

Association between cellular radiosensitivity and G1/G2 checkpoint proficiencies in human cholangiocarcinoma cell lines

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Abstract. Cholangiocarcinoma is a destructive malignancy with a poor prognosis and lack of effective medical treatment. Radiotherapy is an alternative treatment for patients with unresectable cholangiocarcinoma. However, there are limited data on the radiation responsiveness of individual cholangiocarcinoma cells, which is a key factor that influences radiation treatment outcome. In this study, we found that cholangiocarcinoma cell lines differ remarkably in their radiosensitivity. The variation of radiosensitivity of cholangiocarcinoma cells correlates with their p53 status and existing G1 and/or G2 checkpoint defects. We also demonstrated the potential of checkpoint kinase Chk1/2 inhibition on the enhancement of the radiosensitivity of cholangiocarcinoma cells. Thus, this study provides useful information for predicting radiation response and provides evidence for the enhancement of radiotherapeutic efficiency by targeting checkpoint kinase Chk1/2 in some subpopulations of cholangiocarcinoma patients.

Introduction

Cholangiocarcinoma (CCA) is a lethal malignancy that originates from the biliary epithelium. The worldwide incidence of CCA is relatively rare but the incidence and mortality have both gradually increased over the past decades (1-4). CCA

is an aggressive cancer, with a poor survival rate and lack of effective medical treatment (4,5). Up to date, surgical resection is the only potentially curative treatment for CCA, nevertheless, the 5-year survival rate is dismal (6-8). In addition, the majority of CCA patients presents at an advanced stage when surgical therapy is not feasible (5,9,10).

Chemotherapy is considered as a reasonable treatment option for unresectable and advanced CCA (9). However, the clinical benefit of this treatment regime is very low due to remarkably resistant of CCA to common chemotherapy (6,9). The use of several chemotherapeutic drugs such as gemcitabine, cisplatin, leucovorin-mediated 5-fluorouracil or capecitabine has been considered as the active regimens in CCA (11-13). Nevertheless, neither single drug nor combination treatment has prolonged the median survival time to exceed 12 months (14).

Radiotherapy is regarded as an alternative therapy for patients with unresectable CCA and as an adjuvant or neoadjuvant treatment (15-20). Most studies have demonstrated the benefit of radiotherapy in terms of survival outcomes. However, the efficacy of this modality remains very low due to the resistance of CCA cells to the fatal effects of radiation. The responsiveness of cancer cells to radiotherapy is influenced by many factors. This includes such biologic factors as intrinsic radiosensitivity, proliferation status, and genetic alterations in DNA damage checkpoints, DNA repair and cell death pathways (21-24).

The DNA damage checkpoints are cardinal factors in response to radiation-induced DNA damage or DNA damaging chemotherapy. The function of these checkpoints is to facilitate DNA repair and promote cell death in unrepaired cells (25). Most cancer cells encompass multiple defects in DNA damage checkpoints and cell death pathways leading to resistance to radiation-induced cell death (26). Cancer cells with a defect in G1 checkpoint depend on S and G2 checkpoints to repair radiation-induced DNA damage (27). Since cells with damaged DNA can only be transiently arrested in the S phase, G2 checkpoints are key guardians to prevent these cells from attempting the complex process of mitosis (25). It has been proposed that abrogation of G2 checkpoints in cancer cells

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could prevent completion of DNA repair. Therefore, cancer cells with damaged DNA are forced to enter the M phase and subsequently die while attempting to divide (27,28).

Enhancing radioresponsiveness of tumors by using checkpoint kinase inhibitors is theoretically a promising approach to increase efficacy of radiotherapy (29). Thus, several studies have analyzed the effects of combined applications of checkpoint kinase inhibitors and ionizing radiation (30-33). Nevertheless, the enhancement of radioresponsiveness of CCA cells by checkpoint kinase inhibitors has not been investigated.

In this study, we examined the efficiency of G1 and G2 checkpoints of three human CCA cell lines: KKU-100, KKU-M214, and KKU-M055 and correlated existing G1 and/or G2 checkpoint defects with the radiosensitivity of CCA cell lines. Furthermore, we evaluated the potential of checkpoint kinase Chk1/2 inhibition to enhance the radiosensitivity of CCA cell lines. Thus, this study provides useful information for improving radiotherapeutic outcome of CCA patients.

Materials and methods

Cell culture. The human CCA cell lines established from primary tumor of CCA patients with different histological types: KKU-100 and KKU-M055 (poorly differentiated adenocarcinoma), and KKU-M214 (moderately differentiated adenocarcinoma) were obtained from the Liver Fluke and Cholangiocarcinoma Research Center, Khon Kaen University. MMNK1, an immortalized human cholangiocyte cell line was a gift from Dr N. Kobayashi (34). Cells were cultured at 37°C, 5% CO₂ in DMEM/F12, containing 2.5 mM L-glutamine, 10% fetal bovine serum, 0.25% sodium bicarbonate, 40 U/ml penicillin G and 40 µg/ml streptomycin.

Cell irradiation and treatments. Approximately 5x10⁴ cells/well of exponentially growing cells were seeded into 6-well plates. Cells were irradiated at room temperature with a single dose of 0, 2, 4 or 6 Gy at a dose rate of 2.2 Gy/min (Cobalt-60 source; Theratron Phoenix). The source to sample distance was 80 cm. After irradiation, cells were incubated at 37°C in a 5% CO₂ humidified atmosphere. For G1 checkpoint analyses, the cells were cultivated with growth medium containing 10% fetal bovine serum for 12 h. Then, the cells were washed with PBS, cultivated in serum-free medium for 24 h and irradiated with a single dose of 6 Gy. Subsequently, cells were released from starvation by replacement the medium with fresh growth medium containing 10% fetal bovine serum. The cells were collected at different time points for further analysis. For G2 checkpoint, CCA cells were gamma-irradiated with a single dose of 6 Gy and subsequently, the irradiated cells were collected at different time points for cell cycle and western blot analysis. For checkpoint kinase inhibitor treatment, cells were treated with 50 or 100 nM of 1-(2-((S)-piperidin-3-ylcarbonyl)-5-(3-fluorophenyl)thiophen-3-yl) urea (AZD7762) (Selleck Chemicals, USA) for 2 h. Then, the cells were irradiated with a single dose of 2, 4 or 6 Gy, and were collected at different time points for further analysis.

Clonogenic cell survival assays. Cells were seeded in duplicate into a 6-well plate. The cell number seeded per plate

varied with the radiation dose, so that colonies could be counted conveniently. The cells were irradiated with a single dose of 0, 2, 4 or 6 Gy. The cells were allowed to grow for 10-14 days until the surviving cells produced macroscopically visible colonies. The cells were fixed with 95% ethanol for 10 min and then stained with Giemsa for 10 min. Colonies containing more than 50 cells were counted, and survival fractions were calculated as ratios of the amount of colonies formed from treated cells and untreated cells, corrected for plating efficiency.

Cell cycle analysis. Approximately 8x10⁴ cells/well were seeded into a 6-well plate and cultivated for 12 h. Two hours prior to irradiation, the cells were treated with or without 50 or 100 nM of AZD7762. The treated cells were irradiated with a single dose of 6 Gy and collected 24 h after irradiation. Propidium iodide (PI) staining of isolated nuclei for cell cycle analysis was performed as described previously (35). Briefly, the cell pellet was mildly resuspended in a solution containing 584 µg/ml NaCl, 1,000 µg/ml Na-citrate, 10 µg/ml RNase A, 0.3 µg/ml Nonidet P-40 and 50 µg/ml PI. The cell suspensions were incubated for 30 min in the dark at room temperature, followed by the addition of a solution containing 15 mg/ml citric acid, 0.25 mM sucrose and 50 µg/ml PI. The suspension of PI-stained isolated nuclei was analyzed with a FACScan (Becton-Dickinson) flow cytometer.

Terminal deoxynucleotidyltransferase dUTP nick end labeling (TUNEL) assay. Cells were seeded onto sterile glass cover slips and cultured in 6-well plates overnight. The cells were treated with 100 nM µg/ml of AZD7762 or 6 Gy of gamma-irradiation alone or with a combination of both treatments. Forty-eight hours after irradiation, a TUNEL assay was performed according to the manufacturer's protocol (Click-iT[®] TUNEL Alexa Fluor[®] 647 Imaging Assay, Invitrogen). The stained cells were visualized under fluorescent microscope. For each treatment condition, the number of TUNEL-positive stained cells was counted from 30 randomly selected fields and expressed as percentage of the total number of nucleated cells.

Western blot analysis. Total protein was extracted from cells at indicated time points after each treatment as described (35). A total of 30 µg of protein from each sample were separated by SDS-polyacrylamide gels electrophoresis and electro-blotted onto PVDF membranes. The membranes were blocked in TBS-T containing 5% non-fat skim milk for 1 h at room temperature. Then, the membranes were probed with a primary antibody diluted in 3% BSA in TBS-T overnight at 4°C. After washing thrice with TBS-T, the membranes were incubated with a horseradish peroxidase-labeled secondary antibody diluted in blocking buffer for 1 h. The membranes were washed thrice with TBS-T and the immunoreactivity was detected by chemiluminescence (GE Healthcare, Buckinghamshire, UK) on X-ray film.

Statistical analyses. Data are shown as the mean ± SD or SE of three independent experiments. For survival analysis, survival fractions *SF* were normalized to the survival observed in the treatment groups that received no irradiation. Survival curves

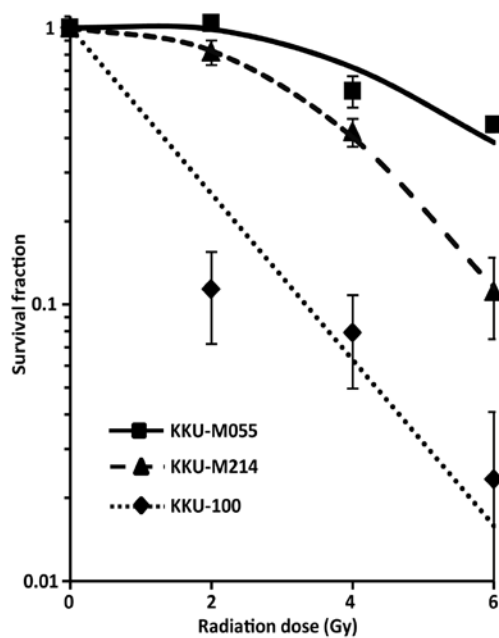


Figure 1. Cellular radiosensitivity of human cholangiocarcinoma cells; KKKU-100 (diamond), KKKU-M055 (rectangle) and KKKU-M214 (triangle). Cells were seeded into 6-well plates and then irradiated with a single dose of 0, 2, 4 or 6 Gy of gamma-radiation at room temperature. Clonogenic survival of gamma-irradiated cells was determined twelve days later. The plot shows the mean surviving fractions \pm standard deviation from three independent experiments.

were fitted to a linear quadratic model of the form $SF = e^{-\alpha d - \beta d^2}$ for the cell lines KKKU-M214 and KKKU-M055, where d is the radiation dose and α and β are constants. Survival of the cell line KKKU-100 was fitted to a linear model of the form $SF = e^{-\alpha d}$. D_{37} values were calculated from three independent experiments and tested for significant differences by ANOVA analysis followed by Tukey's post hoc testing.

Results

Human CCA cells possess different radiosensitivities. We first assessed the radiosensitivity of each CCA cell line. Clonogenic survival assays were performed after gamma irradiated with the dose rate of 2.2 Gy/min. Cell survival curves were plotted and the D_{37} values were calculated. As shown in Fig. 1, the radiosensitivity of CCA cell lines varied from the most radiosensitive to the most radioresistant cell lines KKKU-100 > KKKU-M214 > KKKU-M055 with the D_{37} values of 1.5 ± 0.2 , 4.2 ± 0.2 and 6.1 ± 0.2 Gy, respectively.

P53 status of the human CCA cells in response to radiation damage. As the accumulation of p53 protein following irradiation is an important determinant of cellular radiosensitivity, we further determined p53 levels of each cell line after exposure to radiation. The levels of p53 proteins in CCA cell lines observed as full length in KKKU-100 or truncated as observed in KKKU-M214 and KKKU-M055 and the non-cancerous MMNK1 cell were increased with time of irradiation (Fig. 2). The levels of p53 protein were significantly lower in all CCA cell lines as compared to that of the immortalized cholangiocyte MMNK1 cells.

Radiation-induced apoptosis in human CCA cells. Apoptosis is a common mechanism of cell death in tumors induced by ionizing radiation. Therefore, apoptosis induction in CCA cell lines was determined in this study (Fig. 3). In the most radio-sensitive cell line (KKU-100), apoptosis was strongly induced from 0.37% apoptotic cells in the control cultures to 23.40% in the irradiated cell cultures at 48 h after irradiation. Apoptosis induction in KKKU-M214 cell line was moderate. The amount of apoptotic cells increased from 2.14 to 9.15%. Remarkably, radiation increased the amount of apoptotic cells only slightly from 0.71 to 1.43% in KKKU-M055 cells, which is the most radioresistant cell line.

G1 checkpoint is defective in radioresistant CCA cells. To assess whether CCA cell lines had a defect in G1 checkpoint, the cells were synchronized at early G1 phase by serum deprivation, then were irradiated and released from starvation as described in Materials and methods. Representative results of cell cycle analyses are shown in Fig. 4A. After release from serum starvation, the proportions of the cells in S phase were significantly increased in all CCA cell lines as observed at 24 h then declined to nearly basal level within 48 h. However, in the cells that were irradiated prior to release from serum starvation, only KKKU-100 cells were persisted at the G1 phase while KKKU-M214 and KKKU-M055 cells entered S and G2/M phase dramatically. These results indicate that G1 checkpoint was effective in KKKU-100 cells, but defective in M055 and KKKU-M214 cells. The observations were further underlined by detection of proteins involved in the cell cycle and checkpoint control during G1/S transition. As shown in Fig. 4B, after release from serum starvation, levels of pRb-Ser795, pRb-Ser807/811, cdc25A and cyclin A were clearly induced in all unirradiated CCA cells. Remarkably, these proteins were not induced in KKKU-100 cells that were irradiated prior to release from

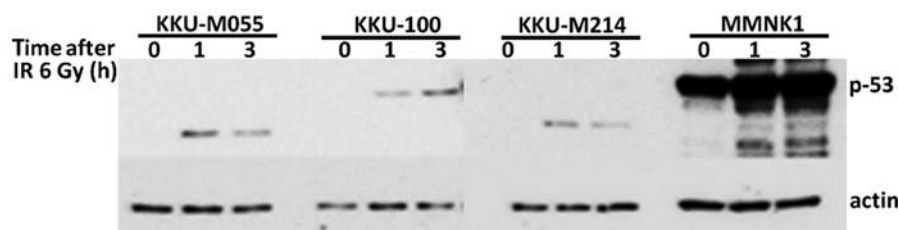


Figure 2. P-53 status of human cholangiocarcinoma cells in response to radiation damage. Gamma-irradiated cells were collected at different time points for total cell lysates extraction. The levels of p-53 protein in human cholangiocarcinoma cell lines (KKU-100, KKKU-M055, KKKU-M214) and an immortalized human cholangiocyte cell line (MMNK1) were determined by western blot analysis. Detection of actin was used as a loading control.

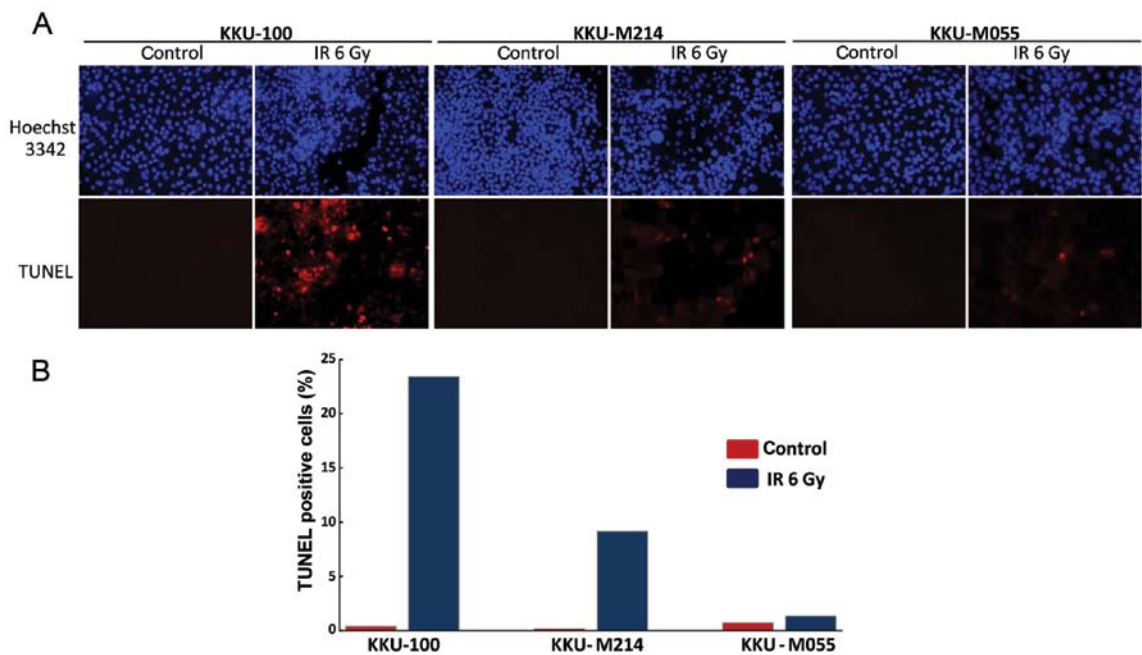


Figure 3. Apoptosis induction in human cholangiocarcinoma cells in response to radiation. The human cholangiocarcinoma cells, KKU-100, KKU-M214 and KKU-M055, were gamma-irradiated with a single dose of 6 Gy. Forty-eight hours after irradiation, TUNEL imaging was performed to detect apoptotic DNA fragments (red). Nuclei were stained by Hoechst 33342 (blue). (A) Representative fluorescence microscopic images of control cells that received no irradiation (control) or 6 Gy gamma-irradiation (IR 6 Gy). (B) Percentages of TUNEL-positive cells counted from 30 fields are shown.

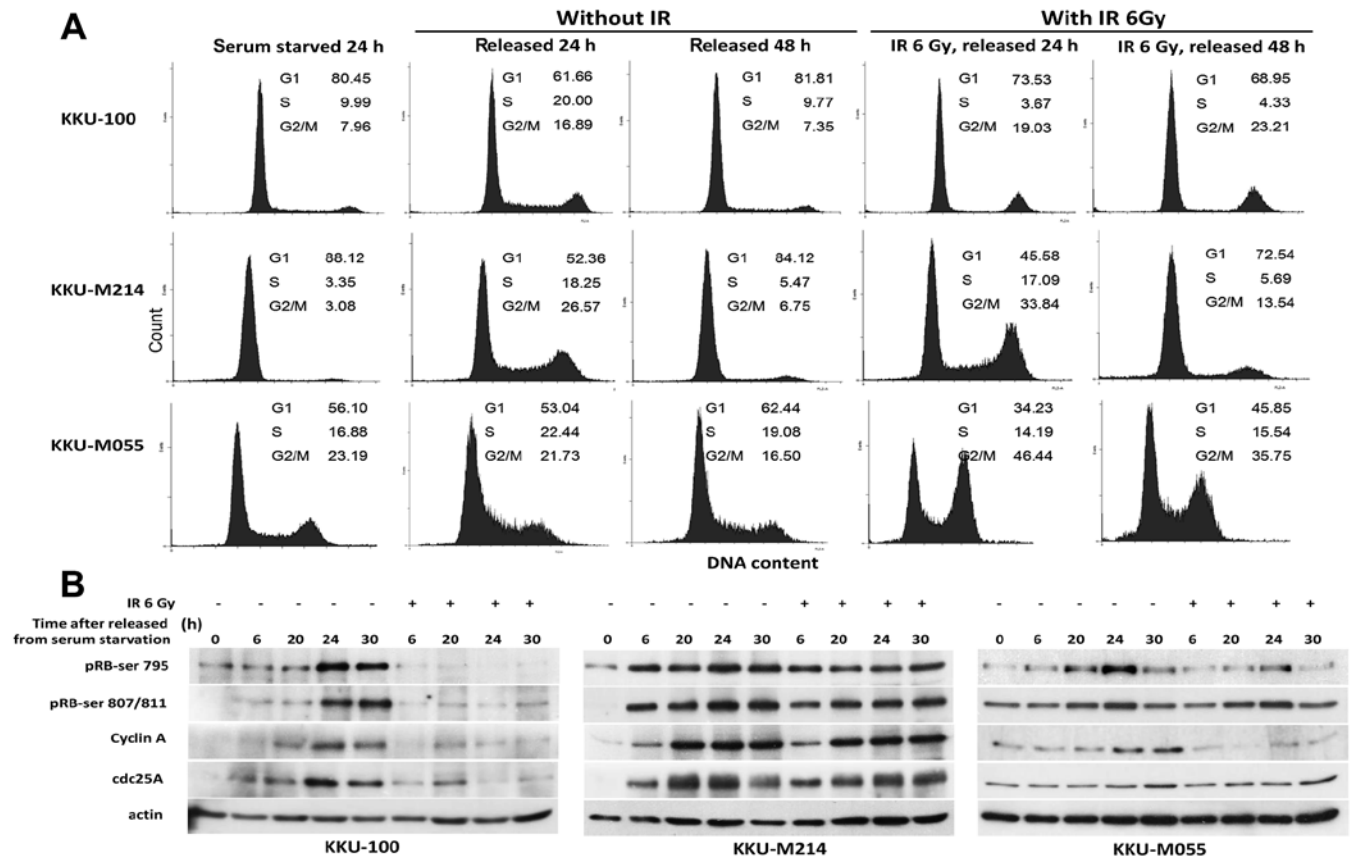


Figure 4. G1 checkpoint status of human cholangiocarcinoma cells in response to radiation damage. The human cholangiocarcinoma cells were cultivated under serum deprivation for 24 h and then gamma-irradiated with a single dose of 6 Gy. Subsequently, the irradiated cells were released from starvation and collected at different time points for cell cycle and western blot analysis. (A) Cell cycle distribution profiles of KKU-100, KKU-M214 and KKU-M055 cells analyzed by flow cytometry. This result is representative of two independent experiments. (B) Levels of p-RB-Ser795, p-RB-Ser807/811, cyclin A and cdc25A in KKU-100, KKU-M214 and KKU-M055 cells that were treated with (+) or without (-) 6 Gy gamma-irradiation. Detection of actin was used as a loading control. Each result is representative of two independent experiments.

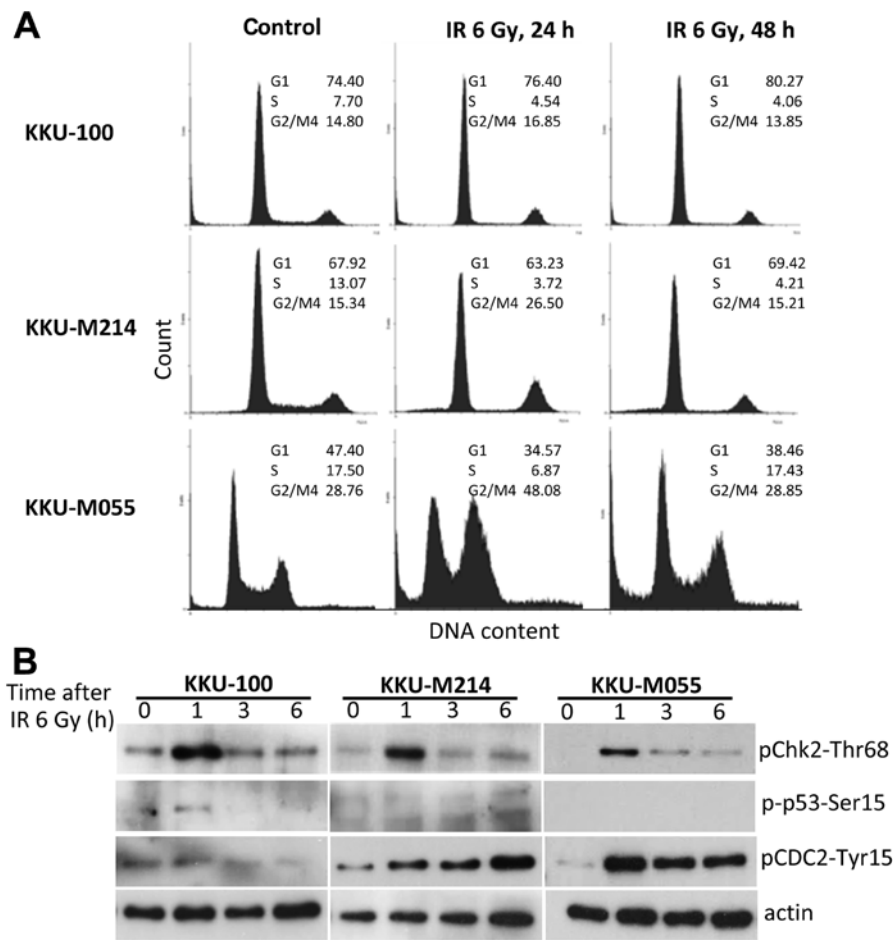


Figure 5. G2 checkpoint status of human cholangiocarcinoma cells in response to radiation damage. The human cholangiocarcinoma cells were gamma-irradiated with a single dose of 6 Gy. Subsequently, the irradiated cells were collected at different time points for cell cycle and western blot analysis. (A) Cell cycle distribution profiles of KKKU-100, KKKU-M214 and KKKU-M055 cells analyzed by flow cytometry. This result is representative of two independent experiments. (B) Levels of p-Chk2-Thr68, p-p53-Ser15 and p-Cdc2-Tyr15 in extracts of KKKU-100, KKKU-M214 and KKKU-M055 cells that were treated with (+) or without (-) 6 Gy gamma-irradiation. Detection of actin was used as a loading control. Each result is representative of two independent experiments.

serum starvation. This finding indicated no progression of KKKU-100 cells from G1 to S phase in response to radiation-induced damage. In contrast, induction of pRb-Ser795 and pRb-Ser807/811 was observed in irradiated KKKU-M214 and KKKU-M055 cells. In addition, cdc25A, and cyclin A were increased with time after irradiation in KKKU-M214 cells. From these results, it can be assumed that KKKU-M214 and KKKU-M055 cells were not halted at G/S phase in response to radiation-induced damage.

Transient activation of the G2 checkpoint in radioresistant CCA cells. To investigate the efficiency of the G2 checkpoint of CCA cells, cell cycle and protein levels of phospho-p53, phospho-Chk2 kinase and phospho-Cdc2 kinase were analyzed. Cell cycle distributions were analyzed 24 and 48 h after irradiation (Fig. 5A). A radiation-induced G2/M block was clearly demonstrated in KKKU-M055 cells by an increase of the G2/M population from 29 to 48% at 24 h after irradiation. However, the G2/M population of KKKU-M055 cells decreased to base line level within 48 h as compared to that of unirradiated control cells. The G2/M population of KKKU-M214 cells was moderately increased from 15 to 27% at 24 h after irradiation and decreased to 15% within 48 h after irradiation. These results indicate that

the G2 checkpoints of KKKU-M055 and KKKU-M214 cells were transiently activated but cells failed to prolong the G2 arrest after gamma-irradiation. Remarkably, no activation of the G2 checkpoint was found in KKKU-100 cells. These findings are consistent with the rapid induction of phospho-Chk2 and phospho-Cdc2 without induction of phospho-p53 in response to radiation damage in KKKU-M055 and KKKU-M214 cells. In KKKU-100 cells, only phospho-Chk2 levels were strongly increased after irradiation, while phospho-Cdc2 and phospho-p53 were slightly elevated (Fig. 5B).

Chk1/2 inhibitor enhances the radiosensitivity of radioresistant CCA cells. The inhibition of Chk1 and Chk2 has been reported to enhance the radiosensitivity of several cancer cell lines. Therefore, the potential of Chk1/2 inhibitor (AZD7762) to enhance radiosensitivity of CCA cells was investigated. In all three cell lines, AZD7762 treatment significantly impacted cellular radiosensitivity (Fig. 6A and Table I). Treatment of the cells with AZD7762 significantly enhanced radiosensitivity of KKKU-M055 and KKKU-M214 cells. The radiation enhancement ratios for KKKU-M055 cells, calculated from D_{37} values are 1.8 and 2.0 for 50 nM and 100 nM AZD7762, respectively. The radiation enhancement

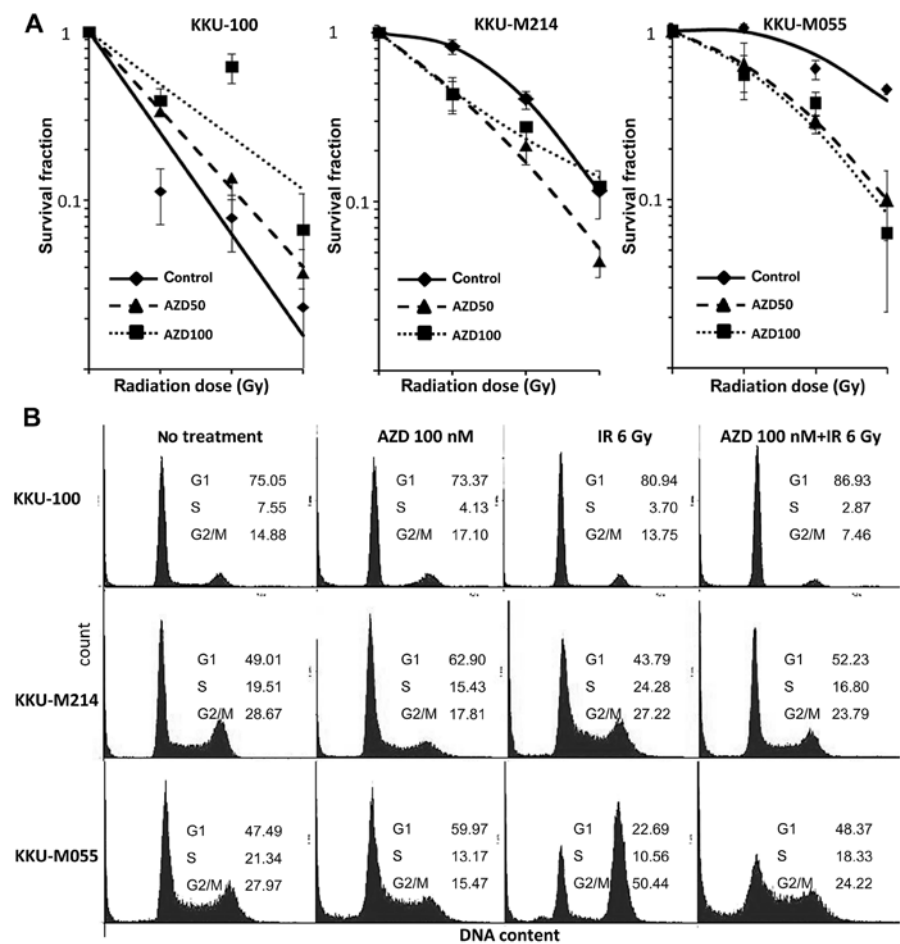


Figure 6. The effect of Chk1/2 inhibitor AZD7762 on the radiosensitivity and the radiation-induced G2 arrest of cholangiocarcinoma cells. (A) Cells were treated with 50 nM of AZD (triangle), 100 nM of AZD (square), or left untreated (control, diamond) for two hours before gamma-irradiation. Clonogenic survival was determined 12 days after gamma-irradiation. The plots show the mean surviving fraction \pm standard deviation of three independent experiments. (B) Cells were seeded into 6-well plates and pre-treated with or without AZD 2 h before gamma-irradiation. Subsequently, the irradiated cells were collected at different time points for cell cycle analysis. Cell cycle distribution profiles of KKKU-100, KKKU-M214 and KKKU-M055 cells analyzed by flow cytometry. Each result is representative for two independent experiments.

Table I. D_{37} values observed in different cell lines after AZD7762 treatment.

Cell line	AZD (nM)	D_{37} (Gy) \pm SD	p-value (vs. no AZD treatment)	p-value (vs. 100 nM AZD treatment)
KKU-100	0	1.5 \pm 0.2		
	50	1.9 \pm 0.1	0.338	
	100	2.7 \pm 0.5	0.006 ^a	0.034 ^b
KKU-M214	0	4.1 \pm 0.2		
	50	2.4 \pm 0.5	0.010 ^a	
	100	2.6 \pm 0.6	0.015 ^a	0.928
KKU-M055	0	6.1 \pm 0.2		
	50	3.4 \pm 0.8	0.004 ^a	
	100	3.1 \pm 0.7	0.002 ^a	0.827

^aStatistically differences where p<0.05 compared with no AZD treatment. ^bStatistically differences where p<0.05 compared 50 nM AZD treatment with 100 nM AZD treatment.

ratios for KKKU-M214 cells are 1.7 and 1.6 for 50 nM and 100 nM AZD7762, respectively. However, a contradictory effect of AZD7762 on the radiosensitivity of KKKU-100 cells was found. AZD7762 treatment significantly reduced radio-

sensitivity of KKU-100 cells, reflected by an increase of the D_{37} value from 1.5 to 1.9 and 2.7 for the concentration of 50, and 100 nM AZD7762, respectively. This result indicates antagonistic effect of AZD7762 on radiation-induced cell killing in KKU-100 cells.

Chk1/2 inhibitor abrogates radiation-induced G2 arrest of CCA cells. Inhibition of Chk1/2 kinases has been shown to abrogate G2 checkpoint in response to radiation-induced DNA damage (36). To investigate the effect of AZD7762 on the radiation-induced G2/M arrest of CCA cells, the cell cycle distribution profiles were analyzed for irradiated cells with or without AZD7762 pretreatment (Fig. 6B).

Radiation-mediated G2 arrest and abrogation of G2 arrest by AZD7762 was merely observed in KKU-100 and KKU-M214 cells. By contrast, G2 arrest was strongly induced by radiation treatment in KKU-M055 cells and treatment of the cells with AZD7762 significantly abrogated G2 arrest.

Discussion

Cellular radiosensitivity is influenced by several factors of DNA damage response and repair processes, cell cycle regulation and cell death pathways (21,23,24). In this study, we demonstrated that three CCA cell lines possessed remarkably different radiosensitivity. The differences were shown partly due to intrinsic factors including the intact p53 and DNA damage checkpoints. Based on this context, KKU-100 cells, shown to be chemotherapeutic resistant (37), were sensitive to radiation. In contrast, chemosensitive cell, KKU-M055, were resistant to irradiation. Moreover, we showed that Chk1/2 inhibitor could abrogate radiation-induced G2 arrest and enhanced radiosensitivity of KKU-M055.

Radiotherapy is regarded as a promising approach to control CCA either as monotherapy or combined with surgical intervention (9,14,19). Although most studies have demonstrated a survival benefit of radiotherapy, the effectiveness of this treatment modality remains very low due to the resistance of CCA cells to the fatal effects of radiation (15,19,20,38). The variation of the radiation responsiveness of individual cancer cells is a key factor that influences radiation treatment outcome (22,39,40). In this study, we demonstrated that the radiosensitivity of CCA cell lines—KKU-100, KKU-M214 and KKU-M055 was considerably different, with D_{37} values of 1.5 Gy of the most radiosensitive cells (KKU-100) versus 6.1 Gy of the most radioresistant cells (KKU-M055). Interestingly, among three CCA cell lines, KKU-100 has been reported to be the most resistant cell line towards chemotherapeutic drugs (37). Therefore, it would be possible that radiotherapy could be an effective treatment in chemotherapy resistant CCA.

The p53 tumor suppressor protein plays an important role in the regulation of the above described processes (41). It has been reported that cells containing wild-type p53 are more sensitive to radiation than mutant p53-expressing cells (42). In this study, an accumulation of p53 protein following radiation-induced DNA damage was found in all three CCA cell lines. Nevertheless, expression of full length p53 protein was found in KKU-100 cells only, whereas KKU-M055 and KKU-M214 cells expressed truncated p53. Thus, it can be speculated that

the different radiosensitivities of the three CCA cell lines may result from alterations of p53 functions in these cells.

Efficiency of DNA damage checkpoints has a major impact on cellular radiosensitivity. However, p53 mediates cellular radiosensitivity only in the G1-phase of the cell cycle (42). We found that KKU-100, the most radiosensitive cell line, expressed full-length p53 and possessed an intact G1 checkpoint. Therefore, radiosensitivity of CCA cells might rely on the function of p53 and the efficiency of the G1 checkpoint. Since various p53 mutations may result in different phenotypes, the impact of p53 and the G1 checkpoint on the radiosensitivity of CCA cells needs to be further investigated.

Evidence of several studies reveals a strong relationship between apoptotic cell death and radiosensitivity of tumors (23). In the present study, radiation is a potent inducer of apoptosis in the most radiosensitive cell line (KKU-100). Conversely, the potential of radiation to induce apoptosis is very weak in the most radioresistant cells (KKU-M055). Therefore, it can be speculated that cell death by apoptosis might determine overall radiosensitivity of CCA cells. Frequently, aberrations in apoptosis are found in cancer cells carrying p53 mutations and result in a radioresistant phenotype (43). In agreement with our findings, KKU-M055 and M214 cells, expressing truncated p53 proteins are more resistant to radiation as compared to KKU-100 cells. However, the role of p53 and apoptosis for determining the radiosensitivity of CCA cells remains to be further clarified.

Several studies revealed that Chk1/2 inhibition offers significant and selective tumor radiosensitization (29). In this study, a radiosensitization effect of the Chk1/2 inhibitor AZD7762 was found in two CCA cell lines (KKU-M055 and M214). These cells express a truncated p53 protein and are defective in the G1 checkpoint. On the other hand, the Chk1/2 inhibitor AZD7762 treatment failed to enhance radiosensitivity of KKU-100 cells. Since AZD7762 abrogates radiation-induced G2 arrest in KKU-M055 cells only, it is unlikely that the abrogation of the G2 checkpoint primarily contributes to AZD7762-mediated radiosensitization of CCA cells. Recently, AZD7762 was reported to radiosensitize p53 mutant breast cancer cells by inhibiting DNA damage repair and promoting radiation-induced apoptosis and mitotic catastrophe (30). Thus, it is possible that the radiosensitization effect of AZD7762 in CCA cells is mediated via DNA damage repair process and/or cell death pathways.

In conclusion, the benefit of radiotherapy in terms of survival outcomes of CCA patients could be improved by technological advancement of radiation therapy delivery and by selecting tumor entities that respond well to this treatment option. Thus, identification of molecular markers for predicting radiation response of this disease is required. In this study, we demonstrated that the variation of radiosensitivity of CCA cells is correlated with their p53 status and existing G1 and/or G2 checkpoint defects. We also demonstrated the potential of checkpoint kinase Chk1/2 inhibition on the enhancement of the radiosensitivity of CCA cells. Thus, this study provides useful information for predicting radiation response and provides evidence for the enhancement of radiotherapeutic efficiency by targeting checkpoint kinase Chk1/2 in some subpopulations of CCA patients.

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