SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 28: 414, 2024

# Predicting recurrence in intermediate- and high-risk stage IB-IIB cervical cancer treated with adjuvant cisplatin and paclitaxel chemotherapy post-radical hysterectomy: The role of TBX2 expression

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Received May 14, 2024; Accepted June 4, 2024

DOI: 10.3892/ol.2024.14547

**Abstract.** Cervical cancer remains a significant global health concern despite advances in prevention and treatment. Current management for intermediate- to high-risk stage IB-IIB cervical cancer post-radical hysterectomy involves irradiation or concurrent chemoradiation, although patients can exhibit several adverse events. Adjuvant chemotherapy has emerged as a promising alternative; however, its efficacy remains unclear. T-box (TBX)2 is a transcription factor that is involved in the regulation of cell cycle progression during cancer and embryonic development. Additionally, elevated expression of TBX2 is associated with resistance to DNA-damaging chemotherapeutic agents, such as cisplatin, carboplatin and doxorubicin. The aim of the present study was to investigate the relationship between TBX2 expression and recurrence in patients receiving adjuvant cisplatin and paclitaxel (TP) chemotherapy post-radical hysterectomy. Additionally, the impact of TBX2 knockdown on cisplatin sensitivity was assessed *in vitro*. A retrospective analysis was performed on 100 patients. Patients were categorized into two groups based on recurrence within 2 years of treatment initiation: The non-recurrent group (n=85) and the recurrent group (n=15). TBX2 expression was assessed immunohistochemically, and multiple logistic regression analysis was performed to identify predictors of recurrence. Additionally, the impact of small interfering RNA-mediated TBX2 knockdown on cervical cancer cell sensitivity to cisplatin was evaluated. TBX2 expression was significantly higher in the recurrent group compared with that in the non-recurrent group (P<0.01). Patients were stratified into low TBX2 expression (weighted score ≤8; n=80) and high TBX2 expression (weighted score ≥9; n=20) groups. The high TBX2 expression group exhibited a higher recurrence rate compared with the low expression group (P<0.01). Multivariate analysis identified TBX2 expression as an independent predictor of recurrence (P<0.01). Moreover, TBX2 knockdown significantly enhanced cervical cancer cell sensitivity to cisplatin *in vitro* (P<0.05). These findings highlight TBX2 expression as a potential predictive biomarker for recurrence amongst patients with intermediate- to high-risk stage IB-IIB cervical cancer receiving adjuvant TP chemotherapy post-radical hysterectomy.

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*Key words:* uterine cervical cancer, adjuvant chemotherapy, cisplatin, paclitaxel, T-box 2, apoptosis

## Introduction

According to the 2022 Global Cancer Statistics, there were 661,021 new cases of cervical cancer and 348,189 deaths attributed to the disease. This ranks cervical cancer as the fourth most common cancer amongst women globally and the fourth leading cause of cancer-associated deaths amongst women worldwide (1). For patients diagnosed with stage IB1-IIA cervical cancer, as classified by the 2008 Federation of Gynecology and Obstetrics (FIGO) staging system (2), the recommended primary treatment involves radical hysterectomy accompanied by pelvic lymphadenectomy, which is endorsed by the guidelines of the National Comprehensive Cancer Network (3) and the Japan Society of Gynecologic Oncology (4). The guidelines of the Japan Society of Gynecologic Oncology suggest that patients diagnosed with stage IIB cervical cancer may also be considered for treatment with radical hysterectomy accompanied by pelvic lymphadenectomy (4). After surgery, adjuvant treatments such as irradiation or concurrent chemoradiation are employed based on risk factors for recurrence evaluated from the resected specimens. These risk factors include lymphovascular space invasion, a larger tumor size, deep cervical interstitial infiltration, parametrial invasion, and lymph node metastasis (5). However, irradiation or concurrent chemoradiation following

radical surgery can lead to adverse events, including urinary disturbance, lower-limb lymphedema, bowel obstruction, sexual dysfunction, ovarian dysfunction, and mental health issues associated with these lasting adverse effects (6-8). Given these concerns, efforts have been made globally to introduce postoperative adjuvant chemotherapy for patients at intermediate or high risk of recurrence (6,7,9,10). However, the current evidence supporting chemotherapy as postoperative adjuvant therapy is limited, and careful consideration is warranted when determining its suitability. Thus, the ability to predict the effectiveness of adjuvant chemotherapy may significantly assist gynecologic oncologists in selecting the appropriate adjuvant treatment, whether it be irradiation or chemotherapy.

T-box (TBX)2, a transcription factor, belongs to the TBX gene family, which plays a critical role in organogenesis and pattern formation across vertebrate and invertebrate species (11). TBX2 is involved in development, cell cycle regulation, and oncogenesis (12,13). Elevated levels of TBX2 expression have been observed in various types of cancer, including esophageal squamous cell (14), endometrial (15), melanoma (16), prostate, breast (17), laryngeal squamous cell carcinoma (18), non-small cell lung (19), gastric (20), and pancreatic cancer (21). Additionally, elevated expression of TBX2 has been associated with resistance to DNA-damaging chemotherapy agents such as cisplatin, carboplatin, and doxorubicin (22-26).

In the present study, the relationship between TBX2 expression and recurrence was investigated in patients with intermediate- and high-risk stage IB-IIB cervical cancer who received adjuvant cisplatin and paclitaxel (TP) chemotherapy following radical hysterectomy. Additionally, the impact of TBX2 knockdown on the sensitivity of cervical cancer cells to cisplatin *in vitro* was assessed.

## Materials and methods

*Patients*. This retrospective analysis included 100 patients who underwent radical hysterectomy for cervical cancer stages IB-IIB (FIGO 2008) and received postoperative adjuvant TP chemotherapy (paclitaxel at 135 mg/m$^2$ and cisplatin at 50 mg/m$^2$ every 3 weeks for 3-6 cycles) between January 1, 2014 and December 31, 2020, at Osaka City University and Osaka Metropolitan University. Clinical data including age, FIGO stage, histology, results of blood sample laboratory analysis, date of primary surgery, initiation and completion of chemotherapy, recurrence, and death from any cause were recorded. Assessment of recurrence during postoperative adjuvant chemotherapy was conducted every 3 cycles using computed tomography (CT) or magnetic resonance imaging (MRI), complemented by physical examinations after each cycle. Upon completion of the treatment regimen, outpatient evaluations, including physical examinations, ultrasonography, and tumor marker measurements, were scheduled every 3 months. Furthermore, in the second postoperative year, recurrence evaluation was performed using CT or MRI. In cases where abnormal clinical findings or elevated tumor markers were observed, imaging assessments were performed without waiting for the 2-year postoperative period. Overall survival was calculated from the date of surgery to the date of death from any cause, while disease-free survival was defined as the time from surgery to the detection of recurrence. For patients still alive at the end of the assessment period, data were censored as of the last confirmed survival date.

To explore the association between TBX2 expression and recurrence, patients were categorized into two groups: Those who did not experience recurrence within 2 years after primary surgery (non-recurrent group), and those who experienced a recurrence within 2 years (recurrent group).

The research was conducted at Osaka City University and Osaka Metropolitan University. All participating patients provided written informed consent for the treatment regimen and the use of their samples in future research endeavors, including the present study. The present study was approved by the Institutional Review Board of Osaka Metropolitan University Hospital (approval no. 2022-102). Osaka Metropolitan University was established in April 2022 through the merger of Osaka City University and Osaka Prefecture University. Currently, only Osaka Metropolitan University exists; its approval in 2022 encompasses the approval for Osaka City University.

*Immunohistochemical staining and scoring*. To assess the expression of TBX2 in cervical cancer surgical specimens, immunohistochemical staining was performed followed by scoring of the tissues. Initially, 4-$\mu$m paraffin-embedded sections were prepared from tissue blocks obtained during surgery of patients with cervical cancer. These sections underwent deparaffinization and rehydration using autoclaving, which involved heating at 121˚C for 20 min. The sections were then incubated overnight at 4˚C with a TBX2 antibody (cat. no. LS-C402301; LifeSpan BioSciences, Inc.) diluted 1:500. This was followed by application of the Dako REAL EnVision Detection System Peroxidase/DAB+, Rabbit/Mouse (cat. no. K5007; Agilent Technologies, Inc.) at room temperature for 3 min for visualization of antibody staining. Tissue sections were counterstained with hematoxylin for 1 min at room temperature to enhance the visibility of the structures. Scoring for TBX2 expression utilized a weighted scoring system as described by Sinicrope *et al* (27) based on the percentage of stained tumor cells and staining intensity. Percentage stained was scored as follows: 0, <5% coverage; 1, 5-25%; 2, 25-50%; 3, 50-75%; and 4, >75%. Intensity was categorized as 1 for weak, 2 for moderate, and 3 for intense. This scoring system allows for a quantifiable assessment of TBX2 expression levels in the tumor tissues

*Cell culture*. The TCS cell line, which comprises human cells derived from uterine cervical squamous carcinoma, were sourced from the RIKEN BioResource Center. These cells were cultured in Minimum Essential Medium from Gibco; Thermo Fisher Scientific, Inc., supplemented with 10% FBS and 1% penicillin-streptomycin solution. Cultures were maintained in a humidified incubator at 37˚C with an atmosphere of 5% $CO_2$.

*TBX2 knockdown and cell survival assays*. For siRNA transfections, Lipofectamine® RNAiMAX (Invitrogen; Thermo Fisher Scientific, Inc.) was used. TBX2-specific siRNA (cat. no. SASI_Hs01_00169003; MilliporeSigma) and control siRNA (cat. no. SIC001_10NMOL; MilliporeSigma) were

used. The si-TBX2 sense sequence was CGCUAUAAGUUC CACAACUdTdT and the antisense sequence was AGUUGU GGAACUUAUAGCGdTdT; the sequence for the control siRNA was not disclosed by the manufacturer. TCS cells were seeded at a density of $1x10^4$ cells/well in 96-well tissue culture plates. Post-seeding, the cells were incubated with media containing either TBX2 siRNA or control siRNA at 37˚C for 24 h. This was followed by exposure to various concentrations of cisplatin (3.125, 6.25, 12.5, or 25 $\mu$M) at 37˚C for an additional 24 h. Subsequently, 10 $\mu$l Cell Counting Kit-8 solution (Dojindo Molecular Technologies, Inc.) was added to each well, and the cells were incubated at 37˚C for 1 h. The absorbance at 450 nm was then measured using a microplate reader (Corona Electric, Co., Ltd.). All procedures were performed in strict accordance with the manufacturer's protocol.

*Reverse transcription-quantitative PCR (RT-qPCR).* Following TBX2 siRNA transfection, TBX2 mRNA expression knockdown was confirmed using RT-qPCR. TaqMan primer and probes for TBX2 (cat. no. Hs00911929_m1) and hypoxanthine phosphoribosyl-transferase 1 (HPRT1) (cat. no. Hs02800695_ml), both sourced from Thermo Fisher Scientific, Inc., were utilized as per the manufacturer's instructions. HRPT1 was used as a housekeeping gene as a reference for mRNA expression. Total RNA extraction from TCS cells was performed using a RNeasy Mini Kit according to the manufacturer's protocol (Qiagen GmbH). Subsequently, 1 $\mu$g total RNA was reverse transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. For qPCR analysis, TaqMan Fast Universal PCR MasterMix (Thermo Fisher Scientific, Inc.) was employed. The thermocycling conditions consisted of an initial denaturation step at 95˚C for 20 sec, followed by 40 cycles of denaturation at 95˚C for 3 sec and annealing/extension at 60˚C for 30 sec. The relative changes in gene expression were calculated using the $2^{-\Delta\Delta Cq}$ method (28).

*Statistical analysis.* The data were analyzed using GraphPad Prism version 9 (GraphPad Software, Inc.), and the data are presented as the median and range. Differences between two groups were assessed using a Fisher's exact test or Mann-Whitney U-test. The receiver operating characteristic (ROC) curve was used to establish the cut-off value for weighted scores of TBX2 expression. Prognostic analysis was performed using the Kaplan-Meier method alongside log-rank tests. To identify independent risk factors for recurrence, detected through univariate analysis with a Fisher's exact test, multivariate analysis was used for the logistic regression analysis. P<0.05 was considered to indicate a statistically significant difference. RT-qPCR was performed using five replicates, while cell survival assays were performed using 10 replicates.

## Results

*Patient characteristics.* In the present study, 85 patients were included in the non-recurrent group and 15 patients were included in the recurrent group. There were no significant differences in the age and FIGO stage between the two groups. The recurrent group had a significantly higher proportion of cases exhibiting non-squamous cell carcinoma (non-SCC), positive parametrium invasion, and positive lymph node metastasis (P=0.01, P=0.024, and P=0.026, respectively; Table I). This suggests that non-SCC histology, positive parametrium invasion, and positive lymph node metastasis are risk factors for recurrence during univariate analysis. Specifically, non-SCC histology consisted of eight usual type endocervical adenocarcinoma cases, two adenosquamous carcinoma cases, two endometrioid carcinoma cases, and one clear cell carcinoma case in the non-recurrent group, whereas in the recurrent group, it consisted of four usual type endocervical adenocarcinoma cases, two large cell neuroendocrine carcinoma cases, and one poorly differentiated adenocarcinoma case.

Table I. Clinicopathological characteristics of the patients.

| Characteristic | Non-recurrent group | Recurrent group | P-value |
|---|---|---|---|
| No. of patients | 85 | 15 | |
| Age, years[a] | 55 (27-78) | 55 (39-76) | 0.915[b] |
| FIGO stage | | | 0.159[c] |
| I | 52 | 6 | |
| II | 33 | 9 | |
| Histology | | | 0.01[c,d] |
| SCC | 72 | 8 | |
| Non-SCC | 13 | 7 | |
| Parametrium invasion | | | 0.024[c,e] |
| + | 19 | 8 | |
| - | 66 | 7 | |
| Lymph node metastasis | | | 0.026[c,e] |
| + | 35 | 11 | |
| - | 50 | 4 | |

[a]Median (range); [b]Mann-Whitney U test; [c]Fisher's exact test; [d]P≤0.01; [e]P≤0.05. FIGO, Federation of Gynecology and Obstetrics; SCC, squamous cell carcinoma.

*TBX2 weighted score and cutoff value to predict recurrence.* Immunohistochemical staining demonstrated that TBX2 was predominantly localized in the cell nuclei. Representative images with weighted scores of 0, 6, and 12 are shown in Fig. 1. TBX2 expression was found to be significantly higher in the recurrence group (P<0.01, Fig. 2A). To determine the cutoff value of TBX2 expression for predicting recurrence, ROC curve analysis was performed. Setting the cutoff value for the weighted score at 9 yielded a sensitivity of 66.7% and a specificity of 88.2%. Additionally, the area under the curve was 0.797, with a 95% confidence interval ranging from 0.666 to 0.928 (Fig. 2B).

*TBX2 expression as a predictor of recurrence and overall survival.* After applying a cutoff value of 9, the study population was divided into two groups based on TBX2 expression levels: the low expression group (score ≤8; 80 patients) and the high expression group (score ≥9; 20 patients) of the weighted score. No significant differences were observed between the two groups in terms of age, histology, parametrium invasion,

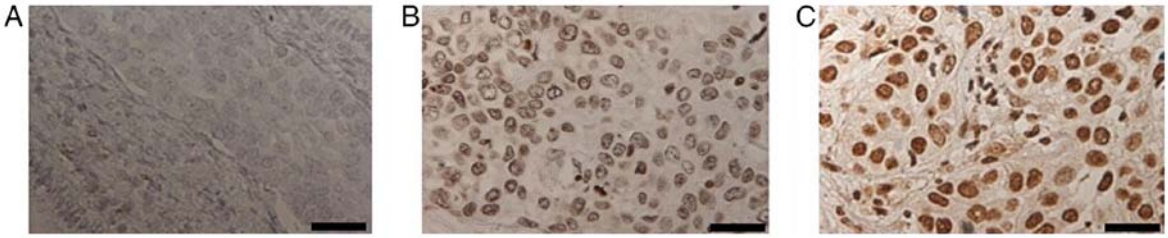


Figure 1. Immunohistochemical staining of T-box 2 in cervical cancer specimens obtained surgically, with hematoxylin counterstaining. Representative images corresponding to weighted scores of (A) 0, (B) 6 and (C) 12. Scale bar, 50 $\mu$m.

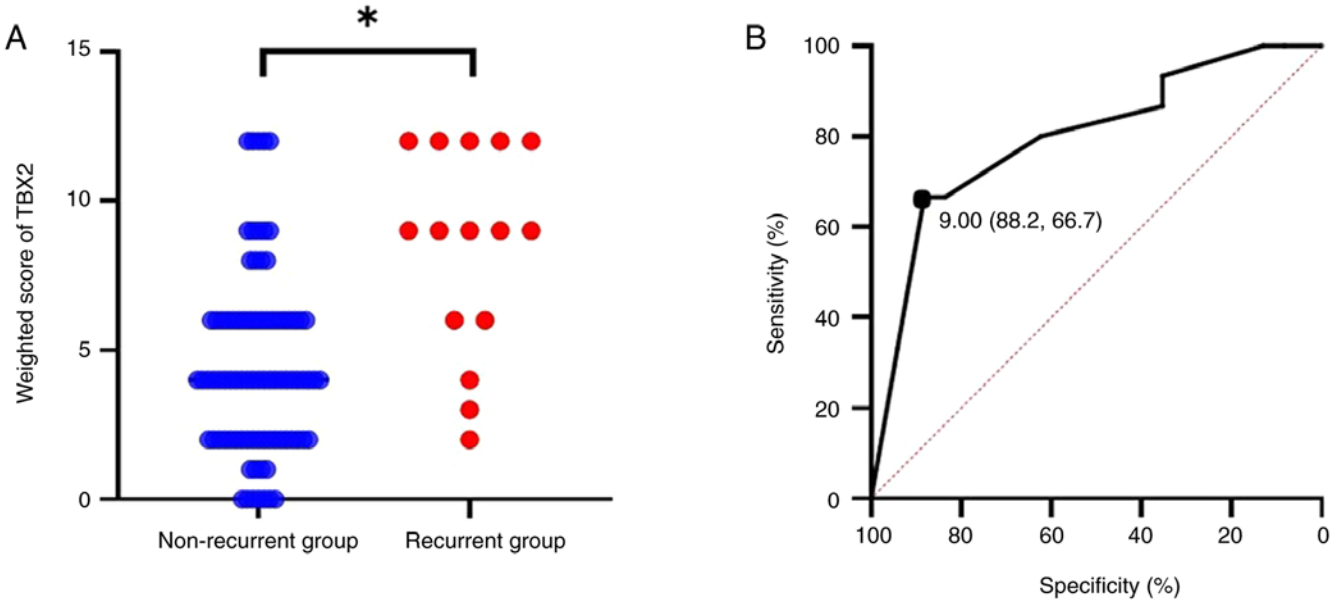


Figure 2. Comparison of TBX2 weighted scores between two groups and ROC curve for prediction of recurrence. (A) Comparative analysis of TBX2 weighted scores between the two groups demonstrated a significantly lower score in the non-recurrent group compared to the recurrent group. (B) A ROC curve was constructed using the weighted scores from both groups. The analysis revealed a cutoff value of 9 for predicting recurrence, with a sensitivity of 66.7% and specificity of 88.2%. The AUC was 0.797, with a 95% confidence interval of 0.666-0.928. *P<0.01. TBX2, T-box 2; AUC, area under the curve; ROC, receiver operator characteristic.

and lymph node metastasis. However, there was a significant difference in the FIGO stage between the groups (P=0.024; Table II). In the low TBX2 expression group, 5 cases (6.25%) experienced recurrence, whereas in the high expression group, 10 cases (50%) exhibited recurrence, and the difference in the incidence of recurrence was significant (P<0.01; Table III). Furthermore, overall survival was significantly longer in the low TBX2 expression group (P<0.01; Fig. 3), suggesting that TBX2 expression can be used to predict overall survival.

*Identification of independent risk factors for recurrence through multivariate analysis*. Multivariate analysis was used to identify independent predictors of recurrence in patients with intermediate- and high-risk stage IB-IIB cervical cancer who received adjuvant TP chemotherapy following radical hysterectomy. According to the analysis, TBX2 expression and histological type were significant risk factors for recurrence (Table IV). The odds ratios associated with high TBX2 expression and non-SCC histological types were 12.3 and 5.01, respectively. High TBX2 expression was identified as the most significant independent risk factor for recurrence in these patients, demonstrating the highest odds ratio.

*Enhancing sensitivity of cervical cancer cells to cisplatin through TBX2 knockdown*. To assess the impact of TBX2 expression knockdown on chemotherapy sensitivity, cervical cancer cell lines were utilized *in vitro*. Transfection of si-TBX2 successfully decreased TBX2 expression in these cells, as evidenced by RT-qPCR analysis, which demonstrated a significant reduction in TBX2 mRNA levels compared to cells transfected with control siRNA (P<0.01; Fig. 4A). After confirming the successful knockdown of TBX2, the viability of cervical cancer cells with TBX2 expression knocked down was compared to that of the control-transfected cells. Notably, at concentrations of ≥12.5 $\mu$M cisplatin, the viability of tumor cells with TBX2 knockdown was significantly lower than that of the corresponding control cells (P<0.05; Fig. 4B). These findings demonstrate that TBX2 knockdown increased the sensitivity of cells to cisplatin.

## Discussion

Despite the introduction of the HPV vaccine, which can decrease the risk of developing cervical cancer by up to 90% with complete vaccine coverage (29), cervical cancer

Table II. Characteristics of the patients according to T-box 2 expression.

| Characteristics | Low expression group | High expression group | P-value |
|---|---|---|---|
| No. of patients | 80 | 20 | |
| Age, years[a] | 54.5 (29-76) | 61.0 (37-76) | 0.93[b] |
| FIGO stage | | | 0.024[c,d] |
| I | 51 | 7 | |
| II | 29 | 13 | |
| Histology | | | 0.114[d] |
| SCC | 67 | 13 | |
| Non-SCC | 13 | 7 | |
| Parametrium invasion | | | 0.053[d] |
| + | 18 | 9 | |
| - | 62 | 11 | |
| Lymph node metastasis | | | 0.454[d] |
| + | 35 | 11 | |
| - | 45 | 9 | |

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

Table III. Association between T-box 2 expression and recurrence.

| TBX2 expression, n (%) | No recurrence (%) | Recurrence (%) | P-value |
|---|---|---|---|
| Low expression[a] | 75 (93.8) | 5 (6.25) | <0.01[b,c] |
| High expression[d] | 10 (50.0) | 10 (50.0) | |

[a]Score, ≤8; [b]P≤0.01; [c]Fisher's exact test; [d]score ≤9.

remains a significant global health threat for women. The current standard of care for patients with intermediate- and high-risk stage IB-IIB cervical cancer post-radical hysterectomy involves either irradiation or concurrent chemoradiation, determined by the risk factors of recurrence assessed using resected specimens (3,4). However, these treatments, particularly following highly invasive gynecologic surgery, can lead to severe side effects that significantly impair a patient's quality of life. These include urinary disturbances, lower-limb lymphedema, bowel obstruction, sexual and ovarian dysfunction, and mental health issues stemming from these chronic conditions (6-8). Given these complications, there has been a global shift towards exploring adjuvant chemotherapy as an alternative, with promising results reported (6,7,9,10). A notable randomized non-inferiority multicenter trial showed no significant difference in 3-year progression-free survival rates, which were 91.9% in both the adjuvant chemotherapy and concurrent chemoradiation therapy (CCRT) groups. Similarly,

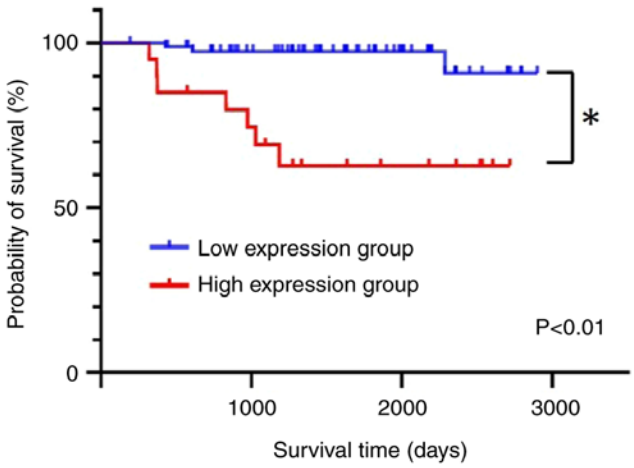


Figure 3. Kaplan-Meier survival analysis demonstrating overall survival. Patients with low TBX2 expression experienced significantly longer overall survival compared to those with high TBX2 expression. *P<0.01. TBX2, T-box 2.

5-year overall survival rates were 90.6% for chemotherapy and 90.0% for CCRT, with the chemotherapy group displaying a trend towards a better quality of life (9). Nevertheless, adjuvant chemotherapy is not yet a standard treatment following radical hysterectomy. If the likelihood of recurrence could be more accurately predicted following adjuvant chemotherapy, this would provide valuable guidance for gynecological oncologists in choosing between chemotherapy and radiation therapy as adjuvant treatments.

The TBX gene family consists of five distinct subfamilies: T, Tbx1, Tbx2, Tbx6, and Tbr1. Within the T subfamily, the T subfamily encompasses T and TBX19; the Tbx1 subfamily encompasses TBX1, TBX10, TBX15, TBX18, TBX20, and TBX22; the Tbx2 subfamily includes TBX2, TBX3, TBX4, and TBX5; the Tbx6 subfamily contains TBX6 and Mga; and the Tbr1 subfamily consists of TBR1, TBR2, and TBX21 (11). TBX genes play pivotal roles in organogenesis and pattern formation across vertebrate and invertebrate species (11). TBX2 is a transcription factor that was found to be involved in the regulation of cell cycle progression during cancer and embryonic development (12,13). TBX2 has been shown to facilitate the evasion of senescence by down-regulating the cell cycle regulators p21 and p14 (16,30,31). In addition to its role in cell cycle regulation, TBX2 also mediates apoptotic signaling pathways through p21 (32). Suppression of p21 reportedly leads to chemoresistance via modulation of the G1/S cell cycle transition and inhibiting apoptosis induced by chemotherapy in lung cancer (33). Additionally, knockdown of TBX2 sensitized cisplatin-resistant breast cancer cells to cisplatin (23), which is consistent with the results of the present study. However, to the best of our knowledge, the association between TBX2 expression and the efficacy of platinum-based adjuvant chemotherapy in patients with intermediate to high-risk stage IB-IIB cervical cancer following radical hysterectomy remains largely unclear.

In the present study, it was found that TBX2 expression levels were associated with both recurrence and overall survival rates among patients with intermediate- to high-risk stage IB-IIB cervical cancer who underwent adjuvant TP chemotherapy following radical hysterectomy. Specifically,

Table IV. Multivariate analysis for detecting independent risk factors for recurrence.

| Variable | Odds ratio | 95% confidence interval | P-value |
|---|---|---|---|
| T-box 2 expression, high/low | 12.3 | 3.0-50.5 | <0.01[a,b] |
| Histology, SCC/non-SCC | 5.01 | 1.06-20.50 | 0.038[b,c] |
| Parametrium invasion, +/- | 1.99 | 0.471-8.390 | 0.350[b] |
| Lymph node metastasis, +/- | 4.58 | 0.985-21.300 | 0.052[b] |

[a]P<0.01; [b]Logistic regression analysis; [c]P<0.05. SCC, squamous cell carcinoma.

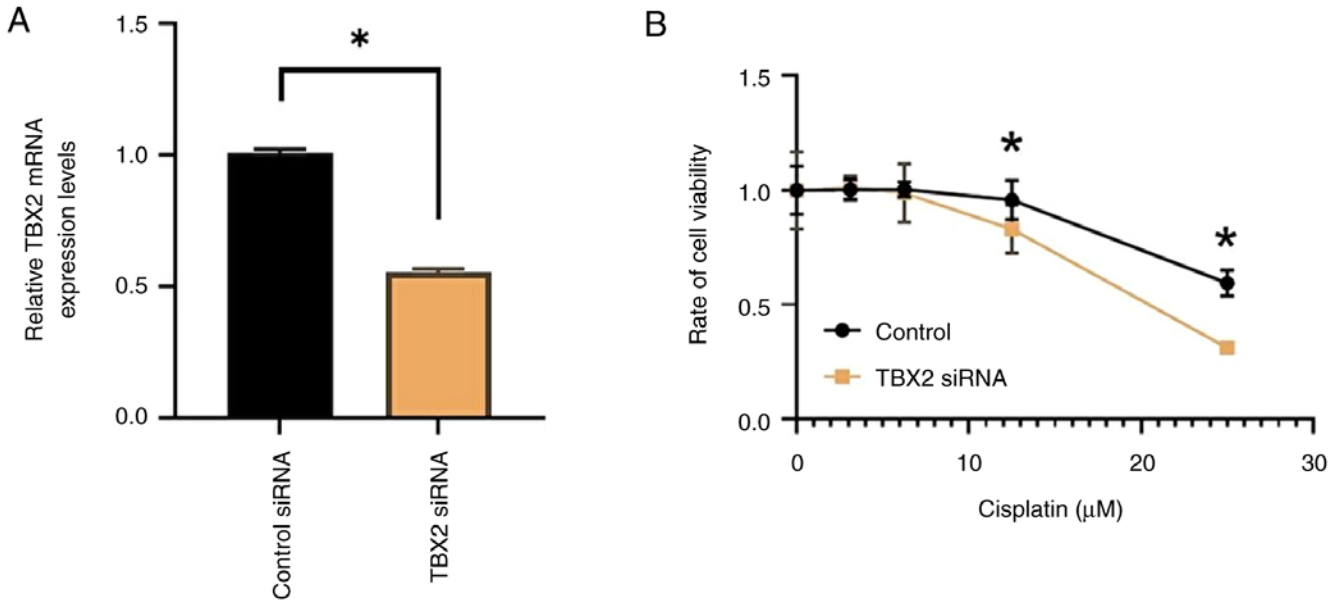


Figure 4. Effects of TBX2 knockdown on cisplatin sensitivity in cervical cancer cells. (A) TBX2 mRNA expression was significantly reduced in cells transfected with TBX2-siRNA compared to control siRNA-transfected cells, indicating successful knockdown. *P<0.01. (B) Treatment with varying concentrations of cisplatin demonstrated significantly decreased cell viability in TBX2 knockdown cells at concentrations of 12.5 $\mu$M and higher. *P<0.05. Data are presented as the mean ± standard deviation. TBX2, T-box 2; siRNA, small interfering RNA.

the high expression group (score ≥9; consisting of 20 patients) exhibited a higher likelihood of recurrence and poorer overall survival outcomes. Multivariate analysis aimed at identifying independent predictors of recurrence in this patient subset revealed that high TBX2 expression emerged as the most significant risk factor, demonstrating the highest odds ratio. Furthermore, the *in vitro* experiments confirmed that TBX2 knockdown using siRNA enhanced the effectiveness of cisplatin against cervical cancer cells.

This study has several limitations. Firstly, its retrospective design may introduce biases that affect the collection, analysis, and interpretation of data. Secondly, it includes a relatively small cohort of 100 patients, which could restrict the generalizability of the findings and reduce the statistical power. Thirdly, the study participants were drawn from specific institutions within a limited geographical area, which may further constrain the applicability of the findings to broader populations or regions. Fourthly, TBX2 expression was assessed using only immunohistochemical techniques and a single scoring system. Fifthly, the study did not examine interactions with other biomarkers, such as CLPTM1L, which is also reported to be associated with the effectiveness of TP chemotherapy (34). Sixthly, the study did not investigate the underlying mechanisms by which TBX2 contributes to chemoresistance. To validate these results, larger, multicenter prospective studies are necessary. Standardizing the techniques for assessing TBX2 expression is crucial for reliable clinical implementation. Further research into the biological mechanisms by which TBX2 contributes to chemoresistance and tumor progression could uncover additional therapeutic targets. Additionally, further research should also investigate additional factors that may interact with TBX2 expression levels to improve the accuracy of predictions regarding recurrence.

To the best of our knowledge, this study is the first to show the correlation between TBX2 expression and recurrence among cervical cancer patients treated with TP as adjuvant chemotherapy. TBX2 shows promise as a valuable clinical marker for gauging the efficacy of TP in this patient cohort, characterized by intermediate- to high-risk stage IB-IIB disease following radical hysterectomy. While adjuvant chemotherapy is gaining traction due to growing evidence of its clinical benefits, it has yet to become a standard guideline-recommended treatment. Therefore, it is crucial to identify reliable indicators that gynecological oncologists can utilize to assess the sensitivity of

adjuvant chemotherapy. By stratifying patients based on TBX2 expression levels, gynecological oncologist can tailor adjuvant treatment plans more effectively. For instance, patients with high TBX2 expression might benefit from more aggressive monitoring and conventional therapeutic strategies including irradiation or concurrent chemoradiation. Conversely, patients with low TBX2 expression, who are at a lower risk of recurrence with adjuvant TP chemotherapy, could choose TP chemotherapy as adjuvant therapies and avoid the side effects related to irradiation or concurrent chemoradiation, thereby improving their quality of life. This study may contribute significantly to the ongoing effort to identify optimal candidates for adjuvant chemotherapy among patients with cervical cancer.

In conclusion, the present study suggests that TBX2 expression may potentially function as a predictive biomarker for recurrence in patients with intermediate- and high-risk stages IB-IIB cervical cancer treated with adjuvant TP chemotherapy post-radical hysterectomy.

**Acknowledgements**

The authors would like to thank Dr Yukimi Kira (Research Support Platform, Osaka Metropolitan University, Graduate School of Medicine in Osaka, Japan) for their technical assistance.

**Funding**

This study received financial support from the JSPS KAKENHI (grant no. 19K09808).

**Availability of data and materials**

The data generated in the present study may be requested from the corresponding author.

**Authors' contributions**

TN, TF and TS conceived and designed the research. TN, EU, YA, TW, RT and MY performed the experiments and collected the data. TN, TF, TY and TS were responsible for data analysis. TN and TF drafted the manuscript. TN and TF confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

**Ethics approval and consent to participate**

The Ethics Committee of Osaka Metropolitan University Hospital (Osaka, Japan) approved the study protocol (approval no. 2022-102). All participants provided written informed consent to take part in this study.

**Patient consent for publication**

Written informed consent was obtained from all participants for the publication of their data.

**Competing interests**

The authors declare that they have no competing interests.

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