Supplementary information

Pulsed low-dose rate radiotherapy has an improved therapeutic effect on abdominal and pelvic malignancies

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Materials and methods

Cell lines

Human pancreatic cancer cell lines PANC-1, BXPC-3 and CFPAC-1 were purchased from Procell Life Science &Technology Co., Ltd.(Wuhan, China), human prostate cancer cell lines PC3, 22Rv1 and DU145 were purchased from Chinese Academy of Sciences (Beijing, China), human normal pancreatic cells HPDE6-C7 was purchased from BeNa Culture Collection (Suzhou, China), human normal prostate cells WPMY-1 was purchased from KeyGEN Biotechnology Co., Ltd. (Nanjing, China), human normal colorectal cells FHC was a gift from the Jiangsu Cancer Institute. PANC-1, CFPAC-1, PC3, DU145, HPDE6-C7 and WPMY-1 cells were cultured in dulbecco's modified eagle medium (DMEM) with 10% fetal bovine serum (FBS, HyClone, USA) and 1% penicillin-streptomycin (Beyotime, China). BXPC-3, 22Rv1 and FHC cells were cultured in RPMI 1640 medium with 10% FBS and 1% penicillin-streptomycin.

Radiation treatment

Cancer cells and normal cells in exponential growth phase were digested with trypsin solution (Beyotime, China) and inoculated into 6-well plates. Conventional culture was suspended until cells adhere to the wall, then all of cancer cells were one-off irradiated with 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1.0 and 2.0 Gy, respectively, at a dose rate of 100 cGy/min, normal cells were irradiated with 2 Gy, which divided evenly into two 1 Gy with different intervals at a dose rate of 300 cGy/min. All cells were irradiated with 6MeV-X ray and the source-surface distance was 100 cm. Besides, the surface of plates was covered with 1cm thick tissue equivalent filler.

Colony formation assay

After digesting with trypsin solution, cancer cells and normal cells in the exponential growth phase were seeded in 6-well plates with appropriate cell density. Cells were irradiated as described above. After radiation, cells were cultured until the visible colonies formed about 10~14 days later. Then cell clones were fixed with 4% paraformaldehyde (VICMED, China) and stained with crystal violet (Beyotime, China). All colonies with over 50 cells were counted. The plating efficiency (PE) was calculated as dividing the number of colonies by the number of

cells plated. Survival fraction (SF) was calculated as dividing the number of colonies of experimental group by the number of colonies of control group. The relevant parameters such as α_s, α_r, dc, β, T1/2 and surviving curves were obtained according to the induced repair model by GraphPad Prism 5.0 software. Each experiment was repeated three times.

Establishment and treatment of animal models

All experiments *in vivo* were carried out according to the guidelines of Jiangsu Council on Animal Care. Male BALB/c nude mice (Vital River, China), 4 to 6 weeks old, were adaptively reared at SPF level Laboratory Animal Center for one week before the experiment began. Xenograft tumors were inoculated in the distal right lower extremity of mice by subcutaneous injection of 5×10^6 PC-3 or PANC-1 cells in 200 μL serum-free DMEM medium. When the volume of subcutaneous tumors reached 100–150 mm^3, the mice were randomly divided into three groups (five per group) for different treatments: control group (without any treatment), conventional radiotherapy group (RT group, 2 Gy/d×3 d at dose rate of 300 cGy/min) and PLDR group (radiotherapy scheme depended on the results of experiments *in vitro*). Only the tumor area was irradiated and the rest of the body was protected by lead shielding. Tumor volumes were calculated by measuring two perpendicular diameters with a calliper every 3 days and by using the formula $V=0.5\times a\times b^2$, where *a* and *b* are the larger and smaller diameters, respectively. Mice were weighted every week. When tumor volumes reached 3000 mm^3 or the surface of tumor ulcerated, mice were euthanized by overdose of carbon dioxide. Tumor growth curves, overall survival curves and body weight change curves of tumor-bearing mice were obtained by GraphPad Prism 5.0 software.

Western Blot

Western blot was used to evaluate the effect of RT and PLDR schedules on the expression levels of DNA damage proteins in human prostate cancer calls (PC3) and human pancreatic cancer cells (PANC-1). The adherent PC3 and PANC-1 cells seeded in 6-well plates were irradiated according to the RT and PLDR schedules as shown below: RT schedule - both PC3 and PANC-1 cells were irradiated with 6 Gy singly at dose rate of 300 cGy/min; PLDR schedule - PC3 cells were irradiated with 6 Gy, which was delivered using 0.2 Gy separated by 2.5 min at dose of 100 cGy/min and PANC-1 cells were irradiated with 6 Gy, which was delivered using 0.2 Gy separated by 3.5 min at dose of 100 cGy/min. Different time points after irradiation, cells were harvested for western blot as previous study. Briefly, membranes were incubated with following primary antibodies at 4℃ overnight: DNA-PKcs (Bioworld, BS1092), KU70 (Proteintech, 10723-1-AP), KU80 (Proteintech, 16389-1-AP), γ-H_2AX (Cell Signaling Technology, 7631S) and GAPDH (Proteintech, 60004-1-Ig). Secondary antibodies were obtained from Vicmed (VA001 or VA002) and incubated with membranes for 1 h at room temperature. Each western blot was repeated three times. The band intensity was analyzed by Image J software.

Statistical Analysis

Statistical analysis was performed using statistical software packages SPSS 25.0. Quantitative data was expressed in mean±standard deviation (SD) (x±s), statistically significant difference was determined using two-sample *t*-tests. Repeated measurement variance analysis was used to compare the differences of tumor volume and body weight of tumor-bearing mice at different time points. Kaplan-Meier (K-M) analysis was used to assess survival curves and Wilcoxon rank-sum test was used to compare survival curves among groups. a=0.05 was the significant level and P<0.05 was considered statistically significant.