

• Basic Research •

Correlation of DNA-dependent protein kinase catalytic subunit expression to radiosensitivity of non-small cell lung cancer cell lines

Yi Pan,¹ Wei-Xiong Li,¹ Jun-Ming Li,¹ Jian-Quan Zhu,² Yi-Qiang Liang¹ and Ai-Lin Guo²

1. Department of Radiation Oncology,
Guangdong Provincial People's Hospital,
Guangzhou, Guangdong, 510080,
P. R. China
2. Department of Research Center,
Guangdong Provincial People's Hospital,
Guangzhou, Guangdong, 510080,
P. R. China

Correspondence to: Wei-Xiong Li
Tel.: 86.20.83827812.51321
Email: weixiong_l@yahoo.com

Grants: Medical Scientific Research Foundation of Guangdong Province (No. A2006014); Natural Science Foundation of Guangdong Province (No. 06020905)

This paper was translated into English from its original publication in Chinese.
Translated by: Ya-Lan Tao and Wei Liu

The original Chinese version of this paper is published in: Ai Zheng (Chinese Journal of Cancer) 28(7); <http://www.cjcsysu.cn/cn/article.asp?id=15838>

Submitted: 2009-01-08
Revised: 2009-03-10

[Abstract] **Background and Objective:** DNA-dependent protein kinase catalytic subunit (DNA-PKcs) plays an important role in repairing irradiation-induced DNA double-strand break (DSB), and affects the radiosensitivity of tissue cells. This study was to detect the expression of DNA-PKcs in different non-small cell lung cancer (NSCLC) cell lines and evaluate its correlation to radiosensitivity. **Methods:** The content and activity of DNA-PKcs in five NSCLC cell lines A549, H1299, L78, PGCL3 and H460 were measured by Western blot and the DNA-PK activity assay. Cell survival was analyzed using clonogenic formation assay. **Results:** The radiosensitivities of five NSCLC cell lines were different. The values of survival fraction at 2 Gy (SF2) were 0.74 in A549 cells, 0.25 in H1299 cells, 0.21 in H460 cells, 0.48 in PGCL3 cells, and 0.58 in L78 cells. The protein levels of DNA-PKcs were 3.26 ± 0.98 in A549 cells, 0.51 ± 0.07 in L78 cells, 0.51 ± 0.11 in H1299 cells, 0.86 ± 0.23 in H460 cells, and 2.60 ± 0.76 in PGCL3 cells. The activity values of DNA-PKcs were 8.30 ± 1.03 in A549 cells, 2.45 ± 0.52 in H1299 cells, 0.11 ± 0.02 in H460 cells, 4.13 ± 0.87 in PGCL3 cells, and 0.42 ± 0.07 in L78 cells. In adenocarcinoma and large cell carcinoma cell lines, SF2 were correlated to DNA-PKcs content ($P < 0.05$, $r = 0.95$) and activity ($P = 0.03$, $r = 0.98$). **Conclusion:** DNA-PKcs is an important factor to predict the radiosensitivity in adenocarcinoma and large cell lung cancer cell lines. **Key words:** DNA-PKcs, lung neoplasm, radiosensitivity

Radiotherapy is one of the main treatments for non-small cell lung cancer (NSCLC), but the radiosensitivity of NSCLC patients of various histological types is different. How to determine radiation dose according to radiosensitivity is an urgent issue. DNA-dependent protein kinase (DNA-PK), a serine/threonine protein kinase activated by double-stranded DNA, plays an important role in repairing radiation-induced double-strand break (DSB) repair. DNA-PK dysfunction leads to the decline of repairing capability and enhancement of radiation sensitivity in cells. DNA-PK is consisted of catalytic subunit (DNA-PKcs) and regulatory subunit (Ku) of DNA-PK. Recent studies suggested that DNA-PKcs may play a more crucial role in DSB repairing.¹⁻² This study was to detect the expression of DNA-PKcs in different NSCLC cell lines and evaluate its correlation to radiosensitivity.

Materials and Methods

Cell lines and cell culture. Lung adenocarcinoma cell lines A549 and H1299, lung squamous carcinoma cell line L78, large lung cell carcinoma cell lines PGCL3 and H460 were provided by Department of Research Center, Guangdong Provincial Peoples Hospital. All cells were routinely cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), penicillin and streptomycin at 37° C in a humidified atmosphere of 5% CO₂, and passaged every 2-3 days.

Radiation parameters. Elekta Precise linear accelerator, which yields 6 MV X-ray at 300cGy/min was used as a source of radiation. The source-to-target distance was 100 cm and the radiation field was 20 cm × 20 cm with 1 cm wax block for dose compensation.

Clone formation assay. Exponentially growing cells were treated with 0.25% trypsin to prepare single-cell suspension. After counted, varying amounts of cells were diluted gradiently according to radiation dose, seeded into 60 mm culture dishes and irradiated. For A549 cell line, 200, 200, 400, 800, 2000 and 6000 cells were irradiated by 0, 1, 2, 4, 6 and 8 Gy, respectively; for other cell lines, 200, 200, 500, 2000, 5000 and 10 000 cells by 0, 1, 2, 4, 6 and 8 Gy, respectively. Then cells were cultured for 10-14 days till colonies appeared. Then the colonies were fixed with methanol for 15 min, and stained with crystal violet for 10 min. The colonies with cell count of >50 were counted. Triplicate parallel experiments were performed. All data were analyzed using GraphPade Prism 5.0 software. Survival curves were plotted as the log of survival fraction versus radiation dose with the single-hit multitarget model. The values of mean lethal dose (D0), quasi-threshold (Dq) and survival fraction at 2 Gy (SF2) were calculated.

Western bolt detection of DNA-PKcs expression. Total protein of exponentially growing cells was extracted. Protein concentration was determined with BCA protein assay (BCA-100, Shanghai Biocolor BioScience & Technolgy Company). Total protein (60 μ g)

were separated on 8% SDS-polyacrylamide gel and transferred onto polyvinylidene difluoride membrane. The membranes were blocked with 5% degreased milk for 1 h, and incubated with DNA-PKcs monoclonal antibody ab1832 (ABcam, UK; 1:1000) and mouse anti-GAPDH monoclonal antibody (BOAO Biotech CO., Ltd., 1:3000) at 4°C overnight, respectively, then washed with TPBS for 5 min for 3 times and incubated with HRP-labeled goat anti-mouse secondary antibody SB100 (Jingmei Biotech, 1:6000) at room temperature for 1 h, washed with TPBS again for 3 times, exposed, developed and fixed. GAPDH was used as internal control. Integrated optical density (IOD) of the bands was analyzed with BioGene automatic image analysis system to measure relative expression level of DNA-PKcs protein.

DNA-PK activity detection. Cells were treated with 0.25 % trypsin, centrifuged at 1500 r/min at 4°C for 5 min. The cell pellets were re-suspended in a buffer containing 50 mmol/L NaF, 40 mmol/L NaCl, 20 mmol/L HEPES (PH=7.6), 25% W/V glycerol, 0.2 mmol/L EDTA, 0.5 mmol/L DTT, 0.5 mmol/L PMSF, 0.5 μ g/mL PepstainA, 1 mg/mL Trypsin inhibitor, 0.5 μ g/mL Aprotinin, and 1 μ g/mL Leupeptin, then placed in liquid nitrogen for 1 min incubated at 30°C for 1 min (repeated 3 times). The mixture was centrifuged at 1500 r/min for 5 min at 4°C. The supernatant was retained for DNA-PK activity detection using the TECT DNA-PK assay kit according to the manufacturers protocol (Promega, V7870).

Statistical analysis. SPSS12.0 software was used for statistical analysis. All data are expressed as mean ± SD. Results were analyzed using Pearson correlation.

Results

Cell survival. The D0 values were (1.62 ± 0.21) Gy for A549 cells, (1.19 ± 0.09) Gy for H1299 cells, (1.29 ± 0.13) Gy for H460 cells, (1.53 ± 0.04) Gy for PGCL3 cells, and (2.40 ± 0.16) Gy for L78 cells; the Dq values were (2.30 ± 0.33) Gy for A549 cells, (0.43 ± 0.15) Gy for H1299 cells, (<0.01 ± 0.00) Gy for H460

Table 1 Effect of irradiation on survival of non-small cell lung cancer cell lines

Cell line	Survival rate (%)					
	0 Gy	1 Gy	2 Gy	4 Gy	6 Gy	8 Gy
A549	100±0.00	82.61± 5.89	74.12±7.77	28.13±16.20	12.22±3.85	2.36±0.86
H1299	100±0.00	52.83±18.00	24.73±2.25	3.87± 1.49	1.22±0.06	0.36±0.08
H460	100±0.00	45.38± 5.23	20.51±2.95	4.72± 0.39	0.61±0.14	0.08±0.03
PGCL3	100±0.00	70.92±10.32	47.58±0.79	10.33± 1.05	4.05±0.37	1.44±0.19
L78	100±0.00	78.22± 5.35	58.24±2.09	30.54± 1.35	11.12±1.72	4.75±0.31

All values are presented as mean±SD of three experiments.

cells, (1.15 ± 0.02) Gy for PGCL3 cells, and (1.02 ± 0.07) Gy for L78 cells; the SF2 values were (0.74 ± 0.08)% for A549 cells, (0.25 ± 0.02)% for H1299 cells, (0.21 ± 0.03)% for H460 cells, (0.48 ± 0.01)% for PGCL3 cells, and (0.58 ± 0.02)% for L78 cells (Table 1).

Protein expression of DNA-PKcs. The relative expression level of DNA-PKcs were 3.26 ± 0.98 in A549 cells, 0.51 ± 0.11 in H1299 cells, 0.86 ± 0.23 in H460 cells, 2.60 ± 0.76 in PGCL3 cells, and 0.51 ± 0.07 in L78 cells (Fig. 1). When analyzing the five cell lines, no correlation between SF2 and IOD radio was found (p=0.23); when analyzing A549, H1299, H460, and PGCL3 cell lines, SF2 was correlated to IOD radio (p<0.05, r=0.95).

Activity of DNA-PK . The activity values of DNA-PKcs were 8.30 ± 1.03 in A549 cells, 2.45 ± 0.52 in H1299 cells, 4.13 ± 0.87 in PGCL3 cells, 0.11 ± 0.02 in H460 cells, and 0.42 ± 0.07 in L78 cells. When analyzing the five cell lines, no correlation between DNA-PKcs activity and SF2 was found (p=0.21); when analyzing A549, H1299, H460, and PGCL3 cell lines, SF2 were positively correlated to DNA-PKcs activity (p=0.03, r=0.98), suggesting that A549 cells with the highest DNA-PKcs activity was radio-resistant, while H460 cells with the lowest DNA-PKcs activity was radiosensitive.

Discussion

DNA is the main target of radiation. DSB is the important mechanism of killing cells by irradiation.³ DSB repairing capability of tissues directly affects their radiosensitivity. DNA-PK, an essential enzyme of DSB repairing, consists of the catalytic subunit DNA-PKcs and the

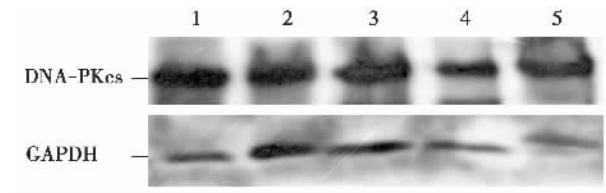


Figure 1 The expression of DNA-dependent protein kinase catalytic subunit protein in non-small cell lung cancer cell lines detected by Western blot

Lane 1: A549 cells; lane 2: L78 cells; lane3: H1299 cells; lane 4: H460 cells; lane 5: PGCL3 cells.

regulatory subunit Ku.

DNA-PKcs consists of 4127 amino acid residues, weighs about 470 ku, and its gene locates at 8q11, which is involved in DSB recognition and repair via combing with a heterodimeric component of Ku. DNA-PKcs could be activated by DNA terminal without Ku and directly connected both ends of DNA breaks,^{1,2} suggesting that DNA-PKcs may play a more important role than Ku in DSB repairing. Anderson et al.⁴ analyzed gene sequence of radiosensitive malignant glioma cell line M059J, and found that chromosome 8 showed no expression and activity of DNA-PKcs, while homologous wild-type cell line M059K exhibited normal DNA-PK activity and radiosensitivity. Beamish et al.⁵ found that gene mutation of DNA-PKcs induced severe combined immunodeficiency in mice, and cells showed deficiency in DSB repair and high radiosensitive. Ortiz et al.⁶ presumed that up-regulated DNA-PKcs expression was the phenotype of radioresistant bladder carcinoma cells. All researches prompted that DNA-PKcs is closely associated with radiosensitivity.

The five NSCLC cell lines in our study showed different radiosensitivity: large cell

carcinoma cell line H460 was the most radiosensitive; adenocarcinoma cell line A549 was radio-resistant. Cell lines of the same histological type also showed different radiosensitivity: between two adenocarcinoma cell lines, H1299 was more radiosensitive than A549; between two large cell carcinoma cell lines, H460 was more radiosensitive than PGCl3. The five NSCLC cell lines also showed different DNA-PKcs protein expression: adenocarcinoma A549 cells showed the highest DNA-PKcs content; adenocarcinoma H1299 cells and squamous carcinoma L78 cells showed the lowest DNA-PKcs content. Cell lines of the same histological type also showed different DNA-PKcs content: between two large cell carcinoma cell lines, PGCl3 showed higher DNA-PKcs content than H460; between two adenocarcinoma cell lines, A549 showed higher DNA-PKcs content than H1299. In adenocarcinoma and large cell carcinoma cell lines, SF2 was positively correlated to DNA-PKcs content ($p < 0.05$, $r = 0.95$) and activity ($p = 0.03$, $r = 0.98$). This result suggests that DNA-PKcs is an important factor to predict the radiosensitivity of adenocarcinoma and large cell carcinoma cell lines. Qu et al.⁷ got the same results when studying the relationship between the radiosensitivity of nasopharyngeal carcinoma cell lines and DNA-PKcs. Zhuang et al.⁸ found that SF2 was positively correlated to DNA-PKcs content, but not related with Ku80 in cervical cancer and breast cancer cell lines, suggesting that DNA-PKcs expression level could better represent cell radiosensitivity than Ku80 expression level.

However, in our study, when the analyses included squamous carcinoma cell line, SF2 had no correlations to DNA-PKcs content and activity. Squamous carcinoma L78 cells were radioresistant although they had low DNA-PKcs

content and activity, suggesting that DNA-PKcs is not the only factor affecting the radiosensitivity of cells. We did not further explore whether this result was related to histological type of cells or one specific cell line because only one squamous carcinoma cell line was used in this study.

In conclusion, DNA-PKcs is an important factor that affect the radiosensitivity of NSCLC cells, and may be a potential target of gene therapy to increase the radiosensitivity of NSCLC.

References

- [1] Hammarsten O, Chu G. DNA-dependent protein kinase: DNA binding and activation in the absence of Ku [J]. *Proc Natl Acad Sci USA*, 1998,95(2):525-530.
- [2] West RB, Yaneva M, Lieber MR. Productive and nonproductive complexes of Ku and DNA-dependent protein kinase at DNA termini [J]. *Mol Cell Biol*, 1998,18(10):5908-5920.
- [3] Yanold J. Molecular aspects of cellular responses to radiotherapy [J]. *Radiother Oncol*, 1997,44(1):1-7.
- [4] Anderson CW, Dunn JJ, Freimuth PI, et al. Frameshift mutation in PRKDC, the gene for DNA-PKcs, in the DNA repair-defective, human, glioma-derived cell line M059J [J]. *Radiat Res*, 2001,156(1):2-9.
- [5] Beamish HJ, Jessberger R, Riballo E, et al. The C-terminal conserved domain of DNA-PKcs, missing in the SCID mouse, is required for kinase activity [J]. *Nucleic Acids Res*, 2000,28(7):1506-1513.
- [6] Ortiz T, Lopez S, Burguillos MA, et al. Radiosensitizer effect of wortmannin in radioresistant bladder tumoral cell lines [J]. *Int J Oncol*, 2004,24(1):169-175.
- [7] Qu S, Zhu XD, Li DR, et al. Relationship between the expression of DNA-PKcs and radiosensitivity in nasopharyngeal carcinoma cell lines [J]. *Zhong Liu Fang Zhi Yan Jiu*, 2007,34(4):237-240. [in Chinese]
- [8] Zhuang L, Yu SY, Huang XY, et al. Potentials of DNA-PKcs, Ku80, and ATM in enhancing radiosensitivity of cervical carcinoma cells [J]. *Ai Zheng*, 2007,26(7):724-729. [in Chinese]