Integrative Cancer Therapies
9(1) 59–65

DOI: 10.1177/1534735409357758
http://ict.sagepub.com

Relative Biological Effectiveness and Cell-Killing Efficacy of Continuous Low-Dose-Rate ^{125}I Seeds on Prostate Carcinoma Cells In Vitro

Anyan Liao, MD,[1] Junjie Wang, MD, PhD,[1] Jidong Wang, MD,[1] Hongqing Zhuang, MD,[1] and Yong Zhao, PhD[2]

Abstract

The aim of this study was to determine the effects of ^{125}I seeds on prostate carcinoma (PC3) cells. The relative biological effectiveness of ^{125}I seeds on PC3 cells with respect to ^{60}Co γ rays was 1.4. Both 4 Gy of ^{60}Co γ ray and ^{125}I seed irradiation increased the percentage of cells in G_2 phase, but there was no significant difference between these 2 types of radiation. Significantly, ^{125}I seeds induced higher apoptotic rates of PC3 cells compared with ^{60}Co γ ray irradiation. Furthermore, Bcl-2 expression, but not caspase-3 activity, in PC3 cells was downregulated after irradiation with ^{125}I seed or ^{60}Co γ rays.

Keywords

^{125}I seeds, PC3 cell, cell proliferation, cell cycle, cell apoptosis, caspase-3, Bcl-2

Introduction

Clinical evidence demonstrates that prostate cancer treatment using ^{125}I seeds is more effective than that using other radiation modalities. Lung cancer, head and neck cancer, pancreatic carcinoma, and prostate cancer have been treated using interstitial implants.[1-5] Interstitial brachytherapy shows a greater percentage of local control and a lower percentage of complications.[6-11] Its advantages can be attributed to both physical and biological aspects. For example, the local radiation distribution is enhanced, that is, the tumor to normal tissue dose ratio is less than 1. Moreover, tumor repopulation can be reduced because of the shorter treatment time by administering fractionated radiotherapy with external beam radiotherapy (EBRT).[12] The reduction of oxygen-enhancement ratio with decrease in dose rate may partially decrease the radioresistance of hypoxic cells.[13] However, the radiobiological mechanism of continuous low-dose-rate (CLDR) irradiation is not very clear. In our present study, ^{125}I seeds at a low-dose-rate directly caused more cell death than that of ^{60}Co γ rays irradiation in human prostate cancer cells (PC3), indicating that ^{125}I seeds may have potential clinical applications in prostate cancer therapy.

Materials and Methods

Reagents

Cell culture media was provided by the Zoology Institute of the Chinese Academy of Sciences. Propidium iodide and annexin V were purchased from Cell Signaling Company (Cell Signaling Technology, Beverly, MA). All other materials were obtained from the Zoology Institute of the Chinese Academy of Sciences.

Cell Lines and Cell Culture

The androgen-independent human prostate cancer cell line (PC3 cell) was kindly provided by the Beijing Institute for Cancer Research. It was cultured in RPMI 1640, which is supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, and 100 U/mL penicillin/streptomycin. The

[1]Peking University 3rd Hospital. Beijing, China
[2]Chinese Academy of Sciences, Beijing, China

Corresponding Author:
Junjie Wang, Department of Radiation Oncology, Peking University 3rd Hospital, Beijing 100083, China
Email: doctorwangjunjie@yahoo.com.cn

cells were incubated in a humidified atmosphere consisting of 5% CO_2 and 95% air at 37°C. PC3 cells in exponential growth were irradiated.

Irradiation Conditions

^{125}I seeds were used for CLDR irradiation at a dose rate of 2.517 cGy/h (Model-6711; provided by Zhe Jiang province, Ningbo Jun An Pharmaceutic Inc., Ningbo, China). In the irradiation apparatus, 14 ^{125}I seeds were placed at equal intervals in recesses around the circumference of a polystyrene disc having a diameter of 35 mm and thickness of 3 mm. This disc was placed above the cell culture dish and was supported by polystyrene foam. The distance between ^{125}I seed source and the cells was 1.2 cm.[14,15] During irradiation, the source and culture dish were placed inside an incubator covered with a lead lid. The distance between the ^{60}Co γ ray source and culture dish was 1.5 m and the dose rate was 2.215 Gy/min.

Colonogenic Survival Assay

Cells from exponentially growing stock cultures were plated on to Corning tissue-culture dishes having a diameter of 35 mm. The cells were seeded 18 hours prior to irradiation in order to allow them to attach to the tissue culture dish and reach exponential growth phase. The irradiation time ranged from 1 to 60 hours for CLDR irradiation and for several minutes for high-dose-rate irradiation. Cells in the control and irradiation groups were exposed to different radiation dosages (0, 0.5, 1, 1.5, 2, 4, 6, and 8 Gy). Cells were cultured in a humidified atmosphere consisting of 95% air and 5% CO_2 at 37°C during CLDR irradiation. High-dose-rate irradiation and unirradiated controls were maintained under identical conditions. After irradiation, the cells were washed with trypsin and counted. At least 3 culture dishes per experimental group were used, and the cells were allowed to grow under the afore-mentioned conditions for 14 days. The colonies were then fixed with 96% ethanol and stained with 15% Giemsa. The colonies with more than 50 cells were selected. The surviving fractions after irradiation were defined as the fraction of cells in the irradiated colonies relative to that in the untreated colonies.

Cell Survival Curve

The survival rate of the cell colonies after irradiation was evaluated by the colony-forming assay. The plating efficiency (PE) and survival fraction (SF) were calculated as follows: PE = (colony number/inoculating cell number) × 100%. SF = PE (tested group)/PE (0-Gy group) × 100%. A dose–survival curve was obtained for each experiment and used for calculating several survival parameters. Parallel samples were set at each radiation dosage. The cell-survival curve was plotted with the Graphpad 4.0 software, using the formula $SF = 1 - (1 - e^{-D/D0})^N$. The multitarget, single-hit model was applied to calculate cellular radiosensitivity (mean lethal dose, D_0), capacity for sublethal damage repair (quasithreshold dose, D_q), and extrapolation number (N). The D_{10} values were used to calculate the relative biological effectiveness (RBE). Here $D_{0(\text{Co-60})}$ is the dose required to reduce the cell survival count to D_0 value by using ^{60}Co γ ray, and D_0(I-125) value is the dose required to produce the same effect using ^{125}I seeds under a given irradiation condition.

Cell Proliferation

PC3 cells in exponential growth were exposed to grade dose of irradiation from ^{125}I seeds or ^{60}Co γ ray at various doses (0, 1, 2, 4, 6, and 8 Gy). After culturing for 24, 48, 72, and 96 hours in an incubator, PC3 cells were collected and stained with trypan blue to monitored for cell number.

Cell Cycle Analysis

PC3 cells in exponential growth were exposed to 4 Gy ^{125}I seeds or ^{60}Co γ rays radiation. After culturing for 12, 24, 48, and 72 hours, cells used for cell-cycle testing were stained with propidium iodide after ethanol fixation and analyzed by fluorescence-activated cell sorting (FACS) using Coulter EPICS and ModFit software (Verity Software House, Topsham, MN). Each test was performed 3 times.[16]

Cell Apoptosis Analysis

PC3 cells in exponential growth were irradiated using ^{125}I seeds and by ^{60}Co γ rays with various doses (2, 4, 6, and 8 Gy). After 48 hours, cells were trypsinized, acridine orange stained, and determined under fluorescence microscope. At the same time, cells were counted and washed twice with cold phosphate-buffered saline. Cells used for apoptosis tests were stained with propidium iodide and annexin V for 15 minutes in the dark. Agarose gel electrophoresis of DNA and flow cytometry were used to detect apoptosis in PC3 cells (Verity Software House, Topsham, MN). Each test was performed 3 times.[16]

Caspase-3 Activity Assay

PC3 cells in exponential growth were irradiated by ^{125}I seeds and by ^{60}Co γ rays at various doses (2, 4, 6, and 8 Gy). After 48 hours, caspases-3 activity was assayed by caspase-3 assay kit. We analyzed 3 samples for each dose.

Bcl-2 Expression

PC3 cells in exponential growth were irradiated by ^{125}I seeds and by ^{60}Co γ rays at various doses (2, 4, 6, and 8 Gy). After

48 hours of irradiation, indirect immunofluorescence assay was performed to detect Bcl-2 gene expression.

Data Analysis

All data are presented as mean ± SD. The *t* test was used to compare the means of the control and experimental groups. *P* values <.05 were considered statistically significant.

Results

Radiobiological Parameters of ^{125}I Seeds and ^{60}Co γ Rays on PC3 Cells

Data showed that cell-killing effects were related to dose rate. On the basis of the colony-forming assay, the cell survival curves of PC3 cells after irradiation with ^{125}I seeds and ^{60}Co γ ray were plotted (Figure 1). The radiobiological parameters for PC3 cells irradiated by ^{125}I seeds were as follows: $D_0 = 2.243$, $D_q = 0.87$, $N = 1.618$, and SF2 = 0.5, whereas the radiobiological parameters after irradiation with ^{60}Co γ rays were as follows: $D_0 = 2.824$, $D_q = 1.08$, $N = 1.587$, and SF2 = 0.7. The RBE for ^{125}I seeds compared with that of ^{60}Co γ rays is 1.4 (Table 1).

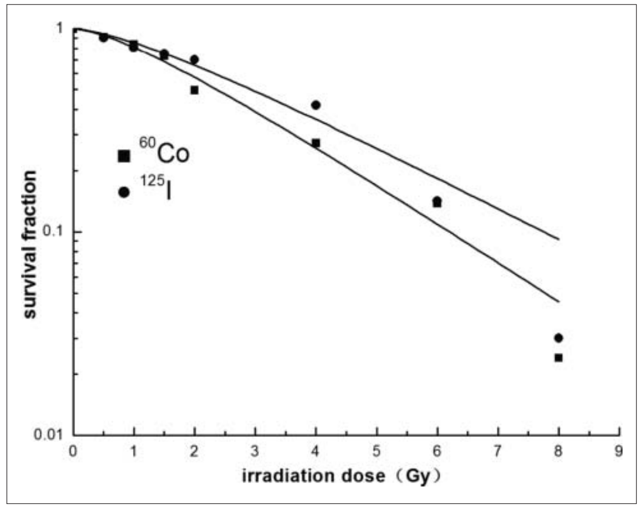

Figure 1. Cell survival curves of ^{125}I seeds and ^{60}Co γ ray irradiation in prostate carcinoma (PC3) cells were plotted on the basis of colony-forming assay
The radiobiological parameters of PC3 cells irradiated by ^{125}I seeds were as follows: $D_0 = 2.243$, $D_q = 0.87$, $N = 1.618$, and SF2 = 0.5. The parameters in the case of ^{60}Co γ rays were as follows: $D_0 = 2.824$, $D_q = 1.08$, $N = 1.587$, and SF2 = 0.7. The relative biological effectiveness (RBE) for ^{125}I seeds with respect to ^{60}Co γ rays is 1.4.

Table 1. Radiobiological Parameters of ^{125}I Seeds or ^{60}Co γ Ray Irradiation

	D_0	D_q	N
^{60}Co group	2.824	1.08	1.587
^{125}I group	2.243	0.87	1.618

Abbreviations: D_0, mean lethal dose; D_q, quasi-threshold dose; N, extrapolation number.

The Direct Effects of ^{125}I Seeds or ^{60}Co γ Rays on Cell Growth of PC3 Cells

PC3 cell survival fraction was detected by trypan blue at 48 hours after PC3 cells exposed to various doses (1, 2, 4, 6, and 8 Gy) of ^{125}I seeds and ^{60}Co γ rays. The survival fraction of PC3 cells after different doses of irradiation is summarized in Table 2. The survival fraction was significantly decreased in a dose-dependent manner. At the same dose, significantly fewer surviving cells were detected when cells were exposed to ^{125}I compared with ^{60}Co. On the other hand, at 24, 48, 72, and 96 hours culturing after PC3 cells were radiated with 4 Gy of ^{125}I seeds and ^{60}Co γ rays, PC3 cells were collected and stained with trypan blue to monitored for cell viability. The survival curve of PC3 cells at different time points after 4-Gy irradiation is shown in Figure 2. Significantly decreased cell numbers were observed in both types of radiation. However, at the same dose, the survival fractions of ^{125}I seeds were always lower than ^{60}Co γ rays. The effect of ^{125}I seeds on the survival of PC3 cells was thus more efficient.

Table 2. Survival Fraction of Prostate Carcinoma (PC3) Cells After Irradiation With Either ^{125}I Seeds or ^{60}Co γ Rays[a]

	Radiation Dose (Gy)				
	0	2	4	6	8
^{60}Co	99.5 ± 0.4	88.4 ± 0.3	76.2 ± 1.4	64.3 ± 1.9	52.5 ± 1.2
^{125}I	99.3 ± 0.4	88.3 ± 0.4	66.5 ± 1.5[b]	47.1 ± 2.0[b]	22.38 ± 0.7[c]

[a]Data are shown as %($x \pm s$).
[b]$P < .05$ (compared with ^{60}Co group).
[c]$P < .01$ (compared with ^{60}Co group).

The Effect of ^{125}I Seeds on Cell Cycle of PC3 Cells

The cell cycle of PC3 cells was detected by flow cytometry at 48 hours after 4 Gy radiation. As shown in Figure 3, there was a significant increase in the percentage of G_2 cells after ^{60}Co γ ray or ^{125}I irradiation. As the percentage of G_2 cells increased, the percentage of G_1 cells decreased. The percentage of cells in the S phase remained constant. There is no significant different in cell cycle phase between cells received ^{60}Co γ ray or ^{125}I seeds, indicating that the different

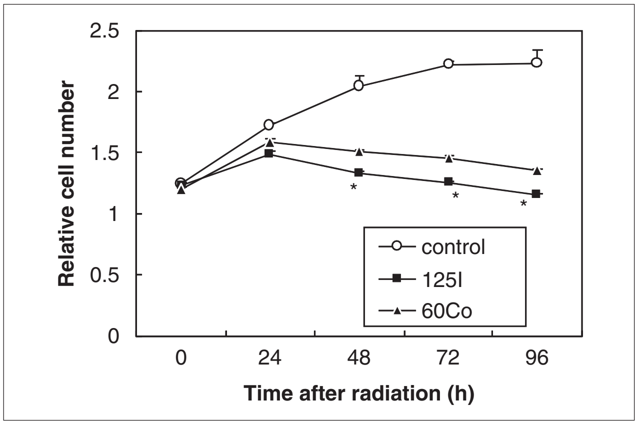

Figure 2. Survival curves of prostate carcinoma (PC3) cells at different time points after high-dose-rate and low-dose-rate irradiation

Cells exposed to 4 Gy of ^{125}I seeds or ^{60}Co γ rays. *$P < .05$ compared with cells exposed to ^{60}Co γ rays.

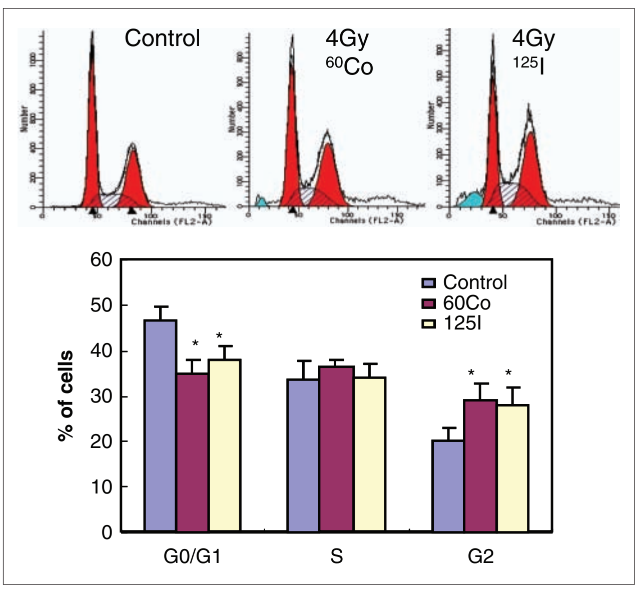

Figure 3. The cell division phase of prostate carcinoma (PC3) cells exposed to either ^{125}I seeds or ^{60}Co γ rays

Cell cycle was determined by flow cytometry. *$P < .05$ compared with cells exposed to ^{60}Co γ rays.

killing efficiencies of ^{125}I and ^{60}Co may not be caused by different effects on cell cycle but in other ways.

Cellular Apoptosis of PC3 Cells Caused by ^{125}I Seeds or ^{60}Co γ Rays

After 48 hours of irradiation, an increased apoptotic rate of PC3 cells was observed when PC3 cells received either ^{60}Co γ rays or ^{125}I irradiation (Figure 4). However, the death rate of PC3 cells exposed to ^{125}I seed irradiation was greater than that of PC3 cells exposed to ^{60}Co γ rays irradiation at 4 Gy.

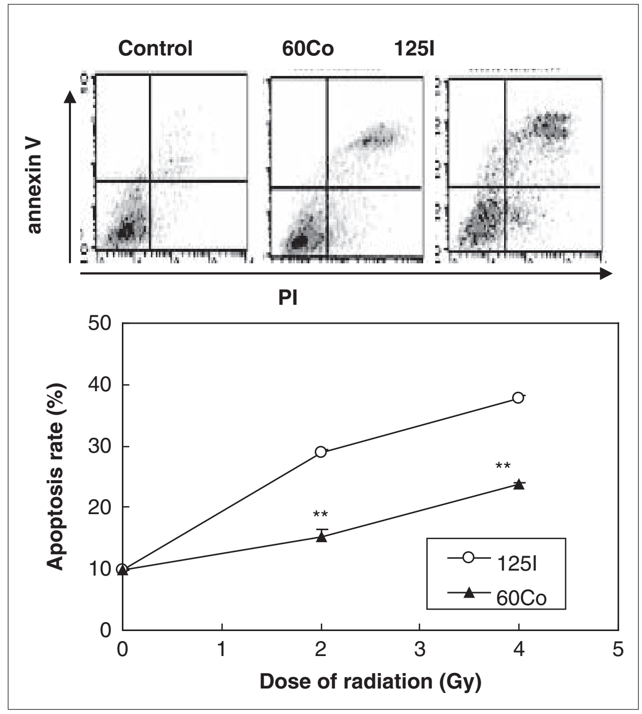

Figure 4. Apoptosis of prostate carcinoma (PC3) cells exposed to either ^{125}I seeds or ^{60}Co γ rays

PC3 cells were exposed to different doses of ^{125}I seed or ^{60}Co γ rays. Cell death was determined by 2-color flow cytometry at 48 hours after radiation. **$P < .05$ compared with cells exposed to ^{60}Co γ rays.

Caspase-3 and Bcl-2 Expression in PC3 Cells After ^{125}I Seeds or ^{60}Co γ Irradiation

To determine the involvement of caspase-3 and Bcl-2 in cell death caused by radiation in the present system, they were detected at 48 hours after radiation. Caspase-3 activity in PC3 cells exposed to ^{125}I seed or ^{60}Co γ ray irradiation did not change significantly (Table 3). However, Bcl-2 gene expression was significantly downregulated in the experimental group by 48 hours after ^{125}I seed or ^{60}Co γ rays irradiation, suggesting the role of Bcl-2 in the present system (Table 4).

Discussion

For the treatment of localized carcinoma of the prostate, both fractionated high-dose-rate radiation—administered as EBRT—and protracted low-dose rate-radiation—administered as brachytherapy—have been used.[17,18]

Table 3. Caspase-3 Activity in Prostate Carcinoma (PC3) Cells at 48 Hours After Irradiation With ^{125}I Seeds or ^{60}Co γ Rays (2 to 8 Gy)[a]

	^{60}Co Group	^{125}I Group
Control	0.54 ± 0.07	0.55 ± 0.03
2 Gy	0.54 ± 0.05	0.54 ± 0.06
4 Gy	0.53 ± 0.09	0.53 ± 0.09
6 Gy	0.53 ± 0.05	0.54 ± 0.04
8 Gy	0.53 ± 0.08	0.52 ± 0.02

[a]Significant differences were not observed compared with the control group.

Table 4. Bcl-2 Expression in Prostate Carcinoma (PC3) Cells at 48 Hours After ^{125}I Seeds or ^{60}Co γ Rays Irradiation ($x \pm SD$)

	^{60}Co Group	^{125}I Group
Control	15.4 ± 0.4	15.4 ± 0.4
2 Gy	13.1 ± 0.3	13.1 ± 0.9a
4 Gy	11.1 ± 0.3	10.1 ± 0.8a
6 Gy	7.2 ± 0.9	6.9 ± 0.7a
8 Gy	4.3 ± 0.5	4.1 ± 0.5a

[a]$P < .01$ (compared with the control group).

Furthermore, each treatment method has recently benefited from technical innovations that have the potential to reduce treatment-associated side effects. For EBRT, the use of conformal radiation therapy permits more accurate targeting of tumor tissue, and as a result, there is an increased total radiation exposure to the affected areas and decreased radiation exposure to normal tissues, such as the rectum and bladder, which are adjacent to the targeted prostate region.[19] Similarly, for brachytherapy, technical improvements in planning and accurate placement of interstitial seed implants also permit increased radiation exposure to the tumor tissue with relatively lesser effects on normal tissues. It is important to determine the radiobiological implications of using new radioisotopes such as ^{125}I seeds. In our study, we assessed RBE, cell cycle, and apoptosis of the androgen-independent PC3 cells after exposure to brief high-dose-rate radiation and to protracted low-dose-rate radiation exposure, which are analogous to EBRT and seed brachytherapy, respectively.

We determined the cell-killing efficacy of ^{125}I seeds and ^{60}Co γ rays and investigated their effects on cell proliferation using colony-forming assay, which is the conventional method used in such analysis. Our study revealed that PC3 cells were more sensitive to prolonged low-dose-rate radiation treatment than to high-dose-rate radiation treatment. On the basis of the radiobiological parameter results, we observed that the CLDR irradiation administered by ^{125}I seeds was more beneficial when compared with high-dose-rate irradiation.

Most studies have shown that the RBE of ^{125}I seeds ranged from 1.3 to 1.5. The results of our study are consistent with those of the previous reports.[20-22] A mechanism that has been proposed for the increased sensitivity of cancer cells postirradiation is their arrest in the G_2 phase.[23-26] However, our present study did show significant difference for cell cycle phase between PC3 cells exposed to ^{125}I seeds and those exposed to ^{60}Co γ rays. However, more significantly enhanced cell death was caused by ^{125}I seeds than ^{60}Co γ rays in PC3 cells. Thus, our results indicate that apoptosis may be playing a central role in the cytotoxic effects of radiation when cells are exposed to CLDR irradiation by ^{125}I seeds.

A key molecular mechanism of radiation-induced cell death is via the activation of the apoptotic pathway.[27-30] Apoptosis represents an efficient and ubiquitous cellular-suicide pathway that involves the activation and inhibition of complex genetic events in response to cytotoxic stimuli. Triggering of the apoptotic process is regulated by several intracellular factors, such as the members of the Bcl-2 gene family, bax, cytochrome C, and caspases.[31-37] Our results indicated that Bcl-2 expression was significantly downregulated after ^{125}I seed or ^{60}Co γ ray irradiation (2, 4, 6, and 8 Gy). The caspase-3 activity in PC3 cells exposed to ^{125}I seed or to ^{60}Co γ rays did not change significantly 48 hours after irradiation. It is essential to confirm that CLDR increased the radiosensitivity of PC3 cells via the Bcl-2 signal transduction pathway.[38-41]

In summary, our present data determined that the RBE of ^{125}I seeds in PC3 cells is 1.4. The enhanced RBE by ^{125}I seeds may be mainly caused by enhanced cell death but not by alteration of cell cycle. This study offered basic radiation biological parameters for ^{125}I seeds in prostate cancer PC3 cells and supporting beneficial effects of using CLDR irradiation.

Acknowledgments

The authors wish to thank Dr Dong-Mei Tian for his critical reading of the manuscript, Ms Jing Wang and Ms Jian-Xia Peng for their expert technical assistance, and Ms Qing-Huan Li for her excellent laboratory management.

Declaration of Conflicting Interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research and/or authorship of this article:

National Natural Science Foundation of China (Grant No. 39900167)

References

1. Yu Y, Anderson L, Li ZF, et al. Permanent prostate seed implant brachytherapy: Report of the American Association of Physicists in Medicine Task Group No. 64. *Med Phys.* 2005;26:2054-2076.
2. Harrison LB. Application of brachytherapy in head and neck cancer. *Semin Surg Oncol.* 1997;13:177-184.
3. Purohit RS, Shinohara K, Meng MV, Carroll PR. Imaging clinically localized prostate cancer. *Urol Clin North Am.* 2003; 30:279-293.
4. Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ. Cancer statistics. *CA Cancer J Clin.* 2003;53:5-26.
5. Montemaqqi P, Dobelbower R, Crucitti F, et al. Interstitial brachytherapy for pancreatic cancer: report of seven cases treated with ^{125}I and a review of the literature. *Int J Radiat Oncol Biol Phys.* 1991;21:451-457.
6. Nath R, Anderson LL, Luxton G, Weaver KA, Wilianson JF, Meigooni AS. Dosimetry of interstitial brachytherapy sources: Recommendations of the AAPM Radiation Therapy Committee Task Group No. 43. *Med Phys.* 1995;22:209-234.
7. Reniers B, Vynckier S, Verhaegen F. Theoretical analysis of microdosimetric spectra and cluster formation for Pd-103 and I-125 photon emitters. *Int J Radiat Oncol Biol Phys.* 2004; 49:3781-3795.
8. Yue N, Chen Z, Nath R. Edema-induced increase in tumour cell survival for I-125 and Pd-103 prostate permanent seed implants a mathematical model. *Int J Radiat Oncol Biol Phys.* 2005;47:1185-1204.
9. Nickers P, Coppens L, Jansen N, Deneufbourg JM. ^{192}Ir low dose rate brachytherapy for boosting locally advanced prostate cancers after external beam radiotherapy: a phase II trial. *Radiother Oncol.* 2006;79:329-334.
10. Merrick GS, Butler WM, Galbreath RW, Lief JH. Five-year biochemical outcome following permanent interstitial brachytherapy for clinical T1-T3 prostate cancer. *Int J Radiat Oncol Biol Phys.* 2001;51:41-48.
11. Richard E, Grimm PD, Blasko JC, Sylvester JE, Meier RM, Cavanagh W. 10-year biochemical (prostate specific antigen) control of prostate cancer with ^{125}I brachytherapy. *Int J Radiat Oncol Biol Phys.* 2001;51:31-40.
12. Rivard MJ, Butler WM, Devlin PM, et al. American Brachytherapy Society recommends no change for prostate permanent implant dose prescriptions using iodine-125 or palladium-103. *Brachytherapy.* 2007;6:34-37.
13. Armpilia CI, Dale RG, Coles IP, et al. The determination of radiobiologically optimized half-lives for radio nuclides used in permanent brachytherapy in plants. *Int J Radiat Oncol Biol Phys.* 2003;55:378-385.
14. Aird G, Folkard M, Mayes CR, Bownes PJ, Lawson JM, Joiner MC. A purpose built iodine-125 plaque for low dose rate low energy irradiation of cell lines in vitro. *Br J Radiol.* 2001;74:56-61.
15. Ling CC, Li WX, Anderson LL. The relative biological effectiveness of I-125 and Pd-103. *Int J Radiat Biol Phys.* 1995;32:373-378.
16. Sambrook J, Russell D. *Molecular Cloning: A Laboratory Manual*. 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2000:1235-1262.
17. King CR, Dipetrillo TA, Wazer DE. Optimal radiotherapy for prostate cancer: predictions for conventional external beam, IMRT, and brachytherapy from radiobiologic models. *Int J Radiat Oncol Biol Phys.* 2001;46:165-172.
18. Merrick GS, Butler WM, Galbreath RW, Lief JH, Adamovich E. Relationship between percent positive biopsies and biochemical outcome after permanent interstitial brachytherapy for clinically organ-confined carcinoma of the prostate gland. *Int J Radiat Oncol Biol Phys.* 2002;52:664-673.
19. Nickers P, Thissen B, Jansen N, Deneufbourg JM. ^{192}Ir or ^{125}I prostate brachytherapy as a boost to external beam radiotherapy in locally advanced prostatic cancer: a dosimetric point of view. *Radiat Res.* 2006;78:47-52.
20. Wang JZ, Mayr NA, Nag S, et al. Effect of edema relative biological effectiveness and dose heterogeneity on prostate brachytherapy. *Med Phys.* 2006;33:1025-1032.
21. Lehnert S, Reniers Brigitte, Verhaegen F. Relative biologic effectiveness in terms of tumor response of ^{125}I implants compared with ^{60}Co gamma rays. *Int J Radiat Oncol Biol Phys.* 2005;63:224-229.
22. Ichard T. Relative biological effectiveness enhancement of a ^{125}I brachytherapy seed with characteristic X rays from its constitutive materials. *Med Phys.* 2002;29:1397-1402.
23. Mitchell R, Joiner C. Effect of subsequent acute-dose irradiation on cell survival in vitro following low dose-rate exposures. *Int J Radiat Biol*. 2002;78:981-990.
24. Scott SL, Gumerlock PH, Beckett L, Goldberq Z. Survival and cell cycle kinetics of human prostate cancer cell lines after single and multifraction exposures to ionizing radiation. *Int J Radiat Oncol Biol Phys.* 2004;59:219-227.
25. Enns L, Bogen KT, Wizniak J, Murtha AD, Weinfeld M. Low-dose radiation hypersensitivity is associated with p53-dependent apoptosis. *Mol Cancer Res.* 2004;2:557-566.
26. Joiner MC, Marples B, Lambin P, Short SC, Turesson I. Low-dose hypersensitivity: current status and possible mechanisms. *Int J Radiat Oncol Biol Phys.* 2001;49:379-389.
27. Nakamura H, Yasui Y, Saito N, Tachibana A, Komatsu K, Ishizaki K. DNA repair defect in AT cells and their hypersensitivity to low-dose-rate radiation. *Radiat Res.* 2006;165: 277-282.
28. Collis SJ, Schwaninger JM, Ntambi AJ, et al. Evasion of early cellular response mechanisms following low level radiation-induced DNA damage. *J Biol Chem.* 2004,279: 49624-49632.
29. Carlsson J, Håkansson E, Eriksson V, et al. Early effect of low dose-rate radiation on cultured tumor cells. *Cancer Biother Radiopharm.* 2003;18:663-670.

30. Krueqer SA, Joiner MC, Weinfeld M, Piasentin E, Marples B. Role of apoptosis in low-dose hyper-radiosensitivity. *Radiat Res.* 2007;167:260-267.
31. Michael, Wang L, Handly JR. Ionizing radiation-induced mitogen-activated protein (MAP) kinase activation in DU-145 prostate carcinoma cells: MAP kinase inhibition enhances radiation-induced cell killing and G2/M-phase arrest. *Radiat Res.* 2000;153:371-383.
32. Tang DG, Li L, Chopra DP, Porter AT. Extended survivability of prostate cancer cells in the absence of trophic factors: increased proliferation evasion of apoptosis,and the role of apoptosis proteins. *Cancer Res.* 1998;58:3466-3479.
33. Lowe SL, Rubinchik S, Honda T, Mcdonnell TJ, Dong JY, Norris JS. Prostate-specific expression of Bax delivered by an adenoviral vector induces apoptosis in LNCaP prostate cancer cells. *Gene Ther.* 2001;818:1363-1371.
34. Shi CJ, Zeng HH, Li HW, Yang FG, Wu XQ, Yu LZ. Induction of apoptosis in prostate cancer cell line PC-3 by BBSKE, a novel organoselenium compound, and its effect in vivo [in Chinese]. *Zhonghua Yi Xue Za Zhi.* 2003;83:1984-1988.
35. Szostak MJ, Kaur P, Amin P, Jacobs SC, Kyprianou N. Apoptosis and bcl-2 expression in prostate cancer: significance in clinical outcome after brachytheray. *J Urol.* 2001;165:2126-2130.
36. Bondar VM, McConkey DJ. Anoikis is regulated by BCL-2-independent pathways in human prostate carcinoma cells. *Prostate.* 2002;51:42-49.
37. Keledjian K, Kyprianou N. Anoikis induction by quinazoline based alpha 1-adrenoceptor antagonists in prostate cancer cells: antagonistic effect of bcl-2. *J Urol.* 2003;169:1150-1156.
38. Mirzaie-Joniani H, Eriksson D, Sheikholvaezin A, et al. Apoptosis induced by low-dose-rate radiation. *Cancer.* 2002;94:1210-1214.
39. Kroger LA, Denardo GL, Gumerlock PH, et al. Apoptosis-related gene and protein expression in human lymphoma xenografts (Raji) after low dose rate radiation using 67Cu-217-BAT-Lym-l radioimmunotherapy. *Cancer Biother Radiopharm.* 2001;16:213-225.
40. Yacoub A, Mckinstry R, Hinman D, Chung T, Dent P, Hagan MP. Epidermal growth factor and ionizing radiation up-regulate the DNA repair gens XRCC1 and ERCC1 in DU145 and LNCaP prostate carcinoma through MAPK signaling. *Radiat Res.* 2003;159:439-452.
41. Bromfield GP, Meng A, Warde P, Bristow RG. Cell death in irradiated prostate epithelial cells: role of apoptotic and clonogenic cell kill. *Prostatic Dis.* 2003;61:73-85.