

T-box 2 expression is a useful indicator of the response to neoadjuvant chemotherapy for patients with locally advanced uterine cervical squamous cell carcinoma

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Abstract. Platinum-based concurrent chemoradiotherapy is the standard treatment for patients with locally advanced uterine cervical squamous cell carcinoma. Reducing the tumor size by administering neoadjuvant chemotherapy (NAC) is beneficial for successful hysterectomy, resulting in a more favorable prognosis. Therefore, identifying biomarkers that predict the effectiveness of NAC in patients with cervical squamous cell carcinoma remains a priority. Cancer cells widely express T-box 2 (TBX2), which contributes to the resistance to DNA-damaging chemotherapeutic agents. The present study aimed to determine the association between TBX2 protein expression in tumor tissues and the efficacy of NAC in locally advanced uterine cervical squamous cell carcinoma using immunohistochemistry. Data from 46 patients with locally advanced uterine cervical squamous cell carcinoma were classified into two groups based on their effective or ineffective response to NAC treatment. In addition, the effect of small interfering RNA-mediated knockdown of TBX2 on the sensitivity of cervical cancer cells to cisplatin was investigated *in vitro*. The results revealed that there were no significant differences in patient clinicopathological features between the NAC effective and NAC ineffective groups. The overall survival of the NAC effective group was significantly improved compared with the NAC ineffective group ($P=0.007$). Tumors from the NAC effective group also had significantly downregulated TBX2 expression levels compared with those from the NAC ineffective group ($P=0.0138$). Of note, decreased TBX2 expression was indicated to be significantly associated with higher sensitivity to NAC ($P=0.009$). The low TBX2 expression group had a more favorable overall survival

compared with the high TBX2 expression group ($P=0.049$). Furthermore, knockdown of TBX2 expression significantly increased cancer cell sensitivity to cisplatin *in vitro*. In conclusion, the results of the present study suggested that TBX2 expression may be a useful predictor of the response to NAC in patients with locally advanced uterine cervical squamous cell carcinoma.

Introduction

Cervical cancer, one of the most common types of malignancy worldwide, is the fourth leading cause of cancer-associated mortality among females (1). Despite significant advances in prevention, screening and diagnostic methods, certain cases are diagnosed at locally advanced stages, such as Federation of Gynecology and Obstetrics (FIGO) stages IIIA, IIIB and IVA. Platinum-based concurrent chemoradiotherapy (CCRT) is currently used as the standard treatment strategy for patients with advanced disease (2-4). However, compared with patients with early-stage disease, the prognosis of these patients is unfavorable, with a 5-year survival rate of <60% (5,6). Reducing the tumor size by administering neoadjuvant chemotherapy (NAC) was indicated to facilitate the success of hysterectomy, resulting in improved prognosis (7,8); therefore, the use of NAC for the treatment of cervical cancer has attracted significant attention in recent years (8). However, if NAC fails to sufficiently reduce the tumor size to perform hysterectomy, the treatment strategy is altered to radiation therapy, which delays the initiation of core treatment and results in unfavorable prognosis (9,10). Thus, if it were possible to predict the response to NAC, this would have the potential to become one of the major strategies to treat patients with locally advanced cervical squamous cell carcinoma, providing a greater variety of treatment options for patients. Therefore, to identify eligible patients to receive NAC, there is an urgent requirement to discover biomarkers indicating the response to NAC in patients with locally advanced cervical squamous cell carcinoma.

The T-box (TBX) gene family consists of five subfamilies, including T, Tbx1, Tbx2, Tbx6 and Tbx1. TBX genes have a crucial role in organogenesis and pattern formation in vertebrate and invertebrate species (11). Transcription factors of the T-box

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families serve essential roles throughout development (12). Increased expression levels of TBX2 and TBX3, which are included in the Tbx2 subfamily, are thought to be associated with the oncogenic process. TBX2 is a transcription factor involved in embryonic development, cell cycle regulation and cancer (13,14). Cancer cells widely express TBX2 and it has been indicated to allow cancer cells to bypass senescence by suppressing the cell cycle regulators p21 and p14 (15-17). Suppressing p21 reportedly resulted in chemoresistance by modulating the G₁/S cell cycle transition and inhibiting chemotherapy-induced apoptosis in lung cancer (18). In addition, TBX2 expression levels were indicated to be upregulated in melanoma (16), breast cancer (17,19), prostate cancer (20), non-small cell lung cancer (21), gastric cancer (22), laryngeal squamous cell carcinoma (23) and pancreatic cancer (24). Furthermore, ectopic TBX2 expression was associated with resistance to DNA-damaging chemotherapeutic agents, such as doxorubicin and cisplatin (25-27). However, to the best of our knowledge, the relationship between TBX2 expression and platinum-based chemotherapy in cervical squamous cell carcinoma has remained largely elusive.

The present study investigated the value of TBX2 expression as an indicator of the effectiveness of NAC by determining the relationship between TBX2 expression in tumors and the response to NAC in patients with locally advanced uterine cervical squamous carcinoma. Furthermore, the effect of small interfering RNA (siRNA/si)-mediated knockdown of TBX2 expression on the sensitivity of cervical cancer cells to cisplatin was evaluated *in vitro*.

Materials and methods

Patient study. A total of 46 patients with FIGO stage IIIA and IIIB uterine cervical squamous cell carcinoma who underwent NAC at Osaka City University Hospital (Osaka, Japan) between April 1995 and March 2010, were retrospectively evaluated. The inclusion criteria were as follows: Patients were diagnosed as uterine cervical squamous cell carcinoma histologically, stages IIIA and IIIB, patients who underwent NAC and patients with available medical records to analyze. Patients for whom sufficient medical records were unavailable were excluded. A punch biopsy of tumor tissue was performed before the administration of chemotherapy (cisplatin). Clinical variables including age, FIGO stage, tumor size and the effect of NAC treatment were obtained for each patient. Patients were divided into two groups based on their response to NAC: i) NAC effective group (n=25), which included patients who were successfully treated with NAC, underwent a hysterectomy and received radiotherapy; and ii) NAC ineffective group (n=21), which comprised patients who experienced NAC treatment failure and only received radiotherapy. The effect of NAC was evaluated by pelvic examination and computed tomography or magnetic resonance imaging. Successful NAC was defined as the stage being reduced to stage I or II, making a hysterectomy possible, while in cases with unsuccessful NAC, the tumor size was not sufficiently reduced to perform a hysterectomy. All patients were administered 50, 75 or 100 mg/m² cisplatin (Bristol Myers Squibb), which was based on the renal function and age of the patients (28), intra-arterially via balloon-occluded arterial infusion three times over 30 min.

Written informed consent was obtained from all patients prior to the tumor biopsy and the experimental protocol was approved by the Institutional Review Board of Osaka City University Hospital (Osaka, Japan; approval no. 4276).

Immunohistochemical (IHC) staining. The sections (4 μm thick) were prepared from the paraffin-embedded tissue blocks. The sections were deparaffinized and endogenous peroxidase activity was blocked by immersing in 3% hydrogen peroxidase in methanol. Antigen retrieval was performed by immersing the samples in 10 mM citrate buffer (pH 6.0) and heating the samples in an autoclave at 110°C for 20 min. IHC staining was performed using the Dako LSAB2 Peroxidase kit (cat. no. K0675; Agilent Technologies, Inc.). In brief, after blocking endogenous peroxidase activity and performing antigen retrieval, tissue sections were incubated with a rabbit polyclonal anti-TBX2 antibody (1:50 dilution; cat. no. LS-C402301; LifeSpan BioSciences, Inc.) in a humidity chamber at 4°C overnight. Following incubation with the primary antibody, the sections were incubated with biotinylated goat anti-mouse and anti-rabbit immunoglobulin G secondary antibodies included in the Dako LSAB2 Peroxidase kit (cat. no. K0675; Agilent Technologies, Inc.) at room temperature for 10 min. The sections were subsequently incubated with a streptavidin-peroxidase complex and 3,3'-diaminobenzidine was used as the chromogen. The specificity control was prepared in the same way with omission of the primary antibodies.

TBX2 expression levels were quantitatively scored according to the weighted scoring method established by Sinicrope *et al.* (29). The IHC score was determined using a light microscope (magnification, x400) based on the average percentage of stained tumor cells, which was scored using a scale of 0-4 as follows: 0 (<5%), 1 (5-25%), 2 (25-50%), 3 (50-75%) and 4 (>75%). The intensity of cell staining was scored as 1+ (weak), 2+ (moderate) and 3+ (intense). Weighted scores were calculated by multiplying the score of the stained tumor cell percentage and the score of the staining intensity.

Cell lines and culture. The CaSki cervical cancer cell line (cat. no. IFO50007) was purchased from the Japanese Collection of Research Bioresources Cell Bank. Cells were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin, and maintained in an incubator with 5% CO₂ at 37°C.

Small interfering (si)RNA transfection and cell survival assay. CaSki cells were seeded into 96-well plates (2x10³ cells/well) and divided into two groups: i) Treated group, in which cells were transfected with TBX2-specific siRNA (customized siTBX2; Sigma-Aldrich; Merck KGaA); and ii) control group, in which cells were transfected with nontargeting siRNA (Mission® Universal Negative Control #1; cat. no. SIC001-10NMOL; Sigma-Aldrich; Merck KGaA). The siTBX2 sequence was as follows: Sense, 5'-CCAAUG AACUGCAGAGCAU[dT][dT]-3' and antisense, 5'-AUGCUC UGCAGUUCAUUGG[dT][dT]-3'. siTBX2 transfections were performed using Lipofectamine® RNAiMax (Invitrogen; Thermo Fisher Scientific, Inc.) strictly following the manufacturer's protocol. After confirming cell adhesion in both

Table I. Clinicopathological features of patients in the NAC effective and NAC ineffective groups.

| Variable | NAC effective group (n=25) | NAC ineffective group (n=21) | P-value |
|---|----------------------------|------------------------------|--------------------|
| Age, years | | | |
| Mean ± SD | 49.3±13.2 | 53.7±11.2 | 0.241 ^a |
| Range | 24-69 | 37-68 | |
| Body height, cm | | | |
| Mean ± SD | 154.5±6.67 | 152.1±5.09 | 0.182 ^a |
| Range | 138-166 | 143-162 | |
| Body weight, kg | | | |
| Mean ± SD | 53.01±8.76 | 48.73±8.19 | 0.096 ^a |
| Range | 37-78 | 38-67 | |
| International Federation of Gynecology and Obstetrics stage | | | 1 ^b |
| IIIA | 1 | 0 | |
| IIIB | 24 | 21 | |
| Tumor size, mm | | | 0.464 ^a |
| Mean ± SD | 46.6±16.8 | 50.2±12.5 | |

^aUnpaired Student's t-test; ^bFisher's exact test. NAC, neoadjuvant chemotherapy; SD, standard deviation.

groups, the control group was provided with fresh medium containing nontargeting siRNA transfection complexes, whereas the transfection group was treated with fresh medium containing siTBX2 transfection complexes. Following 24 h of incubation at 37°C, the cells in each group were incubated for an additional 48 h at 37°C in fresh medium containing 0, 2.5, 5.0, 7.5, 10 or 50 µM cisplatin. Cell viability was subsequently measured using a Cell Counting Kit (CCK)-8 assay (Dojindo Molecular Technologies, Inc.). In brief, 10 µl CCK-8 and 100 µl RPMI-1640 were added to each well, followed by incubation at 37°C for 2 h. The absorbance of each well was measured at a wavelength of 450 nm using a microplate reader (Corona Electric Co., Ltd.). The percentage of viable cells in comparison with the control cells was evaluated using dose-response curves.

Reverse transcription-quantitative PCR (RT-qPCR). RT-qPCR was performed using TaqMan chemistry. The procedure was performed according to the manufacturer's protocol. TaqMan primer and probes for TBX2 (cat. no. Hs00911929_m1) and hypoxanthine phosphoribosyl-transferase 1 (cat. no. Hs02800695_m1) were obtained from Thermo Fisher Scientific, Inc. Total RNA was extracted from CaSki cells using the RNeasy Mini kit (Qiagen GmbH). Total RNA (1 µg) was reverse transcribed into cDNA using a High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific, Inc.). qPCR was subsequently performed on an ABI 7500 Fast Real-Time PCR detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.) using TaqMan Fast Universal PCR Master Mix (Thermo Fisher Scientific, Inc.). The following thermocycling conditions were used for qPCR: Initial denaturation at 95°C for 20 sec, followed by 40 cycles of 95°C for 3 sec and 60°C for 30 sec. The $2^{-\Delta\Delta C_q}$ method was used to calculate the relative changes in gene expression for the RT-qPCR experiments (30).

Statistical analysis. Statistical analysis was performed using EZR version 1.3 software (Saitama Medical Center, Jichi Medical University) (31). Values are expressed as the mean ± standard deviation. Statistically significant differences between groups were determined using unpaired Student's t-tests. Fisher's exact test was used to determine the association between categorical variables in the different patient groups. The Kaplan-Meier method and log-rank tests were used for survival analysis. Mann-Whitney U-tests were used to compare the IHC weighted scores. A receiver operating characteristic (ROC) curve was used to determine the cutoff value of TBX2 score to predict the effect of NAC treatment. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics. The 46 patients in the present study were divided into two groups based on treatment efficacy: NAC effective group (n=25) and NAC ineffective group (n=21). Differences in age, body height, body weight, FIGO stage and tumor size were analyzed and no statistically significant differences in these variables were identified between the two groups (Table I).

TBX2 protein expression levels in cervical squamous cell carcinoma tissues. IHC was used to analyze TBX2 protein expression levels in cervical squamous cell carcinoma tissues and the weighted scores of both groups were calculated (Fig. 1A-E). TBX2 was indicated to be predominantly located in the cell nuclei. The weighted score of the NAC ineffective group was significantly increased compared with that in the NAC effective group ($P = 0.0138$; Fig. 1E). A ROC curve was used to determine the cutoff value of TBX2 scores to predict the effectiveness of NAC treatment (Fig. 1F). The ROC curve indicated that with a cutoff value of 6, TBX2 scores were

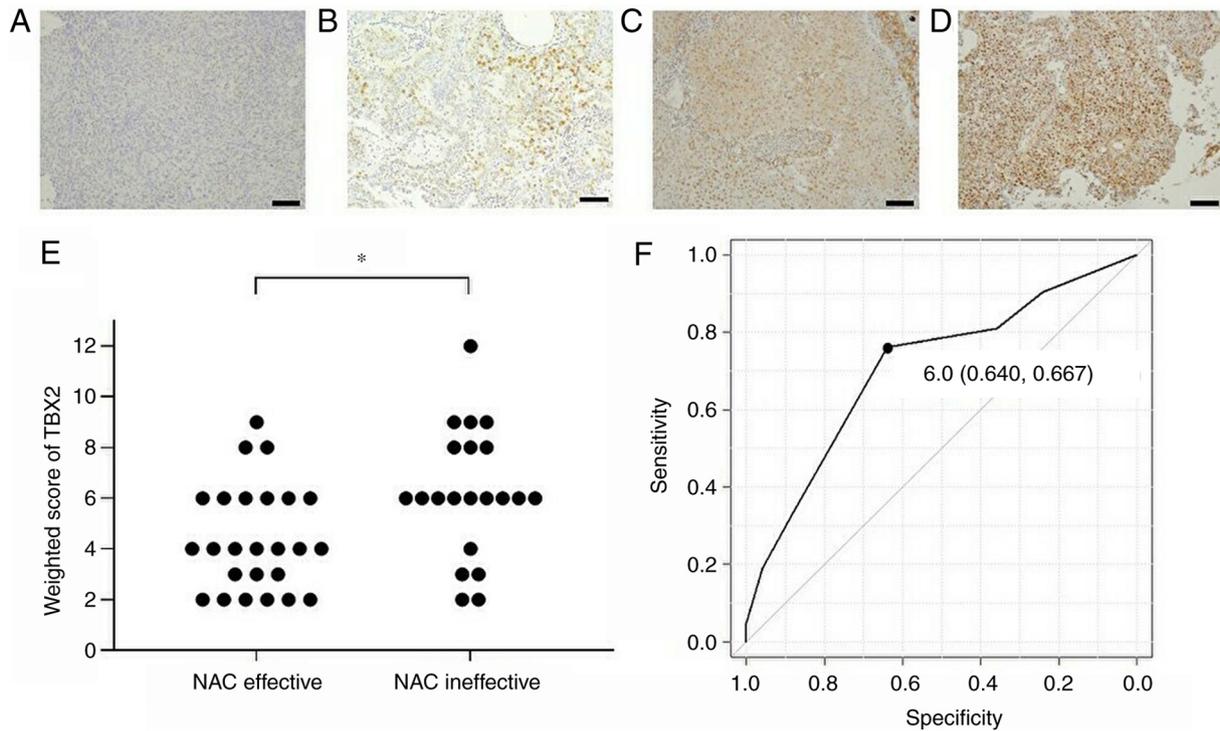


Figure 1. IHC staining for TBX2 expression in punch biopsy samples obtained prior to the initiation of treatment. (A) Negative control IHC staining was performed without the primary antibody. Representative sections with a weighted score of (B) 4, (C) 6 and (D) 12 following staining with an anti-TBX2 primary antibody. Hematoxylin was used for counterstaining (scale bars, 50 μ m). (E) Weighted scores for TBX2 protein expression levels in punch biopsy samples obtained prior to the initiation of treatment. TBX2 expression levels were significantly downregulated in the NAC effective group compared with the NAC ineffective group. * $P=0.0138$ (Mann-Whitney U-test). (F) Receiver operating characteristic curve for determining the TBX2 cutoff score to evaluate the effectiveness of NAC. With a cutoff value of 6, the specificity was 64.0% (0.640) and the sensitivity was 66.7% (0.667). The area under the curve was 0.662 with a 95% confidence interval of 0.505-0.819. TBX2, T-box 2; NAC, neoadjuvant chemotherapy; IHC, immunohistochemistry.

able to predict the effectiveness of NAC, with a specificity of 64% and sensitivity of 67%. The area under the curve was 0.662 with a 95% confidence interval of 0.505-0.819. The patients were subsequently divided into the following two groups based on their weighted scores: Low TBX2 expression (weighted score, ≤ 4 ; $n=21$) and high TBX2 expression (weighted score, ≥ 6 ; $n=25$). No statistically significant differences were observed in the patient characteristics between these two groups (Table II). These results suggested that TBX2 expression may be associated with the efficacy of NAC.

Association between NAC sensitivity and TBX2 expression.

Whether there was a difference in NAC effectiveness between the low TBX2 expression and high TBX2 expression groups was subsequently evaluated. In the low TBX2 expression group, NAC was effective in 76.2% of cases. By contrast, in the high TBX2 expression group, NAC was effective in 36% of patients. Of note, this difference in effectiveness of NAC between the two groups was statistically significant ($P=0.009$; Table III). These results indicated that the low TBX2 expression group may be more sensitive to NAC compared with the high TBX2 expression group.

Survival analysis. The patients' follow-up period varied from 124 to 5,015 days. The overall survival of patients in the NAC effective group was significantly improved compared with that in the NAC ineffective group ($P=0.007$; Fig. 2A). In addition, the low TBX2 expression group had a more favorable

overall survival compared with the high TBX2 expression group ($P=0.049$; Fig. 2B). These results suggested that TBX2 expression may help predict the prognosis of patients with locally advanced uterine cervical squamous cell carcinoma who received NAC treatment.

Effect of TBX2 knockdown on the cisplatin sensitivity of cervical cancer cells. RT-qPCR analysis revealed that TBX2 mRNA expression levels were significantly downregulated following transfection with siTBX2 compared with control cells (Fig. 3A). Of note, CaSki cells transfected with siTBX2 had a significantly enhanced sensitivity to cisplatin compared with control cells ($P<0.01$; Fig. 3B). These results suggested that TBX2 may be involved in the mechanism through which cisplatin exerts cytotoxic effects on cancer cells.

Discussion

NAC is a useful treatment option for patients with cervical cancer; therefore, a significant amount of research has focused on the use of NAC for patients with cervical cancer in recent years. For instance, Sala *et al.* (8) reported that NAC improved the survival outcome for patients with stage IB2-IVA uterine cervical cancer in a multicenter retrospective analysis. Chen *et al.* (32) demonstrated that NAC reduced the probability of lymph node metastasis for patients with stage IB1-IIB uterine cervical cancer. In addition, de Vincenzo *et al.* (33) reported that the administration of NAC followed by conization in

Table II. Clinicopathological features of patients in the TBX2 low and high groups.

| Variable | Low TBX2 expression (score ≤ 4) (n=21) | High TBX2 expression (score ≥ 6) (n=25) | P-value |
|--|---|--|--------------------|
| Age, years | | | |
| Mean \pm SD | 50.4 \pm 12.7 | 52.0 \pm 12.6 | 0.659 ^a |
| Range | 24-69 | 29-68 | |
| Body height, cm | | | |
| Mean \pm SD | 154.0 \pm 7.22 | 153.0 \pm 5.00 | 0.573 ^a |
| Range | 138-166 | 143-162 | |
| Body weight, kg | | | |
| Mean \pm SD | 53.10 \pm 9.61 | 49.34 \pm 7.60 | 0.146 ^a |
| Range | 37-78 | 38-67 | |
| International Federation of Gynecology and Obstetrics stage | | | 1 ^b |
| IIIA | 1 | 0 | |
| IIIB | 20 | 25 | |
| Tumor size, mm | | | 0.439 ^a |
| Mean \pm SD | 46.2 \pm 15.2 | 50.0 \pm 15.0 | |

^aUnpaired Student's t-test; ^bFisher's exact test. TBX2, T-box 2; SD, standard deviation.

Table III. Number of patients with low (score of ≤ 4) and high (score of ≥ 6) TBX2 expression in the NAC effective and NAC ineffective groups.

| TBX2 expression | NAC effective group | NAC ineffective group | P-value |
|-----------------|---------------------|-----------------------|--------------------|
| Low | 16 (76.2) | 5 (23.8) | 0.009 ^a |
| High | 9 (36.0) | 16 (64.0) | |

^aFisher's exact test. Values are expressed as n (%). TBX2, T-box 2; NAC, neoadjuvant chemotherapy.

stage IB2-IIA1 cervical cancer resulted in improved fertility. Sun *et al* (34) also reported that treatment with NAC provided an improved quality of life for patients with stage IB2-IIA cervical cancer.

Platinum-based CCRT is currently used as a standard treatment strategy for patients with locally advanced uterine cervical squamous cell carcinoma (2-4). However, the prognosis of these patients remains unfavorable, suggesting that improvements in the available treatments are required. Effective NAC treatment has been reported to reduce the tumor size, allow patients to undergo hysterectomy and potentially improve patient prognosis (7,8). However, if NAC is ineffective, there are currently no alternatives to surgical resection other than radiotherapy, which may lead to unfavorable prognosis due to the delay in the initiation of core treatment (35). Therefore, identifying biomarkers that are able to predict the efficacy of NAC remains important for selecting patients that are likely to benefit from NAC treatment.

Cisplatin and other platinum-containing drugs exert antitumor effects by covalently binding to DNA in cancer cells (36), which facilitates apoptosis by inhibiting DNA replication (37). Usually, platinum-based chemotherapy is initially effective; however, cancer cells may later develop resistance to these agents (38). Potential mechanisms of platinum resistance include decreased cellular uptake of cisplatin (39,40), increased cisplatin detoxification capacity (41), enhanced DNA damage repair capacity (42,43), deactivation of apoptotic signaling pathways (44) and other epigenetic modifications that occur at both the cellular and molecular levels (45,46).

TBX2 is a transcription factor that was discovered to be involved in the regulation of cell cycle progression during cancer and embryonic development (13,14). TBX2 was indicated to be widely expressed in cancer cells and permits them to bypass senescence by suppressing the cell cycle regulators p21 and p14^{ARF} (15-17). In addition to its role in cell cycle regulation, p21 also mediates apoptotic signaling pathways (47). Several studies have demonstrated that p21 has a proapoptotic role in cancer (48). For instance, p21 induction by RNA activation enhanced antitumor activity by promoting cell cycle arrest and apoptotic cell death in bladder cancer cells (48). Transcriptional activation of p21 inhibited the viability of hepatocellular carcinoma cells and significantly increased cell apoptosis by downregulating the expression levels of anti-apoptotic proteins, including survivin and Bcl-xL, and upregulating the expression of executioner caspase-3 and -9 (49). In addition, TBX2 has been reported to contribute to cancer cell resistance to therapeutic agents, such as cisplatin (25,26), by modulating the G₁/S cell cycle transition and inhibiting chemotherapy-induced apoptosis via suppression of the cell cycle regulator p21 (18). Those reports regarding cancer cell resistance to cisplatin are consistent with the present results and they support the present results.

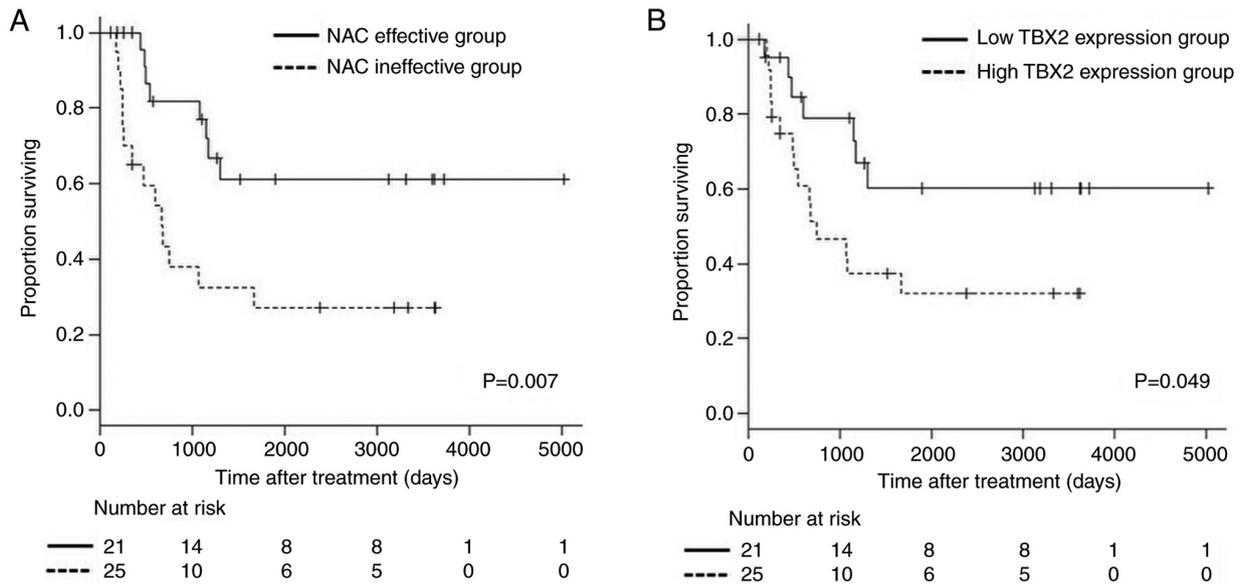


Figure 2. Kaplan-Meier analysis of overall survival of the patients. (A) Comparison of survival between the NAC effective group (n=25) and NAC ineffective group (n=21). Solid line, NAC effective group; dashed line, NAC ineffective group. The NAC effective group had a significantly improved overall survival compared with the NAC ineffective group (P=0.007; log-rank test). (B) Comparison of survival between the low TBX2 expression group (n=21) and high TBX2 expression group (n=25). Solid line, low TBX2 expression; dashed line, high TBX2 expression. The low TBX2 expression group had significantly improved overall survival compared with the high TBX2 expression group (P=0.049; log-rank test). Any censored datapoints represent missing follow-up data. TBX2, T-box 2; NAC, neoadjuvant chemotherapy.

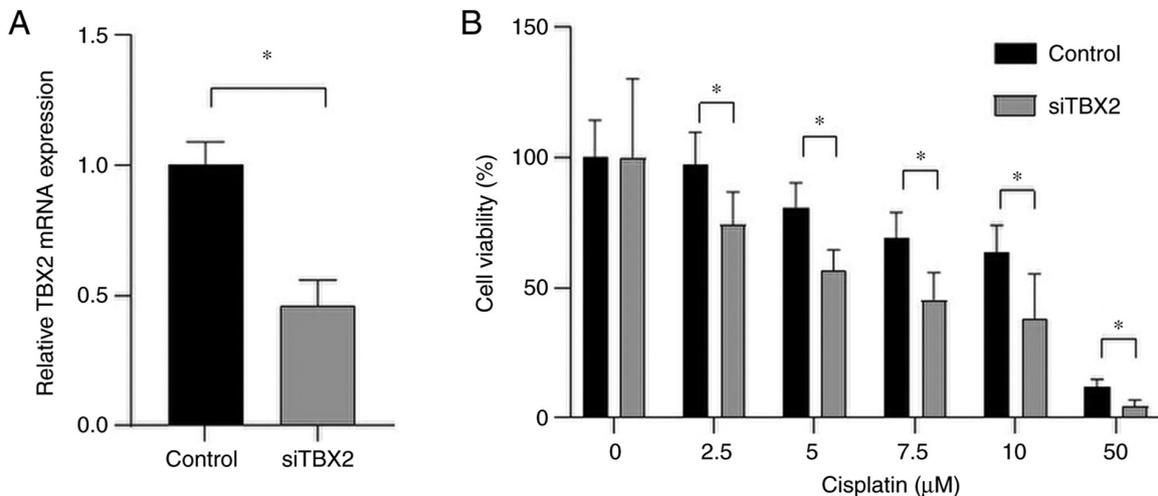


Figure 3. Effect of siTBX2 on TBX2 mRNA expression levels and cell viability in CaSki cervical cancer cells. (A) TBX2 mRNA expression levels were significantly downregulated following knockdown of TBX2 in CaSki cells. The mRNA expression levels were normalized to hypoxanthine phosphoribosyltransferase 1 mRNA. (B) Viability of CaSki cells in the presence or absence of siRNA specific for TBX2 after incubation for 24 h with 0, 2.5, 5.0, 7.5, 10 or 50 μM cisplatin. *P<0.01 vs. Control (unpaired Student's t-test). Values are expressed as the mean ± standard deviation. TBX2, T-box 2; si/siRNA, small interfering RNA.

The results of the present study predicted NAC efficacy in patients with locally advanced uterine cervical squamous cell carcinoma by determining TBX2 expression levels in histological samples obtained prior to the initiation of treatment. Patients with downregulated TBX2 protein expression levels were more susceptible to NAC treatment and more likely to undergo successful surgery following NAC treatment, resulting in improved prognosis.

To the best of our knowledge, the present study was the first to suggest the potential of determining TBX2 expression in tumors to predict the efficacy of NAC treatment in patients with

locally advanced uterine cervical squamous cell carcinoma. In addition, these results may improve the response to NAC treatment in patients with any stage of cervical squamous cell carcinoma by making it possible to select eligible patients that are likely to benefit from NAC treatment.

However, only 46 patients were included in the present retrospective study; therefore, the major limitations of the present study are the small number of patients used and the retrospective design. And even though human papillomavirus (HPV) infection status is a crucial factor when investigating uterine cervical cancer, those data of the

patients included in the present study were not available. Further studies involving a larger number of cases and data including the HPV infection status are required to address this issue and validate the present findings. Furthermore, the study was performed at a single institution without any external validation. In addition, the present study remains a hypothesis-exploratory study. Therefore, future studies are required to be performed at multiple centers to validate the present results.

In conclusion, the results of the present study indicated that TBX2 expression may represent a useful predictor of the response to NAC for patients with locally advanced uterine cervical squamous cell carcinoma. These results may be applied to patients with any stage of cervical squamous cell carcinoma to predict whether they may benefit from NAC treatment.

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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 upon reasonable request.

Authors' contributions

YI, TF and TS designed the study. YI, HM, SN, YA, MS and MY performed the experiments and collected the data. YI, TF, TY and TS analyzed the data. YI and TF wrote the manuscript. YI and TF confirm the authenticity of all the raw data. All authors 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. 4276; Osaka, Japan). Written informed consent was obtained from all patients prior to participation.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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