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ORIGINAL ARTICLE

Basic Study

Biological effects of low-dose-rate irradiation of pancreatic carcinoma cells *in vitro* using ^{125}I seeds

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Abstract

AIM: To determine the mechanism of the radiation-induced biological effects of ^{125}I seeds on pancreatic carcinoma cells *in vitro*.

METHODS: SW1990 and PANC-1 pancreatic cancer cell lines were cultured in DMEM in a suitable environment. Gray's model of iodine-125 (^{125}I) seed irradiation was used. *In vitro*, exponential phase SW1990, and PANC-1 cells were exposed to 0, 2, 4, 6, and 8 Gy using ^{125}I radioactive seeds, with an initial dose rate of 12.13 cGy/h. A clonogenic survival experiment was performed to observe the ability of the cells to maintain their clonogenic capacity and to form colonies. Cell-cycle and apoptosis analyses were conducted to detect the apoptosis percentage in the SW1990 and PANC-1 cells. DNA synthesis was measured *via* a tritiated thymidine (^{3}H-TdR) incorporation experiment. After continuous low-dose-rate irradiation with ^{125}I radioactive seeds, the survival fractions at 2 Gy (SF2), percentage apoptosis, and cell cycle phases of the SW1990 and PANC-1 pancreatic cancer cell lines were calculated and compared.

RESULTS: The survival fractions of the PANC-1 and

SW1990 cells irradiated with ^{125}I seeds decreased exponentially as the dose increased. No significant difference in SF2 was observed between SW1990 and PANC-1 cells (0.766 ± 0.063 *vs* 0.729 ± 0.045, $P < 0.05$). The ^{125}I seeds induced a higher percentage of apoptosis than that observed in the control in both the SW1990 and PANC-1 cells. The rate of apoptosis increased with increasing radiation dosage. The percentage of apoptosis was slightly higher in the SW1990 cells than in the PANC-1 cells. Dose-dependent G2/M cell-cycle arrest was observed after ^{125}I seed irradiation, with a peak value at 6 Gy. As the dose increased, the percentage of G2/M cell cycle arrest increased in both cell lines, whereas the rate of DNA incorporation decreased. In the ^{3}H-TdR incorporation experiment, the dosimetry results of both the SW1990 and PANC-1 cells decreased as the radiation dose increased, with a minimum at 6 Gy. There were no significant differences in the dosimetry results of the two cell lines when they were exposed to the same dose of radiation.

CONCLUSION: The pancreatic cancer cell-killing effects induced by ^{125}I radioactive seeds mainly occurred *via* apoptosis and G2/M cell cycle arrest.

Key words: ^{125}I radioactive seeds; Biological effects; Pancreatic cancer; SW1990; PANC-1

Core tip: We compared the radiobiological effects observed in SW1990 and PANC-1 pancreatic cancer cell lines after irradiation with ^{125}I seeds through a clonogenic cell survival assay and ^{3}H-TdR incorporation experiment. Continuous low-dose-rate irradiation with ^{125}I seeds was found to induce apoptosis and G2/M cell cycle arrest.

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INTRODUCTION

Pancreatic cancer is a fatal disease that is a serious health problem worldwide; the overall five-year survival rate for patients with this disease is approximately 5%. Risk factors for pancreatic cancer include smoking, family history of chronic pancreatitis, advancing age, vitamin D deficiency, and occupational exposure[1]. In general, mortality is nearly the same as morbidity[1]. Due to the absence of a capsule, the tumor can easily infiltrate the surrounding tissue; local invasion and distant metastasis of the tumor occurs rapidly in most patients. Patients are usually diagnosed at an advanced stage, with the effectiveness of chemotherapy being very limited for such patients. Interstitial brachytherapy is an ideal treatment that can prevent recurrence, prolong life expectancy, and improve the quality of life in advanced patients[2]. The most commonly used radioactive seeds for interstitial brachytherapy are ^{131}Cs and ^{103}Pd, among others. As reported that the relative biological effectiveness of ^{103}Pd *vs* 250 kVp X-rays and iodine-125 (^{125}I) *vs* ^{60}Co X-rays are 1.24 and 1.39, respectively[3-5]. ^{125}I emits 27.4 keV X-rays and 35.5 keV γ-rays, which increase the radiosensitivity of the tumor[6-8]. ^{125}I has a $T_{1/2}$ of 60.1 d and can therefore provide continuous irradiation for nearly 200 d. The advantages of ^{125}I have led to its widespread application in China[9-11]. There are various pancreatic cancer cell lines available, including PANC-1, SW1990, P3, Capan-1, Capan-2, AsPC-1, and Hs766T. Among them, PANC-1 and SW1990 are used most often in experiments[12-15]. According to the American Type Culture Collection, the SW1990 line was established from a spleen metastasis of a grade Ⅱ pancreatic adenocarcinoma derived from the exocrine pancreas, and PANC-1 originated from the epithelium of the pancreas duct. The difference between the radiobiological effects induced by ^{125}I seeds on SW1990 and PANC-1 cells has not been described in the literature. In this study, we compared the radiobiological effects observed in SW1990 and PANC-1 cells after irradiation using ^{125}I seeds through clonogenic cell survival assay and tritiated thymidine (^{3}H-TdR) incorporation experiment.

MATERIALS AND METHODS

Cell lines and cell culture

Cells from the SW1990 and PANC-1 pancreatic cancer cell lines (kindly provided by the Chinese Academy of Sciences Shanghai cell library) were cultured in DMEM supplemented with 100 IU/mL penicillin, 100 mg/mL streptomycin, and 10% heat-inactivated fetal bovine serum (Biotrom Company, Germany) in a humidified atmosphere of 95% air and 5% CO_2 at 37 ℃.

^{125}I seeds

^{125}I radioactive seeds (BT-125-I) were purchased from the Shanghai Xinke Limited Corporation. We used Gray's model of ^{125}I seed irradiation[16,17], as shown in Figure 1. The model consists of 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 located around a 35 mm circumference. In the cell culture plaque, identical recesses were created, also around a 35 mm circumference. The absorbed dose and the exposure time could be calculated. The initial dose rate was 12.13 cGy/h and the accumulated doses were 2, 4, 6, and 8 Gy. The entire model was housed in an incubator throughout the study.

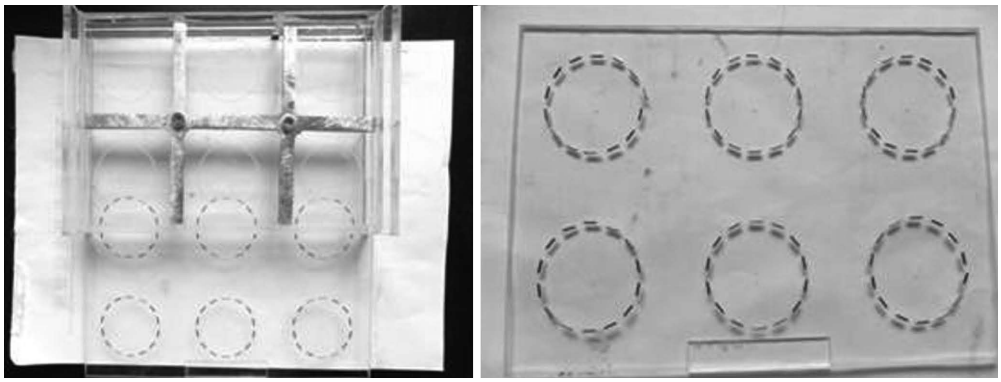

Figure 1 Appliance used for irradiation with ^{125}I radioactive seeds.

Cell irradiation

SW1990 and PANC-1 cells in the exponential phase of growth were irradiated in a tissue culture flask (35 mm in diameter) using the model described above. After irradiation, the cells were incubated for a further 21 d at constant temperature and humidity.

Clonogenic survival experiment

Clonogenic survival is defined as the ability of cells to maintain their clonogenic capacity and to form colonies[18]. Cells in the control and irradiation groups were briefly exposed to various radiation dosages (0, 2, 4, 6, and 8 Gy). After incubation for 14 d, the colonies were stained and manually counted. Colonies that contained more than 50 cells were regarded as survivors. The plating efficiency (PE) and survival fraction (SF) were calculated as follows: PE = (number of colonies/number of inoculating cells) × 100% and SF = PE (tested group)/PE (0 Gy group) × 100%. The dose-survival curves were fitted using the linear quadratic model SF = $e^{-\alpha D-\beta D\wedge 2}$. A dose-survival curve was obtained for each experiment and the values of SF2 (the fitted SF value at 2 Gy) were calculated based on the dose-survival curves of the PANC-1 and SW1990 cells in the presence of ^{125}I seeds.

Cell cycle and apoptosis analysis

An annexin-V/propidium iodide (PI) apoptosis kit was purchased from Invitrogen Corporation. The cells of the control group and the CLDR treated groups were exposed to various radiation dosages (0, 2, 4, 6, and 8 Gy). The cells were collected 48 h after irradiation. For the detection of apoptotic cells, the cells were trypsinized, stained with acridine orange, and observed under a fluorescence microscope. At the same time, the cells were counted. The cells used for the apoptosis analysis were stained with PI and annexin V. The cells used for cell cycle analysis were stained with PI after ethanol fixation. Each analysis was performed 3 times.

^{3}H-TdR incorporation experiment

DNA synthesis was measured *via* a ^{3}H-TdR incorporation experiment. Thymidine phosphorylase was added during cell proliferation to mark the TdR with the radioactive nuclide ^{3}H and to introduce the resulting ^{3}H-TdR into the culture system, where it could be incorporated into DNA molecules. The same amounts (about 1 × 10^{5}) of SW1990 and PANC-1 cells were plated and irradiated with ^{125}I seeds at doses of 2, 4, 6, and 8 Gy. A volume of 10 μL of ^{3}H-TdR was injected into each culture system after irradiation. A liquid scintillation counter was used to estimate the relative dosimetry for each group, with the value corresponding to the control group set to 1. After 24 h of culture, the amounts of ^{3}H-TdR that had been incorporated were estimated and compared between the control group and treated groups for both cell lines.

Statistical analysis

The data were plotted as the mean ± standard deviation, with $P < 0.05$ being considered significant. SAS 9.1 (NC, USA) software was used to acquire the cell survival curves.

RESULTS

Clonogenic survival rates

PANC-1 and SW1990 cells were irradiated using ^{125}I seeds at doses of up to 8 Gy and their survival fractions were measured based on colony formation. Figure 2 presents the dose–response curves for the cell-killing effects of irradiation with ^{125}I seeds on these 2 human pancreatic cancer cell lines. The survival fractions of the PANC-1 and SW1990 cells that were irradiated with ^{125}I seeds decreased exponentially with an increasing dose of radiation. The SF2 value for SW1990 was 0.766 ± 0.063 and the SF2 value for PANC-1 was 0.729 ± 0.045. No significant differences between the two cell lines were observed.

The apoptosis rates of SW1990 and PANC-1 cells

The results indicated that for a higher absorbed dose, the ^{125}I seeds induced a higher percentage of apoptosis in both the SW1990 and PANC-1 cells (Figure 3). The percentage of apoptosis was slightly higher in the SW1990 cells than in the PANC-1 cells, but the

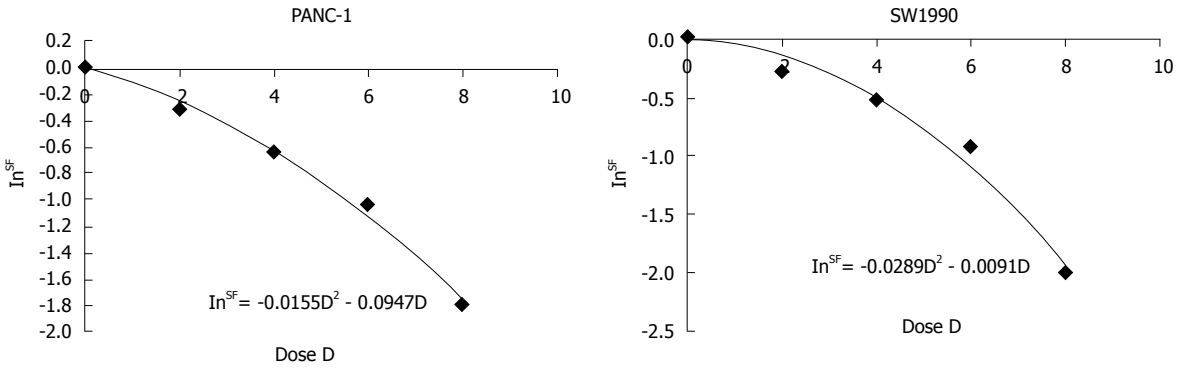

Figure 2 Survival curves for PANC-1 and SW1990 cells after irradiation using ^{125}I seeds. The vertical ordinate represents the Napierian logarithm of survival fraction (SF) and the horizontal ordinate represents the radiation dose.

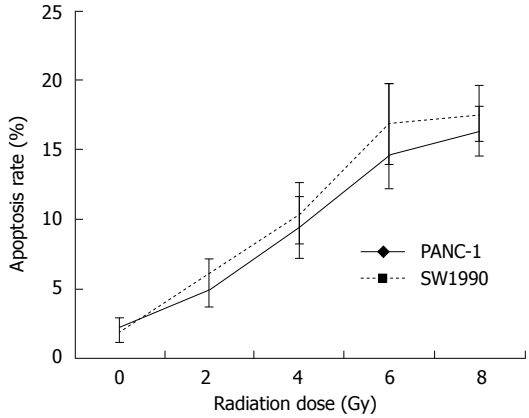

Figure 3 Apoptosis rates of PANC-1 and SW1990 cells after irradiation with ^{125}I seeds at various doses.

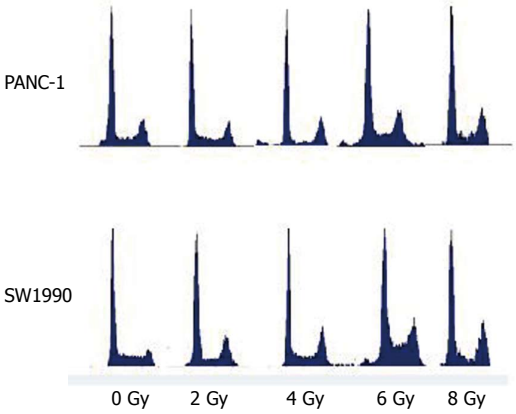

Figure 4 The cell cycle phases of PANC-1 and SW1990 cells after irradiation with ^{125}I seeds at various doses.

difference was not significant.

Cell cycle analysis of SW1990 and PANC-1 cells

The results of cell cycle analysis performed *via* flow cytometry are presented in Table 1 and Figure 4. The results indicated that dose-dependent G2/M cell-cycle arrest occurred after irradiation with ^{125}I seeds, with a peak value at 6 Gy.

^{3}H-TdR incorporation experiment

In the ^{3}H-TdR incorporation experiment, the dosimetry results for both the SW1990 and PANC-1 cells decreased as the radiation dose increased, with the lowest dosimetry readings at 6 Gy. There were no significant differences in the dosimetry results between the two cell lines at the same radiation dose. Additionally, the dosimetry readings at 2 Gy exhibited no significant difference compared with the control group. This result suggested that a cumulative dose of 2 Gy may not have been sufficient to suppress DNA synthesis. The dosimetry readings at 4 Gy were higher than those at 6 Gy and 8 Gy for both cell lines ($P < 0.05$) and there were no significant differences between the readings at 8 Gy and 6 Gy ($P > 0.05$). However, the dosimetry readings for the SW1990 and PANC-1 cells at 8 Gy were slightly higher than those at 6 Gy, especially for the SW1990 cells (Figure 5).

DISCUSSION

Interstitial brachytherapy with radioactive seeds has a history spanning more than 100 years. ^{125}I, ^{103}Pd, and ^{131}Cs are the most commonly used types of radioactive seeds. The advantages of interstitial brachytherapy using radioactive seeds are the low dose rates and conformal irradiation[19,20], which increases the dose applied within the target area, thereby decreasing the incidental radiation injury to normal tissues and the attendant complications[21,22]. Hennequin *et al*[23] have demonstrated that low-dose-rate brachytherapy offers certain radiobiological advantages compared with external beam radiotherapy: subtle damage repair during irradiation, leading to the relative protection of healthy tissues; lack of tumor cell repopulation; cell cycle redistribution; and a low oxygen enhancement ratio. Radioactive seeds were first used for the treatment of prostate cancer and demonstrated high efficacy[24,25]. Irradiation therapy may encourage tumor cells to remain in the sensitive resting period, thereby resulting in tumor cell apoptosis, and cause damage to their DNA, thereby killing the cancer cells. In this study, we assessed the radiobiological effects of ^{125}I

Table 1 Percentage of PANC-1 and SW1990 cells in each phase of the cell cycle after irradiation with ^{125}I seeds at various doses

	Gy	*n*	G1 (%)	S (%)	G2/M (%)	G2/G1 (%)
PANC-1	0	3	49.22 ± 3.92	25.38 ± 1.8	21.31 ± 1.91	1.94 ± 0.16
	2	3	51.34 ± 4.14	26.63 ± 3.9	22.48 ± 2.13	1.95 ± 0.12
	4	3	52.94 ± 3.83	28.12 ± 1.2	26.24 ± 2.15	1.98 ± 0.19
	6	3	55.83 ± 4.28	30.82 ± 2.2	33.21 ± 2.21	1.99 ± 0.15
	8	3	54.03 ± 3.21	27.83 ± 3.5	28.14 ± 3.81	1.99 ± 0.13
SW1990	0	3	43.79 ± 4.52	37.31 ± 2.8	19.08 ± 1.36	1.93 ± 0.14
	2	3	42.47 ± 3.27	36.03 ± 4.2	20.08 ± 1.72	1.94 ± 0.09
	4	3	40.98 ± 3.94	38.42 ± 3.2	24.36 ± 1.18	1.96 ± 0.12
	6	3	41.99 ± 4.39	30.82 ± 2.2	31.99 ± 3.29	1.98 ± 0.19
	8	3	52.08 ± 3.80	32.83 ± 3.5	27.63 ± 2.94	1.97 ± 0.20

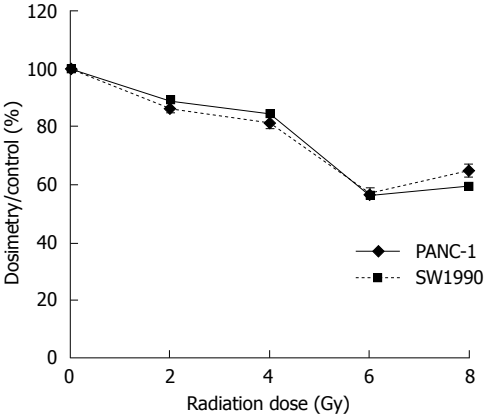

Figure 5 The DNA incorporation rates of PANC-1 and SW1990 cells after irradiation with ^{125}I seeds at various doses.

seeds on human pancreatic cancer cells *in vitro*.

Clonogenic survival experiments can reflect the ability of cells to proliferate *in vitro*[26]. The clonogenic survival experiment performed in this study revealed similar SF2 values between the two investigated pancreatic cancer cell lines after irradiation at doses of 0, 2, 4, 6, and 8 Gy using ^{125}I radioactive seeds. This result indicated that the cellular radiosensitivity of the two pancreatic cancer cell lines to continuous low-dose-rate irradiation produced by ^{125}I seeds was similar.

Our results demonstrated that a higher absorbed dose of ^{125}I induced a higher percentage of apoptosis in both the SW1990 and PANC-1 cells. This finding indicated that apoptosis may play an important role in the therapeutic effects exerted by the application of continuous low-energy ^{125}I irradiation to SW1990 and PANC-1 cells. A study by Jian *et al*[27] also demonstrated that cell apoptosis was the key factor in ^{125}I seed brachytherapy in a pancreatic carcinoma xenograft. Organisms exposed to ionizing radiation (IR) exhibit increased production of reactive oxygen species (ROS), which are determining factors in the induction of apoptosis[28]. ROS damage critical cellular components such as DNA, proteins, and lipids, eventually causing cellular apoptosis[29]. Therefore, the apoptosis induced by ^{125}I irradiation is a key mechanism underlying the therapeutic effects of ^{125}I seed implantation in the treatment of pancreatic cancer.

As suggested by the data obtained in our cell cycle analysis, continuous low-energy ^{125}I irradiation causes the arrest of both SW1990 and PANC-1 cells in the G2/M phase. It is well known that progression through the various phases of the cell cycle is a tightly regulated process that involves the activities of various cyclins and cyclin-dependent kinases, which are specific to different cell cycle phases[30]. Our results indicate that dose-dependent G2/M cell cycle arrest occurs after ^{125}I seed irradiation, with a peak value at 6 Gy.

Previous studies have shown that apoptosis and G2/M cell-cycle arrest are the predominant mechanisms involved in the inhibition of tumor growth[31-33]. Several studies[34] that have focused on the operational mechanism of ^{125}I radioactive seeds in the treatment of prostate cancer have demonstrated that this inhibition is achieved through the down-regulation of BCL-2 and certain influences on caspase. Some scholars[35,36] believe that a change in DNA methyltransferase is also a key element. The results of our study were consistent with those of previous studies. In our study, the percentages of apoptosis and G2/M cell cycle arrest increased with increasing irradiation dose. Our ^{3}H-TdR phosphorylase incorporation experiment explored the decrease in DNA synthesis after irradiation. We found that continuous low-dose-rate irradiation at 6 Gy using ^{125}I seeds is effective for tumor inhibition *in vitro*. Although many issues remain to be addressed, we believe that with further developments in fundamental research, the applications of ^{125}I radioactive seed implantation in clinical practice will continue to be explored.

CONCLUSION

We demonstrated that two human pancreatic cancer cell lines (PANC-1 and SW1990) exhibited similar radiosensitivity to continuous low-dose-rate irradiation with ^{125}I seeds and that a dose of 6 Gy is effective for tumor inhibition *in vitro*. The radiobiological effects induced by continuous low-dose-rate irradiation with ^{125}I seeds were most likely due to apoptosis and G2/M cell-cycle arrest.

COMMENTS

Background

Pancreatic cancer is a fatal disease and a serious health problem worldwide. Patients are usually diagnosed at an advanced stage, with the effectiveness of chemotherapy for such patients being very limited. Interstitial brachytherapy is an ideal treatment that can prevent recurrence, prolong life expectancy, and improve the quality of life in advanced patients.

Research frontiers

This study focused on whether continuous low-dose-rate irradiation with iodine-125 (^{125}I) seeds would cause radiation-induced biological effects on pancreatic carcinoma cells *in vitro*. The mechanism(s) through which this would occur were also explored.

Innovations and breakthroughs

This experiment assessed the radiobiological effects of ^{125}I seeds on human pancreatic cancer cells *in vitro*. The difference between the radiobiological effects induced by irradiation with ^{125}I seeds on SW1990 and PANC-1 cells has not been described in the literature. This study demonstrated that the survival fractions of PANC-1 and SW1990 cells irradiated with ^{125}I seeds decreased exponentially with an increasing dose of radiation. Furthermore, the ^{125}I seeds induced a higher percentage of apoptosis than that observed in the control in both SW1990 and PANC-1 cells.

Applications

The ^{125}I seeds induced a higher percentage of apoptosis than that observed in the control for both the SW1990 and PANC-1 cells. This finding indicated that apoptosis may play an important role in the therapeutic effects exerted by continuous low-energy ^{125}I irradiation in the treatment of pancreatic cancer.

Terminology

Clonogenic survival experiment, cell cycle and apoptosis analyses, and tritiated thymidine (^{3}H-TdR) incorporation experiment were the major experiments conducted to assess the ratios of apoptosis and cell cycle arrest and to perform comparisons between the different cell lines and radiation doses.

Peer-review

This study was well-designed and clarified that the radiobiological effects induced in pancreatic cancer cells by continuous low-dose-rate irradiation with ^{125}I seeds were mainly caused by apoptosis and G2/M cell-cycle arrest. These conclusions will be very useful in guiding the treatment of tumors *via* interstitial brachytherapy using ^{125}I seeds.

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