Inhibition of CK2 to overcome paclitaxel resistance in gastric cancer

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Inhibition CK2 to overcome paclitaxel resistance in gastric cancer

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ABSTRACT

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Despite advances in treatment, gastric cancer (GC) remains among the most fatal malignancies. Paclitaxel has been used treatment for GC, however, it has limited clinical efficacy owing to drug resistance development. Casein kinase (CK) 2 activation has been implicated in the proliferation of various tumor types and resistance to chemotherapy. Herein, we investigated the mechanistic basis for the association between CK2 activation and paclitaxel resistance. CK2 expression was evaluated by immunohistochemistry in formalin-fixed, paraffin-embedded tumor specimens from 59 advanced GC patients treated with paclitaxel as second-line therapy. Patients with high CK2 expression (29/59, 39%) showed lower disease control rate (47.7 % vs. 72.3 %, p=0.017) and shorter progression-free

survival (2.8 vs. 4.8 months, p=0.009) than patients with low expression.

CK2 protein expression was associated with sensitivity to paclitaxel in 49 GC cell lines. In SNU-1 line which showed paclitaxel resistance, high CK2 expression, and sensitivity to the CK2 inhibitor, 5-[(3-chlorophenyl)amino]-benzo[c]-2,6 naphthyridine-8-carboxylic acid (CX-4945), combination therapy with CX-4945 and paclitaxel exerted synergistic antiproliferative effects and inhibited of down signaling of phosphatidylinositol 3-kinase/AKT signaling. These results demonstrate that CK2 activation is related to paclitaxel resistance and that CX-4945 in combination with paclitaxel could be a treatment of choice for paclitaxel resistance in GC

Keywords: gastric cancer, paclitaxel, drug resistance, casein kinase II

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I. INTRODUCTION

Gastric cancer (GC) is major health problem worldwide, with high incidence and a poor prognosis.[1] Surgical resection in combination with adjuvant chemotherapy is the only curative treatment strategy for localized GC.[2,3] However, recurrence is common, and for advanced or metastatic GC, chemotherapy is the first treatment option. Although clinical trials have sought to improve survival rates in GC patients, the median overall survival for metastatic disease is only about 1 year.[4] Therefore, there is an urgent need for new strategies for improving treatment and survival.

Paclitaxel is effective for advanced GC treatment and has response rates in the range of 15% to 28% when used as monotherapy.[5-9] It is most commonly used as second-line therapy in GC and showed good toxicity profiles in stomach cancer.[10] However, the

median duration of paclitaxel response in GC is only about 3 months, and eventually patients have paclitaxel resistance.[11]

The molecular mechanism underlying paclitaxel resistance is not well understood. Common mechanisms of drug resistance are overexpression of P-glycoprotein, and protein kinase C-α (PKC-α), as well as mitogen-activated protein kinase and phosphatidylinositol-3-kinase (PI3K)/AKT signaling pathways.[12-15] The PI3K/AKT pathways is specially activated following paclitaxel treatment, whereas PI3K inhibition sensitized tumors to paclitaxel and included cell death via mitotic arrest.[16-18]

Casein kinase (CK) 2 recently has proposed anticancer drug target is a constitutively active serine/threonine kinase that has pro-survival/anti-apoptotic functions.[19] Given that CK2 is the overexpressed in multiple cancers and is implicated in many non-oncogenic processes required to sustain the cancer phenotype, its selective inhibition is an attractive strategy for cancer treatment.[19-21] CK2 has been shown to phosphorylate AKT1 at Ser129 to promote cell survival by generating a constitutively active form of the protein.[22] It also phosphorylates and stabilizes phosphatase and tensin homolog, thereby inhibiting its activity and inducing PI3K-mediated survival signaling and oncogensis.[23] 5-[(3-chlorophenyl)amino]-benzo[c]-2,6 naphthyridine-8-carboxylic acid (CX-4945) is small

molecule, inhibitor of CK2 whose biological activity has been investigated *in vitro* and *in vivo*.[24] Key attributes of CX-4945 include potent inhibition of CK2 enzymatic activity and a highly selective kinase profile.[25] We speculated that paclitaxel resistance is associated CK2 activation and PI3K/AKT signaling, and that combining CK2 inhibition with paclitaxel chemotherapy can improve GC treatment efficacy.

To test this hypothesis, we investigated the association between CK2 expression and paclitaxel resistance, and evaluated whether CX-4945 in combination with paclitaxel can overcome paclitaxel resistance in GC.

II. MATERIALS AND METHODS

1. Clinical specimens and chemotherapy protocol

We used paraffin blocks of surgical specimens or endoscopic biopsied specimens of 59 advanced GC patients obtained before paclitaxel therapy at Yonsei University Health System (Seoul, Korea). All patients received paclitaxel (175mg/m^2 on day 1 every 3 weeks, or 70 - 80 mg/m^2 on day 1, 8, and 15 every 4 weeks) as a second-line chemotherapeutic agent. Paclitaxel was given until disease progression or occurrence of intolerable toxicities. Tumor assessments were performed every two cycles, and disease response was categorized as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) according to the Response Evaluation Criteria in Solid Tumor (RECIST, v.1.1).[26]

2. Iimmunohistochemical analysis of CK2 and phosphorylated-AKT

CK2 and phosphorylated (p-) AKT expression was evaluated by immunohistochemistry using anti-CK2 (Abcam, Cambridge, UK; 1:50) and anti- p-AKT (Cell Signaling Technology, Danvers, MA, USA, 1:40) antibodies. Sections cut at

a thickness of 4 μm and mounted on slides were deparaffinized in xylene, rehydrated and treated with 2.5% H_2O_2 for 10 min to block endogenous peroxidase activity. Heat-induced antigen retrieval was carried out for 2 min in 10 mmol/l citrate buffer (pH 7.0). After washing with phosphate-buffered saline (PBS), sections were incubated with primary antibody for 90 min at room temperature. After washing with PBS, sections were incubated at room temperature for 30 min with an anti-mouse secondary antibody followed by washing with PBS. Immunoreactivity was detected by incubation for 1 min with 0.5% 3,3-diaminobenzidine solution. After washing with PBS, the sections were stained with Harris hematoxylin, and three areas per slide were analyzed to confirm tissue diagnosis. The numbers of tumor cells with membrane and cytoplasmic labeling of CK2 and cytoplasmic and nuclear labeling of p-AKT were counted. Scoring was performed by a pathologist blinded to patients' clinical information. Protein expression was interpreted with weighted histoscore method.[27] The intensity of protein expression was scored as 0 (negative), 1 (light brown), 2 (brown), and 3 (dark brown). The final score was calculated as follows: - (0 × % of negative cells) + (1 × % of light brown cells) + (2 × % of brown cells) + (3 × % of dark brown cells). Patients were subdivided into following four groups based on the final score: 0 (negative), 1+ (final score; 1–100), 2+ (final score 101–200),

and 3 + (final score 201–300). High CK2 and p-AKT expression was defined as a score ≥ 1+.

3. Cell culture and reagents

A total of 49 GC cell lines were used in this study. The YCC series was established by the Yonsei Cancer Center (Cancer Metastasis Research Center, Seoul, Korea) from the ascites or peripheral blood of advanced GC patients. Other cell lines were obtained from the Korean Cell Line Bank (Seoul National University, Seoul, Korea) and the American Type Culture Collection (American Type Culture Collection, Manassas, VA, USA).

Cells were maintained in Roswell Park Memorial Institute-1640 medium (Lonza, Walkersville, MD, USA) or Eagle's minimal essential medium (Lonza, Walkersville, MD, USA) and supplemented with 10% fetal bovine serum (Omega Scientific, Tarzana, CA, USA), 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mmol/l glutamine in a 5% CO2-humidified atmosphere at 37°C. Cell lines were expanded and cryopreserved in liquid nitrogen in our laboratory.

CX-4945 was synthesized by and supplied without cost by Cylene Pharmaceuticals (San Diego, CA, USA). Paclitaxel was purchased from Sigma-Aldrich (St. Louis, MO,

USA).

4. Cell growth inhibition assay

Cell proliferation was evaluated with 3-(4,5-dimethylthiazol-2yl)-2.5-diphenyltetrazolimu bromide (MTT) assay. Cells were seeded at the density of 5×10^3 cells into a 96-well plate and incubated at 37°C for 24 h. Paclitaxel and CX-4945 were serially diluted with medium and added to each well. After 72 h of incubation, 50 ml (2 mg/ml) of the MTT solution was added, and followed by incubation for an additional 4 h. After centrifugation at 400 × *g* for 10 min, the medium containing MTT was removed and the remaining MTT-formazan crystals were dissolved by adding 150 μl of dimethylsulfoxide. After 10 min incubation with shaking, the absorbance at 570 nm was measured with a multi-well microplate read. Results are expressed as percentage cell survival, which was calculated which the following formula: ¼ [(mean absorbance of test wells − standard absorbance) / (mean absorbance of control wells − standard absorbance)] × 100. Control wells were treated with the medium alone (without the drug). At least 3 replicates were prepared for each treatment and the average of these data was used for data analyses. Synergy was assessed using the New Bliss Independence Model.[28]

Combined percentage inhibition $Y_{ab,\ P}$ was predicted using the complete addition of probability theory as $Y_{ab,\ P} = Y_a + Y_b - Y_aY_b$ (drug A at dose a inhibits Y_a% of tumor growth and drug B at dose b inhibits Y_b% of tumor growth). The observed combined percentage inhibition $Y_{ab,\ O}$ was then compared as $Y_{ab,\ P}$, $Y_{ab,\ O} > Y_{ab,\ P}$, $Y_{ab,\ O} = Y_{ab,\ P}$, and $Y_{ab,\ O} < Y_{ab,\ P}$ indicated that effects were synergistic, independent (additive), and antagonistic, respectively.

5. Detection of CK2 activity in cell lysates

GC lines were seeded in 6-well plates overnight, then collected at 80% confluence by scraping into ice-cold PBS. CK2 activity in cell lysates was determined using a CK2 kinase assay kit (CycLex Co., Nagano, Japan) according to the manufacturer's instructions.

6. CK2 RNA extraction

Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. First strand cDNA was synthesized from 2 μg total cellular RNA with oligo (dT) using a cDNA synthesis kit (Fermentas, Hanover,

MD, USA). Quantitative reverse transcription polymerase chain reaction (RT-PCR) was performed on a 7500 fast real-time PCR system (Applied Biosystems, Foster City, CA, USA) using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). The primers used were as follows: CK2a, 5'-TGTCCGAGTTGCTTCCCGATACTT-3' and 5'-TTGCCAGCATACAACCCAAACTCC-3' and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), 5'-CCATGGAGAAGGCTG GGG-3' and 5'-CAAAGTTGTCAT GGATGACC-3'. Relative copy number was determined using the comparative *Ct* method. GAPDH served as the internal control for normalization.

7. Cell cycle analysis

Cell cycling was evaluated by flow cytometry analysis of cells stained with propidium iodide (PI) (BD Biosciences, San Jose, CA, USA). SNU-1 cells were left untreated, or treated for 36 h with paclitaxel (0.1 or 1 μM), CX-4945 (5 or 1 μM) typsinized, fixed overnight with 70% ethanol at −20°C and incubated for 30 min in the dark at room temperature with 200 μl PI solution. After washing with PBS, cells were resuspended in 500 μl PBS analyzed on a FACS Calibur system (BD Biosciences, San Jose, CA, USA).

8. Western blot analysis

SNU-1 cells were washed twice in PBS, lysed in ice-cold buffer composed of 1% Nonidet P-40, 10% (v/v) glycerol, 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 2.5 mM EDTA, 100 μM Na_3VO_4, 1% aprotinin, 1% leupeptin, and 1 mM perfluoro-1-butanesulfonyl fluoride for 30 min at 4°C and centrifuged 13,000 rpm for 20 min at 4°C. The supernatant was collected and total protein concentration was quantified by the Bradford assay and stored at −70°C until use. For western blotting, proteins were mixed with 5 × sample buffer and boiled at 95°C for 5 min, then resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis using 7.5% or 10% polyacrylamide gels, followed by transfer to a polyvinylidene difluoride membrane (GE Healthcare, Piscataway, NJ, USA) by electroblotting. The membrane was blocked with 5% skim milk for 1 h at room temperature and incubated overnight with primary antibodies against the following proteins: p-CK2 (pS/pTDXE, #8738), AKT (Ser473, #9272), p-AKT (Ser473, #4058), mammalian target of rapamycin (mTOR) (7C10, #2983), p-mTOR (Ser2448, #2971), p70S6K (#9202), p-p70S6K (Thr387, #9234), and cleaved PARP1 (Asp214, #9541) (all from Cell Signaling Technology); anti-p-AKT (Ser129, ab133458, Abcam, Cambridge, UK); and CK2 (1AD9, sc-12738, Santa Cruz

Biotechnology, Santa Cruz, CA, USA). Horseradish peroxidase-conjugated anti-mouse and -rabbit IgG (Santa Cruz Biotechnology) were used as secondary antibodies. The membrane was developed by enhanced chemiluminescence (GE Healthcare, Piscataway, NJ, USA). Anti-alpha tubulin antibody (#T6199; Sigma-Aldrich) was used as a loading control. Protein band signal intensities were quantitated with ImageJ software (National Institutes of Health, Bethesda, MD, USA).

9. Statistical analysis

Clinical characteristics and treatment outcomes of patients were compared according to CK2 expression with the $\chi 2$ test and Fisher's exact test. Survival estimates were calculated using the Kaplan-Meier method. Progression-fee survival (PFS) was calculated from the date chemotherapy was started to the earliest date of disease progression or death from any other cause. And overall survival (OS) was defined as the time from the start of paclitaxel treatment to death from any cause. Differences in PFS and OS according to CK2 and p-AKT expression were compared with the log-rank test. Significant effects between treatment groups or between treatment and control groups were evaluated with using the Mann-Whitney U test. Data were analyzed SPSS, v 23.0

(SPSS Inc., Chicago, IL, USA) and plots and curves were generated with Prism v.5 software (GraphPad Inc., San Diego, CA, USA).

III. RESULTS

1. Patient characteristics

A total of 59 patients for whom tumor tissue specimens were available for CK2 and p-AKT expression analysis were included in this study. The median age was 59 years (range, 53-68 years), and 62.7% was male. About 60% patients were underwent gastrectomy before paclitaxel treatment (Table 1), eight (13.6%) were positive human epidermal receptor 2 (HER2).

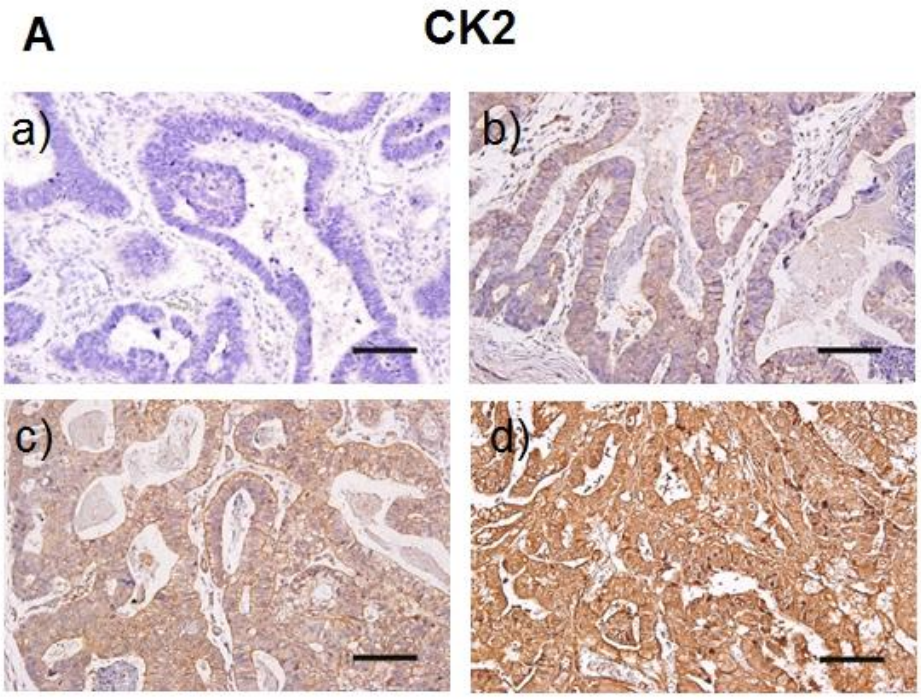
Table 1. Patients' clinicopathologic characteristics

Characteristics		Number	%
Age			
	Median	59	
	IQR	53-68	
Sex			
	Male	37	62.7
	Female	22	37.3
WHO classification			
	Adenocarcinoma well differentiated	6	10.2
	Adenocarcinoma moderately differentiated	15	25.4
	Adenocarcinoma poorly differentiated	26	44.1
	Signet ring cell	9	15.5
	Others	3	5.2
Prior gastrectomy			
	Yes	36	61
	No	23	39
HER2 status			
	Positive	8	13.6
	Negative	39	66.1
	Unknown	12	20.3
Disease			
	Measurable	30	62.7
	Non-measurable	22	37.3
Metastatic location			
	Distant Lymph node	16	27.1
	Liver	13	22
	Peritoneum	37	62.7
	Lung	3	5.1
	Bone	2	3.4

HER2, human epidermal receptor 2; IQR, interquartile range; WHO, World Health Organization

2. Expression of CK2 and p-AKT in GC patients

Median CK2 and p-AKT immunoreactivity scores were 50 (range, 0-210) and 110 (range, 0-270), respectively. At a cutoff score of 1+, CK2 and p-AKT expression was high in 23 (40%) and p-AKT in 40 (67.8%) of the 59 patients, respectively (Figure 1).



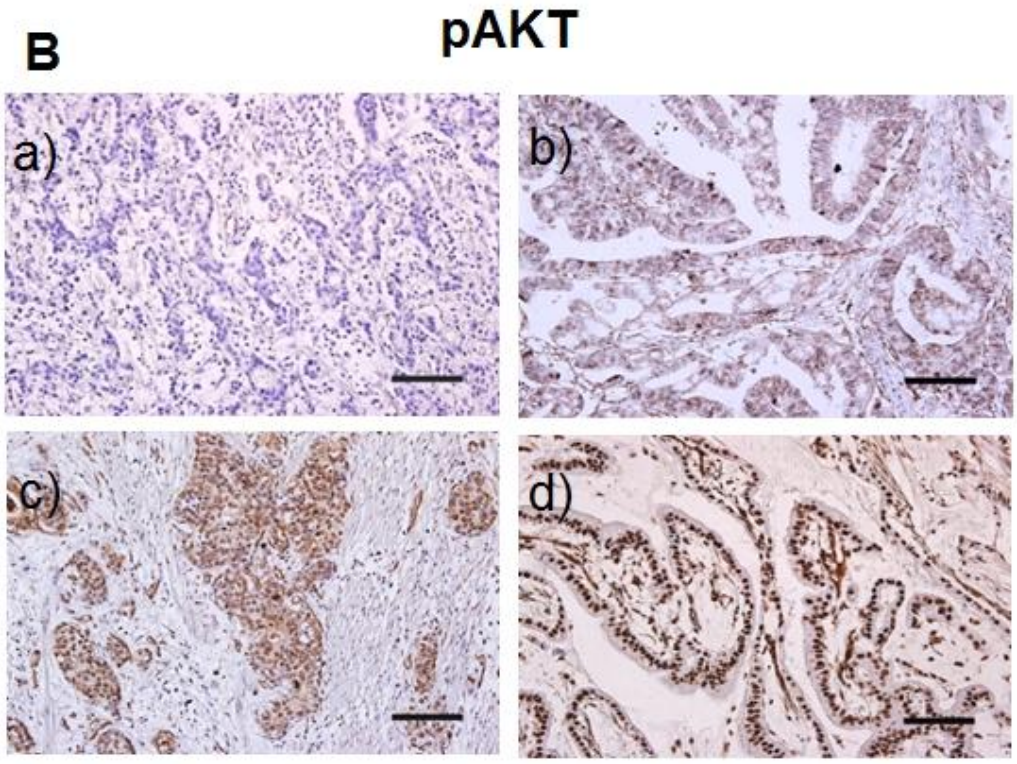


Figure 1. CK2 and p-AKT protein expression in tumor tissue from gastric cancer patients. (A) CK2 and (B) p-AKT immunoreactivity was graded as a) negative, b) weak (light brown), c) moderate (brown), or d) strong (dark brown). Original magnification: 200x, Scale bar: 50μm.

3. Association between CK2 and p-AKT expression and paclitaxel response

Response to paclitaxel was evaluated 56 of 59 patients. Two patients could not undergo a second cycle of chemotherapy due to decreased performance status, and one patient refused chemotherapy after the first treatment cycle. The overall response rate (ORR) was 10.2% and the disease control rate (DCR = CR + PR + SD) was 62.7%. There was no association between ORR or DCR and p-AKT expression. However, patients with

high CK2 levels had lower DCR (47.8% vs. 72.3%, $p = 0.017$) (Table 2).

Table 2. Response according to expression of CK2 and p-AKT

	All (%)	CK2			p-AKT		
		Positive (n=23)	Negative (n=36)	p-value	Positive (n=40)	Negative (n=19)	p-value
CR	0	0	0	0.004	0	0	0.295
PR	6 (10.2)	4 (17.4)	2 (5.6)		2 (5)	4 (21.1)	
SD	31 (52.5)	7 (30.4)	24 (66.7)		22 (55)	9 (47.4)	
PD	19 (32.2)	12 (52.2)	7 (19.4)		14 (35)	5 (26.3)	
NE	3 (5.1)	0	3 (8.3)		2 (5)	1 (5.3)	
ORR	6 (10.2)	4 (17.4)	2 (5.6)	0.181	2 (5)	4 (21.1)	0.077
DCR	37 (62.7)	10 (47.8)	26 (72.3)	0.017	24 (60)	13 (68.5)	0.503

CR, complete response; DCR, disease control rate; NE, not evaluable or not assessed; ORR, overall response rate; p-AKT, phosphorylated AKT; PD, progressed disease; PR, partial response; SD, stable disease

4. Association between CK2 and p-AKT expression and patients survival following paclitaxel therapy

In all patients, the median PFS and OS were 3.5 months [95% confidential interval (CI); 2.7 - 4.3 months] and 8.9 months (95% CI; 6.3 - 10.8 months), respectively. There was no difference in PFS according to age and sex, and no difference in PFS and OS according to p-AKT expression. However, PFS was longer in patients with low as

compared with high CK2 expression (median PFS, 4.1 vs. 2.8 months, p = 0.009). OS was not associated with CK2 expression (Figure 2).

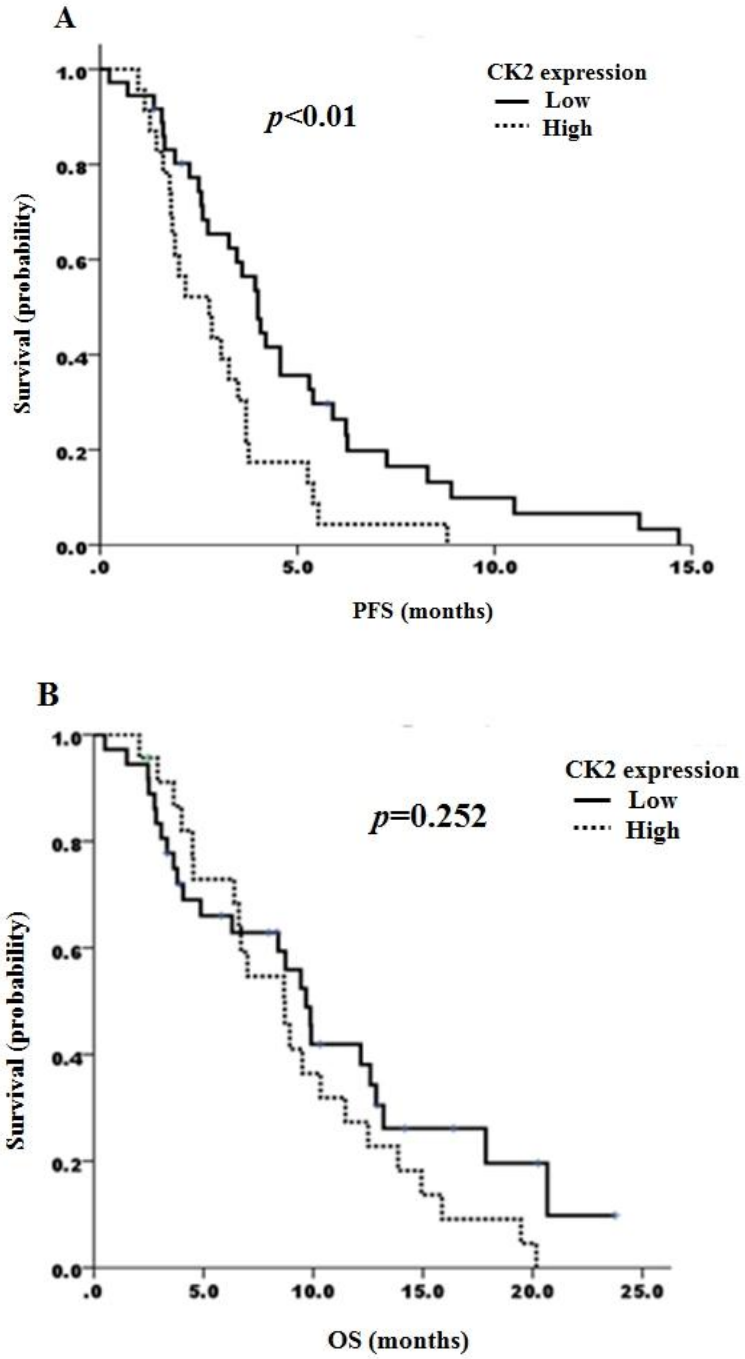


Figure 2. Kaplan-Meier survival curve according to CK2 expression. (A) PFS in 59

patients; the median values for patients with low and high CK2 expression were 4.1 months versus 2.8 months (p=0.009). (B) There was no difference of OS according to CK2 level.

5. Paclitaxel sensitivity in 49 GC cell lines

Since CK2 protein expression was associated with paclitaxel resistance in tumor tissue of gastric cancer patients, we investigated the association between paclitaxel sensitivity and CK2 expression in 49 gastric cancer cell lines. The antiproliferative effect of paclitaxel was evaluated with the MTT assay. The YCC-30 cell line was the most sensitive to paclitaxel treatment with half-maximal inhibitory concentration (IC50) of 0.0003 μM; in contrast, YCC-25, YCC-28, and YCC-33 cells were highly resistant to the effects of paclitaxel IC50 ≥10 μM. The cut-off level of paclitaxel sensitivity/resistance is 0.1 μM Catalogue of Somatic Mutations in Cancer (http://cancer.snager.ac.uk/cosmic). According to this value, 18 cell lines (36.7%) were resistant to paclitaxel (Figure 3).

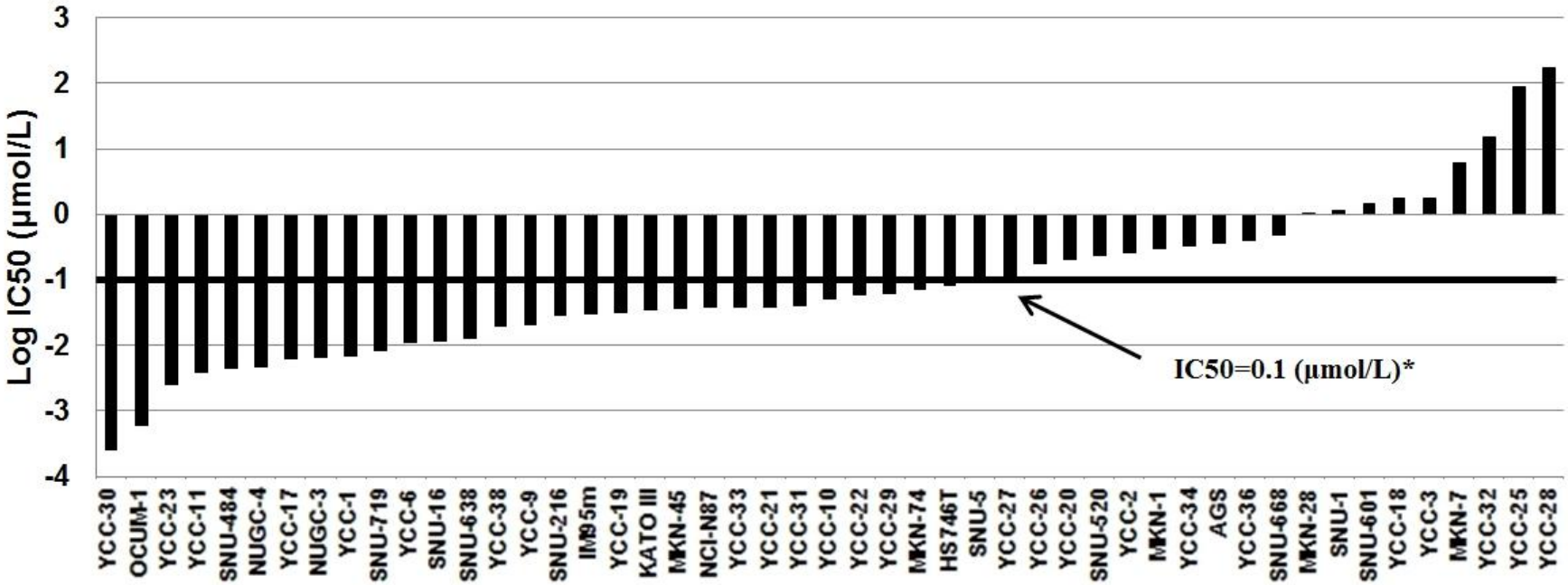
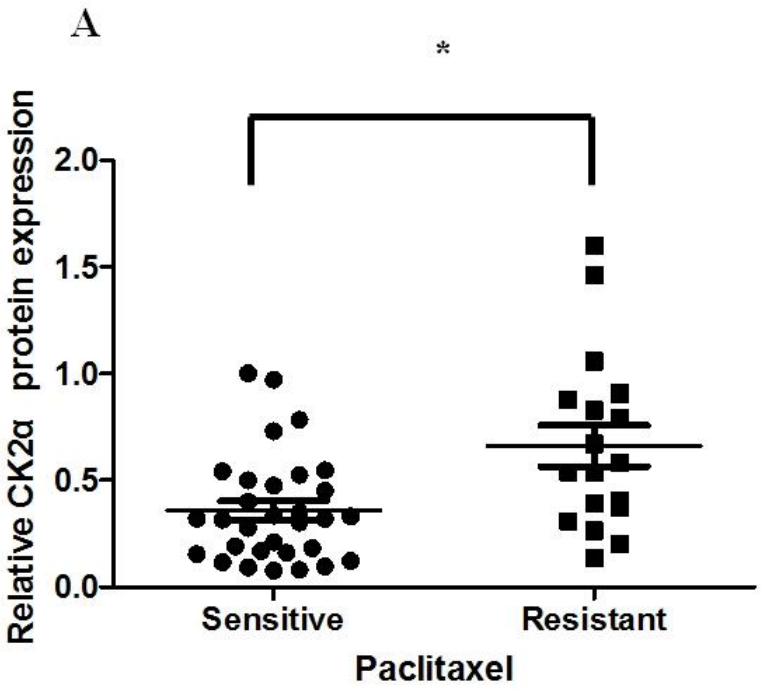
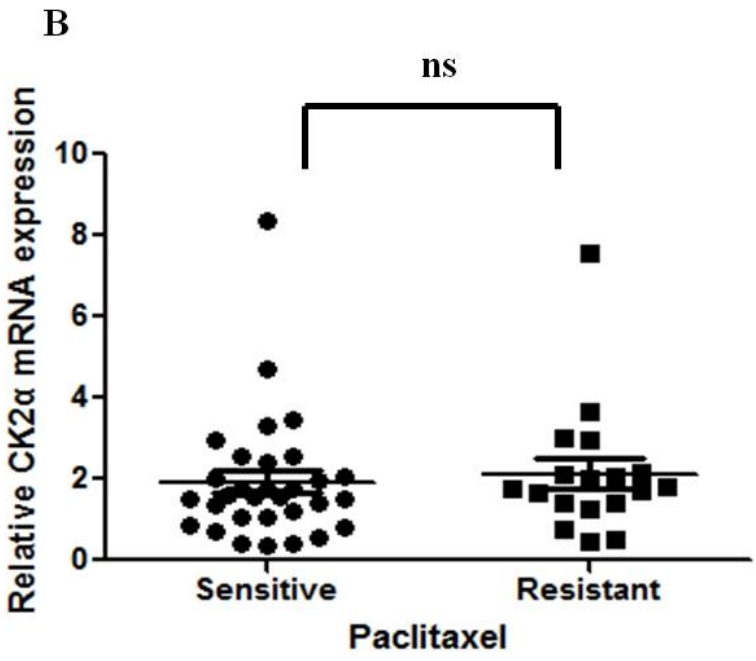


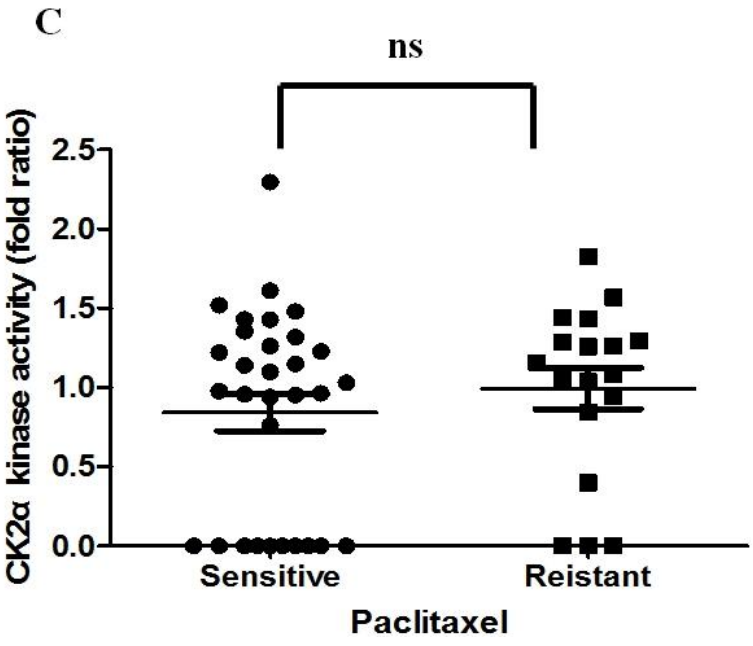
Figure 3. Antiproliferative effect of paclitaxel against 49 gastric cell lines. GC cell lines (n = 49) were treated with increasing doses of paclitaxel and IC50 was determined with the MTT assay. The most sensitive cell line was YCC-30 (IC50 = 0.0003 μM), whereas SNU-1 cells showed the highest resistance (IC50 = 7.2 μM). *Cutoff level for resistance to paclitaxel defined according to the Catalogue of Somatic Mutations in Cancer database.

6. Association between CK2 expression and paclitaxel sensitivity

We evaluated CK2 mRNA and protein expression, and CK2 activity in 49 GC cell lines. Paclitaxel-resistant cell lines had higher CK2 protein expression than those that were sensitivity the the drug (p=0.041, Figure 3A). However, neither CK2 mRNA nor activity was correlated with paclitaxel sensitivity (Figure 4B and 4C). p-AKT expression was not associated with paclitaxel resistance (Figure 4D).







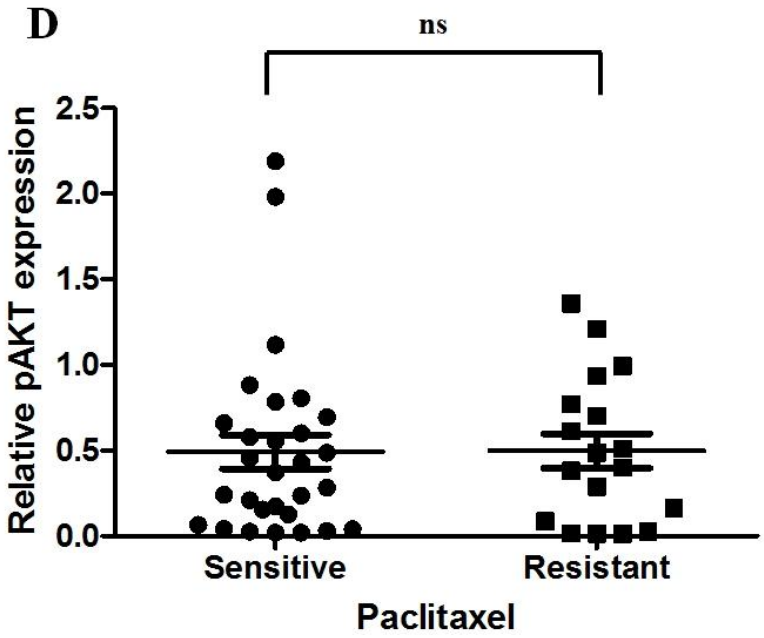
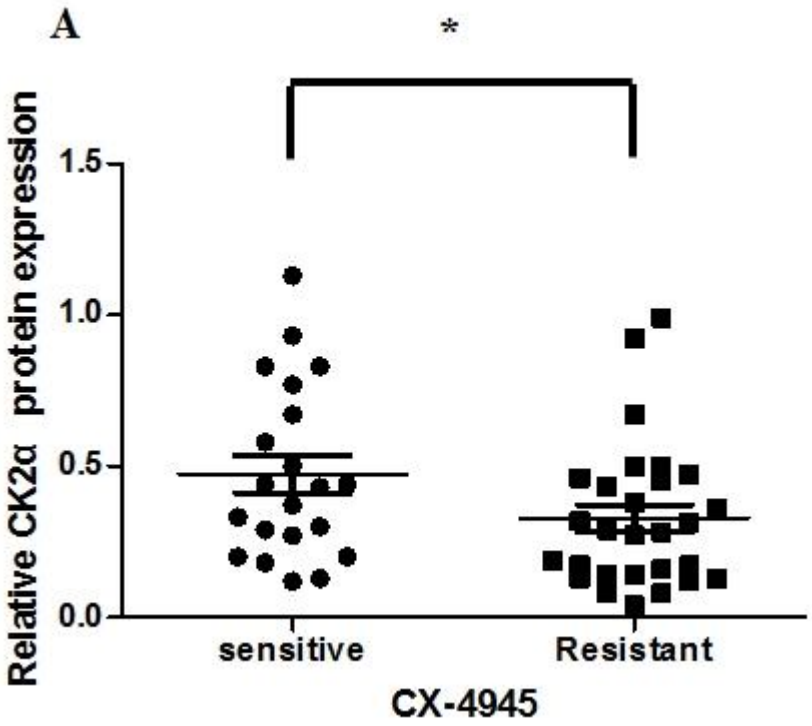


Figure 4. Association between CK2 expression and paclitaxel sensitivity. (A) CK2 protein levels in 49 gastric cell lines were evaluated by western blotting and compared between paclitaxel-sensitive and -resistant cells. (B) CK2 mRNA expression was assessed by quantitative reverse transcription PCR and compared between paclitaxel-sensitive and -resistant lines. (C) CK2 kinase activity was evaluated by the CK2 kinase assay and compared between paclitaxel-sensitive and -resistant lines. (D) p-AKT expression was assessed by western blotting and compared between paclitaxel-sensitive and -resistant cell lines. The mRNA and protein levels were normalized to those of Jurkat cells. *$p < 0.05$; ns, not significant.

7. Association between CK2 expression and CX-4945 sensitivity in GC cells

We treated the GC cell lines with CX-4945 and found that the IC50 ranged from 2.7 to >100 μM. The cell lines were divided into CX-4945-sensitive and -resistant groups, with a cut-off CX-4945 IC50 of 10 μM. According to this value, 20 cell lines were sensitive to

CX-4945. High CK2 protein expression was correlated with CX-4945 sensitivity (p = 0.058); however, there was no association between CK2 mRNA or activity and CX-4945 sensitivity (Figure 5).



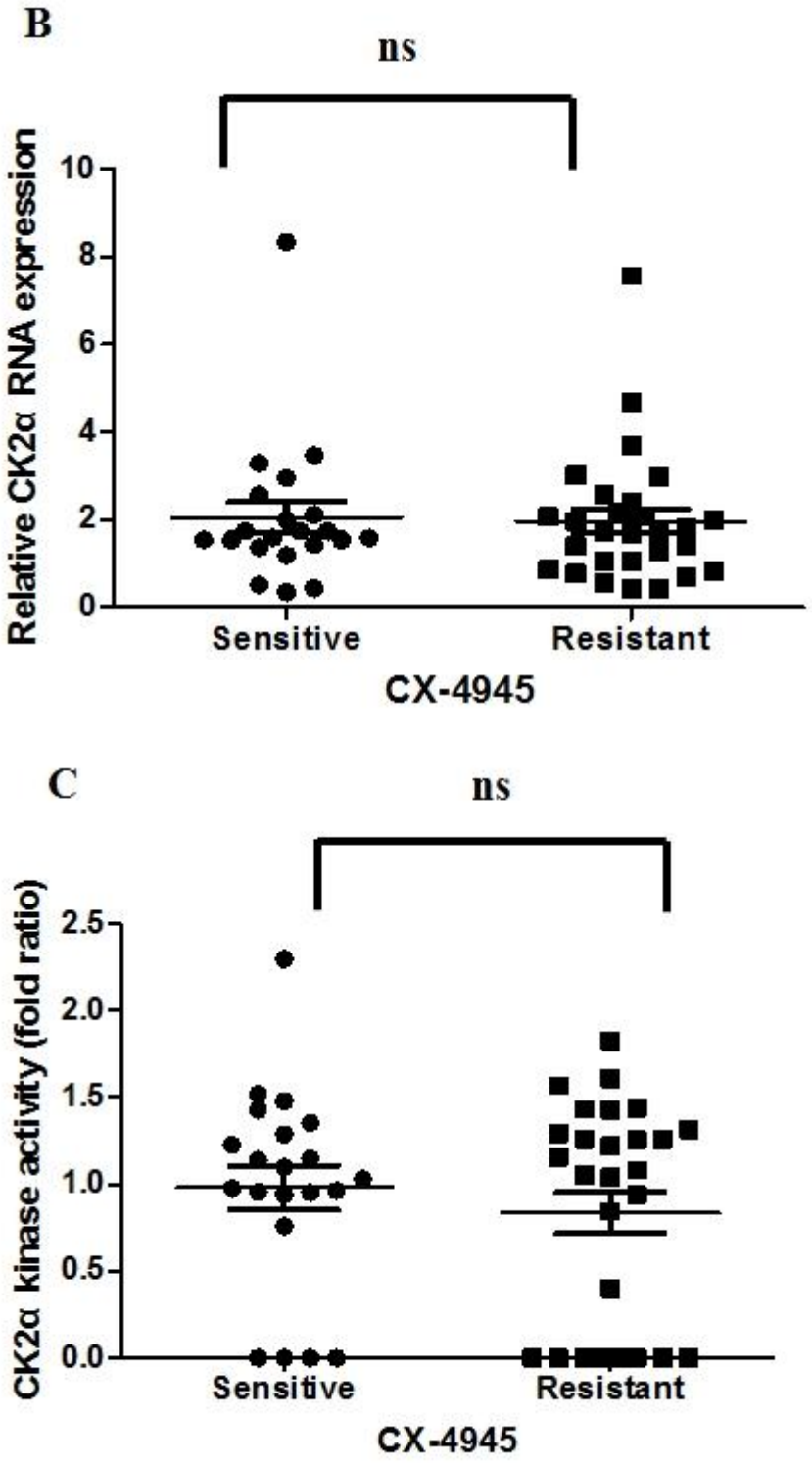


Figure 5. Association between CK2 expression and CX-4945 sensitivity. (A) CK2 protein levels in 49 gastric cell lines were evaluated by western blotting and compared between

CX-4945-sensitive and -resistant cells. (B) CK2 mRNA expression was assessed by quantitative reverse transcription PCR and compared between CX-4945-sensitive and -resistant lines. (C) CK2 kinase activity was evaluated by the CK2 kinase assay and compared between CX-4945-sensitive and -resistant lines. The mRNA and protein levels were normalized to those of Jurkat cells

8. Synergistic effect of paclitaxel and CX-4945 on proliferation of paclitaxel resistant SNU-1 cells

The above results indicated that CK2 expression was associated with paclitaxel resistance and that CX-4945 was effective in cell lines with CK2 protein expression. We therefore, speculated that combined treatment of paclitaxel and CX-4945 could overcome paclitaxel resistance. We tested our hypothesis by treating paclitaxel-resistant (IC50 = 1.122 μM, Figure 3), CX-4945-sensitive (IC50 = 7.2 μM) SNU-1 cells with paclitaxel (0.1 or 1 μM monotherapy), CX-4945 (5 or 10 μM monotherapy), or both (0.1 μM paclitaxel, and 5 or 10 μM CX-4945). The combination of both drug had synergistic inhibitory effect on cell proliferation relative to CX-4945 or paclitaxel monotherapy according to New Bliss Independence Model ($Y_{ab,\ O} = 0.767 > Y_{ab,\ P}=0.478$) (Figure 6)

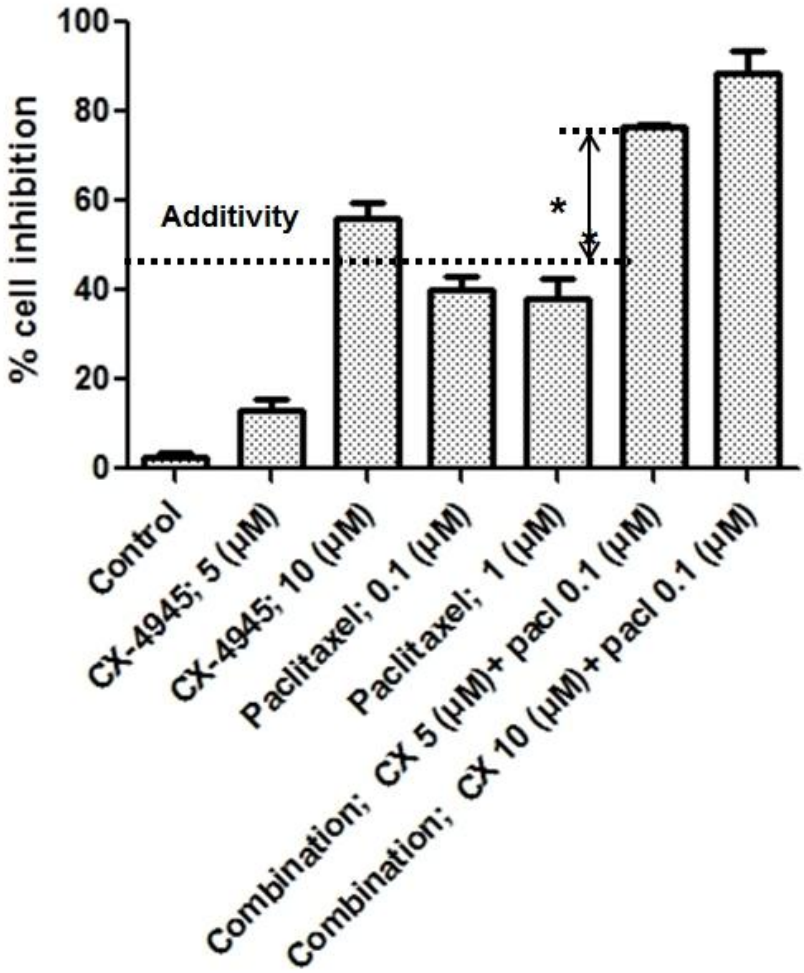


Figure 6. Synergistic antiproliferative effects of paclitaxel and CX-4945 in SNU-1 cells. The additivity line is equivalent to the theoretical combined antiproliferative effects of drugs A and B [%A + %B (100 −%A/100] in which %A and %B are the percentages of cells killed by drugs A and B, respectively, at a given concentration. *28.9%.

We investigated whether paclitaxel, CX-4945, or their combination inhibited SNU-1 cell-cycle progression of SNU-1 by flow cytometry. The proportion of cells in G2/M phase was increased by concomitant treatment with both drugs (Figure 7). Notably, G2/M

arrest was markedly increased in these cells treated with paclitaxel combined with CX-4945.

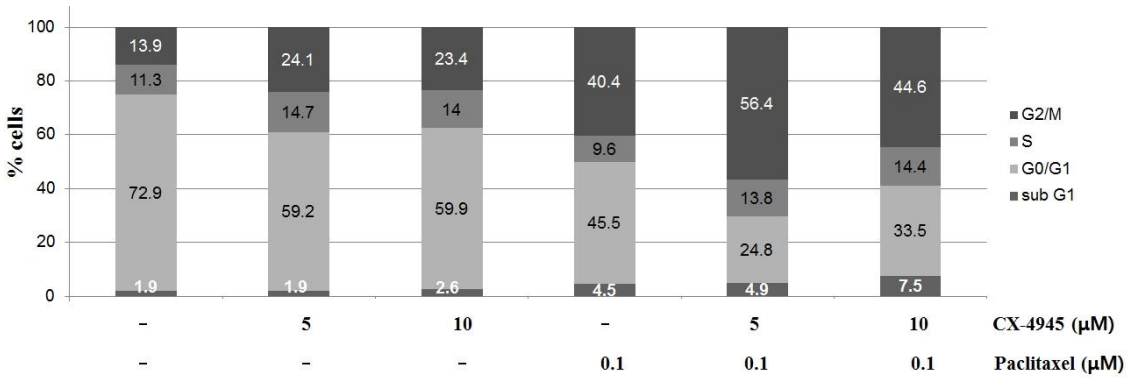


Figure 7. Cell cycle analysis of paclitaxel and CX-4945 in SNU-1 cells. SNU-1 cells were treated with CX-4945 (5 or 10 μM), paclitaxel (0.1 or 1 μM), and paclitaxel (0.1 μM) combined with CX-4949 (5 or 10 μM) for 36 h, and cell cycle distribution was analyzed by flow cytometry. The fraction of cells in sub-G1, G0/G1, S, and G2/M phases of the cell cycle is shown. Combined treatment of paclitaxel and CX-4945 induced G2/M arrest

9. Effects of paclitaxel combined with CX-4945 on CK2 expression, PI3K/AKT signaling, and apoptosis in SNU-1 cells

The effects of combined paclitaxel and CX-4945 treatment on CK2 expression, PI3K/AKT signaling, and apoptosis were analyzed by western blotting analysis in SNU-1 cells. CX-4945 decreased phosphorylation of CK2, AKT and downstream signaling protein p70S6K, while paclitaxel has opposite effect. CX-4945 combined

with paclitaxel decreased p-CK2, p-AKT, p- p70S6K expression. The effect of CX-4945 on apoptosis was also investigated by evaluating cleaved poly (ADP-ribose) polymerase (PARP)-1 protein expression. CX-4945 and paclitaxel monotherapy induced cleaved PARP-1 expression; this effect was potentiated by combination treatment with both drugs (Figure 8).

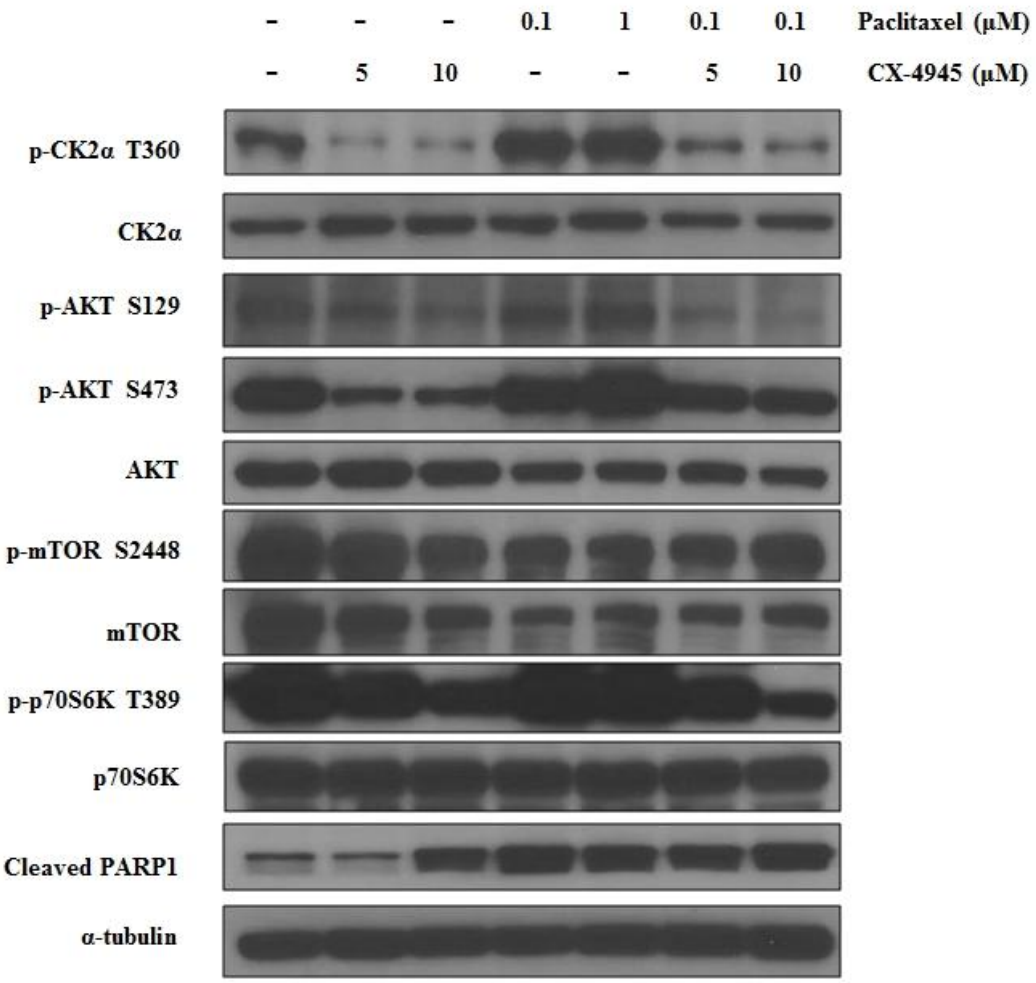


Figure 8. Effect of CX-4945 combined with paclitaxel on the CK2 expression,

PI3K/AKT signaling, and apoptosis in SNU-1 cells. SNU-1 cells were treated with vehicle, CX-4945 (5 or 10 μM), paclitaxel (0.1 or 1 μM), and paclitaxel (0.1 μM) combined with CX-4949 (5 or 10 μM) for 48h. Western blotting was conducted to assess AKT signaling

IV. DISCUSSION

In this study, we examined the relationship between CK2 expression and paclitaxel resistance in GC. CK2 is a ubiquitous serine/threonine kinase that regulates a variety of cellular processes including cell cycling, proliferation, and apoptosis.[29] CK2 overexpression is correlated with tumorigenesis in various types of cancer as well as with the degree of tumor invasion; more over, patients with elevated levels of CK2 had lower overall survival in surgically resected GC.[21,30,31] Interestingly, CK2 was shown to exert anti-apoptotic effects via upregulation of AKT.[22] Given that PI3K/AKT activation is associated with paclitaxel resistance, we speculated that CK2 is also related to paclitaxel resistance via the PI3K/AKT pathway in GC. [16,17,32]

To test our hypothesis, we investigated CK2 expression in the tumor tissue of GC patients treated with paclitaxel as second-line chemotherapy. As predicted, CK2 overexpression was correlated with paclitaxel resistance. Patients with higher CK2 expression levels had significantly shorter survival and lower DCR from paclitaxel therapy as compared to those with low CK2 expression. The association of between CK2 expression and paclitaxel resistance was confirmed *in vitro* using GC cell lines.

CX-4945 is a potent and selective small-molecule inhibitor of CK2 that is active against

a broad range of malignancies, including breast, colon, pancreas, prostate, ovarian, and lung cancer.[33] The efficacy of CX-4945 against GC has been not previously reported. We identified 20 GC cell lines among 49 that were CX-4945 sensitive cell lines, with a CX-4945 IC50 cut-off level of 10 μM. Cell lines with high CK2 protein expression trended to be sensitive to CX-4945. Combination treatment of SNU-1 which overexpress CK2 protein and show paclitaxel resistance, and CX-4945 sensitivity with paclitaxel and CX-4945 had a synergistic effect on proliferation relative to cell treated with either drug alone. Combination treatment and CX-4945 monotherapy also decreased the phosphorylation of of CK2, AKT, and the downstream factor p70S6K, whereas paclitaxel monotherapy had the opposite effect. This suggests that PI3K/AKT activation by paclitaxel may contribute to paclitaxel resistance. Indeed, PI3K/AKT signaling was suppressed by CK2 inhibition, leading to cell cycle arrest and apoptosis.

Given our observation that CK2 activation is related with paclitaxel resistance, CX-4945 in combination with paclitaxel could be a treatment of choice in paclitaxel-resistant GC. However, the inhibition of CK2 to overcome paclitaxel resistance requires confirmation xenograft model before clinical trials are initiated. In addition, paclitaxel sensitivity must be evaluated, according to the different molecular subtypes of GC

which have been shown to be associated with distinct clinical outcomes[34,35] Two genes, involved in tubulin processing-*tubulin gamma complex-associated protein 4 and N-acetyltransferase 10*- were related to poor prognosis in the Stem-A subtype of ovarian cancer, which exhibits elevated microtubule activity and is sensitive to several microtubule polymerization-inhibiting drugs; such as vincristine and vinorelbine.[36] It is possible that GC subtype also have variable sensitivity to microtubule inhibitors.

V. CONCLUSION

The results presented here suggest that high CK2 expression is negative predictive marker for paclitaxel response in GC. Paclitaxel resistance was related to CK2 expression through the PI3K/AKT signaling pathway. Our findings suggested that CK2 inhibition with CX-4945 which induces G2/M cell cycle arrest and increases apoptosis by suppressing PI3K/AKT signaling in combination with paclitaxel can be effective against paclitaxel resistant GC.

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ABSTRACT (IN KOREAN)

위암에서 paclitaxel 저항 극복 방법으로서 CK2의 억제 효과

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정민규

진행성 위암은 치료 발전에도 불구하고, 여전히 예후가 않좋은 암중에 하나이다. 파클리탁셀은 위암의 주된 항암제 중에 하나이나, 저항기전으로 인해 그 효과는 제한적이다. 최근, Casein kinase 2 (CK2)의 활성화는 다양한 암종에서 종양의 증식과 연관되고, 항암제 저항성과 관계되는 연구가 보고되고 있다. 따라서, 본 연구는 위암에서, 파클리탁셀의 저항성과 CK2 활성화에 대해 조사하고자 하였다. 2차 약제로 파클리탁셀로 치료 받은 59명의 진행성 위암환자를 대상으로, 치료 전 종양조직을 이용하여 면역 화학 조직 염색법으로 CK2 발현을 조사하였다. CK2가 과발현 된 환자는 (29/59, 39%), CK2가 발현되지 않은 환자에 비해 질병 조절율이 통계학적으로 낮았으며 (47.7 % vs.

72.3 %, p=0.017), 무진행 생존율도 짧았다 (2.8 months vs. 4.8 months, p=0.009).

49 개의 위암 세포주를 이용하여 파클리탁셀 및 CK2 억제제인 CX-4945 에 대한 항증식효과를 조사한 결과 CK2 단백질의 과발현과 파클리탁셀의 저항성과 상관관계가 있었다. 49 개의 위암세포주 중에 파클리탁셀에 저항성이 있으며, CK2 단백질이 과발현을 보이며, CX-4945 에 민감한 세포 주인 SNU-1 세포주를 선택하여, 파클리탁셀과 CX-4945 와 병합 치료하였을 경우 항종양 상승효과가 있었으며, PI3K/AKT 경로를 통해 억제됨을 확인 하였다.

결론적으로, CK2 과발현은 위암에서 파클리탁셀의 저항성과 관련이 있었으며, paclitaxel 과 CK2 억제제인 CX-4945 를 병합치료는 파클리탁셀의 저항성을 극복하는 치료 방법이 될 수 있을 것이다.

핵심되는 말: 위암, 파클리탁셀, 약물 저항성, casein kinase II

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