Fyn expression is associated with the response of patients with locally advanced uterine cervical squamous cell carcinoma to neoadjuvant chemotherapy

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Abstract. Neoadjuvant chemotherapy (NAC) followed by surgery is the current standard of treatment for locally advanced uterine cervical cancer; however, its value and outcomes remain contested. Identifying biomarkers that allow for the prediction of the effect of NAC efficacy before initiation of treatment is essential, in order to assist in choosing the optimum therapeutic regimen to maximize the beneficial outcomes of treatment. In the present retrospective study, 44 patients with locally advanced uterine cervical squamous cell carcinoma who underwent NAC were divided into two groups: A NAC successful group and a NAC failure group, depending on the efficacy of NAC. Subsequently, the association between Fyn expression, a non-receptor tyrosine kinase that is a member of the Src family kinases, and NAC efficacy was determined; Fyn expression was detected by immunohistochemistry and assessed using a weighted scoring method. Additionally, the effect of Fyn knockdown on the sensitivity of a uterine cervical cancer cell line to cisplatin was determined. Notably, there were no significant differences between the two groups of patients regarding their characteristics. Regarding overall survival, the NAC successful group had a significantly longer survival time than the NAC failure group (P=0.01). Furthermore, the expression levels of Fyn in tumor tissues were significantly lower in the NAC successful group compared with those in the NAC failure group (P=0.003). The patients were subsequently divided into two groups (high expression group and low expression group) according to a cutoff value of 3, which was determined by producing a receiver operating characteristic curve from the weighted scores. The low expression group was significantly more sensitive to NAC than the high expression group (P<0.001). *In vitro* experiments revealed that Fyn knockdown significantly enhanced the sensitivity of uterine cancer cells to cisplatin (P<0.05). In conclusion, Fyn expression may be a potentially useful biomarker for predicting the response to NAC in patients with locally advanced uterine cervical squamous cell carcinoma, and may also be a promising molecular target for the management of uterine cancer.

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Introduction

Uterine cervical cancer has the fourth highest incidence and the fourth highest mortality rate of all cancers, with 604,125 new cases and 341,831 deaths in 2020 worldwide (1). Screening and prevention of cervical cancer have improved through human papillomavirus typing and vaccination; however, patients with locally advanced stages [Federation of Gynecology and Obstetrics (FIGO) stages IB2-IVA] are still commonly diagnosed in clinical practice. Currently, for locally advanced uterine cervical cancer, especially for stages IIIB-IVA, concurrent platinum-based chemoradiotherapy (CCRT) is the standard treatment strategy (2). However, the 5-year relative survival rate for patients with stage IIIB-IVB uterine cervical cancer is <60%, which is considerably lower than that for patients with early-stage uterine cervical cancer, for whom survival is >90% (3). Neoadjuvant chemotherapy (NAC) followed by surgery is considered one of the treatment options for patients with locally advanced uterine cervical cancer, even though its value remains contested. NAC has the potential to reduce the tumor size and allow for a hysterectomy for patients with stages IIIB-IVA uterine cancer in which hysterectomy is normally not possible, and this course can improve a patient's prognosis (4-6). However, if the NAC treatment does not effectively shrink the tumor size, the patients instead have to undergo radiotherapy, and this delays the initiation of the core treatment, and thus a worse prognosis. Therefore, if the effectiveness of NAC for locally advanced uterine cancer patients can be predicted, it may be possible to accurately choose the optimal candidate for NAC to improve outcomes. To this end, there is an urgent need for the identification of biomarkers that can easily be assessed prior to initiation of treatment, which can predict the effects of NAC for locally advanced uterine cancer patients.

The Src family of kinases (SFKs) play crucial roles in regulating a wide variety of cellular functions such as proliferation, migration, invasion, differentiation, survival angiogenesis, and motility in several types of cancers (7). Fyn, a non-receptor tyrosine kinase that is a member of the SFKs, is a 59 kDa protein, the gene for which is located on chromosome 6q21 (8). Fyn has been reported to phosphorylate and prevent the breakdown of PI3K enhancer-activating Akt (PIKE-A), which is an inhibitor of apoptosis (9). The overexpression of Fyn leads to the promotion of the antiapoptotic activity of Akt (10). Therefore, Fyn is considered an important molecule that can confer resistance to anti-cancer agents that exert their effects by inducing apoptosis. This fact can be applied to the chemotherapy using platinum-based anti-cancer agents for cervical carcinoma.

However, to the best of our knowledge, there is no research on whether Fyn affects the outcomes of chemosensitivity to platinum-based anti-cancer agents in cervical squamous cell carcinoma. The present study is the first to focus on chemosensitivity to a platinum-based anti-cancer agent in cervical squamous cell carcinoma based on Fyn expression, and the results revealed the value of Fyn expression as a biomarker in predicting the efficacy of NAC in patients with locally advanced uterine cervical squamous carcinoma.

Materials and methods

Patients. The present study is a retrospective study in which a total of 44 patients were enrolled. The inclusion criteria were as follows: i) Patients diagnosed with FIGO Stage IIIB (FIGO 2008) uterine cervical cancer whose cancer was pathologically confirmed by punch biopsy specimens before the initiation of treatment; ii) patients who underwent NAC using cisplatin at the Osaka City University Hospital (Osaka, Japan) between April 1996 and April 2010; iii) aged 20-70 years old; and iv) the medical records were available to analyze. Patients whose medical records were deemed inadequate for analysis were excluded. Information on the clinical factors such as FIGO stage, age, the effect of NAC, body mass index, serum squamous cell carcinoma (SCC) antigen value, and tumor size were collected before the initiation of treatment.

To compare which factors contributed to NAC efficacy, the patients were grouped depending on the efficacy of NAC into two groups: A NAC success group and a NAC failure group. NAC success was defined as a decrease in tumor size to that expected in stage I or II, and for whom a hysterectomy was made possible, and as failure when NAC failed to decrease the tumor size and a hysterectomy was not made possible, and thus radiation therapy had to be performed. NAC was performed using cisplatin. The total amount of cisplatin (Bristol Myers Squibb) administered was 50, 75, or 100 mg/m^2 (dependent on the renal function). Cisplatin was injected intra-arterially using balloon-occluded arterial infusion over 30 min three times every 4 weeks (4). In the NAC success group, a hysterectomy and consecutive radiation therapy were performed after NAC and in the NAC failure group, radiation therapy alone was performed after NAC.

All patients enrolled in this study provided written informed consent for the treatment and the use of their samples in this research prior to the initiation of NAC and the Institutional Review Board of Osaka City University Hospital approved this study (approval no. 2019-01).

Immunohistochemical (IHC) staining and scoring. For IHC, 4 μm thick sections generated from the paraffin-embedded tissue blocks were used. Before IHC staining, tissues were deparaffinized and the endogenous peroxidase activity of the samples was quenched using 3% hydrogen peroxide in methanol, after which antigen retrieval was performed by immersing the samples in Target Retrieval Solution, pH 9.0 (cat. no. S2367; Agilent Technologies, Inc.) and heating in an autoclave at 121˚C for 20 min. The DAKO LSAB2 Peroxidase kit (cat. no. K0675; Agilent Technologies, Inc.) was used for IHC according to the manufacturer's protocol. A rabbit monoclonal anti-Fyn antibody (cat. no. ab184276; 1:250 dilution) was used as the primary antibody at 4˚C overnight and biotinylated goat immunoglobulin G antibodies included in the DAKO LSAB2 Peroxidase kit were used as secondary antibodies at room temperature for 10 min. The slides were immersed in DAB solution to develop the stain at room temperature for 10 min and were counterstained with hematoxylin and 0.3% ammonia water at room temperature for 1 min.

Fyn expression was quantified using an established weighted scoring method (11). In this method, two independent factors were employed to generate the score, multiplying the score of the stained tumor cell percentage and the score of the staining intensity to obtain a weighted score for each sample. The score of the stained tumor cells was based on the average percentage of stained tumor cells and assigned as follows, 0 (<5%), 1 (5-25%), 2 (25-50%), 3 (50-75%), 4 (>75%). The score of the staining intensity was based on the intensity of staining and assigned as follows, 1 (weak), 2 (moderate), and 3 (intense).

Cell culture. CaSki cells (human papillomavirus-related cervical squamous cell carcinoma, cat. no. IFO50007) were incubated in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS and 1% penicillin in a humidified incubator at 37˚C with 5% CO_2.

Fyn knockdown and cell survival assays. Fyn siRNA transfections were performed using Lipofectamine® RNAiMax (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. A Fyn-specific siRNA (Fyn siRNA: cat. no. sc-29321; Santa Cruz Biotechnology, Inc.) or control siRNA (control siRNA-A: cat. no. sc-37007; Santa Cruz Biotechnology, Inc.) were used. The sense sequence of Fyn siRNA is CAUCGAGCGCAUGAAUUAU, and the antisense sequence was AUAAUUCAUGCGCUCGAUG which are provided in 5'→ 3'orientation. The sequence of control siRNA is confidential. CaSki cells were incubated in 96-well plates ($2x10^3$ cells/well) and divided into two groups: A treated group, in which Fyn siRNA transfection was performed, and a control group, in which control siRNA was transfected. After cell adhesion, in the treated group, cells were incubated with fresh medium containing Fyn siRNA transfection complexes, and in the control group, cells were incubated with fresh medium containing control siRNA at 37˚C for 24 h. Next, the cells in both groups were incubated for 24 h at 37˚C in fresh medium containing 10, 25, or 50 μM cisplatin. To determine cell viability, 10 μl Cell Counting Kit-8 solution (Dojindo Molecular Technologies, Inc.) and 100 μl RPMI-1640 medium were added to each well of both groups, and the cells were

Table I. Patient characteristics.

Characteristic	NAC successful group	NAC failure group	P-value
n	23	21	
Age, years[a]	47 (24-59)	55 (37-68)	0.241[b]
BMI[a]	21.7 (14.6-29.6)	21.3 (12.7-27.1)	0.891[b]
SCC antigen, ng/ml[a]	8.45 (0.7-187.0)	11.1 (1.6-49.3)	0.539[b]
Tumor size before NAC, mm[a]	43.5 (25-80)	48.0 (35-78)	0.257[b]
Tumor size after NAC, mm[a]	0 (0-45)	35.5 (5-51)	<0.001[b]

[a]Data are presented as the median (range). [b]Mann-Whitney U test. NAC, neoadjuvant chemotherapy; SCC, squamous cell carcinoma.

incubated 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.).

Reverse transcription-quantitative PCR (RT-qPCR). RT-qPCR was performed to verify the successful knockdown of Fyn mRNA expression after transfection of Fyn siRNA. TaqMan chemistry was used to perform RT-qPCR according to the manufacturer's protocol with TaqMan primer and probes for Fyn (cat. no. Hs00941613_m1) and hypoxanthine phosphoribosyl-transferase 1 (cat. no. Hs02800695_ml, Thermo Fisher Scientific, Inc.) which was used as an internal control (12). First, total RNA was extracted from cells using a RNeasy Mini kit (Qiagen GmbH). Next, total RNA (1 μg) was reverse transcribed into cDNA using a High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific, Inc.). Finally, qPCR was performed using TaqMan Fast Universal PCR MasterMix (Thermo Fisher Scientific, Inc.). The following thermocycling conditions were used for the qPCR: Initial denaturation at 95˚C for 20 sec; followed by 40 cycles at 95˚C for 3 sec and 60˚C for 30 sec. All procedures were performed in accordance with the manufacturer's protocol. The relative changes in gene expression were calculated using the $2^{-\Delta\Delta Cq}$ method (13).

Statistical analysis. Values are expressed as the mean ± standard deviation. A Fisher's exact test was used for determining the association between categorical variables in the two different groups, and a Mann-Whitney U-test was used for comparing the median and mean values between the two different groups. A Receiver Operating Characteristic (ROC) curve was generated to determine the cutoff value of the Fyn score to predict the effect of NAC treatment. The Kaplan-Meier method and log-rank tests were used to compare the survival between the two different groups. Three replicants were performed in RT-qPCR, and ten replicates were performed in cell survival assays. P<0.05 was considered to indicate a statistically significant difference. GraphPad Prism Version 8 (GraphPad Software, Inc.) was used for all statistical analyses.

Results

Patient characteristics and overall survival. There were 23 patients in the NAC success group and 21 patients in the NAC failure group. Comparison of the patients' characteristics between the two groups showed no significant differences in age, BMI, serum SCC value, and tumor size before NAC (Table I). Tumor size after NAC was significantly larger in the NAC failure group than in the NAC successful group (P<0.001; Table I). Regarding the overall survival, the NAC success group had significantly better overall survival than the NAC failure group (P=0.01; Fig. 1).

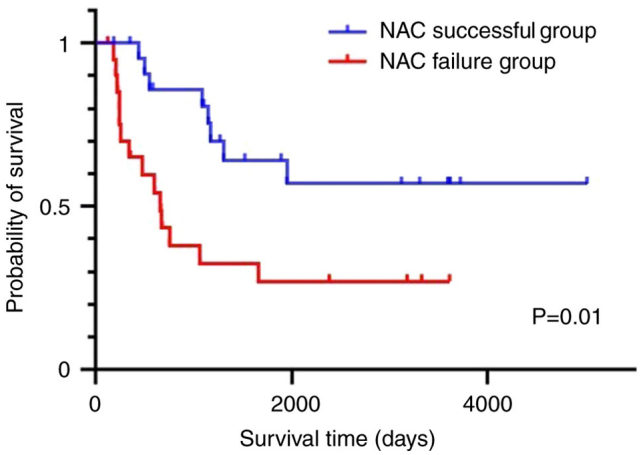

Figure 1. Kaplan-Meier survival analysis. Comparison of the overall survival between the NAC successful group and the NAC failure group. The overall survival of the NAC successful group was significantly better than that of the NAC failure group (P=0.01). NAC, neoadjuvant chemotherapy.

Fyn expression and Fyn cutoff values for predicting NAC efficacy. The expression of Fyn was compared between the two groups using IHC. Fyn protein expression was observed primarily at the cell membrane and in the cytoplasm (Fig. 2). The difference in expression was evaluated using weighted scores, and the results showed that the expression in the NAC failure group was significantly higher than in the NAC success group (P=0.003; Fig. 3A). Next, we evaluated the cutoff value of the weighted score to predict the efficacy of NAC using a ROC curve. The ROC curve showed that a cutoff value of 3 predicted the NAC efficacy with a sensitivity of 82.6% and specificity of 71.4%, with an area under the curve of 0.759 and a 95% confidence interval of 0.613-0.905 (Fig. 3B).

Contribution of Fyn expression to NAC efficacy. Based on the cut off value of 3, patients were divided into two groups, a

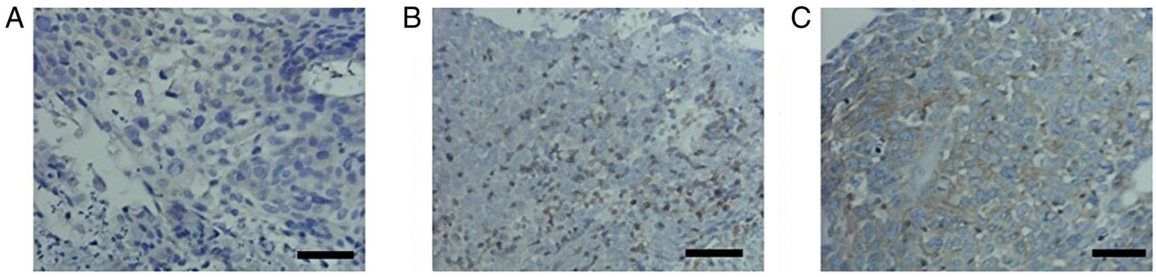

Figure 2. Immunohistochemical staining of Fyn in uterine cervical squamous cell carcinoma specimens counterstained with hematoxylin. Representative images of a weighted score of (A) 0, (B) 6, and (C) 12. Scale bar, 50 μm.

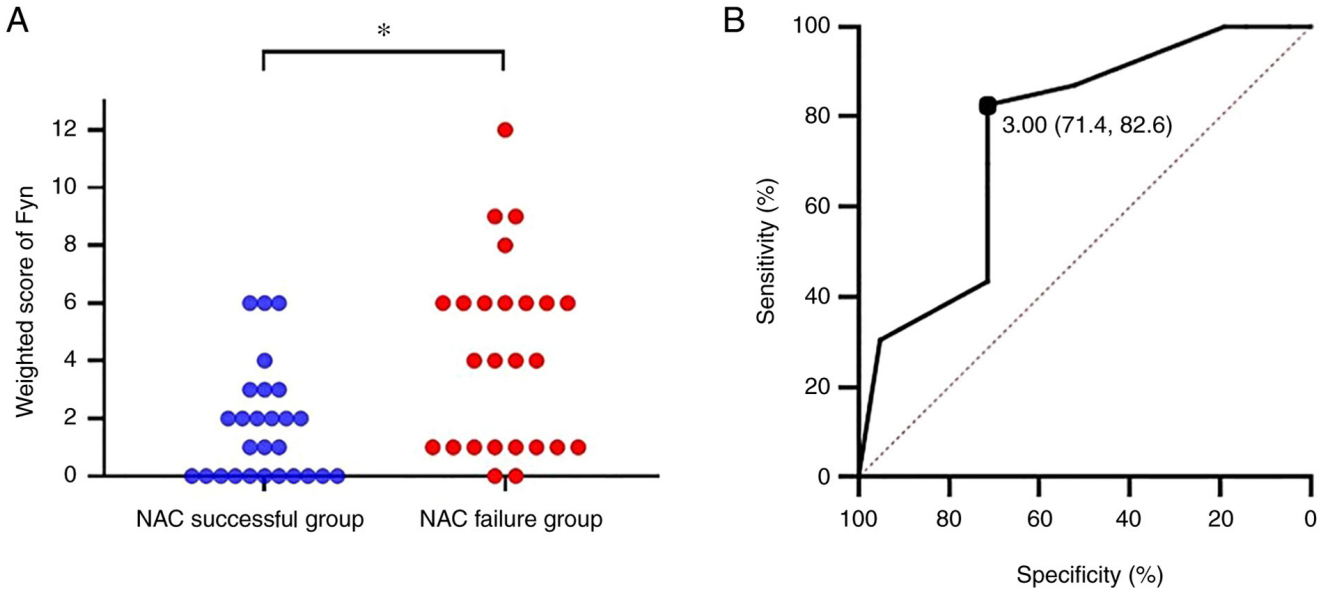

Figure 3. Analysis of the weighted score of Fyn. (A) Comparison of the weighted score of Fyn between the NAC successful group and the NAC failure group. The score in the NAC successful group was significantly lower than that in the NAC failure group (P=0.003). (B) Receiver operator characteristic curve for determining the Fyn cutoff score for predicting the effectiveness of NAC, indicating a cut off value of 3 with a sensitivity of 82.6% and specificity of 71.4% was optimal, with an area under the curve value of 0.759 and a 95% confidence interval of 0.613-0.905.

low expression group (n=25) in which the weighted score was ≤3 and a high expression group (n=19) in which the weighted score ≥4. The rate of NAC success between the two groups was compared. In the low expression group, the success rate was 76% and the failure rate was 24%, in the high expression group, the success rate was 21.1% and the failure rate was 78.9% (Table II). The success rate in the low expression group was significantly higher than that in the high expression group (P<0.001), which was evaluated with the Fisher's exact test determining the association of categories in two group variables (NAC successful group and NAC failure group). This indicated that high expression of Fyn contributed to reduced sensitivity to NAC in local advanced uterine cervical cancer patients.

Contribution of Fyn knockdown to the cisplatin sensitivity of cervical cancer cells. Next, the effect of Fyn knockdown on cisplatin sensitivity was evaluated *in vitro* using human cervical cancer cells. Fyn expression was knocked down by transfection of si-Fyn. The downregulation of Fyn mRNA expression was confirmed by RT-qPCR. As shown in Fig. 4A, Fyn mRNA expression was significantly suppressed in the treated cells compared with the control cells transfected with the control siRNA (P<0.05). After confirmation of knockdown of Fyn, the effect of Fyn knockdown on the sensitivity to cisplatin in the uterine cervical cancer cells was determined. The cell viability of cells in which Fyn was knocked down was significantly lower than in the cells transfected with the control siRNA when treated with 10, 25, or 50 μM cisplatin (Fig. 4B). These results indicate that Fyn knockdown contributed to the enhancement of cisplatin sensitivity on uterine cervical cancer cells.

Discussion

NAC followed by surgery is considered the standard treatment option for patients with locally advanced uterine cervical cancer, and there are numerous studies revealing the effectiveness of NAC for patients with locally advanced uterine cervical cancer. Nguyen *et al* (5) performed a meta-analysis that showed that dose-intense cisplatin-based NAC followed by surgery increases survival in stage IB2-IVA uterine cervical cancer patients. Mori *et al* (14) reported that NAC using paclitaxel and carboplatin followed by surgery was a promising mode of

Table II. Association between Fyn expression and NAC efficacy.

Fyn expression	NAC successful group	NAC failure group	P-value
Low expression, n (%)	19 (76)	6 (24)	<0.001[a]
High expression, n (%)	4 (21.1)	15 (78.9)	

[a]Fisher's exact test; NAC, neoadjuvant chemotherapy.

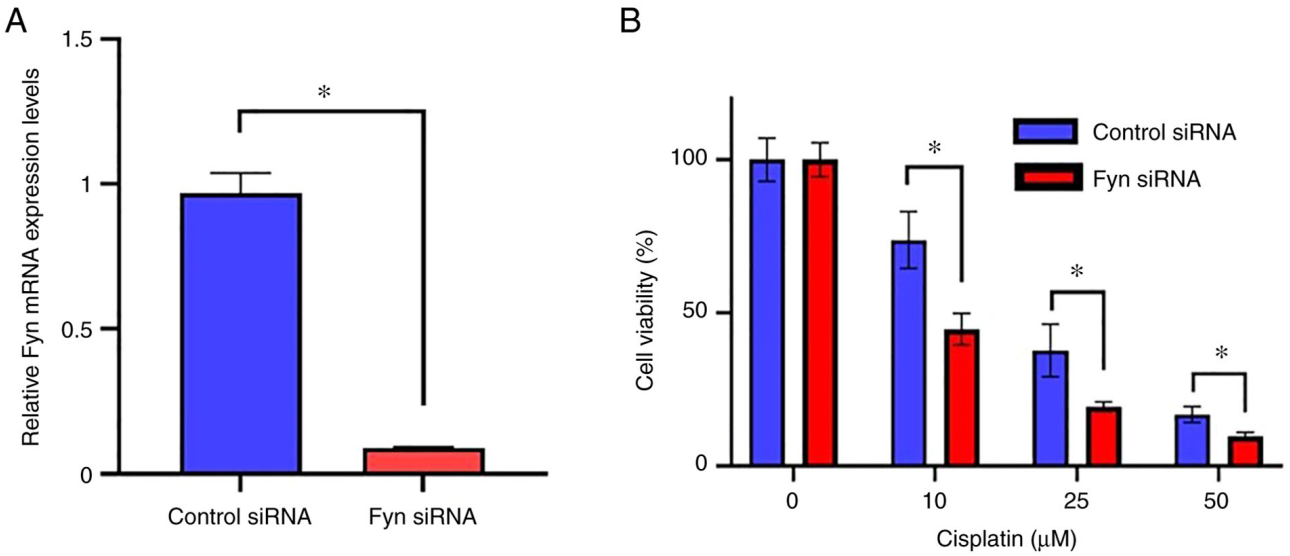

Figure 4. Effect of Fyn knockdown on the sensitivity to cisplatin *in vitro*. (A) Relative Fyn mRNA expression levels in the control and Fyn knockdown cells. After transfection of si-Fyn, Fyn mRNA expression levels were significantly lower than that in the control cells (P<0.05). (B) Comparison of cell viability of uterine cervical cancer cells between the control siRNA group and Fyn siRNA group after treating cells with several different concentrations of cisplatin. The cell viability of the si-Fyn group was significantly lower than that of the control group when treated with 10, 25, or 50 μM cisplatin. Data are presented as the mean ± SD. *P<0.05. siRNA, small interfering RNA.

therapy for stage IB2-IIIB uterine cervical cancer patients with a good probability of improving the prognosis. Shoji *et al* (15) reported that NAC using cisplatin and irinotecan followed by surgery was a useful therapeutic strategy for management of stage IB2-IIIB uterine cervical cancer with a good probability of improving the prognosis. Finally, Sala *et al* (6) demonstrated that NAC followed by surgery improved survival outcomes for stage IB2-IVA uterine cervical cancer patients, and suggested that NAC followed by surgery was an effective alternative treatment option to CCRT standard treatment strategy.

Currently, for locally advanced uterine cervical cancer, especially for patients with stage IIIB-IVA cancer, CCRT is the standard treatment strategy (2); however, the prognostic outcomes of these patients have remained poor (3). Therefore, there is an urgent need to establish an effective treatment strategy for these patients. One candidate strategy to improve the prognosis is NAC followed by surgery. However, if NAC is not effective, surgery cannot be performed and the only treatment option available is radiotherapy, which may result in unfavorable treatment outcomes due to the delay before the initiation of the core treatment. Therefore, if the efficacy of NAC can be predicted in advance, the patients who will benefit from NAC effectively can be selected before the initiation of treatment. To this end, it is crucial to identify biomarkers that can be used in predicting NAC efficacy.

Tyrosine kinases can be divided into two groups, receptor tyrosine kinases, and non-receptor tyrosine kinases. Receptor tyrosine kinases include vascular endothelial growth factor receptor (VEGFR), epidermal growth factor receptor (EGFR), and mesenchymal-epithelial transition factor, and they receive signals via soluble ligands. Non-receptor tyrosine kinases include families such as Src, Abl, focal adhesion kinase, and the Janus kinase (16). Dysregulation of the activation of these tyrosine kinases causes cancer by altering cellular growth, function, and shape which are hallmarks of malignancy (17). The SFKs consists of the following members: c-Src, Fyn, Lck, Yes, Lyn, Fgr, Blk, and Hck. Among these, Fyn, c-Src, and Yes are expressed ubiquitously, whereas the others exhibit restricted tissue expression (7,18,19). Fyn is a non-receptor tyrosine kinase that is localized to the inner side of the cell membrane; however, when activated, it is translocated to other cellular components such as the nucleus (20). Fyn has been reported to exhibit a wide variety of biological functions, such as signal transduction in the nerves thus contributing to the regulation of brain function, signal transduction through T cell receptors, and adhesion-mediated signal transduction under physiological conditions. Additionally, Fyn has been reported to contribute to the development and progression of several malignancies through regulation of cell growth, cell death, cell motility, morphogenic transformation, and migration, and

is considered an essential factor in the development, progression, and metastasis of cancer (8,21). Fyn has been reported to be involved in a wide variety of malignancies such as breast cancer, prostate cancer, glioma, melanoma, squamous cell carcinoma of the head and neck, chronic myeloid leukemia, cholangiocarcinoma, gastric cancer, thyroid cancer, and esophageal squamous cell carcinoma (8,21-27).

Fyn has been reported to be involved in the receptor tyrosine kinase (EGFR, VEGF, platelet-derived growth factor receptor, fibroblast growth factor) pathway in cancer. Fyn transmits signals through Ras-independent pathways (via PIK3/Akt, STAT3, FAK, β-catenin, VAV1, paxillin, and/or SHC) and Ras-dependent pathways (via Ras/MEK/ERK) (28). Through involvement in these pathways, Fyn mediates the growth factor-induced anti-apoptotic effects of Akt/PKB (8). As a result, there are also several reports showing the correlation between Fyn expression and sensitivity to anti-cancer agents due to the regulation of apoptosis by Fyn. Knockdown of Fyn facilitates doxorubicin-induced apoptosis and increases the sensitivity to doxorubicin in doxorubicin-resistant cells (29). Additionally, the levels of Fyn expression markedly influence the efficacy of PP2, which is an SFK inhibitor, by inducing apoptosis (9). Furthermore, there are several reports on the correlation between Fyn expression and sensitivity to anti-cancer agents. Fyn was reported to be upregulated in tamoxifen-resistant estrogen receptor-positive breast cancer cell lines, and when Fyn expression was knocked down the estrogen receptor-positive breast cancer cell lines became sensitive to tamoxifen (30). Upregulated expression of Fyn has been reported to be involved in resistance to imatinib in chronic myeloid leukemia (31). Fyn upregulation has also been reported to exert negative effects on chemosensitivity to gemcitabine in pancreatic ductal adenocarcinoma via regulation of miR-125a-3p (32). Fyn is considered an important molecule that can confer resistance to anti-cancer agents, and it exerts its effect predominantly by inducing apoptosis (9,29).

The antitumor effects of platinum-based anti-tumor drugs including cisplatin are achieved by covalently binding to the DNA of cancer cells (33). There are several mechanisms that contribute to the resistance of platinum-based anti-tumor drugs such as inactivation of apoptotic signaling pathways (34,35), enhanced DNA damage repair capacity (36,37), increased cisplatin detoxification (38), decreased cellular uptake of cisplatin (39,40), and other epigenetic modifications that occur at the molecular and cellular levels (41,42).

Thus far, several biomarkers that exhibit potential for predicting the efficacy of NAC to uterine cervical cancer patients, such as uncoupling protein 2, protein arginine methyltransferase, and T-box 2, have been identified (43-45). In the current study, high Fyn expression was shown to be negatively associated with the effectiveness of NAC using cisplatin for locally advanced uterine cervical cancer, and knockdown of Fyn increased the sensitivity to cisplatin in uterine cervical cancer cells *in vitro*. It is hypothesized that the accumulation of these findings will allow for a more accurate prediction of the effects of NAC treatment. If the efficacy of NAC can be predicted using biopsy specimens prior to the initiation of treatment, the optimal treatment strategy can be predicted for patients. Taking pathological samples from the uterine cervix via punch biopsy is a routine procedure in the clinic, thus there is no need for further invasive procedures for the patients.

To the best of our knowledge, this study is the first to show the association between Fyn expression and the efficacy of NAC for patients with locally advanced uterine cervical squamous cell carcinoma. In the present study, Fyn was shown to contribute to the prognosis of patients; however, as this study is a retrospective study with a relatively small number of cases from a single institute, further larger prospective studies with patients from multiple institutes, ideally from several different countries are required to confirm the results presented here.

In conclusion, this study showed that Fyn expression may be a potentially useful predictive biomarker of the response to NAC for patients with locally advanced uterine cervical squamous cell carcinoma that is easy to evaluate using biopsy specimens. The results also suggest that Fyn may be a promising molecular target for the management of uterine cancer.

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Availability of data and materials

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

Authors' contributions

SN, TF and TS designed the study. SN, TN, EU, YA, KI and MY performed the experiments and collected the data. SN, TF, TY and TS analyzed the data. SN and TF wrote the manuscript. SN 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. 2019-01; 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.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
2. Gennigens C, De Cuypere M, Hermesse J, Kridelka F and Jerusalem G: Optimal treatment in locally advanced cervical cancer. Expert Rev Anticancer Ther 21: 657-671, 2021.
3. Siegel RL, Miller KD, Fuchs HE and Jemal A: Cancer statistics, 2022. CA Cancer J Clin 72: 7-33, 2022.
4. Ishiko O, Sumi T, Yasui T, Matsumoto Y, Kawamura N, Ogita S, Kamino T, Nakamura K and Yamada R: Balloon-occluded arterial infusion chemotherapy, simple total hysterectomy, and radiotherapy as a useful combination-therapy for advanced cancer of the uterine cervix. Oncol Rep 7: 141-144, 2000.
5. Nguyen VT, Winterman S, Playe M, Benbara A, Zelek L, Pamoukdjian F and Bousquet G: Dose-intense cisplatin-based neoadjuvant chemotherapy increases survival in advanced cervical cancer: An up-to-date meta-analysis. Cancers (Basel) 14: 842, 2022.
6. Sala P, Bogliolo S, Barra F, Fazio A, Maramai M, Cassani C, Gardella B, Babilonti L, Giannelli F, Mammoliti S, *et al*: Neoadjuvant chemotherapy followed by radical surgery versus concurrent chemo-radiotherapy in the treatment of locally advanced cervical cancer: A multicenter retrospective analysis. J Invest Surg 35: 308-314, 2022.
7. Yeatman TJ: A renaissance for SRC. Nat Rev Cancer 4: 470-480, 2004.
8. Elias D and Ditzel HJ: Fyn is an important molecule in cancer pathogenesis and drug resistance. Pharmacol Res 100: 250-254, 2015.
9. Noronha G, Barrett K, Boccia A, Brodhag T, Cao J, Chow CP, Dneprovskaia E, Doukas J, Fine R, Gong X, *et al*: Discovery of [7-(2,6-dichlorophenyl)-5-methylbenzo [1,2,4] triazin-3-yl]-[4-(2-pyrrolidin-1-ylethoxy)phenyl]amine-a potent, orally active Src kinase inhibitor with anti-tumor activity in preclinical assays. Bioorg Med Chem Lett 17: 602-608, 2007.
10. Fresno Vara JA, Cáceres MA, Silva A and Martín-Pérez J: Src family kinases are required for prolactin induction of cell proliferation. Mol Biol Cell 12: 2171-2183, 2001.
11. Sinicrope FA, Ruan SB, Cleary KR, Stephens LC, Lee JJ and Levin B: bcl-2 and p53 oncoprotein expression during colorectal tumorigenesis. Cancer Res 55: 237-241, 1995.
12. Valadan R, Hedayatizadeh-Omran A, Alhosseini-Abyazani MN, Amjadi O, Rafiei A, Tehrani M and Alizadeh-Navaei R: Data supporting the design and evaluation of a universal primer pair for pseudogene-free amplification of HPRT1 in real-time PCR. Data Brief 4: 384-389, 2015.
13. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
14. Mori T, Hosokawa K, Sawada M, Kuroboshi H, Tatsumi H, Koshiba H, Okubo T and Kitawaki J: Neoadjuvant weekly carboplatin and paclitaxel followed by radical hysterectomy for locally advanced cervical cancer: Long-term results. Int J Gynecol Cancer 20: 611-616, 2010.
15. Shoji T, Takatori E, Furutake Y, Takada A, Nagasawa T, Omi H, Kagabu M, Honda T, Miura F, Takeuchi S, *et al*: Phase II clinical study of neoadjuvant chemotherapy with CDDP/CPT-11 regimen in combination with radical hysterectomy for cervical cancer with a bulky mass. Int J Clin Oncol 21: 1120-1127, 2016.
16. Boggon TJ and Eck MJ: Structure and regulation of Src family kinases. Oncogene 23: 7918-7927, 2004.
17. Vlahovic G and Crawford J: Activation of tyrosine kinases in cancer. Oncologist 8: 531-538, 2003.
18. Frame MC: Src in cancer: Deregulation and consequences for cell behaviour. Biochim Biophys Acta 1602: 114-130, 2002.
19. Thomas SM and Brugge JS: Cellular functions regulated by Src family kinases. Annu Rev Cell Dev Biol 13: 513-609, 1997.
20. Campbell EJ, McDuff E, Tatarov O, Tovey S, Brunton V, Cooke TG and Edwards J: Phosphorylated c-Src in the nucleus is associated with improved patient outcome in ER-positive breast cancer. Br J Cancer 99: 1769-1774, 2008.
21. Saito YD, Jensen AR, Salgia R and Posadas EM: Fyn: A novel molecular target in cancer. Cancer 116: 1629-1637, 2010.
22. Huang C, Zhou J, Nie Y, Guo G, Wang A and Zhu X: A new finding in the key prognosis-related proto-oncogene FYN in hepatocellular carcinoma based on the WGCNA hub-gene screening trategy. BMC Cancer 22: 380, 2022.
23. Jiang P, Li Z, Tian F, Li X and Yang J: Fyn/heterogeneous nuclear ribonucleoprotein E1 signaling regulates pancreatic cancer metastasis by affecting the alternative splicing of integrin β1. Int J Oncol 51: 169-183, 2017.
24. Lyu SC, Han DD, Li XL, Ma J, Wu Q, Dong HM, Bai C and He Q: Fyn knockdown inhibits migration and invasion in cholangiocarcinoma through the activated AMPK/mTOR signaling pathway. Oncol Lett 15: 2085-2090, 2018.
25. Zhang X, Huang Z, Guo Y, Xiao T, Tang L, Zhao S, Wu L, Su J, Zeng W, Huang H, *et al*: The phosphorylation of CD147 by Fyn plays a critical role for melanoma cells growth and metastasis. Oncogene 39: 4183-4197, 2020.
26. Yu J, Zhou Z, Wei Z, Wu J, OuYang J, Huang W, He Y and Zhang C: FYN promotes gastric cancer metastasis by activating STAT3-mediated epithelial-mesenchymal transition. Transl Oncol 13: 100841, 2020.
27. Liu D, Gao M, Wu K, Zhu D, Yang Y and Zhao S: LINC00152 facilitates tumorigenesis in esophageal squamous cell carcinoma via miR-153-3p/FYN axis. Biomed Pharmacother 112: 108654, 2019.
28. Comba A, Dunn PJ, Argento AE, Kadiyala P, Ventosa M, Patel P, Zamler DB, Núñez FJ, Zhao L, Castro MG and Lowenstein PR: Fyn tyrosine kinase, a downstream target of receptor tyrosine kinases, modulates antiglioma immune responses. Neuro Oncol 22: 806-818, 2020.
29. Mi H, Wang X, Wang F, Li L, Zhu M, Wang N, Xiong Y and Gu Y: miR-381 induces sensitivity of breast cancer cells to doxorubicin by inactivation of MAPK signaling via FYN. Eur J Pharmacol 839: 66-75, 2018.
30. Elias D, Vever H, Lænkholm AV, Gjerstorff MF, Yde CW, Lykkesfeldt AE and Ditzel HJ: Gene expression profiling identifies FYN as an important molecule in tamoxifen resistance and a predictor of early recurrence in patients treated with endocrine therapy. Oncogene 34: 1919-1927, 2015.
31. Grosso S, Puissant A, Dufies M, Colosetti P, Jacquel A, Lebrigand K, Barbry P, Deckert M, Cassuto JP, Mari B and Auberger P: Gene expression profiling of imatinib and PD166326-resistant CML cell lines identifies Fyn as a gene associated with resistance to BCR-ABL inhibitors. Mol Cancer Ther 8: 1924-1933, 2009.
32. Liu G, Ji L, Ke M, Ou Z, Tang N and Li Y: miR-125a-3p is responsible for chemosensitivity in PDAC by inhibiting epithelial-mesenchymal transition via Fyn. Biomed Pharmacother 106: 523-531, 2018.
33. Bose RN: Biomolecular targets for platinum antitumor drugs. Mini Rev Med Chem 2: 103-111, 2002.
34. Siddik ZH: Cisplatin: Mode of cytotoxic action and molecular basis of resistance. Oncogene 22: 7265-7279, 2003.
35. Wang Q, Shi S, He W, Padilla MT, Zhang L, Wang X, Zhang B and Lin Y: Retaining MKP1 expression and attenuating JNK-mediated apoptosis by RIP1 for cisplatin resistance through miR-940 inhibition. Oncotarget 5: 1304-1314, 2014.
36. Martin LP, Hamilton TC and Schilder RJ: Platinum resistance: The role of DNA repair pathways. Clin Cancer Res 14: 1291-1295, 2008.
37. Liu RY, Dong Z, Liu J, Yin JY, Zhou L, Wu X, Yang Y, Mo W, Huang W, Khoo SK, *et al*: Role of eIF3a in regulating cisplatin sensitivity and in translational control of nucleotide excision repair of nasopharyngeal carcinoma. Oncogene 30: 4814-4823, 2011.
38. Surowiak P, Materna V, Kaplenko I, Spaczyński M, Dietel M, Lage H and Zabel M: Augmented expression of metallothionein and glutathione S-transferase pi as unfavourable prognostic factors in cisplatin-treated ovarian cancer patients. Virchows Arch 447: 626-633, 2005.
39. Galluzzi L, Senovilla L, Vitale I, Michels J, Martins I, Kepp O, Castedo M and Kroemer G: Molecular mechanisms of cisplatin resistance. Oncogene 31: 1869-1883, 2012.
40. Morimoto A, Serada S, Enomoto T, Kim A, Matsuzaki S, Takahashi T, Ueda Y, Yoshino K, Fujita M, Fujimoto M, *et al*: Annexin A4 induces platinum resistance in a chloride-and calcium-dependent manner. Oncotarget 5: 7776-7787, 2014.
41. Liu RY, Dong Z, Liu J, Zhou L, Huang W, Khoo SK, Zhang Z, Petillo D, The BT, Qian CN and Zhang JT: Overexpression of asparagine synthetase and matrix metalloproteinase 19 confers cisplatin sensitivity in nasopharyngeal carcinoma cells. Mol Cancer Ther 12: 2157-2166, 2013.

42. Shen DW, Pouliot LM, Hall MD and Gottesman MM: Cisplatin resistance: A cellular self-defense mechanism resulting from multiple epigenetic and genetic changes. Pharmacol Rev 64: 706-721, 2012.
43. Imai K, Fukuda T, Wada T, Kawanishi M, Tasaka R, Yasui T and Sumi T: UCP2 expression may represent a predictive marker of neoadjuvant chemotherapy effectiveness for locally advanced uterine cervical cancer. Oncol Lett 14: 951-957, 2017.
44. Shimomura M, Fukuda T, Awazu Y, Nanno S, Inoue Y, Matsubara H, Yamauchi M, Yasui T and Sumi T: PRMT1 expression predicts response to neoadjuvant chemotherapy for locally advanced uterine cervical cancer. Oncol Lett 21: 150, 2021.
45. Inoue Y, Fukuda T, Nanno S, Awazu Y, Shimomura M, Matsubara H, Yamauchi M, Yasui T and Sumi T: T-box 2 expression is a useful indicator of the response to neoadjuvant chemotherapy for patients with locally advanced uterine cervical squamous cell carcinoma. Oncol Lett 22: 755, 2021.