding age, FIGO stage (2008), histology, lymph node metastasis, and parametrium invasion, there were no significant differences between the two groups (Table II). In the low-expression group, 56 patients (94.9%) did not exhibit recurrence and only 3 patients (5.1%) exhibited a recurrence. In the high expression group, 20 patients (62.5%) did not exhibit a recurrence and 12 patients (37.5%) exhibited recurrence, showing that patients in the high expression group were more likely to exhibit a recurrence than in the low expression group (P<0.010; Table III). Regarding overall survival, patients in the low expression group had a better prognosis (P<0.01; Fig. 3), suggesting that CLPTM1L expression could be used to predict overall survival.

*Multivariate analysis for detecting independent risk factors of recurrence*. To identify independent risk factors for recurrence, multivariate analysis using logistic regression was performed. Multivariate analysis revealed high CLPTM1L expression and histology of non-SCC were significantly associated with recurrence (P<0.010 and 0.042, respectively; Table IV). The odds ratio for recurrence of high CLPTM1L and histology of non-SCC were 7.990 and 4.660, respectively. This suggests that high CLPTM1L expression and histology of non-SCC are independent risk factors for recurrence of intermediate- and high-risk stage IB-IIB cervical cancer undergoing radical hysterectomy followed by TP as adjuvant chemotherapy.

*Contribution of CLPTM1L knockdown to the sensitivity of cervical cancer cells to cisplatin*. To determine the effect of CLPTM1L knockdown on the sensitivity to chemotherapy,

Table II. Characteristics of the patients stratified by cleft lip and palate transmembrane protein 1-like protein expression.

| Characteristics | Low expression group | High expression group | P-value |
|---|---|---|---|
| No. of patients | 59 | 32 | |
| Median age, years (range) | 54 (29-76) | 56 (27-78) | 0.930[a] |
| FIGO stage, n | | | 0.659[b] |
| I | 35 | 17 | |
| II | 24 | 15 | |
| Histology, n | | | 0.281[b] |
| SCC | 49 | 23 | |
| Non-SCC | 10 | 9 | |
| Lymph node metastasis, n | | | 0.051[b] |
| Positive | 37 | 13 | |
| Negative | 22 | 19 | |
| Parametrium invasion, n | | | 0.100[b] |
| Positive | 45 | 19 | |
| Negative | 14 | 13 | |

[a]Mann-Whitney U test; [b]Fisher's exact test. FIGO, Federation of Gynecology and Obstetrics; SCC, squamous cell carcinoma.

Table III. Association between CLPTM1L expression and recurrence.

| CLPTM1L expression | No recurrence, n (%) | Recurrence, n (%) | P-value |
|---|---|---|---|
| Low expression, score ≤4 | 56 (94.9) | 3 (5.1) | <0.010[a,b] |
| High expression, score ≥6 | 20 (62.5) | 12 (37.5) | |

[a]P<0.010. [b]Fisher's exact test. CLPTM1L, cleft lip and palate transmembrane protein 1-like protein.

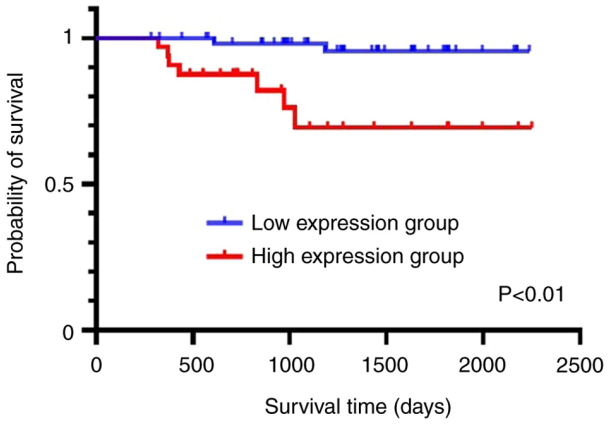


Figure 3. Overall survival analysis using Kaplan-Meier curves. The overall survival in the low CLPTM1L expression group was significantly longer than that in the high CLPTM1L expression group (P<0.01; log-rank test). CLPTM1L, cleft lip and palate transmembrane protein 1-like protein.

human cervical cells were used *in vitro*. CLPTM1L expression was knocked down by transfection of si-CLPTM1L. Knockdown of CLPTM1L mRNA was confirmed by RT-qPCR. CLPTM1L mRNA expression was significantly suppressed compared with cells transfected with the control siRNA (P<0.05; Fig. 4A). After confirmation of successful knockdown of CLPTM1L with siRNA, the viability of cervical cancer cells with CLPTM1L expression knocked down was compared with the control transfected cells. In cells treated with ≥3.1 $\mu$M cisplatin, the viability of tumor cells in which CLPTM1L was knocked down was significantly lower than the respective control cells (P<0.05; Fig. 4B). These results indicate that knockdown of CLPTM1L enhances sensitivity to cisplatin.

## Discussion

Even though radiation therapy, including whole-pelvic irradiation, or chemoradiation is recommended as adjuvant therapy for patients with intermediate- and high-risk stage IB-IIB cervical cancer undergoing radical hysterectomy, chemotherapy has been increasingly chosen as adjuvant therapy (10). This is due to the adverse events associated with radiation therapy following radical surgery such as lower-limb lymphedema, urinary disturbance, bowel obstruction, sexual dysfunction, and mental health issues caused by the lasting issues of other adverse events, and these are frequently more severe than the extent of adverse events associated with chemotherapy (10). Additionally, the efficacy of adjuvant chemotherapy has been increasingly reported (6,10,29). Additionally, one of

Table IV. Multivariate analysis to detect independent risk factors for recurrence.

| Variables | Odds ratio | 95% confidence interval | | P-value |
|---|---|---|---|---|
| | | Lower | Upper | |
| CLPTM1L expression (high vs. low) | 7.990 | 1.850 | 34.400 | <0.010[a] |
| Lymph node metastasis (positive vs. negative) | 2.940 | 0.679 | 12.700 | 0.149 |
| Histology (SCC vs. non-SCC) | 4.660 | 1.060 | 20.500 | 0.042[b] |
| Parametrium invasion (positive vs. negative) | 3.100 | 0.791 | 12.100 | 0.105 |

[a]P<0.01, [b]P<0.05. CLPTM1L, cleft lip and palate transmembrane protein 1-like protein; SCC, squamous cell carcinoma.

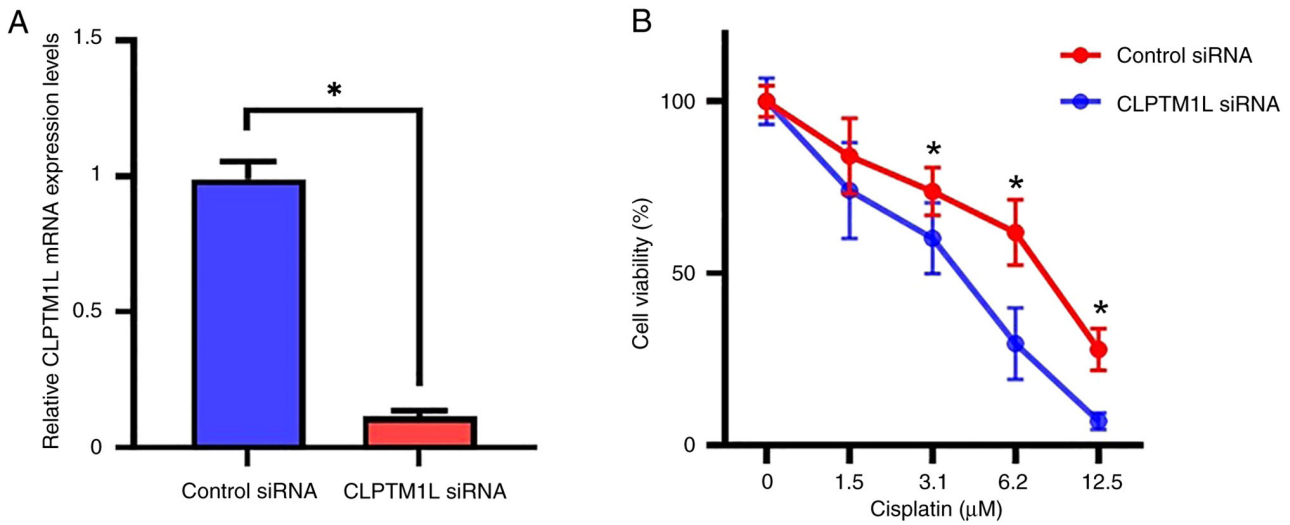


Figure 4. CLPTM1L knockdown and its effect on the sensitivity to cisplatin in cervical cancer cells. (A) CLPTM1L mRNA expression levels in the control siRNA-transfected cells and the CLPTM1L siRNA-transfected cells indicated successful knockdown of CLPTM1L (P<0.05). (B) Cell viability after treating cells with various concentrations of cisplatin. Cell viability was significantly reduced in the CLPTM1L knockdown cells transfected with CLPTM1L siRNA compared with the control cells transfected with control siRNA when treated with ≥3.1 $\mu$M cisplatin (P<0.05). Data are presented as the mean ± standard deviation. *P<0.05 (Mann-Whitney U-test; control siRNA vs. CLPTM1L siRNA). CLPTM1L, cleft lip and palate transmembrane protein 1-like protein; siRNA, small interfering RNA.

the advantages of using chemotherapy as adjuvant therapy is that if radiation therapy is not performed as adjuvant therapy during the primary treatment, it can be used if loco-regional recurrence occurs (4,10,30). However, adjuvant chemotherapy following radical hysterectomy is not a standard treatment yet; if the probability of recurrence following adjuvant chemotherapy could be predicted, this may assist gynecological oncologists in deciding which adjuvant therapy (chemotherapy or radiation therapy) to use.

CLPTM1L was first identified in cisplatin resistance ovarian tumor cells, and recent reports noted that genome-wide association studies revealed a correlation between CLPTM1L expression and lung cancer, pancreatic cancer, prostate cancer, bladder cancer, glioma, melanoma, basal cell carcinoma, and cervical cancer (13-22). Understanding the function of CLPTM1L in healthy organs or tissues requires further study; however, CLPTM1L has been reported to play a crucial role in fetal development and neonatal survival but is not essential in adult animals (27). The function of CLPTM1L in cancer is well understood, where it exerts an anti-apoptotic effect (21,23,31-33). CLPTM1L has been reported to play a crucial role in the activation of the PI3K/AKT pathway by Akt phosphorylation, which regulates proliferative and survival signals in cancer cells including apoptotic signals, and also upregulates the expression of Bcl-xL (21,32), an anti-apoptotic Bcl-2 family member (21,34). Therefore, over-expression of CLPTM1L demonstrate tumor-specific cytoprotective and chemoresistive function by suppressing apoptosis, which is induced by genotoxic agents in cancers (21,31-33). Knockdown of CLPTM1L using siRNA has been reported to decrease Akt phosphorylation and reduce Bcl-xL expression leading to increased chemosensitivity to cisplatin in the cancer cells (31-33).

In the present study, high expression of CLPTM1L was associated with recurrence and a poor prognosis of cervical cancer. There were other factors other than the levels of expression of CLPTM1L, which contribute to recurrence in the univariate analysis in the current study including non-SCC histology, lymph node metastasis, and parametrium invasion. In multivariate analysis, the levels of expression of CLPTM1L were still an independent risk factor for the recurrence along with non-SCC histology. Non-SCC histology has

been reported to be one of risk factors for poor prognosis of patients with cervical cancer (35). The results of this study are comparable to the report. In this research, three cases experienced cancer recurrence despite low expression of CLPTM1L. Two cases of them had non-SCC histology of mucinous carcinoma and the other one case had parametrium invasion, which may contribute to the recurrence. Following knockdown of CLPTM1L, the viability of cervical cancer cells treated with cisplatin for 48 h was significantly decreased compared to the control group, which indicated that knockdown of CLPTM1L enhanced the sensitivity of cervical cancer cells to cisplatin. It indicates that CLPTM1L might be one of useful biomarkers to predict the recurrence and also one of candidates of therapeutic target which can enhance the efficacy of chemotherapy in patients with intermediate- and high-risk stage IB-IIB cervical cancer undergoing radical hysterectomy followed by TP as adjuvant chemotherapy.

The present study has some limitations. First, this was a retrospective study with a relatively small number of cases from a single institute. Additionally, the expression levels of CLPTM1L did not predict recurrence with 100% accuracy, highlighting the likely involvement of several other factors in recurrence. Thus, larger prospective studies including patients from several institutions are required to confirm the results of this study. Additionally, studies that explore other factors that may be combined with CLPTM1L expression levels are required to enhance the sensitivity and specificity of predicting recurrence.

To the best of our knowledge, this study is the first study to show the association between CLPTM1L expression and the recurrence of patients with cervical cancer who underwent TP as adjuvant chemotherapy. CLPTM1L may serve as a valuable clinical biomarker for predicting the recurrence of cervical cancer treated by radical hysterectomy followed by TP. Adjuvant chemotherapy is increasing in popularity due to the increase in the body of literature highlighting its clinical efficacy as adjuvant chemotherapy; however, there remain cases that are not sensitive to this regimen. Thus, it is of significant importance to explore reliable indicators that gynecological oncologists can use to assess the sensitivity to adjuvant chemotherapy. The present study may contribute to achieving the goal of identifying the optimal candidate for adjuvant chemotherapy in patients with cervical cancer.

In conclusion, this study revealed that CLPTM1L expression may be a predictive biomarker of recurrence in patients with intermediate- and high-risk stage IB-IIB cervical cancer undergoing radical hysterectomy followed by TP as adjuvant chemotherapy.

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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Authors' contributions

YA, TF and TS designed the study. YA, TN, EU, SN, KI and MY performed the experiments and collected the data. YA, TF, TY and TS analyzed the data. YA and TF wrote the manuscript. YA and TF confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

## Ethics approval and consent to participate

The present study protocol was approved by the Institutional Review Board of Osaka City University Hospital (approval no. 2021-150; Osaka, Japan). Written informed consent for participation in the current study was obtained from all patients.

## Patient consent for publication

Written informed consent for publication was obtained from all patients.

## Competing interests

The authors declare that they have no competing interests.

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