CANCER BIOTHERAPY AND RADIOPHARMACEUTICALS
Volume 24, Number 4, 2009

DOI: 10.1089/cbr.2008.0563

Original Articles

The Direct Biologic Effects of Radioactive ^{125}I Seeds on Pancreatic Cancer Cells PANC-1, at Continuous Low-Dose Rates

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Abstract

The relative biologic effectiveness of model 6711 ^{125}I seeds (Ningbo Junan Pharmaceutical Technology Company, Ningbo, China) and their effects on growth, cell cycle, and apoptosis in human pancreatic cancer cell line PANC-1 were examined in the present study. PANC-1 cells were exposed to the absorbed doses of 1, 2, 4, 6, 8, and 10 Gy either with ^{125}I seeds (initial dose rate, 2.59 cGy/h) or with ^{60}Co γ-ray irradiation (dose rate, 221 cGy/min), respectively. Significantly greater numbers of apoptotic PANC-1 cells were detected following the continuous low-dose-rate (CLDR) irradiation of ^{125}I seeds, compared with cells irradiated with identical doses of ^{60}Co γ-ray. The D_0 for ^{60}Co γ-ray and ^{125}I seed irradiation were 2.30 and 1.66, respectively. The survival fraction after ^{125}I seed irradiation was significantly lower than that of ^{60}Co γ-ray, with a relative biologic effectiveness of 1.39. PANC-1 cells were dose dependently arrested in the S-phase by ^{60}Co γ-rays and in the G2/M phase by ^{125}I seeds, 24 hour after irradiation. CLDR irradiation by ^{125}I seeds was more effective in inducing cell apoptosis in PANC-1 cells than acute high-dose-rate ^{60}Co γ irradiation. Interestingly, CLDR irradiation by ^{125}I seeds can cause PANC-1 cell-cycle arrest at the G2/M phase and induce apoptosis, which may be an important mechanism underlying ^{125}I seed–induced PANC-1 cell inhibition.

Key words: ^{125}I seed, interstitial brachytherapy, relative biologic effectiveness, apoptosis, cell cycle

Introduction

Interstitial brachytherapy is currently one of the most important forms of radiotherapy, of which permanent implantation of radioactive seeds has become one of the most popular approaches.[1,2] Interstitial brachytherapy has been widely accepted and is commonly applied in the early stages of prostate cancer treatment; it presents a low complication rate and efficacy comparable to radical surgery and external beam radiotherapy (EBRT). Basic research has demonstrated that several cell lines, including the PC-3 cell line, display hyper-radiosensitivity to continuous low-dose-rate (CLDR) irradiation. Both dose rate and dose-dependent radiation damage repair may influence the cell-killing effects of radioactive seeds' relative biologic effectiveness (RBE).[3–6] Though controversial, it is generally accepted that permanent implantation of radioactive seed-mediated interstitial brachytherapy is a promising strategy in pancreatic cancer treatment—with microinvasion, ease of performance, and confirmative efficacy. For pancreatic cancer, particularly, the radioactive seed-implanting technique is predicted to achieve good palliative analgesia and localized control.[7–9] However, there are no reports on the effects of radioactive seeds on pancreatic cancer cells. In this study, we investigated the *in vitro* apoptotic potential and RBE of ^{125}I seeds on human pancreatic cancer cells of the PANC-1 cell line. This study provides basic data on the effects of radioactive ^{125}I seeds on pancreatic cancer cells and the technique's potential impact on the treatment of pancreatic cancer in clinical practice.

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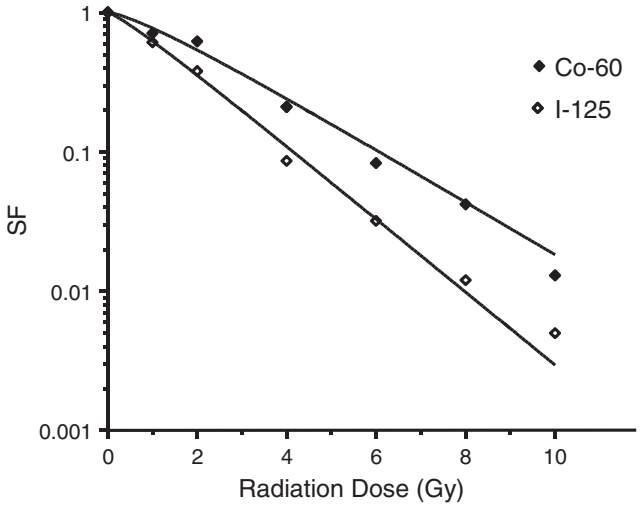

FIG. 1. The dose-survival curve of PANC-1 cells irradiated with ^{125}I seeds or ^{60}Co γ-rays. The dose-survival curve was produced based on the one-hit multitarget model. The two curves show significant differences ($F = 6.12$; $p < 0.05$).

Materials and Methods

Cell Lines and Culture

The human pancreatic cancer cell line, PANC-1, was kindly provided by Xiaohang Zhao, M.D., Ph.D., in the Cancer Hospital/Institute, Chinese Academy of Medical Sciences (Beijing, China). PANC-1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) medium, which was composed of 10% calf serum, 1% penicillin and streptomycin (penicillin, 100 U/mL; streptomycin, 100 mg/mL), and 1% glutamine. Cells were cultured in a 37°C incubator with 5% CO2. Under these conditions, the doubling time of cancer cells was about 52 hour. The medium was replaced two to three times per week.

^{125}I seed and ^{60}Co γ-ray irradiation

An in-house model for *in vitro* iodine-125 seed irradiation (shown in Fig. 1) was developed for this study. The model consisted of a 3-mm-thick polystyrene panel, with a lower-seed plaque layer and an upper-cell culture plaque layer. In the seed plaque, 14 seeds with the same activity were equally spaced within recesses (4.5×0.8 mm) around a 35-mm diameter (D) circumference. For the cell-culture plaque, similar recesses were made around a 35-mm D circumference. The center of the cell-culture plaque was vertically aligned above the seed plaque, so that a 35-mm Petri dish could be placed on it during the experiment. The height (H) between the seed plaque and the bottom of the Petri dish was 12 mm, with a D/H ratio of 2.9. The purpose of this design was to obtain a relatively homogeneous dose distribution at the bottom of the Petri dish. The polystyrene assembly was encased in a 3-mm-thick lead chamber with a vent hole, so that the whole model could be kept in the incubator during the study. The incubator played a protective role by maintaining constant cell-culture conditions.

Model 6711 ^{125}I seeds were provided by the Ningbo Junan Pharmaceutical Technology Company (Ningbo, Zhe Jiang province, China). The activity of the single seed used in this study was 92.5 MBq (2.5 mCi), which translates to an initial dose rate of 2.77 cGy/h to model cells. The dose uniformity of the irradiation model in the cell plane was 1.34—the actual maximum/minimum dose—which was in agreement with other investigators' results.[10,11] The model was validated by using thermoluminescent dosimetry (TLD) measurement. The absorbed doses for various exposure times, in various culture planes, were also measured and verified. The exposure times for delivering doses of 1, 2, 4, 6, 8, and 10 Gy were 36, 73.7, 154.6, 245.8, 345.1, and 460.1 hours (i.e., 1.50, 3.07, 6.44, 10.24, 14.38, and 19.17 days), respectively.

^{60}Co γ-ray irradiation was performed at the Department of Radiation Medicine, Peking University (Beijing, China). The distance between the radiation source and cell plane was 1.5 m, with the dose rate of 221 cGy/min. The absorbed dose was equivalent to CLDR treatment. PANC-1 cells in exponential growth were exposed to a range of 1, 2, 4, 6, 8, and 10 Gy of irradiation. During CLDR treatment, the irradiation model was always placed in the incubator. A blank control was set up under the same conditions as the irradiation group, with the exception of "no-irradiation" conditions.

Colony formation

Cells in exponential growth were digested with trypsin into a single-cell suspension and seeded into 100-mm culture plates at various dilutions. Cells were distributed evenly in 10-mL medium and maintained in an incubator at 37°C for 14 days. Then, the cells were fixed with methanol and stained with Giemsa. Colonies with more than 50 cells were counted and the plating efficiency (PE) was calculated as per the following formula: PE = number of colonies/number of seeded cells×100%.

Dose-survival curve and RBE

The PANC-1 cells were exposed to ^{125}I seed CLDR and ^{60}Co γ-ray irradiation. There were three samples in each dose group; the total dose received per group ranged from 1, 2, 4, 6, 8, to 10 Gy, respectively. After irradiation, cells were digested and seeded in 100-mm plates at various cell densities. Then, the cells were cultured in 35-mm plates for 14 days, and the formed colonies were counted. The survival fraction (SF) was calculated as per the following formula: SF = number of colonies/number of seeded cells×PE (plating efficiency). The dose-survival curve was fitted based on the single-hit multitarget theory formula, $SF = 1 - (1 - e^{-D/D_0})^N$, as reported in an earlier study,[12] with cell-survival percentage plotted on the y-axis and absorbed dose on the x-axis. In addition, the N, D_0, D_{37}, and D_q were calculated based on the dose-survival curve of PANC-1 cells for ^{125}I seeds and ^{60}Co γ-rays. The RBE for ^{125}I was calculated as: RBE = D_0 of ^{60}Co γ ray/D_0 of ^{125}I seed.

Cell death induced by irradiation at various absorbed doses and different time points

PANC-1 cells in exponential growth were exposed to ^{125}I seeds and ^{60}Co γ-rays at the absorbed doses of 1, 2, 4, 6, 8, and 10 Gy, respectively. After irradiation, cells were cultured for 12 hour, following which the cells were collected and stained with trypan blue. The percentage of dead cells was counted

Table 1. Survival Fraction of PANC-1 After Various Doses of ^{60}Co γ-Ray and ^{125}I Seed Irradiation

Radiation dose (Gy)	Cell number/flask	Mean colony counts/flask		Mean survival fraction (SF)	
		^{60}Co	^{125}I	^{60}Co	^{125}I
1.0	200	102	88	0.710	0.610
2.0	400	179	109	0.620	0.380
4.0	1000	151	62	0.210	0.086
6.0	2000	120	46	0.083	0.032
8.0	5000	151	43	0.042	0.012
10.0	10,000	94	36	0.013	0.005

Data are presented as the mean ($n = 3$).

for each delivered dose as: number of dead cells/total number of cells collected $\times$100%. The mean was calculated from three parallel samples for each dose point. In addition, for samples irradiated with the absorbed dose of 4 Gy, the cells were continuously cultured for 12, 24, 48, and 72 hour. The dead cells at various time points were counted and compared by trypan blue staining; there were three parallel samples at each time point.

Measurement the cell apoptosis by flow cytometry

PANC-1 cells in exponential growth were exposed to ^{125}I seeds and ^{60}Co γ-ray at the absorbed doses of 2, 5, and 8 Gy, respectively. After irradiation, cells were continuously cultured for 24 hour, following which, the cells were digested with trypsin and centrifuged. The supernatant was discarded, and the cells were resuspended for counting. The cell concentration was adjusted to 2–5$\times 10^5$ cells/mL for flow-cytometry analysis. Briefly, a 1-mL cell suspension was centrifuged at 1000 rpm and the supernatant was discarded. The step was repeated two to three times. Cells were resuspended in 200-μL binding buffer, followed by the addition of 10 μL of Annexin V-FITC (Fluorescein isothiocyanate). Samples were incubated in dark at room temperature or at 4°C for 30 minutes, followed by the addition of 300-μL binding buffer. Samples were then stored at 4°C for detection. Five (5) minutes before analysis, 5 μL propidium iodide (PI) was added for chromatin staining. Cells were double stained with Annexin V and PI for apoptosis analysis. The blank control cells, Annexin V-FITC single-stained and PI single-stained samples, were also prepared.

Measurement of the cell cycle by flow cytometry

PANC-1 cells in exponential growth were exposed to ^{125}I seeds and ^{60}Co γ radiation at the absorbed doses of 2, 5, and 8 Gy. After irradiation, cells were continuously cultured for 24 hour. Then, the cells were digested with trypsin and centrifuged. The supernatant was discarded, and the cells were resuspended for counting. The cell concentration was adjusted to 2×10^5–1×10^6 cells/mL. Cells were washed with cold phosphate buffered saline (PBS) one to two times and fixed with 3 mL of 70% alcohol, at 4°C overnight. The cells were then centrifuged at 1000 rpm for 5 minutes. The fixation solution was discarded, and cells were washed with cold PBS one to two times. Cells were resuspended in 400 μL of PBS, followed by the addition of 50 μL of 500 μg/mL PI (final concentration, 50 μg/mL). Cells were incubated in the dark at 37°C for 30 minutes and stored at 4°C until cell-cycle analysis by flow-cytometry assay.

Statistical analysis

GraphPad Prism software (version 4.0; GraphPad Software, Inc., La Jolla, CA) was adopted to produce the dose-survival curve. The statistical analysis was performed with SPSS (version 11.5; SPSS Inc., Chicago, IL). Measurement data are presented as the mean $\pm$ standard deviation ($\bar{x} \pm$ SD). The difference between the means of different groups was analyzed with a pair-designed t-test; a value of $p < 0.05$ was considered significant.

Results

Dose-survival curve and RBE

The mean colony numbers and survival fractions of PANC-1 cells at various doses of ^{60}CO γ and ^{125}I seed irradiation are given in Table 1. The fitted dose-survival curve is given in Figure 1. Analysis of Variance analysis revealed a significant difference between the two curves ($F = 6.12$; $p < 0.05$). Based on the dose-survival curve, the N, D_0, D_{37}, and D_q of ^{125}I seeds and ^{60}Co γ-ray were 1.23 versus 1.42, 166 versus 230, 195 versus 295, and 29 versus 65 cGy, respectively. The RBE of ^{125}I seeds was 2.30/1.66 = 1.39.

Cell death induced by irradiation at various absorbed doses and different time points

When the two sources of irradiation were compared, the death rates were significantly different when the absorbed dose was above 4 Gy, while the difference was not significant at absorbed doses below 4 Gy. Results indicated that when the absorbed dose was above 4 Gy, ^{125}I seeds were more potent at inducing apoptosis than ^{60}Co γ-ray irradiation in PANC-1 cells. There was no significant difference in the apoptosis of PANC-1 cells treated by these two types of irradiation when the absorbed dose was below 4 Gy (Fig. 2).

Cell death at various time points following ^{125}I seeds or ^{60}Co γ-ray irradiation is shown in Figure 3. A significantly higher cell-death rate was observed in the ^{125}I seed group, compared to the ^{60}Co γ-ray irradiated group, at 12, 24, 48, and 72 hour postexposure ($p < 0.05$ or <0.01). The difference was most significant at 24 hour post exposure ($p < 0.01$); at 48–72 hour postexposure, the differences between ^{125}I seeds and ^{60}Co γ-ray irradiation were still significant, though not as pronounced as at 12 hour postexposure ($p < 0.05$). A possible

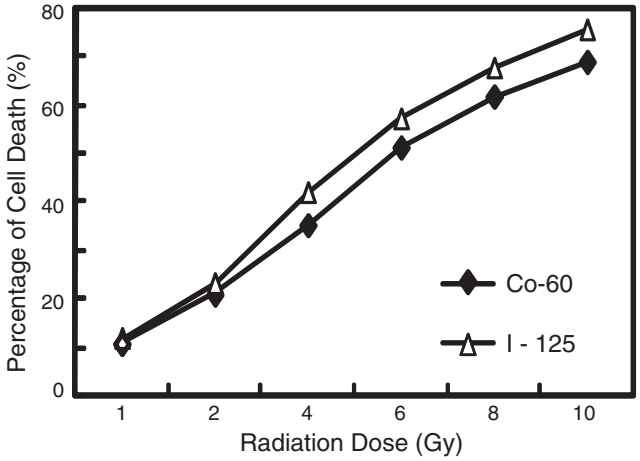

FIG. 2. Cell-death rate of PANC-1 after irradiation with ^{125}I seeds and ^{60}Co γ-rays. Determined by two-color flow cytometry, as mentioned in Materials and Methods. $p < 0.05$, compared with the identical group.

explanation for this phenomenon may be that cell apoptosis occurred during CLDR irradiation, and peaked at 24 hour postexposure, while high-dose-rate (HDR) irradiation caused immediate necrosis in some cells. Generally, cell death induced by apoptosis occurs after irradiation, usually peaking at 48–72-h postexposure.

Cell apoptosis induced by ^{125}I seed and ^{60}Co γ-ray irradiation

Results indicate that with an increase in absorbed dose, ^{125}I seeds induce a higher percentage of apoptosis, with a peak at 5 Gy. When the dose increased to 8 Gy, cell apoptosis remained high, though somewhat lower than at 5 Gy. Compared with ^{60}Co γ-ray irradiation, ^{125}I seeds at various doses produced a significantly higher percentage of apoptosis. Upon ^{60}Co γ-ray irradiation, cells exhibited a higher percentage of necrosis in a dose-dependent manner. Though ^{125}I seed irradiation also caused increased percentage of necrosis with an increase in absorbed dose, it was not as high as the necrosis induced by ^{60}Co γ-irradiation. At the absorbed dose of 2 Gy, there was no significant difference between the two types of irradiation. But, when the absorbed dose increased to 5 and 8 Gy, the difference was significant (Fig. 4). These data indicate that there are different mechanisms involved in cell death induced by CLDR and HDR irradiation.

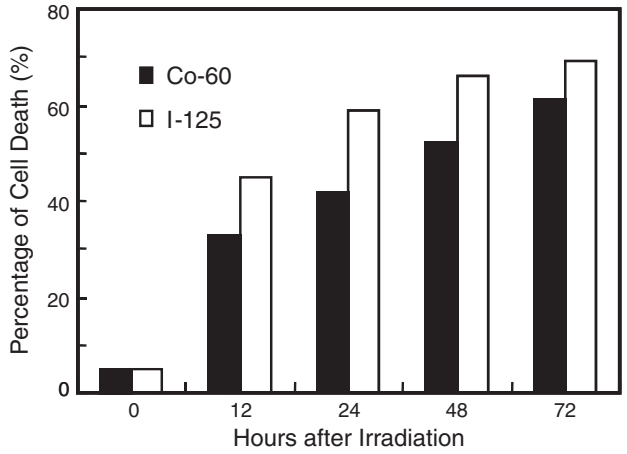

FIG. 3. The cell death of PANC-1 cells at different time points after radiation. Cell death was detected at different time points after radiation, as mentioned in Materials and Methods. $p < 0.05$, compared with the identical group.

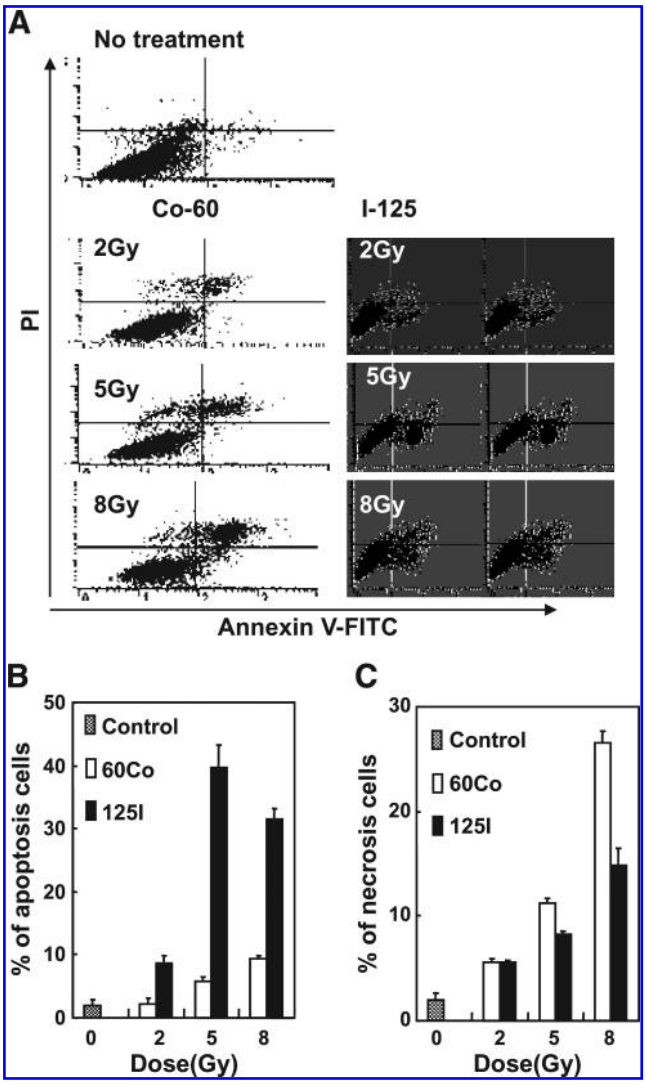

FIG. 4. The apoptosis and necrosis of PANC-1 after ^{60}Co γ-ray and ^{125}I seed irradiation. (**A**) Flow-cytometry assay for cell apoptosis and necrosis after ^{60}Co γ-ray and ^{125}I seed radiation, at different doses. (**B**) Cell-apoptosis assay by flow cytometry. (**C**) Cell-necrosis assay by flow cytometry.

Cell-cycle alteration after ^{125}I seed and ^{60}Co γ-ray irradiation

The results of cell-cycle assay by flow cytometry are given in Figure 5. Results indicate a dose-dependent S-phase arrest of PANC-1 cells 24 hour after ^{60}Co γ-ray irradiation. However, ^{125}I seed irradiation induced a higher percentage of G_2/M cell-cycle arrest in a dose-dependent manner. There was a significant difference discernable between corresponding doses for the ^{125}I seed–and ^{60}Co γ-ray–treated groups ($p < 0.01$), respectively; ^{125}I seed irradiation at 8 Gy

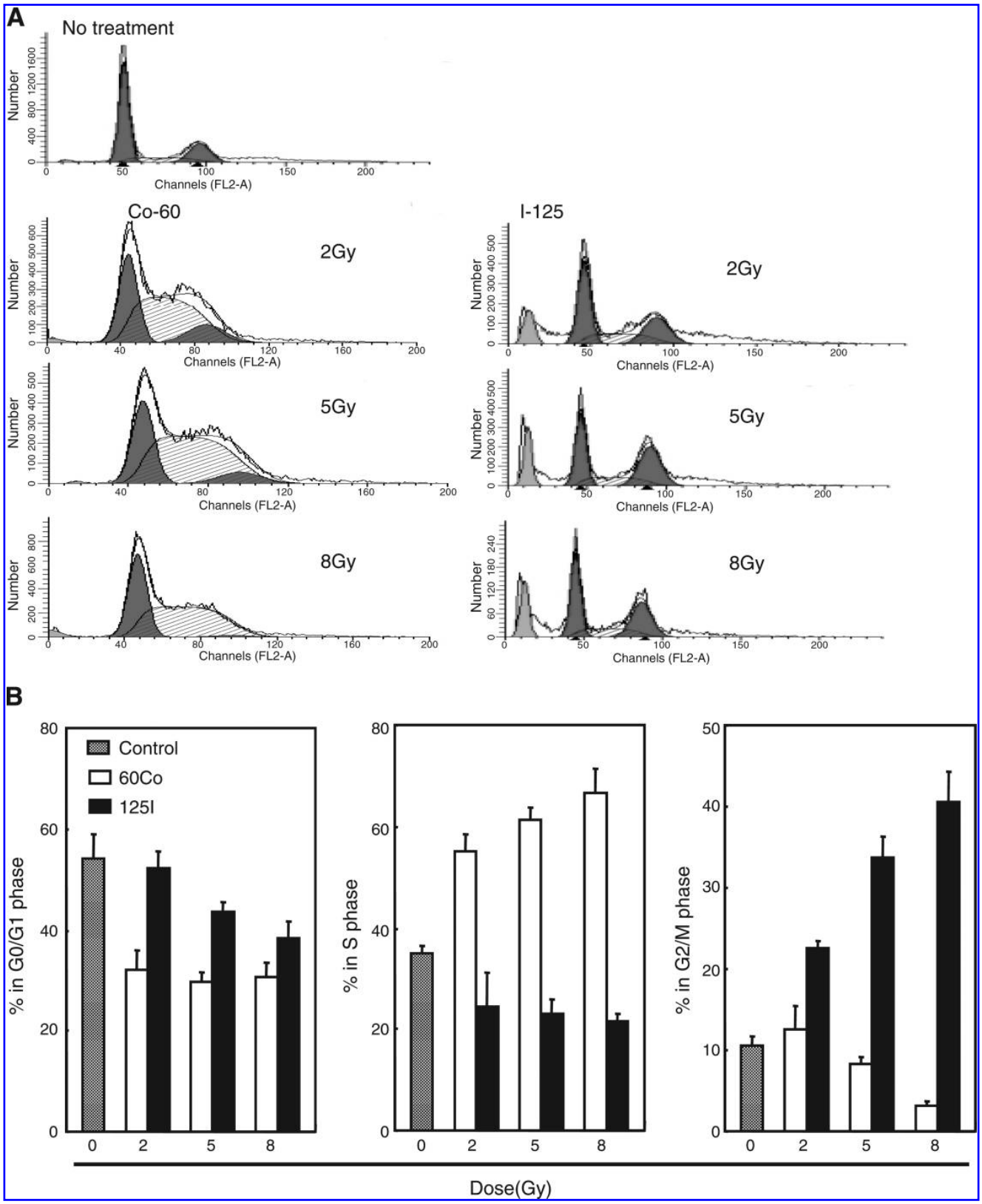

FIG. 5. The cell cycle of PANC-1 cells at different time points after irradiation. (**A**) The cell cycle was detected by Prodium iodide staining and assayed by flow cytometry, as mentioned in Materials and Methods. (**B**) Percentage of cell-cycle arrest at the Go/G_1, S-, and G_2/M phases, in PANC-1 cells, following ^{60}Co γ-ray and ^{125}I seed irradiation at different doses.

resulted in a G2/M arrest in 40.83% of the total cell population, 3.8 times higher than in the ^{60}Co γ-ray–irradiated group.

Discussion

Colony formation is the standard method to evaluate radiation-related RBE. Radioactive ^{125}I seed CLDR radiation and ^{60}Co γ-ray irradiation (i.e., HDR) are both commonly used radiotherapy approaches that are widely accepted in clinical practice; this study contrasts the apoptotic rate and cell-cycle distribution in PANC-1 cells exposed to both sources of irradiation, at equivalent absorbed doses. The RBE for both radiation sources was also compared with a view to provide a foundation for the clinical treatment of pancreatic cancer with ^{125}I seed interstitial brachytherapy. Some studies have demonstrated that the RBE of ^{125}I seeds, at a dose rate ranging from 3 to 900 cGy/h, was 1.0–1.5, when compared with ^{192}Ir, ^{226}Ra, and ^{60}Co γ-ray.[13,14] Ling et al.[6] reported a relative biologic effectiveness of 1.4 in rat embryo cell mouse cells exposed to ^{125}I seed irradiation, with an initial dose rate of 7 and 14 cGy/h. Nath et al. observed an RBE of 1.08 ± 0.07 in the Chinese hamster lung cell line, CCL-16, treated with ^{125}I seeds, compared with 250-keV X-rays, at the dose rate of 6.9 cGy/h.[14] We report comparable results in the current study, in an *in vitro* model for irradiation, using the pancreatic cancer cell line, PANC-1: An RBE of 1.39 was observed while comparing the efficacy of ^{125}I seeds and ^{60}Co γ irradiation at various doses. These findings indicate that ^{125}I seeds will exert a similar biologic effect within a certain dose range, which may further explain the clinical practice of disregarding RBE, even though the ^{125}I seeds differ in activity. These results, in conjunction with data presented in earlier reports, also suggest that ^{125}I seeds produce constant RBE, regardless of target-cell types, which may expand the scope of the technique's clinical applicability.

RBE is associated with many factors, among which linear energy transfer and dose rate play important roles.[14–16] ^{125}I seeds produce low-energy radiation, with a mean photon energy of 28.37 keV. Therefore, ^{125}I seeds have a theoretically higher RBE, which has been reported by several groups. In the HDR range, the radiation's potential to cause cell death increases with dose, while CLDR radiation possesses anticancer potential, because the damaged cell's intrinsic repair mechanism is not activated. Additionally, CLDR exposure induces perturbation of cell cycle, arresting cell cycle at the G_2/M phase, in which cells are sensitive to irradiation. In the present study, the initial dose rate of ^{125}I seeds for irradiation was 2.59 cGy/h, lower than that typically used in clinical treatment (7 cGy/h), which should exert a higher RBE.

Pancreatic cancer is highly malignant by nature, with a poor prognosis, and it is insensitive to cytotoxic agents and radiotherapy in clinical practice. PANC-1 cells originate from an epithelioid carcinoma derived from a patient with pancreatic ductal adenocarcinoma[17] and are resistant to radiotherapy and chemotherapy. In the current study, according to the dose-survival curve, the D_0 value for ^{60}Co γ-ray irradiation was 2.298, while that for ^{125}I seeds was 1.66, indicating the resistance of PANC-1 to irradiation. The lower D_0 for ^{125}I seeds than for ^{60}Co γ-rays suggests more sensitivity of PANC-1 to ^{125}I seed irradiation, compared to ^{60}Coγ irradiation. In the single-hit multitarget model, the extrapolation number, N, stands for the number of target cells sensitive to radiation or the cell number targeted for death; Dq is the quasithreshold dose, representative of the survival curve, and it reflects the ability of cells to repair sublethal damage. In this study, the N-value and Dq for ^{125}I seeds were 1.23 and 0.29, respectively. The N-value and Dq for ^{60}Co γ-ray were 1.42 and 0.65, respectively. According to the single-hit multitarget theory, ^{125}I seeds exert more potent cell-killing effects than ^{60}Co γ-rays in PANC-1 cells. This may result from the combinational actions of repair ability, reoxygenation of anoxic cells, and cell-cycle arrest.[18] For these reasons, some researchers believe that ^{125}I seeds are more suitable for treating cancers resistant to EBRT.

The treatment of unresectable pancreatic cancer continues to remain a major challenge. It is difficult to deliver high-dose radiotherapy to the tumor by using EBRT in combination with intensity-modulated radiation therapy (IMRT), due to the risk of toxicity to adjacent organs. Local persistence and/or recurrence of disease at the primary site remain a significant problem even while using high external beam radiation doses of 65–70 Gy and/or intraoperative electron beam irradiation (IOERT) combined with systemic chemotherapy.[19,20] Initial experience with ^{125}I seed implantation for pancreatic cancer indicated improved local control of the primary tumor with its ability to deliver higher effective doses of radiation to localized tumor volumes. The major problem appeared to be the high rate of perioperative morbidity and mortality.[21,22] So, we implemented ^{125}I seed implants (under the guide of intraoperative ultrasound) for 27 patients with unresectable pancreatic cancer at our hospital (the study was initiated in October 2003). The matched peripheral doses (MPDs) of ^{125}I seed implantation ranged from 110 to 160 Gy. Excellent palliation of pain and local control was achieved, while the overall survival also improved, with less toxicity. The key advantage to utilizing an intraoperative ultrasound guide is that the operator has a clear vision of the tumor location, size, and relationship with other adjacent structures, which determines that the seeds are properly distributed, spatially, and ensures optimal direct dose distribution. The dose inhomogeneity of ^{125}I seeds was no impediment, according to our clinical experience, when compared with the relatively homogeneous dose distribution of EBRT. The benefit of sharp dose fall-off far outweighs the cost of dose heterogeneity in tumor volume for ^{125}I seeds implant.

The development and clinical application of radioactive ^{125}I seeds have provided renewed impetus to interstitial brachytherapy. It can improve the survival and local control rates in treatment of many types of cancers, such as head and neck, intracranial, lung, pancreatic, and rectal cancer.[23] However, the mechanism of ^{125}I seeds in cancer treatment remains unresolved. The current study compares the cell-killing effects of the indigenous model 6711 ^{125}I seeds and ^{60}Co γ-ray, on pancreatic cancer cells, by colony formation assay. When the absorbed dose was above 4 Gy, the cell-death rate in the ^{125}I seed–irradiated group was significantly higher than the group treated with ^{60}Co γ-rays. When the absorbed dose was lower than 4 Gy, there was no significant difference between the two sources of irradiation in terms of cell-death rate. These facts indicate that only when CDLR reached a certain dose level (i.e., exposure time) did ^{125}I

seeds exhibit an enhanced ability to kill cancer cells, surpassing the cell-death rate induced by ^{60}Co γ irradiation. At radiation levels of 4 Gy, ^{125}I seeds produced higher cell death rate than ^{60}Co γ-rays at 12, 24, 48, and 72 hour. The difference was most pronounced at 24 hour and declined by 48–72 hour. This may reflect that, at continuous LDR radiation, apoptosis-induced cell death occurs during irradiation, peaking by 24 hour following exposure, while ^{60}Co γ-ray–induced cell apoptosis occurs postirradiation, peaking at 48–72 hour postexposure. However, the total numbers of apoptotic cells induced by ^{60}Co γ-ray irradiation were lower than those produced with ^{125}I seed irradiation. These facts indicate that ^{125}I seeds at CLDR produce higher killing effects on the PANC-1 cell line than ^{60}Co γ-ray at HDR.

Previous studies have reported that CLDR induces cancer inhibition mainly through apoptosis, and G_2/M arrest is more common in CLDR exposure.[24,25] On the one hand, G_2/M arrest slows down cell-cycle progression, enhancing biologic activity by accumulated absorbed dose in the prolonged G_2/M phase. On the other hand, G_2/M arrest provides the higher chance to repair cell damage. Therefore, the overall effects of G_2/M arrest on cell apoptosis may stem from the juxtaposition these two actions. In this study, PANC-1 apoptosis did not increase much following 2, 5, and 8 Gy ^{60}Co γ-ray radiation, while cell necrosis increased with dose increase. In contrast, following ^{125}I seed radiation, cell apoptosis increased dose dependently, peaking at 5 Gy, while necrosis did not increase much. Cell-apoptosis rates were significantly different between these two types of radiation, indicating that CLDR induced PANC-1 inhibition mainly through apoptosis pathway in a dose-dependent manner.

Cell-cycle analysis showed that PANC-1 cells were arrested in the S-phase within 24 hour, following high-dose ^{60}Co γ-ray irradiation. In contrast, at 24 hour postexposure to ^{125}I seeds at CLDR, PANC-1 cells were arrested in the G_2/M phase. These facts indicate that cell-cycle progression was arrested at different checkpoints by these two types of radiation. S-phase arrest blocks the duplication of damaged DNA, thus avoiding transfer of DNA damage to newly synthesized DNA. G_2/M arrest prevents cells from division before the damaged DNA is repaired, avoiding the misread genetic information to be delivered to the next generation.[26] S-phase arrest triggers the death or genomic instability of damaged cells, enhancing cell necrosis. G_2/M phase arrest directs damaged cells to the apoptotic pathway.[27] It has been verified in fundamental research[28] that cells will be arrested in the G_1, S- or G_2/M phase, to initiate the postdamage repair once the cell-cycle checkpoints have been activated by radiation-induced DNA damage. Once the self-protection mechanism is impaired, cells lose the ability to perform normal repair and, hence, enter apoptosis; this mechanism can significantly enhance sensitivity to treatment. ^{125}I seed irradiation at CLDR will arrest cells in the G_2/M phase. Meanwhile, continuous radiation impairs the cell's ability to repair the damage, promoting cell apoptosis, which is consistent with the apoptosis data from flow-cytometry analysis.

Conclusions

In summary, CLDR irradiation by ^{125}I seeds is more powerful than acute HDR ^{60}Co γ-ray irradiation in inducing cell death of PANC-1 cells (RBE = 1.39). Interestingly, CLDR irradiation by ^{125}I seeds can induce PANC-1 cell-cycle arrest at the G_2/M phase, which may be one of the important mechanisms for ^{125}I seeds inhibiting PANC-1 cells. The present study may potentially impact the clinical practice of ^{125}I seeds to treat pancreatic cancer. Further investigation should clarify the optimum dose and exposure duration and reveal the genes involved in the process.

Acknowledgments

The authors would like to thank Hongzhi Zhang, Ph.D., and Ruijie Yang, Ph.D., for their technical assistance and Ms. Qinghuan Li for her excellent laboratory management. This work was partly supported by a grant from the National Natural Science Foundation of China (no. 39900167).

Disclosure Statement

No competing financial interests exist.

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