Targeting casein kinase 2 (CK2) to overcome cisplatin resistance through combination treatment with a CK2 inhibitor, CX-4945, in gastric cancer

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Directed by Professor Sun Young Rha

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ABSTRACT

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Gastric cancer is the most commonly diagnosed form of cancer in Korea. Although the benefit of radiation therapy combined with chemotherapy in gastric cancer has been established, challenges to providing accurate and safe treatment remain. The current strategy for treatment of gastric cancer includes surgery with chemotherapy in cases of potentially curable disease and chemotherapy alone for advanced cases. Platinum-based antineoplastic drugs are chemotherapeutic agents usually used to treat gastric cancer; these include cisplatin and oxaliplatin. Cisplatin is particularly effective against gastric cancer as it triggers apoptosis by inducing DNA damage through DNA crosslinking. Initial responsiveness to platinum is high, but the majority of cancer patients will eventually relapse with cisplatin-resistant

disease. Many mechanisms of cisplatin resistance have been proposed, including changes in cellular uptake and efflux of the drug, increased detoxification of the drug, and inhibition of apoptosis. In particular, increased DNA repair is a drug-targetable mechanism and is useful as part of the treatment strategy for cisplatin-resistant cancer.

Casein kinase 2 (CK2) plays a critical role in multiple cellular processes, such as DNA repair, maintenance of cell viability, protection of cells from apoptosis, and cell cycle regulation. The expression levels of CK2 have been analyzed in various cancer tissues from patients. Although overexpression of CK2 is known to be significantly correlated with poor prognosis and survival in various cancers, its role in gastric cancer is still being studied. For this reason, research on the correlation of CK2 expression and DNA repair mechanisms in gastric cancer is important. CK2 acts as a key regulator of the cisplatin-induced DNA damage repair process. The combination of cisplatin and a CK2 inhibitor may enhance cisplatin-induced DNA damage and prove useful in the treatment of gastric cancer.

CK2 has emerged as a promising target for therapeutic intervention in the treatment of cancer. Therefore, inhibition of CK2 is a reasonable way to ameliorate cisplatin resistance in gastric cancer.

Key words: gastric cancer, cisplatin resistance, casein kinase 2, CX-4945, DNA repair

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I. INTRODUCTION

According to clinical data from the National Cancer Center in 2013, GC (13.4%) is the second most common cancer, behind thyroid cancer (18.9%). The prognosis of gastric carcinoma is poor, with a 5 yr survival rate of 5.8%. Gastric cancer may spread from the stomach to other parts of the body, and many cases cannot be cured.[1,2]

For this reason, gastric cancer is known to have a high incidence and low survival rate in Korea. Multiple types of treatment, such as chemotherapy, radiotherapy and/or surgery, are often combined to treat gastric cancer.[3-5] Chemotherapy drugs kill cancer cells, but they can also damage normal cells. This damage to normal

cells causes side effects. Nowadays, targeted therapy, a type of chemotherapy that attacks cancer cells while doing less damage to normal cells, is increasingly employed.[6] Targeted therapy is often employed to treat lung, breast, and colorectal cancer, but its use in gastric cancer still has limitations. This is because targeted molecules for gastric cancer treatment are still lacking. Trastuzumab (Herceptin®, Eoche, Switzerland) is approved to treat certain types of stomach cancer that express only 10% of the normal level of HER-2.[7-9] Although many targeted agents have been evaluated in various cancers, targeted molecular studies are still required to improve gastric cancer treatment.[10]

Cisplatin is the most widely used chemotherapeutic agent for gastric cancer and has been extensively studied in anticancer therapy because of its ability to covalently bind to DNA. Also, cisplatin is still used in clinical therapies and treatment for patients with many types of cancers, including head and neck, testicular, ovarian, lung, colorectal, and gastric cancer. Damage to DNA caused by cytotoxic activity is known to result from platinum-DNA adducts, which form inter-/intra-strand cross-links that activate the apoptotic pathway, resulting in cell death.[11] One of the mechanisms of resistance to cisplatin is directly related to cisplatin-induced DNA adducts as cancer cells acquire the ability to repair damaged DNA.[12] Moreover, activation of DNA repair pathways in cancer cells can promote resistance to the cisplatin-induced DNA damage process.[13,14] Enhancing the efficacy of cisplatin by blocking DNA damage repair is a promising therapeutic strategy for restoration of cisplatin resistance in gastric cancer.[15,16]

A targeted therapy molecule involved in DNA repair, CK2, is being studied in cancer treatment.[17] CK2 has been implicated in cell cycle control, DNA repair, regulation of circadian rhythms, and other cellular processes.[17-19] These processes need to retain normal functioning in normal cells, as abnormal functioning makes normal cells malignant.[20] Cancer progression results from the development of

abnormal properties in normal cells, which has been confirmed in breast, ovarian, and lung cancers.[21,22] This phenomenon is correlated with CK2 overexpression and high levels of activity in various cancers. In particular, CK2 overexpression in human cancers is associated with DNA repair and tumor progression. According to *in vitro* and *in vivo* studies, CK2 is important in cancer progression and CK2 is a promising molecule for targeted therapy.[23] For example, cell death pathways triggered by DNA damage can be regulated by CK2.[24] The CK2-dependent DNA repair response may potentiate the ability of DNA-targeted chemotherapeutic agents to kill tumor cells.[25] However, the roles of CK2 expression and activity are still unknown in gastric cancer, so it is necessary to continue studying CK2 in order to overcome cisplatin resistance in gastric cancer.

CX-4945 (Silmitasertib, Senhwa biosciences, California, U.S.A) is a potent, selective, and ATP-competitive inhibitor of both isoforms of the CK2 catalytic subunit, CK2α and CK2α'.[26] The anticancer activity of CX-4945 correlates with the suppression of CK2-regulated PI3K/Akt signaling, cell cycle arrest, and induction of apoptosis. Recently, CX-4945 has been advanced to the testing stage in human clinical trials in ovarian cancer, breast cancer, and cholangiocarcinoma patients.[21,23,27,28] The phase I trial addressed the safety and tolerability of increasing doses of CX-4945 in combination with gemcitabine plus cisplatin. The subsequent phase II trial is a randomized study of antitumor activity in cholangiocarcinoma patients, which compares the standard-of-care protocol of gemcitabine plus cisplatin against treatment with CX-4945 in combination with gemcitabine plus cisplatin at the combination MTD determined in the phase I trial.[27,29] The development of CX-4945 and its warranted advancement into clinical trials will allow for therapeutic targeting of CK2 in gastric cancer.[30]

In this study, we measured CK2 expression and activity in gastric cancer cells, which were inhibited by the CK2 inhibitor CX-4945 and cisplatin. Moreover, a

synergistic effect of CX-4945 combined with cisplatin triggered DNA damage in cisplatin-resistant cells. These data indicate that CK2 is a useful target for reversing cisplatin resistance in gastric cancer cells.

II. MATERIALS AND METHODS

1. Drug

The CK2 inhibitor CX-4945 (a small molecule inhibitor of CK2) was given to us by Senhwa Biosciences (San Diego, CA, USA). Cisplatin (cis-Diamineplatinum (II) dichloride) was purchased from SIGMA-ALDRICH (St. Louis, MO, USA).

2. Cell culture

49 types of gastric cancer cells were used in this study. 4 cell lines were obtained from the ATCC (American Type Culture Collection, Rockville, Maryland, U.S.A), 11 cell lines were obtained from the Korean Cell Line Bank (Seoul, Korea), 9 cell lines were purchased from the JCRB Cell Bank (Japanese Cancer Research Resources Bank, Japan), and 25 cell lines were established by the CMRC (Cancer Metastasis Research Center, Yonsei University College of Medicine, Seoul, Korea) from advanced gastric cancer patients through isolation of ascites or peripheral blood. Cells were cultured in Eagle's Minimum Essential Medium (EMEM), RPMI-1640 containing 10% fetal bovine serum (Lonza, Basel, Switzerland), 100 units/ml penicillin, and 100 μg/ml streptomycin (Lonza, Basel, Switzerland). Cultured cells were incubated at 37˚C in an atmosphere with 5% CO_2.

3. Cell viability assay

Cells (8×10^3) were seeded onto a 96-well plate. After 24 hr, cisplatin and CX-4945 were applied at specific doses. The treatment doses were 1.25, 2.5, 5, 10, 20

μM cisplatin and 0.1, 1, 5, 10, 20 μM CX-4945. Following 3 days of incubation, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was added and the cells were further incubated at 37˚C for 4 hr. Absorbance was read at a wavelength of 570 nm and analyzed using Calcusyn software (Biosoft, U.K).

Concurrent addition, pre-addition, and post-addition were used for combination treatment. Concurrent schedule: same dose for 72 hr. Pre-addition schedule: 4 hr treatment with CX-4945 followed by 68 hr treatment with cisplatin. Post-addition schedule: 24 hr treatment with cisplatin followed by 48 hr treatment with CX-4945. After 3 days of incubation, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (St. Louis, MO, USA) solution was added and the cells were further incubated at 37˚C for 4 hr. Absorbance was read at a wavelength of 570 nm and analyzed with Calcusyn software (Biosoft, U.K).

4. Quantitative real-time PCR (qPCR) analysis

Total RNA was extracted from the cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The reverse transcription reaction was performed with 2 μg of RNA and oligo (dT). The newly synthesized cDNA was amplified on a Stratagene Mx3005P system (Stratagene, La Jolla, CA, USA) using a QuantiTect SYBR Green PCR kit (Qiagen, Valencia, CA, USA). Primers specific for CK2α, CK2α' and GAPDH were designed as follows: CK2α forward 5'-TGT CCG AGT TGC TTC CCG ATA CTT-3' and reverse 5'-TTG CCA GCA TAC AAC CCA AAC TCC-3'; CK2α' forward 5'-AGC CCA CCA CCG TAT ATC AAA CCT-3' and reverse 5'-ATG CTT TCT GGG TCG GGA AGA AGT-3'; GAPDH forward 5'-CCA TGG AGA AGG CTG GGG-3' and reverse 5'-CAA AGT TGT CAT GGA TGA CC-3'. Amplification cycles were: 95℃ for 10 min, then 40 cycles at 95℃ for 30 sec, 60℃ for 10 sec and 72℃ for 30 sec, followed by

72℃ for 10 min.

5. Western blot analysis

Total protein (50 μg) was separated by SDS-PAGE (12% polyacrylamide gel) and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were incubated in blocking solution consisting of 5% skim milk in TBST at room temperature for 1 hr before being incubated at 4℃ overnight with primary antibodies specific for CK2α (anti-CK2α, Santa Cruz Biotechnology, CA, USA). This antibody recognizes both CK2 catalytic subunits α and α'. Peroxidase-conjugated antibodies (anti-mouse, Jackson ImmunoResearch, West Baltimore Pike West Grove, PA, USA) were used as secondary antibodies. The reagent for the enhanced ECL kit was used for detection and images were taken with X-ray film. Data were normalized to α-tubulin levels (anti-α-tubulin, St. Louis, MO, USA).

6. Enzyme activity assay

CK2 activity was determined by employing a CK2 activity kit (CycLex® CK2 (Casein kinase II) Kinase Assay) (CycLex, Nagoya, Japan). Samples were plated onto recombinant p53 pre-coated 96-well plates. Recombinant CK2 (10 μl) was placed in each well and 90 μl Kinase Reaction buffer was added before the cells were incubated at 30˚C for 30 min. After the wells were washed 5 times with washing buffer, 100 μl HRP-conjugated Detection Antibody (TK4D4) was added and the cells were incubated for 30 min at RT. After the wells were washed 5 times with washing buffer again, 100 μl Substrate Reagent was placed into each well and the cells were incubated for 15 min. Then, 100 μl stop solution was added and absorbance was analyzed. A machine capable of measuring absorbance in 96-well

plates at dual wavelengths of 450/595nm could also be used.

7. RNA sequencing analysis

RNA sequencing (RNA-seq) allows for quantitative measurement of CSNK2A1 (CK2α) and CSNK2A2 (CK2α') gene expression levels. Expression levels are measured in fragments per kilobase of exon model per million mapped reads (FPKM). Gene expression levels were normalized to VPS29.

8. Bliss independence model

The Bliss independence model was used to analyze cisplatin and CX-4945 combination data. Combined inhibition was calculated by $E_{bliss} = E_A + E_B - E_A \times E_B$ as an additive, synergistic, or antagonistic effect. E_A and E_B are the fractional inhibition caused by drug A and drug B alone, respectively, at specific concentrations. E_{bliss} is the predicted combinatory response of the two drugs in the absence of drug-drug interactions. If the experimentally measured fractional inhibition is less than E_{bliss}, the combination efficacy is synergistic. On the other hand, if the experimentally measured fractional inhibition is greater than E_{bliss}, the combination efficacy is antagonistic. If E_{bliss} and the experimentally measured inhibition are equal, the combination efficacy is additive.

9. Statistical analysis

Student's t-tests and one-way ANOVAs were used to analyze the findings of the *in vitro* assay. A *p* value of less than 0.05 was considered statistically significant.

III. RESULTS

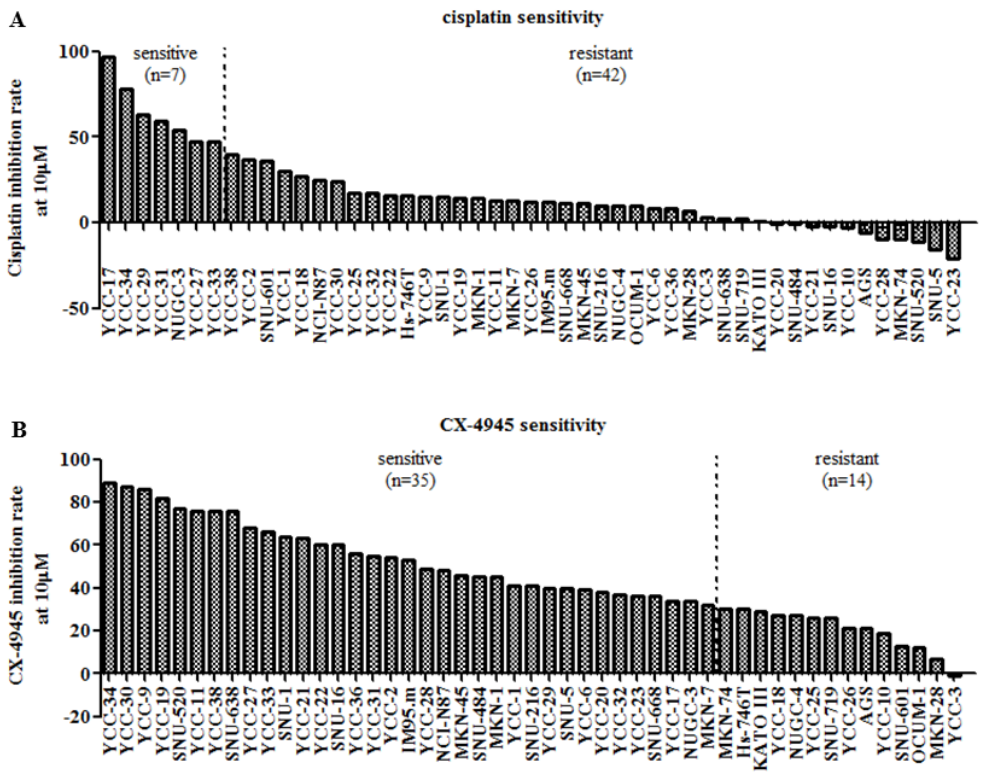
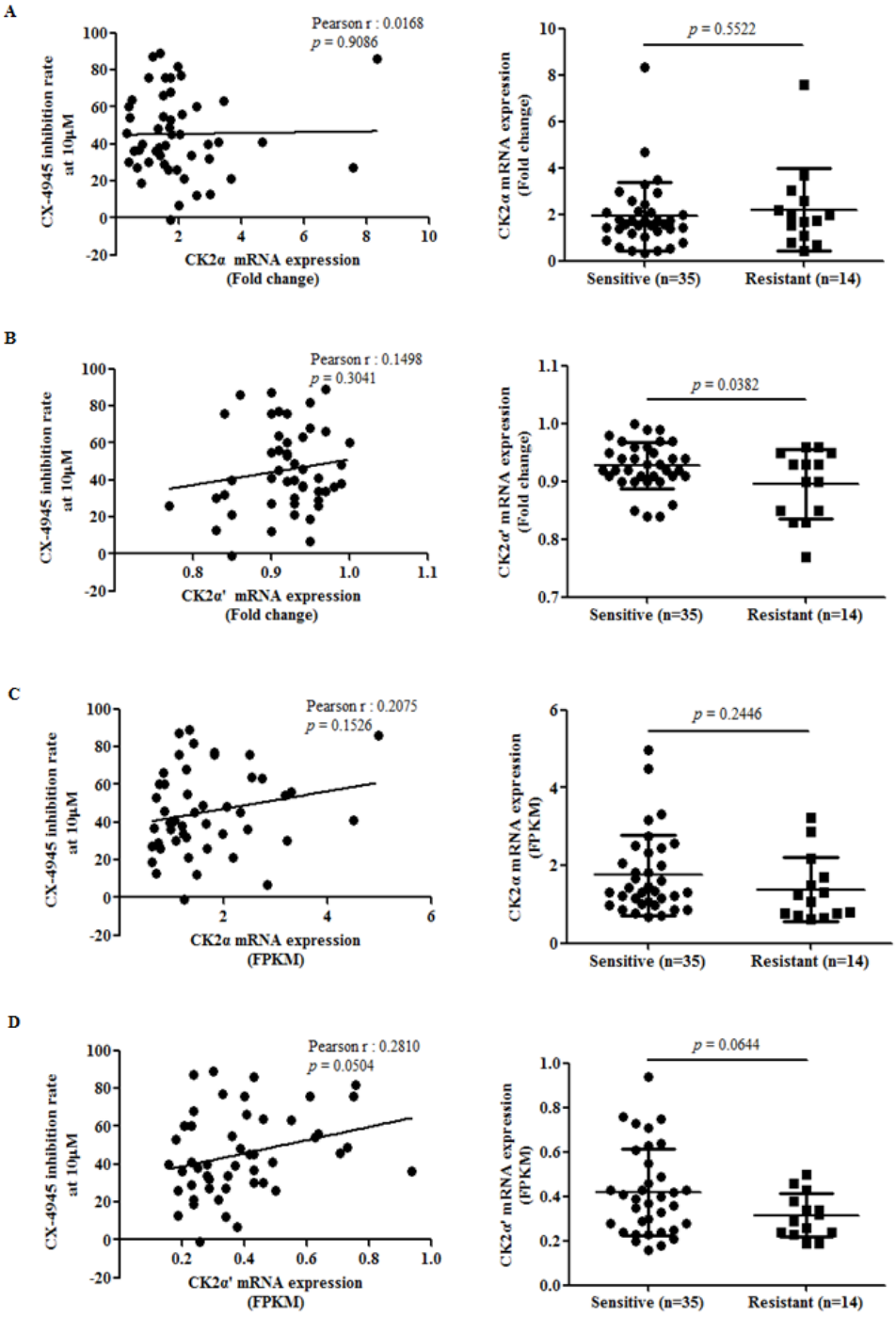


Figure 1. Sensitivity of 49 gastric cancer cell lines to the drugs used (cisplatin and CX-4945). (A). Sensitivity of the 49 gastric cancer cell lines to growth inhibition induced by cisplatin (10 μM). A 40% inhibition in maximal cell growth was our cut-off point for distinguishing sensitive (n=7) from resistant (n=42) lines. (B). Sensitivity of the 49 gastric cancer cell lines to growth inhibition by CX-4945 (10 μM). A 30% inhibition in maximal cell growth was our cut-off point for distinguishing sensitive (n=35) from resistant (n=14) lines.

Results from the 49 gastric cancer cell lines indicated that the cells displayed a wide range of sensitivity to cisplatin and CX-4945. At a cutoff inhibition rate of

40%, 7 cell lines were sensitive to cisplatin and 42 cell lines were resistant to cisplatin (Fig. 1A). Most of the gastric cancer cell lines (85.7%) were resistant to cisplatin. As for CX-4945, at a cutoff inhibition rate of 30%, 35 cell lines were sensitive to CX-4945 and 14 cell lines were resistant to CX-4945. Most of the gastric cancer cell lines (71.4%) were sensitive to CX-4945 (Fig. 1B). The panel of 42 cisplatin-resistant gastric cancer cell lines was further characterized in order to determine potential predictors of combinatory effects of cisplatin and CX-4945.



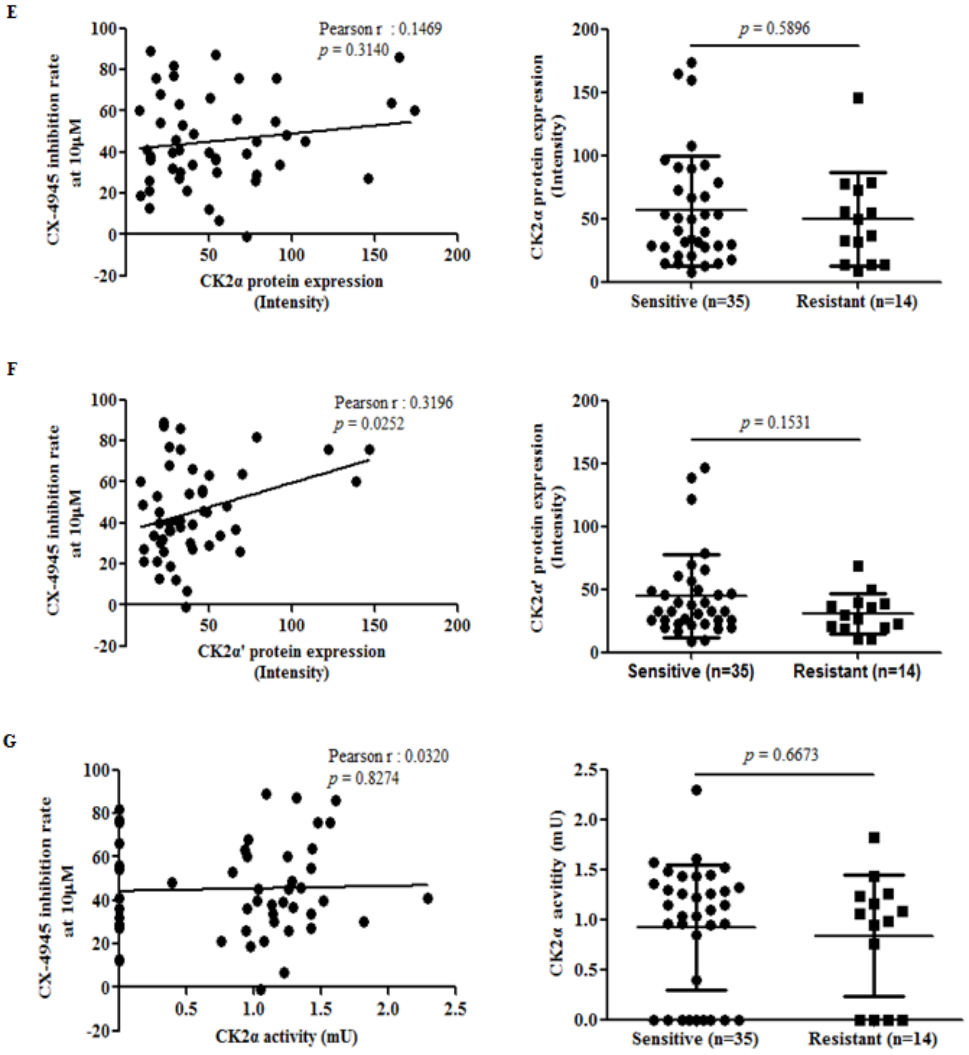


Figure 2. CK2 profiling in 49 gastric cancer cell lines. For these gastric cancer cell lines, mRNA and protein expression levels and enzymatic activity of the CK2 subunits were determined. (A,B,C,D). Correlation between sensitivity to CX-4945 and CK2 subunit mRNA expression in 49 gastric cancer cell lines. (E,F). Correlation between sensitivity to CX-4945 and CK2 subunit protein expression in

49 gastric cancer cell lines. (G). Correlation between sensitivity to CX-4945 and enzymatic activity of CK2 subunits in 49 gastric cancer cell lines.

CK2 expression levels and activity are elevated in many cancers of diverse genetic backgrounds, including breast, lung, prostate, and colorectal cancers and leukemia. Also, overexpression of CK2 in cancer tissues has frequently been linked to disease progression and poor prognosis. When profiled in 49 gastric cancer cell lines, mRNA and protein expression and enzymatic activity of the CK2 subunits exhibited a broad range. An apparent correlation was found between sensitivity to CX-4945 and CK2α' mRNA expression (Fig. 2B,D). Moreover, a significant correlation was found between sensitivity to CX-4945 and CK2α' protein expression, but the sensitive and resistant groups did not exhibit a significant difference in CK2α' protein expression (Fig. 2F). No correlations were observed between sensitivity to CX-4945 and other factors (Fig. 2A,C,E,G). These characteristics identify CK2 mRNA expression as a scientifically validated therapeutic target.

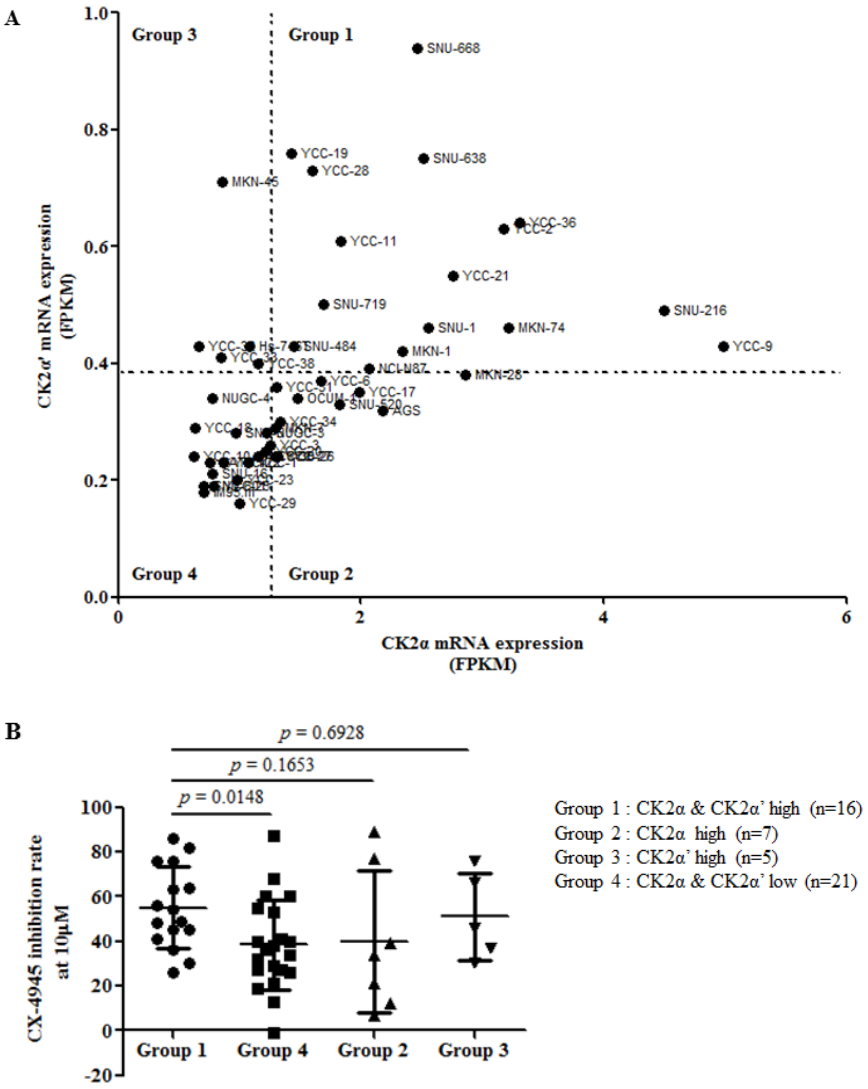


Figure 3. Selection of combination treatment group. (A). Characterization of 4 groups with CK2α and CK2α' mRNA expression analyzed by RNA sequencing. (Cut-off: median expression of CK2α and CK2α'.) Group 1: Both CK2α and CK2α' high group (n=16). Group 2: CK2α high group (n=7). Group 3: CK2α' high group (n=5). Group 4: Both CK2α and CK2α' low group (n=21). (B). Correlation between

sensitivity to CX-4945 and the 4 groups according to CK2α and CK2α' mRNA expression.

According to CK2 profiling, CK2α' mRNA expression was apparently correlated with sensitivity to CX-4945. Although our data did not reveal any clear correlation between sensitivity to CX-4945 and CK2α expression, CK2α expression was significantly higher in various tumors. This identification suggests that both CK2α and CK2α' mRNA expression levels are important in conferring sensitivity to CX-4945. Four groups were identified based on median expression levels (Fig. 3A) and sensitivity was compared between the groups (Fig. 3B). Group 1 was the most sensitive to CX-4945 and Group 4 was the least sensitive to CX-4945 (Fig. 3B). Moreover, Group 1 and Group 4 showed a significant correlation with CX-4945 sensitivity. Therefore, CK2 mRNA expression is a predictive biomarker for CX-4945 sensitivity.

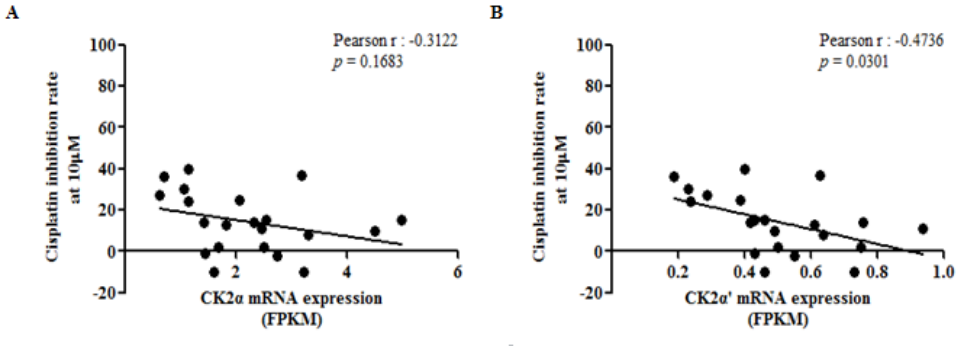


Figure 4. Correlation between CK2 expression and sensitivity to cisplatin. (A). Association between CK2α mRNA expression and sensitivity to cisplatin. (B). Association between CK2α' mRNA expression and sensitivity to cisplatin. Twenty-one cell lines were divided into two groups and evaluated for cisplatin sensitivity. Group 1 consisted of cells that highly expressed both CK2α and CK2α' mRNA (n=16), whereas Group 2 was the cisplatin intermediate resistance group regardless of CK2 expression (n=7). Two cell lines were included in both groups. The cisplatin intermediate group displayed a 20% to 40% inhibition rate for cisplatin at a concentration of 10μM.

Group 1, the CK2α and CK2α' mRNA high group, exhibited a higher degree of sensitivity to CX-4945. Groups 1 and 2 were divided based on sensitivity to cisplatin, because high expression of CK2 activates DNA repair mechanisms. Up-regulation of DNA repair mechanisms makes cells resistant to cisplatin. Although the basal level of CK2 may be low in some cell lines, the reaction to cisplatin increases CK2 expression and function. The effectiveness of cisplatin is slightly affected in the cisplatin intermediate resistance group. Regardless of CK2 expression, accumulation of CK2 activates DNA repair in the cisplatin resistant group. CK2α mRNA expression in the 21 cell lines shows a tendency towards an association with sensitivity to cisplatin (Fig. 4A). However, CK2α' mRNA expression shows a significant correlation with sensitivity to cisplatin (Fig. 4B).

Although the function of CK2α' is less well understood than that of CK2α, CK2α' plays a more critical role in cisplatin resistance than does CK2α. In a previous study, CK2 was shown to act as a key regulator of the DNA repair process, and was involved in the repair of both single- and double-stranded breaks in various human cancer cells overexpressing CK2. These data suggest that inhibition of CK2 could be a reasonable means of rescuing cisplatin resistance.

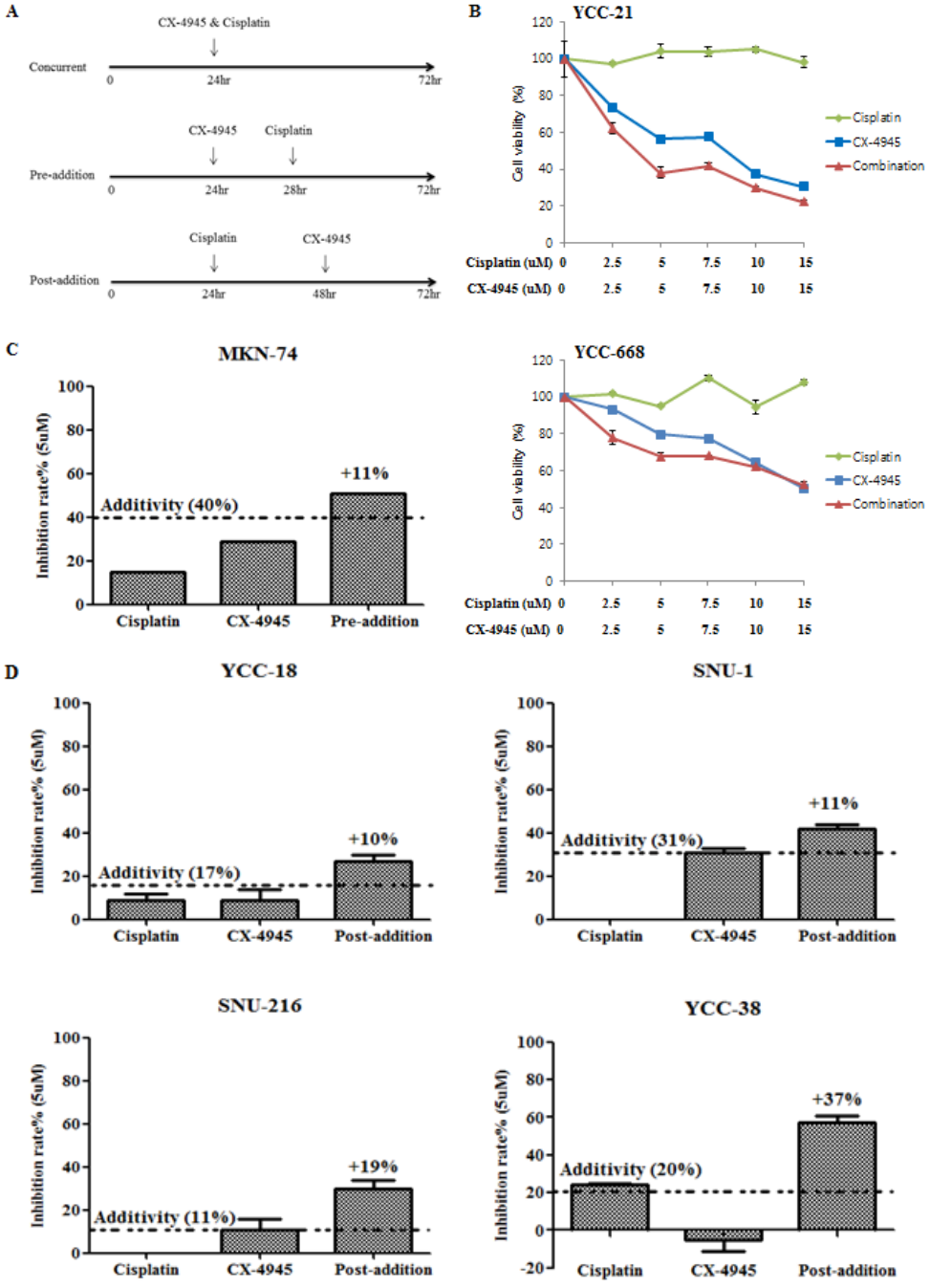


Figure 5. Synergistic effect with concurrent and sequential drug treatment. (A). Combination schedule (concurrent, pre-addition, and post-addition). (B). Synergistic effect in cell lines treated on the concurrent schedule. As a result of this data, dosing for the sequential schedule was determined. (C). Synergistic effect in cell lines treated on the pre-addition schedule. (D). Synergistic effect in cell lines treated on the post-addition schedule. Data are expressed as maximal cell growth at 72 hr. Synergistic effect was analyzed with the Bliss independence model.

Concurrent and sequential (pre-, post-addition) schedules were determined. Concurrent: cisplatin and CX-4945 were applied at the same time. Pre-addition: cisplatin was applied before the CX-4945 half-life (5~12hr) was over. Post-addition: cisplatin was applied prior to CX-4945 to induce DNA damage (Fig. 5A). Both the CK2α and CK2α' mRNA high expression cell lines and the cisplatin intermediate resistance cell lines received combination treatment. Some cell lines revealed a synergistic effect in the concurrent schedule. To select the dose for the sequential schedule, 5μM was identified as having a marked synergistic effect (Fig. 5B). In the sequential schedule, the MKN-74 cell line displayed a dramatic synergistic effect with the pre-addition schedule (Fig. 5C). According to the post-schedule data, the YCC-18, YCC-38, SNU-1, and SNU-216 cell lines displayed a dramatic synergistic effect (Fig. 5D). A different synergistic effect was observed with each schedule. This may relate to CK2 expression and cisplatin sensitivity. Moreover, modification of treatment schedule is important to increasing synergistic effect.

Table 1. Classification of the combined treatment group

Cell line	CK2α	CK2α'	Cisplatin IR	Concurrent	Pre-addition	Post-addition
YCC-9	4.98	0.43	8	-14%	-15%	-10%
MKN-1	2.34	0.42	7	-10%	-31%	-1%
NCI-N87	2.07	0.39	4	-29%	-2%	-17%
YCC-1	1.07	0.23	21	-24%	-28%	-13%
YCC-36	3.31	0.64	0	+5%	-12%	-10%
YCC-21	2.76	0.55	6	+20%	-25%	-8%
SNU-638	2.51	0.75	1	+7%	0%	-9%
SNU-668	2.46	0.94	5	+8%	-15%	-7%
YCC-28	1.60	0.73	22	+22%	-7%	+2%
SNU-484	1.45	0.43	0	+2%	+6%	+5%
SNU-601	0.71	0.19	25	+3%	+6%	-39%
MKN-74	3.22	0.46	0	-9%	+11%	-3%
YCC-11	1.83	0.61	8	-4%	+7%	+1%
SNU-719	1.69	0.5	0	-18%	+5%	-2%
SNU-216	4.5	0.49	19	-10%	-5%	+19%
YCC-2	3.18	0.63	17	-18%	-12%	+1%
SNU-1	2.56	0.46	1	-1%	-8%	+11%
YCC-19	1.43	0.76	20	-5%	-2%	+5%
YCC-30	1.15	0.24	22	-8%	0%	+2%
YCC-38	1.15	0.4	17	-25%	-25%	+37%
YCC-18	0.64	0.29	21	-9%	-17%	+10%

Profiling of the combination group for CK2 expression and synergistic effects. Light green: synergistic effect < 10%, green: synergistic effect ≥ 10%, yellow:

intermediate cisplatin resistance, orange: extreme cisplatin resistance, light purple: CK2α&α' low expression, purple: CK2α&α' high expression. IR: Inhibition of cisplatin at 5μM.

Twenty-one cell lines received combination treatment using the concurrent and sequential schedules. These cell lines were divided into two groups. One is the CK2 mRNA high expression group, and the other is the cisplatin intermediate resistance group. The CK2 high expression group was sensitive to CX-4945, which suggests a synergistic effect with cisplatin. The cisplatin intermediate group may have a synergistic effect with CX-4945, because the CK2-related DNA repair process may be inhibited by CX-4945. The CK2 mRNA high expression group was extremely resistant to cisplatin, which means that CK2-related DNA repair is upregulated in response to cisplatin-induced DNA damage. This group showed a synergistic effect when treated on the concurrent and pre-addition schedules. The cisplatin intermediate group showed low expression of CK2. However, CK2 expression may increase after cisplatin-induced DNA damage is initiated. Therefore, the post-addition schedule produced a synergistic effect in the cisplatin intermediate resistance group.

IV. DISCUSSION

Cisplatin is one of the most effective anticancer agents that is widely used in the treatment of gastric cancer. It is generally considered a cytotoxic drug which kills cancer cells by damaging DNA and inhibiting DNA synthesis. In cancer cells, cisplatin-induced DNA damage activates various pathways to avoid cell death. Clinically acquired resistance can be caused by decreased drug accumulation, which includes reduced uptake or increased efflux of cisplatin, increased drug detoxification by cellular thiols, and increased DNA repair or tolerance to cisplatin-damaged DNA. DNA repair is one of the major concerns in cisplatin resistance mechanisms, and is a very important area of study. Targeting these resistance mechanisms would result in an amelioration of cisplatin resistance in gastric cancer.

CK2 appears to play a central role in the regulation of cell progression and is widely overexpressed in human cancers. CK2 is also known as a key regulator of the repair of both single and double stranded breaks. DNA damage can be regulated by CK2, which involves various repair proteins. Inhibition of CK2 indicated that CK2 is a useful target for reversing cisplatin resistance in gastric cancer. A previous study demonstrated upregulated levels of the CK2 catalytic subunit CK2α in solid tumors; significantly less is known about CK2α'. In CK2 profiling, gastric cancer cells displayed a wide range of mRNA and protein expression and enzymatic activity. CK2α' mRNA and protein expression correlated with sensitivity to CX-4945. These data may suggest a rationale for using CK2α' as a predictive biomarker of sensitivity to CX-4945. When comparing CX-4945 sensitivity between the four groups, Group 1 (CK2α & CK2α' high expression) and Group 4 (CK2α & CK2α' low expression) showed a significant correlation with CX-4945 sensitivity. As a result, Group 1 was chosen to receive combination treatment.

The cisplatin intermediate resistant group displays formation of cisplatin-DNA adducts. This alteration in the structure is recognized by the cellular proteins that repair cisplatin-induced DNA damage. Such damage is primarily repaired via the nucleotide excision repair (NER) system. CK2 is implicated in the phosphorylation of proteins, which are central in mismatch repair, nucleotide excision repair, homologous recombination, and non-homologous end joining. Also, XRCC1 is a key mediator of single-strand break DNA repair, and is involved in the process of cisplatin-induced DNA damage repair in various tumors. XRCC1 was found to identify and bind to DNA inter-strand crosslinks induced by cisplatin. Moreover, CK2 phosphorylates XRCC1 and is required for its stability and efficient DNA repair. These data imply that cells in the cisplatin intermediate resistant group also recruit CK2 and other DNA repair proteins regardless of basal CK2 expression level.

In vitro studies employing CX-4945 in combination with gemcitabine or cisplatin revealed enhanced anti-proliferative effects in A2780 and SKOV-3 ovarian cancer cells. These treatments proceeded on two schedules, the pre-addition and the post-addition schedules. In gastric cancer, concurrent, pre-addition and post-addition were performed to reveal a synergistic effect. There are two patterns of synergistic effects in gastric cancer. The CK2 high expression group displayed a synergistic effect on the concurrent and pre-addition schedules, whereas the cisplatin intermediate resistance group displayed a synergistic effect on the post-addition schedule. The CK2 high expression group may upregulate the DNA repair process, thereby acquiring extreme resistance to cisplatin. Therefore, concurrent or pre-treatment with CX-4945 is important for the synergistic effect. Quick inhibition of CK2 produced a better outcome for CK2 overexpression. After cisplatin treatment, cisplatin-induced DNA damage stimulates the formation of DNA adducts in the cisplatin intermediate resistance group. Then, CK2 may upregulate and phosphorylate other repair proteins for the removal of DNA adducts. Regardless of

CK2 basal level, CK2 may activate DNA repair after cells recognize DNA damage. In this case, treatment with CX-4945 at a later time produced a synergistic effect in combination. Before CX-4945 treatment, CK2 is activated for the DNA repair process. Later CX-4945 treatment produced a better outcome, because cells waited for CK2 activation to repair cisplatin-induced DNA damage.

V. CONCLUSION

The induction of DNA damage by cisplatin is a strategy for the treatment of gastric cancer. Increased DNA repair of cancer cells is an important mechanism of cisplatin resistance. In this study, CK2 was shown to play an important role in cisplatin resistance. Moreover, the synergistic effect will be determined by combination schedule. Treatment schedule is also important to enhancing the synergistic effect. Only a few studies have been done that investigate combinations of cisplatin and CK2 inhibitors. Our results suggest a broad field for future studies on restoring cisplatin resistance in gastric cancer. It also suggests that interactions between treatment schedule and DNA repair by CK2 is a promising treatment strategy.

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ABSTRACT (IN KOREAN)

위암에서 cisplatin과 CX-4945의 병행요법을 통한 cisplatin 내성 극복

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김 현 명

한국에서 위암의 발생률(13.4%)이 높게 나타나고 좋지 않은 생존율과 예후들 때문에 광범위한 치료방법들이 개발되고 있으며 현재 위암의 치료방법으로 수술, 항암치료, 방사선치료 등이 주로 이용되고 있다. 그 중 platinum agent의 한 종류인 cisplatin은 항암치료제로 널리 쓰이고 있으며, 위암치료에 효과를 보이고 있다. 하지만 cisplatin 치료요법 시 약제에 대한 내성이 큰 제한 점이며, 특히 cisplatin의 DNA 손상을 떨어뜨리는DNA 복구기전이 내성이 생기는 중요한 특성 중 하나이다.

이러한 DNA 복구기능을 하는 효소 중에 CK2 (Casein kinase 2)라고 하는 serine/threonine kinase가 있으며cellular process를 조절하는 기능을 갖고 있다. 다른 암 종에서 CK2의 과 발현과 높은 활성도가 환자들의 낮은 생존율과 좋지 않은 예후와 연관이 있다고 알려져 있지만, 아직 위암에서는 구체적인 연구가 이뤄지지 않고 있다. 따라서, 위암에서의 CK2의 발현 상태를 알아보고 DNA 복구기전과 상관관계가 있는지 확인해보고자 한다. 또한, cisplatin의 DNA 손상작용을 CK2가 복구함으로써 cisplatin의 기능을 저해하게 되므로 cisplatin과 CK2 억제제인 CX-4945를 병행투여 한다면

cisplatin의 DNA 손상작용을 효과적으로 이끌어 내어 암 억제효과를 증가시킬 수 있을 것으로 예상한다.

따라서 위암에서의 CK2 발현량과 CX-4945의 sensitivity 사이의 상관관계를 확인하고 cisplatin에 내성이 있는 세포 주에서 CX-4945의 병행요법을 통해 DNA 복구와 세포주기를 조절하는 기전 사이의 상관관계를 확인한다.

핵심되는 말: 위암, cisplatin, Casein Kinase 2, CX-4945, DNA 손상작용, DNA 복구기전