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Transglutaminase 2 Inhibitor KCC009 Induces p53-Independent Radiosensitization in Lung Adenocarcinoma Cells

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: The expression of transglutaminase 2 (TG2) is correlated to DNA damage repair and apoptosis through the p53 pathway. The present study aimed to investigate the potential radiosensitization effect and possible mechanisms of the TG2 inhibitor KCC009 in lung cancer *in vitro*.

Material/Methods: A single hit multi-target model was used to plot survival curves and to calculate the sensitizing enhancement ratios in lung cancer wild-type or mutant p53 of H1299 cells. We performed analyses for changes of cell cycling and apoptotic responses of cells; Western blot analysis and real-time SYBR Green PCR assay were used to determine the changes of mRNA/protein expressions; ELISA assay was used for examination of cytochrome c release in cytoplasm.

Results: Our results showed that KCC009 induced radiosensitization in both H1299/WT-p53 and H1299/M175H-p53 cells. KCC009+IR induced G0/G1 arrest in H1299/WT cells and G2/M arrest in H1299/M175H-p53 cells. KCC009+IR also induced apoptosis in both cell lines. In addition, KCC009+IR decreased the TG2 expression, and increased the p53 expression in H1299/WT cells but not in H1299/M175H-p53 cells. KCC009+IR also increased the expression of p21, Bax, p-caspase-3, and decreased Bcl-2 and CyclinD expression in H1299/WT cells. While KCC009+IR induced phosphorylation of caspase-3 and increase Cyt-C level in the cytoplasm of, and decreased CyclinB, Bcl-2 expression in H1299/M175H-p53 cells, we noticed that Cyt-C level in the nucleus decreased in the H1299/WT cells.

Conclusions: KCC009, a TG2 inhibitor, exhibits potent radiosensitization effects in human lung cancer cells expressing wild-type or mutant p53 with different mechanisms.

MeSH Keywords: **Gene Expression • Genes, p53 • Lung Neoplasms**

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Background

Lung cancer is the most common cancer and the leading cause for cancer-related mortality worldwide [1]. The majority of lung cancer is NSCLC (non-small-cell lung cancer), which includes squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. One third of these patients are diagnosed with stage III disease when curative treatment is extremely limited. Despite tremendous progresses in diagnosis and treatment of lung cancer, the overall treatment outcomes remain poor. Tumor aggressiveness in metastatic lesions is the cause of lethality in lung cancer patients, and is responsible for more than 90% of failure for lung cancer treatment [1,2].

Radiotherapy is one of the main treatments for lung cancer patients, and the p53 pathway plays important roles in regulation of radiotherapeutic responses of cancer cells through DNA damage repair, cell cycle regulation, and apoptosis. Cancer cells harboring wild-type (WT) p53 are relatively more susceptible to radiation-induced apoptosis than cells with mutant p53 expression [3–5], and radiation relapse due to p53 dysfunction is a challenge for clinical treatments of lung cancer patients.

Transglutaminase 2 (TG2) is a ubiquitous multifunctional mammalian protein that catalyzes the formation of intermolecular isopeptide bonds between glutamine and lysine residues of selected proteins [6,7]. The enzymatic activity of TG2 is allosterically regulated by several factors, including guanine nucleotides, Ca²⁺, and redox potential [8]. TG2 has been found to be involved in a diverse range of biological processes, including apoptosis, membrane signaling, cell adhesion and extracellular matrix formation, and elevated expression of TG2 was detected in various forms of cancer. In addition, studies also demonstrated that downregulation of TG2 expression or inhibition of TG2 enzymatic activity can convert chemoresistance in cancer cells [9,10]. In this study, we investigated the radiosensitization effects of KCC009, a TG2 inhibitor, in human lung cancer cells, and potential role of p53 in the KCC009-induced enhancement of radiosensitivity in the cancer cells.

Material and Methods

Drugs and reagents

KCC009 (N-((2S)-1-((3-bromo-4,5-dihydroisoxazol-5-yl)methyl)amino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)-3,4-dihydroxybenzamide, C₂₀H₂₀BrN₃O₆, Molecular Weight: 478.299) was synthesized by Shanghai Yi Fang Biotechnology Co., Ltd., and the structure of KCC009 compound is shown in Figure 1. KCC009 was prepared as a 1M stock in dimethyl sulfoxide (DMSO) and stored at –20°C. RPMI-1640 was purchased from Gibco-BRL (Carlsbad, CA, USA), the Cycle Test™

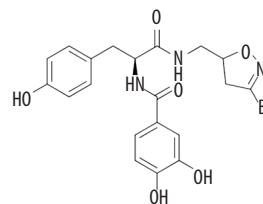


Figure 1. Illustration of the structure of KCC009 compound.

Plus DNA reagent and Annexin V-FITC & Propidium Iodide (PI) Apoptosis Detection kits were obtained from Becton Dickinson and Co., (Franklin Lakes, NJ, USA), AllPrep DNA/RNA/Protein Mini Kit (cat no: 80004) was obtained from QIAGEN (Venlo, Netherlands), Cyt-C-ELISA was obtained from R&D systems Inc., (NJ, USA). Antibodies against caspase-3, Bax, Bcl-2, p21, CyclinB, and CyclinD were obtained from Cell Signaling Technology (Danvers, MA, USA), antiβ-actin antibody was from Santa Cruz Biotechnology, Inc., (Santa Cruz, CA, USA).

Cell culture

Human lung cancer H1299/WT-p53 and H1299/M175H-p53 cells were kindly provided by Dr. Xufeng Chen from University of California Los Angeles [11]. Cells were grown in RPMI-1640 supplemented with 10% fetal bovine serum, 100 µg/mL penicillin and 100 µg/mL streptomycin at 37°C in a 5% CO₂ humidified atmosphere.

Cell proliferation assay

The cell proliferation assays were performed using the MTT method per manufacturer's instructions. Briefly, the cells were seeded in 96-well plates (Costar; Corning Life Sciences, Cambridge, MA, USA) with 5,000 cells/well. Subsequent to an overnight incubation, triplicate wells were treated with various concentrations of KCC009 for 48 hours, and 20 µL MTT solutions (5 mg/mL in phosphate-buffered saline; PBS) were then added to each well and the plate was incubated for 4 hours at 37°C. The MTT formazan was dissolved in 150 µL DMSO and the absorbance was measured with a microplate reader (Multiskan MK3; Thermo Labsystem, Waltham, MA, USA) at a wavelength of 570 nm. Cell proliferation curves were plotted with using the drug concentration as the abscissa axis and the inhibition ratio of the drug as the vertical axis. The 50% growth inhibition (IC₅₀) was calculated according to the curve.

Determination of radiosensitivity

The growing cells were exposed to KCC009 at a concentration of 3.91 µM for 24 hours, and cells were then irradiated with 0, 1, 2, 4, 6, 8, or 10 Gy with using a 6MV X-ray (SIEMENS), and

collected and plated in 60-mm dish with various cell numbers according to the irradiation dose. The cells were maintained in complete medium for 10–14 days and stained with crystal violet. Colonies consisting >50 cells were considered as survival colonies and directly scored using an inverted microscope. The cell survival curve was plotted using the irradiation dosage as the abscissa axis and the survival fraction (SF) as the vertical axis. The average lethal dosage of cells (D0) and the quasi-field dosage (Dq), which indicates the repair ability of cells to sublethal injury, and extrapolation number (N) values were calculated according to the curve. The sensitization enhancement ratio (SER) was calculated according to the following equations: $SF=1-(1-\exp[-D/D0])^N$; $Dq=\ln N/(1/D0)$; and $SER=\text{control group D0 value}/\text{treatment group D0 value}$.

Cell cycle distribution

Cells were treated with radiation, KCC009, or the combination for 48 hours; and DMSO was used as control. The cells were harvested and fixed with 70% ethanol and stored overnight at -20°C . The cells were centrifuged using a Heraeus Labofuge 400 centrifuge (Thermo Fisher Scientific, Waltham, MA, USA) at $300\times g$ and washed twice with PBS, and were then labeled with 50 mg/mL PI with protection from light for 30 minutes prior to analyses by flow cytometry (CellQuest version 3.1; Beckman Coulter, Inc., Brea, CA, USA). Experiments were performed in triplicate.

Apoptosis measurement

Apoptosis was measured by PI/Annexin V double staining. The cells were harvested after treatment for 48 hours, and stained with PI and Annexin V. The apoptotic fraction was detected by flow cytometry (BD FACSCalibur; BD Biosciences, Franklin Lakes, NJ, USA).

Real-time SYBR Green PCR assay for TG2 and p53 gene expression

The cells were harvested after treatment for 48 hours, and RNA was prepared with TRIzol reagent (Invitrogen, USA). The Q-PCR reaction was performed in a 20 μL system containing 10 μL 2 \times iQ SYBR Green Supermix, 0.5 μL of 20 μM forward primer, 0.5 μL of 20 μM reverse primer, 0.8 μL of cDNA, and 8.2 μL double-distilled water (ddH₂O). The real-time PCR was carried out as follows: one cycle of 95°C for 5 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 35 seconds. Fold change = $2^{-\Delta(\Delta\text{Ct})}$, of this equation, $\Delta\text{Ct}=\text{Ct}(\text{target})-\text{Ct}(\text{gapdh})$, $\Delta(\Delta\text{Ct})=\Delta\text{Ct}(\text{treated})-\Delta\text{Ct}$.

Western blot analysis

After 48 hours of the indicated treatments, total cell lysates were prepared and were subjected to SDS-PAGE gel electrophoresis and transferred to a polyvinylidene fluoride membrane

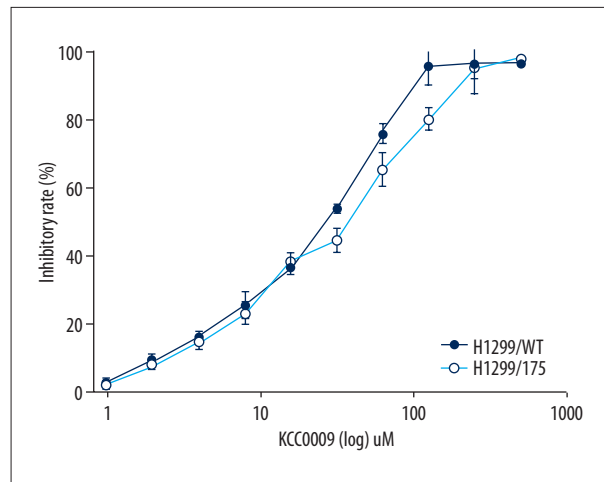


Figure 2. Effects of KCC009 on cell proliferations of H1299/WT-p53 and H1299-M175H-p53 cells. Cell viability was determined with MTT assay. The data represent the average of at least three independent experiments. Error bars indicate standard deviation.

(Seebio Biotech, Inc., Shanghai, China). The membranes were incubated overnight with each primary antibody [1: 1,000] at 4°C with gentle agitation (Wave-SI slim shaker; TAITEC Corporation, Koshigaya, Japan). The membranes were incubated for two hours with a horseradish peroxidase-labeled secondary antibodies (1: 2,000; affinity purified goat anti-mouse IgG and goat anti-rabbit IgG, Santa Cruz Biotech) at room temperature. All membranes were detected using the ECL system (Santa Cruz Biotechnology Inc.).

ELISA analysis for Cyt-C in cytoplasm, nucleus, and mitochondrial

The cells were exposed to KCC009 and/or radiation treatment and incubated for 48 hours. The ELISA kits were applied according to the manufacture's instruction. All extracts were analyzed with both the Mustard ELISA Kit-specific and the Mustard ELISA Kit-total in duplicates.

Results

Effects of KCC009 on cell proliferation of H1299/WT and H1299/M175H-p53 cells

We first determined the potential difference of inhibitory effect of KCC009 on lung cancer cells expression using different types of p53 proteins. The MTT assay results (Figure 2), however, no significant difference for the inhibitory effects of KCC009 were observed on cell proliferation between H1299/WT-p53 and H1299/M175H-p53 ($p>0.05$). The inhibition rates were 15.33 ± 1.46 (%) for H1299/WT-p53 cells, and 14.31 ± 1.90 (%) for

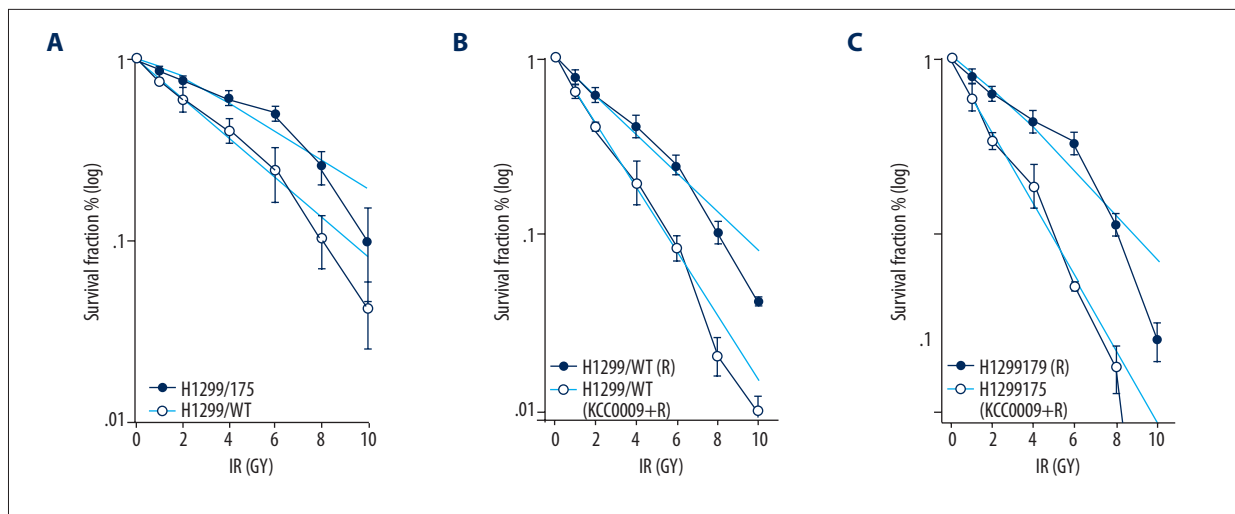


Figure 3. Clonogenic survival assays of indicated cells treated with IR or IR combined with KCC009. Cells were pretreated with 3.91 μ M of KCC009 for 24 hours, and were then irradiated with indicated doses of IR. Colony survival was determined 14–20 days later and survival curves were determined as described in Materials and Methods section.

Table 1. The radiation biology biological parameters calculated by a single hit multi-target model.

Cell	Group	N	Dq	D0	SF2	α	β	SER
h1299/175	IR	1.33	1.94	5.248	0.78	0.10	0.009	1.55
	IR+KCC009	0.958	1.17	3.39	0.54*	0.308	0.002	
h1299/WT	IR	1.00	1.38	3.98	0.6	0.23	0.003	1.65
	IR+KCC009	0.96	0.84	2.41	0.42*	0.44	-0.003	

Table 2. Cell cycle and apoptosis result in different experimental groups.

	H1299/WT (%)				H1299/175 (%)			
	G0+G1	S	G2+M	Apoptosis	G0+G1	S	G2+M	Apoptosis
Control	58.8±2.3	25.1±1.8	16.1±1.8	2.5±1.2	56.1±2.8	26.4±1.5	17.6±1.4	3.5±0.2
KCC009	57.7±1.9	24.1±1.6	18.3±2.3	6.0±0.6	58.5±3.3	23.6±2.4	17.9±1.9	6.3±1.5
IR	64.6±2.5	17.1±1.6	18.3±1.3	17.0±1.1	65.1±3.4	16.3±1.6	18.6±2.1	13.1±2.3
KCC009+IR	77.6*±2.3	11.3±2.2	11.1±2.1	29.1*±2.3	49.7±3.1	19.6±2.4	30.8*±1.7	25.0*±2.4

H1299/M175H-p53 cells when cells were treated with KCC009 at concentration of 3.91 μ M.

KCC009 enhances the radiation sensitivities of H1299/WT-p53 and H1299/M175H-p53 cells. To determine the effect of KCC009 on radiation sensitivity in human lung cancer cells, we performed clonogenic survival analysis. Cells were pretreated with 3.91 μ M KCC009 for 24 hours, and were then irradiated with 0, 2, 4, 6, 8, and 10 Gy of IR. The survived colonies were counted and were plotted for the analysis of the potential radiosensitization effect of KCC009. A multi-target click

mathematical model (Figure 3) simulating the cell SF curve, through which an associated equation and radioactivity parameters, D0 and Dq (Table 1, Figure 3) showed a declined SF2, a decreased Dq and the shoulder of the survival curve is decreased, with an SER value of 1.55 and 1.65, based on D0, indicating a potential radiosensitization effect of KCC009 on lung cancer cells in a p53-independent manner.

KCC009 induces the cell cycle arrest and apoptosis H1299/WT-p53 and H1299/M175H-p53 cells in response to radiation treatment.

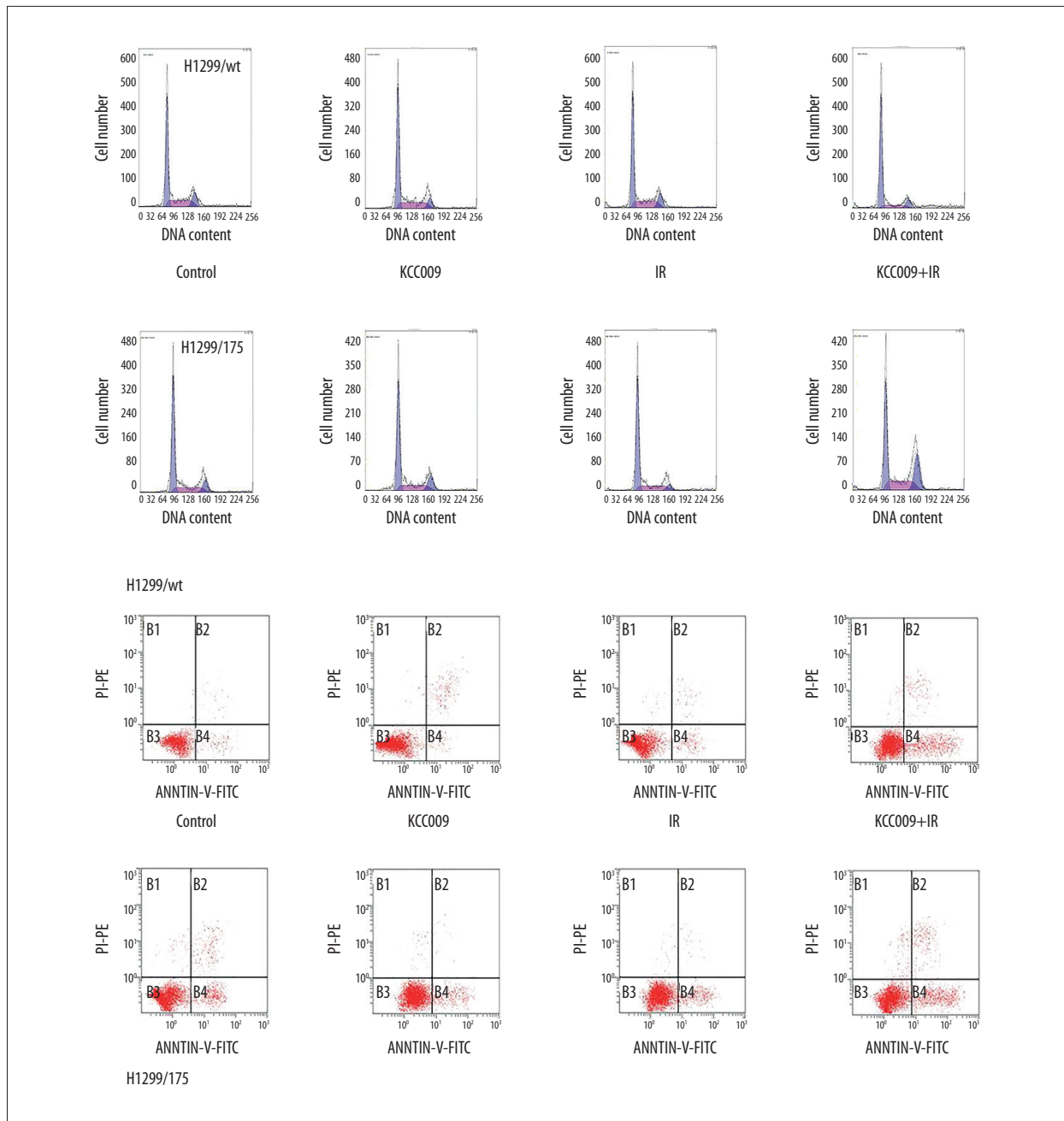


Figure 4. Effects of KCC009 on cell cycling and apoptosis in H1299/WT-p53 and H1299-M175H-p53 cells in response to IR treatment. Cells were treated with 3.91 μ M KCC009 for 24 hours, and irradiated with 6 Gy, or left un-irradiated. Cells were collected 24 hours later after irradiation treatment, and were analyzed by flow cytometry assays. (A) Effect of KCC009 on cell cycling; (B). Effect of KCC009 on apoptosis.

We next determined the effects of KCC009 on cell cycling and apoptosis. The result showed that pretreatment with KCC009 (3.91 μ M for 24 hours) combining IR (6 Gy) induced G0/G1 arrest in H1299/WT-p53 cells, and G2/M arrest in H1299/M175H-p53 cells. Pretreatment with KCC009 combining IR also induced apoptosis in H1299/WT-p53 (17.0 ± 1.1 to $29.1 \pm 2.3\%$) and H1299/M175H-p53 (from 13.1 ± 2.3 to $25.0 \pm 2.4\%$) cells

with statistical significance when compared to the cells receiving IR treatment alone (Table 2, Figure 4).

Effect of KCC009 on TG2 expression

We noticed that IR treatment alone increased the expressions of TG2 and p53 in both cell lines. However, pretreatment with

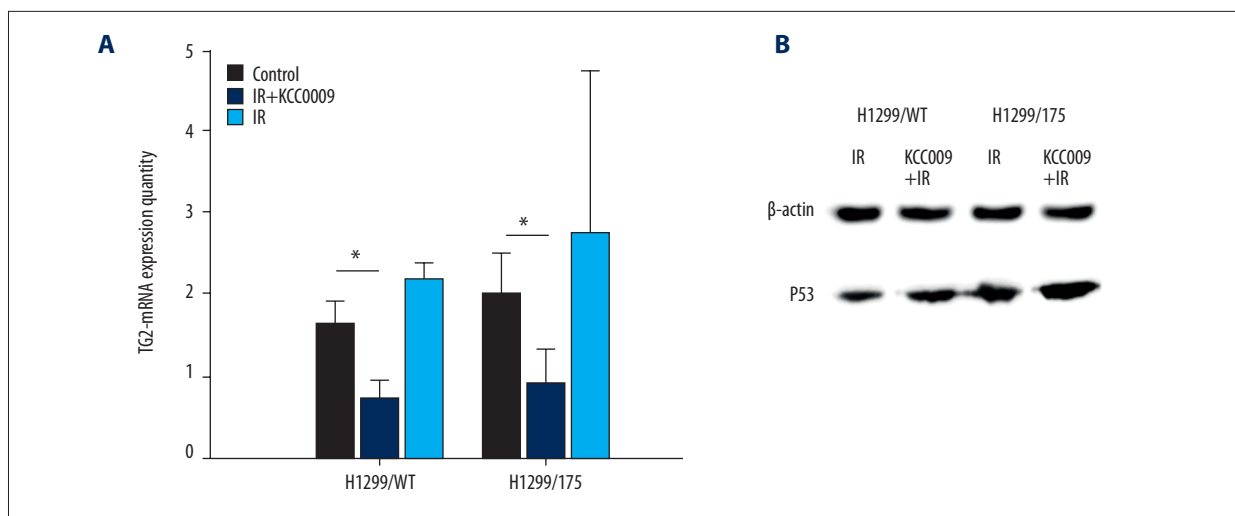


Figure 5. Effects of KCC009 on expressions of TG2 and p53 in irradiated H1299/wt-p53 and H1299-m175H-p53 cells. **(A)** Q-PCR results showing the changes of TG2 mRNA expressions determined in cells treated with IR (6 Gy) or IR (6 Gy) combining with KCC009 (3.91 μ M). Total RNAs were prepared 24 hours after treatments. **(B)** Western blot results showing the effect of KCC009 (3.91 μ M) on p53 expression in irradiated cells receiving 6 Gy of IR. The data represent the average of at least three independent experiments. Error bars indicate standard deviation.

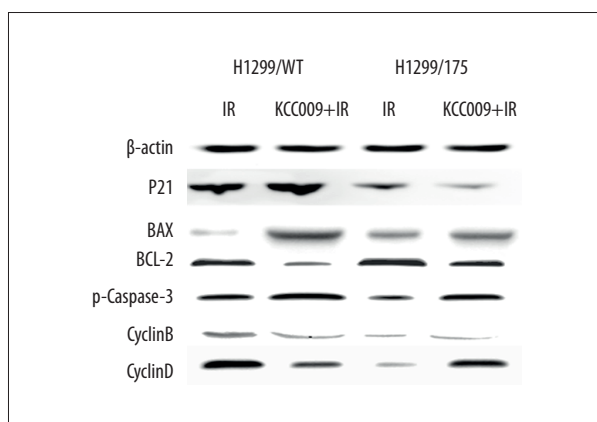


Figure 6. Effects of KCC009 on protein expressions in H1299/WT-p53 and H1299-M175H-p53 cells in response to IR treatment. Representative images of western blot analyses for indicated proteins.

3.91 μ M KCC009 for 24 hours significantly inhibited IR-induced increase of TG2. KCC009 pretreatment also increased the p53 expression slightly in the H1299/WT-p53 cells but not in the H1299/M175H-p53 cells (Figure 5).

Effects of KCC009 on expressions of p21, CyclinB, CyclinD, Bax, p-caspase-3, and Bcl-2, and on localization of Cyt-C in cells in response to IR treatment

We further assessed the molecules that were potentially involved in the KCC009 on cell cycle arrest and apoptosis in irradiated H1299/WT-p53 and H1299/M175H-p53 cells. For this, we determined the expressions of cell cycle checkpoint

regulators p21, CyclinB, CyclinD, and apoptosis regulators Bax, p-caspase-3, and Bcl-2. The result showed that pretreatment of KCC009 (3.91 μ M for 24 hours) increased the expression of p21, Bax, p-caspase-3; and decreased expressions of Bcl-2, and CyclinD in irradiated (6 Gy) H1299/WT-p53 cells, and increased the expression of p-caspase-3; and decreased CyclinB and Bcl-2 expressions in irradiated H1299/M175H-p53 cells (Figure 6).

The ELISA analysis further showed that pretreatment with KCC009 significantly increased the release of Cyt-C into the cytoplasm in cells (from 43.9 ± 3.4 to 78.4 ± 7.3 nM in H1299/WT-p53 cells, and from 38.1 ± 1.9 to 71.8 ± 4.3 nM in H1299/M175H-p53 cells) in response to IR treatment. We also noticed decreased nuclear Cyt-C level in H1299/WT-p53 cells (from 54.4 ± 1.2 to 17.1 ± 1.2) and decreased mitochondrial Cyt-C level (from 63.3 ± 3.3 to 17.4 ± 1.0) in the H1299/M175H-p53 cells when cells were treated with KCC009 combining with IR, with statistical significance (Table 3).

Discussion

Our results presented here revealed that D₀, D_q and N values of irradiated H1299/WT-p53 and H1299-M175H-p53 cells decreased when cells were exposed to KCC009 treatment, and the average lethal dosage of IR decreased when combined with KCC009, indicating that KCC009 could sensitize these cells to IR treatment. The observed decrease of shoulder in cells treated with the combination of KCC009 and radiation suggested a reduced repair ability of cells and sublethal injury in cells with exposure to KCC009.

Table 3. Intracellular distribution of cytochrome C in different experimental groups.

		Cyt-C in Cytoplasm (mean ±SD) nM	Cyt-C in nucleus (mean ±SD) nM	Cyt-C in mitochondrion (mean ±SD) nM
H1299/WT	IR	43.9±3.4	54.4±1.2	61.1±2.8
	KCC009+IR	78.4±7.3	17.1±1.2*	54.9±2.6
H1299/175	IR	38.1±1.9	63.4±4.1	63.3±3.3
	KCC009+IR	71.8±4.3*	58.9±3.8	17.4±1.0*

Radiation induces damage in DNA and causes DNA strand breaks. Single-strand DNA break seems to be a necessary and a sufficient target to activate p53 signaling, resulting in stabilization of p53 protein and its nuclear localization [12,13]. To regulate the expression of cell cycle kinases and the DNA repair gene [14], and cells are thus blocked at the G1/S checkpoint and DNA repair occurs. In cells where DNA damage is limited, the cell can efficiently repairs damaged DNA in a few hours and cells can then reenter the cell cycling; when the damage is too extensive to be repaired, the cell will be driven to apoptosis which may be induced by the induction of the Bax protein, or by the activation of the caspase cascade [15–18]. The p53-dependent G1/S arrest is a transient event, allowing DNA repair before entrance into the S phase. Based on this, it is believed that cells harboring a wild-type p53 have enhanced DNA repair capability, leading to decreased chromosomal aberrations and increased radioresistance, as compared to cells that have lost or inactivated p53 that are more susceptible to radiation induced mitotic [19,20]. We found in this study that exposure to KCC009 significantly increased expressions of p21 (a target of p53 in cells treated with IR [21]) and CyclinD in H1299/WT-p53 cells, and that it decreased expression of CyclinB1, which promotes cells through G2/M into mitosis, in H1299-M175H-p53 cells. Our results further showed that KCC009+IR induced G0/G1 arrest in H1299/WT cells, and G2/M arrest in H1299/M175H-p53 cells, and exposure to KCC009 increased apoptotic responses of these cells to IR treatment. Of interest, cells harboring mutant p53 expressed high levels of CyclinB1, and we noticed that KCC009 decreased the expression of CyclinB1 but significantly restored G2/M arrest in irradiated H1299/M175H-p53 cells, indicating that TG2 inhibitor can cause apoptosis after incomplete DNA repair by radiation [22,23].

Our result also showed that exposure to KCC009 significantly increased the accumulation of Cyt-C in the cytoplasm in irradiated cells. We also found a decreased nuclear level of Cyt-C in H1299/WT-p53 cells, and a decreased mitochondrial level of Cyt-C in H1299/M175H-p53 cells. KCC009+IR also significantly increased the activation of caspase-3 and decreased Bcl-2 expression in H1299/WT-p53 and H1299/M175H-p53 cells when compared to the IR treatment alone. Treatment with KCC009 also increases IR-induced Bax expression in H1299/wt-p53 cells. DNA-damaging agents, such as ionizing radiation, trigger release of cytochrome c from mitochondrial intermembrane spaces [24–27]. Release of cytochrome c is regulated by the balance between antiapoptotic (Bcl-2 and Bcl-xl) and proapoptotic (including Bax and Bak) members of the Bcl-2 family of proteins [28,29], and Bcl-2/Bax ratio is considered to be a crucial factor of cell resistance to apoptosis [30,31]. Following this, caspases act as key components of the apoptotic pathway. Among them, caspase-3 is a frequently activated death protease, catalyzing the specific cleavage of numerous key cellular proteins [32]. These observations thus suggest a role of mitochondrial pathway in KCC009-enhanced apoptosis in irradiated H1299 cells.

Conclusions

Our study showed that KCC009, a TG2 inhibitor, may act as a radiosensitizer in human lung cancer cells in a p53-independent manner, which suggests a potential role of KCC009 as a component of therapeutic regimens benefiting overall lung cancer patients. However, further studies are needed for its clinical application.

References:

- Parkin DM, Bray F, Ferlay J, Pisani P: Global cancer statistics, 2002. *Cancer J Clin*, 2005; 55(2): 74–108
- Jemal A, Siegel R, Xu J, Ward E: Cancer statistics, 2010. *Cancer J Clin*, 2010; 60(5): 277–300
- Lacroix M, Toillon RA, Leclercq G: p53 and breast cancer, an update. *Endocr Related Cancer*, 2006; 13(2): 293–325
- Lowe SW, Schmitt EM, Smith SW et al: p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature*, 1993; 362(6423): 847–49
- Miller LD, Smeds J, George J et al: An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. *Proc Natl Acad Sci USA*, 2005; 102(38): 13550–55
- Griffin M, Casadio R, Bergamini CM: Transglutaminases: Nature's biological glues. *Biochem J*, 2002; 368(Pt 2): 377–96

7. Lorand L, Graham RM: Transglutaminases: Crosslinking enzymes with pleiotropic functions. *Nat Rev Mol cell Biol*, 2003; 4(2): 140–56
8. Stammaes J, Pinkas DM, Fleckenstein B et al: Redox regulation of transglutaminase 2 activity. *J Biol Chem*, 2010; 285(33): 25402–9
9. Verma A, Wang H, Manavathi B et al: Increased expression of tissue transglutaminase in pancreatic ductal adenocarcinoma and its implications in drug resistance and metastasis. *Cancer Res*, 2006; 66(21): 10525–33
10. Yuan L, Siegel M, Choi K et al: Transglutaminase 2 inhibitor, KCC009, disrupts fibronectin assembly in the extracellular matrix and sensitizes orthotopic glioblastomas to chemotherapy. *Oncogene*, 2007; 26(18): 2563–73
11. Chen X, Wong JY, Wong P, Radany EH: Low-dose valproic acid enhances radiosensitivity of prostate cancer through acetylated p53-dependent modulation of mitochondrial membrane potential and apoptosis. *Mol Cancer Res*, 2011; 9(4): 448–61
12. Manna K, Das U, Das D et al: Naringin inhibits gamma radiation-induced oxidative DNA damage and inflammation, by modulating p53 and NF-kappaB signaling pathways in murine splenocytes. *Free Radic Res*, 2015; 49(4): 422–39
13. Tollini LA, Jin A, Park J, Zhang Y: Regulation of p53 by Mdm2 E3 ligase function is dispensable in embryogenesis and development, but essential in response to DNA damage. *Cancer Cell*, 2014; 26(2): 235–47
14. Lindgren T, Stigbrand T, Raberg A et al: Genome wide expression analysis of radiation-induced DNA damage responses in isogenic HCT116 p53+/+ and HCT116 p53-/- colorectal carcinoma cell lines. *Int J Radiat Biol*, 2015; 91(1): 99–111
15. Biddlestone-Thorpe L, Sajjad M, Rosenberg E et al: ATM kinase inhibition preferentially sensitizes p53-mutant glioma to ionizing radiation. *Clin Cancer Res*, 2013; 19(12): 3189–200
16. Hematulin A, Sagan D, Sawanyawisuth K, Seubwai W, Wongkham S: Association between cellular radiosensitivity and G1/G2 checkpoint proficiencies in human cholangiocarcinoma cell lines. *Int J Oncol*, 2014; 45(3): 1159–66
17. Huerta S, Gao X, Dineen S et al: Role of p53, Bax, p21, and DNA-PKcs in radiation sensitivity of HCT-116 cells and xenografts. *Surgery*, 2013; 154(2): 143–51
18. Zolzer F, Jagetia G, Streffer C: G2-block after irradiation of cells with different p53 status. *Strahlenther Onkol*, 2014; 190(11): 1075–79
19. Ballarini F, Bortolussi S, Clerici AM et al: From radiation-induced chromosome damage to cell death: Modelling basic mechanisms and applications to boron neutron capture therapy. *Radiat Prot Dosimetry*, 2011; 143(2–4): 523–27
20. Ianzini F, Bertoldo A, Kosmacek EA et al: Lack of p53 function promotes radiation-induced mitotic catastrophe in mouse embryonic fibroblast cells. *Cancer Cell Int*, 2006; 6: 11
21. Afshar G, Jelluma N, Yang X et al: Radiation-induced caspase-8 mediates p53-independent apoptosis in glioma cells. *Cancer Res*, 2006; 66(8): 4223–32
22. Mishra S, Murphy LJ: The p53 oncoprotein is a substrate for tissue transglutaminase kinase activity. *Biochem Biophys Res Commun*, 2006; 339(2): 726–30
23. Vitale I, Galluzzi L, Castedo M, Kroemer G: Mitotic catastrophe: A mechanism for avoiding genomic instability. *Nat Rev Mol Cell Biol*, 2011; 12(6): 385–92
24. Ambrosi E, Capaldi S, Bovi M et al: Structural changes in the BH3 domain of SOUL protein upon interaction with the anti-apoptotic protein Bcl-xL. *Biochem J*, 2011; 438(2): 291–301
25. He M, Zhao M, Shen B et al: Radiation-induced intercellular signaling mediated by cytochrome-c via a p53-dependent pathway in hepatoma cells. *Oncogene*, 2011; 30(16): 1947–55
26. Kook S, Zhan X, Cleghorn WM et al: Caspase-cleaved arrestin-2 and BID cooperatively facilitate cytochrome C release and cell death. *Cell Death Differ*, 2014; 21(1): 172–84
27. Mondal S, Bhattacharya K, Mallick A et al: Bak compensated for Bax in p53-null cells to release cytochrome c for the initiation of mitochondrial signaling during Withanolide D-induced apoptosis. *PLoS One*, 2012; 7(3): e34277
28. Hengartner MO: The biochemistry of apoptosis. *Nature*, 2000; 407(6805): 770–76
29. Shroff EH, Snyder C, Chandel NS: Bcl-2 family members regulate anoxia-induced cell death. *Antioxid Redox Signal*, 2007; 9(9): 1405–9
30. Reed JC, Miyashita T, Takayama S et al: BCL-2 family proteins: Regulators of cell death involved in the pathogenesis of cancer and resistance to therapy. *J Cell Biochem*, 1996; 60(1): 23–32
31. Sedlak TW, Oltvai ZN, Yang E et al: Multiple Bcl-2 family members demonstrate selective dimerizations with Bax. *Proc Natl Acad Sci USA*, 1995; 92(17): 7834–38
32. Porter AG, Janicke RU: Emerging roles of caspase-3 in apoptosis. *Cell Death Differ*, 1999; 6(2): 99–104