Acta Physiologica Sinica, August 25, 2007, 59 (4): 524-533
http://www.actaps.com.cn

Research Paper

DNA-dependent protein kinase activity and radiosensitivity of nasopharyngeal carcinoma cell lines CNE1/CNE2

HE Yu-Xiang[1,2], ZHONG Ping-Ping[1,2], YAN Shan-Shan[1,2], LIU Li[1,2], SHI Hong-Liu[1], ZENG Mu-Sheng[1], XIA Yun-Fei[1,2,*]

[1]State Key Laboratory of Oncology in Southern China; [2]Department of Radiation Oncology, Cancer Center, Sun Yat-Sen University, Guangzhou 510060, China

Abstract: The present study investigated the relationship between DNA-dependent protein kinase (DNA-PK) and radiosensitivity of nasopharyngeal carcinoma (NPC) cell lines. The dose-survival relationship for NPC cell lines, CNE1 and CNE2, was analyzed using clonogenic formation assay, the activity of DNA-PK of the two cell lines was measured using the Signa TECT DNA-PK assay kit, and the localization and expression of Kus (a heterodimer) and DNA-PKcs protein in CNE1 and CNE2 before irradiation and 15 min, 1 h, 6 h, 12 h, 24 h after 4 Gy irradiation were analyzed by immunofluorescence, laser scanning confocal microscope (LSCM) and Western blot. The results showed that the surviving fraction of CNE1 was higher than that of CNE2 at each dose. The DNA-PK activity of CNE1 was also significantly higher than that of CNE2 before and after irradiation ($P<0.05$), while the expression of total Ku70/Ku80 in CNE1 and CNE2 had no significant difference. Increasing translocation of Ku70 and Ku80 from the cytoplasm to the nuclei in the two cell lines was observed with increase of irradiation time as detected by Western blot, and the immunofluorescence of the DNA-PK complex subunits showed greater nuclear translocation in CNE1 than CNE2 after irradiation. The results suggest that the relatively higher radio-resistance of CNE1 correlates with the higher activity of DNA-PK as compared to that of more radiosensitive CNE2 (or lower radio-resistance) before and after irradiation. Thus, DNA-PK activity may be a useful predictor of radiosensitivity of NPC.

Key words: nasopharyngeal carcinoma; CNE1/CNE2; radiosensitivity; DNA-dependent protein kinase

DNA-PK 的活性与鼻咽癌细胞株 CNE1/CNE2 放射敏感性的关系

贺玉香[1,2]，仲萍萍[1,2]，严珊珊[1,2]，刘 莉[1,2]，史弘流[1]，曾木圣[1]，夏云飞[1,2,*]

中山大学肿瘤医院[1] 华南肿瘤学国家重点实验室；[2] 肿瘤防治中心放射生物室，广州 510060

摘 要：本文主要研究 DNA 依赖的蛋白激酶(DNA-dependent protein kinase, DNA-PK)与鼻咽癌细胞放射敏感性之间的关系。克隆形成实验分析鼻咽癌细胞 CNE1/CNE2 的剂量存活曲线，Signa TECT DNA-PK 试剂盒检测 DNA-PK 活性，免疫荧光及激光显微共聚焦分析放疗前及放疗后 15 min、1 h、6 h、12 h 和 24 h CNE1/CNE2 细胞中 Kus 及 DNA-PKcs 的亚细胞定位，Western blot 分析两株细胞中 Kus 蛋白的表达。结果显示：CNE1 细胞在每个剂量点的存活分数均高于 CNE2 细胞；同时发现放疗前后 CNE1 细胞中的 DNA-PK 活性也均高于 CNE2 细胞，但两株细胞中 Ku70/Ku80 蛋白表达无明显差异；放疗可使 DNA-PK 活性增加，且各个检测时间点 CNE1 细胞增加的幅度大于 CNE2 细胞；DNA-PK 亚基可同时定位于胞浆和胞核，但主要位于胞核，细胞照射后 Ku70、Ku80 和 DNA-PKcs 从胞浆转运到胞核。结果表明：DNA-PK 活性更高可能是 CNE1 细胞较 CNE2 细胞更能抵抗放射的原因之一；放疗所致 DNA-PK 活性增高可能与 DNA-PK 亚基从胞浆转运到胞核有关，而与 Ku 蛋白表达的总量无关。

关键词：鼻咽癌；CNE1/CNE2；放射敏感性；DNA 依赖的蛋白激酶
中图分类号：R739.63

Received 2007-05-02 Accepted 2007-07-03

This work was supported by the National Natural Science Foundation of China (No. 30070237) and National High Technology Research and Development Program of China (No. 2006AA02Z4B4).

*Corresponding author. Tel: +86-20-87343545; Fax: +86-20-87343392; E-mail: xiayunfei@yahoo.com

Radiotherapy is a major treatment modality for nasopharyngeal carcinoma (NPC), and about 60% of five-year survival rate can be obtained by radiotherapy alone. Radioresistance of NPC cells to ionizing radiation is one of the main reasons for the failure of the therapy[1]. Therefore, understanding the mechanism underlying the radioresistance of NPC cells can help us develop new radiosensitizing approaches to improve the treatment efficacy.

It has been established that radiosensitivity is related to DNA double-strand break (DSB) repair system. DNA-dependent protein kinase (DNA-PK), which plays an important role in DSB repair system, is a component trimetric enzyme consisting of a 465- kDa serine/threonine protein kinase catalytic subunit (DNA-PKcs), and a heterodimer called Ku, which comprises 70-kDa (Ku70) and 80/86-kDa (Ku80) proteins as a regulatory subunit[2-4]. The defects in DNA-PK components will lead to decreased DNA-PK activity and less DSB repair, thus increased radiosensitivity. For example, the MO59J cell line with deficiency in DNA-PKcs was 10-fold or more radiosensitive than the MO59K cell line with normal expression of DNA-PKcs[5]. Deficiency in DNA-PKcs expression and DNA repair has also been reported leading to increased radiation sensitivity in the BALB/c mouse[6]. Ku, which functions as an initiator of NHEJ (non-homology end joining), is able to bind DNA-DSBs and recruit DNA-PKcs and other repair factors. The importance of Ku in the response to ionizing radiation is apparent from the extremely increased radiation sensitivity along with deficiency in DSB repair whenever there are mutations in one of its subunits[7,8]. Furthermore, several studies reported that antisense nucleotide targeted to either Ku80, Ku70 or DNA-PKcs messenger RNA could sensitize human fibroblasts, M059K malignant glioma cells, and human non-small cell lung cancer (NSCLC) cell lines to ionizing radiation[9-11]. Some researchers have tried to use Ku as a predictor of radiosensitivity and the prognosis of malignant diseases, such as rectal carcinoma, oropharyngeal, hypopharyngeal carcinomas and carcinoma of the cervix[12-14]. The use of DNA-PKcs as a predictor for chemoradiation therapeutic sensitivity, such as esophageal cancer has also been proposed[15]. Lee *et al*.[16] studied the levels of immunoreactivity for Ku70 and DNA-PKcs in pretreatment biopsy specimens from 66 patients with NPC who were then treated with radiotherapy alone or with concurrent chemotherapy. Their results showed that the level of Ku70 expression could be used as a molecular marker to predict the response to radiotherapy in NPC patients. However, the relationship between DNA-PK expression/activity and radiosensitvity has not been carried out under well-controlled laboratory condition for NPC cell lines exhibiting different degree of radiosensitivity.

The NPC cell lines, CNE1 and CNE2, which were both derived from squamous cell carcinoma[17,18], exhibit prominent difference in morphology and biological behavior. It has been demonstrated that CNE2 is more radiosensitive than CNE1[19-21]. Wang *et al*.[22] found that the expression of ATM (ataxia-telangiectasia mutant), a protein of DNA repair, was higher in CNE1 than that in CNE2, and thus it was suggested that ATM was one of the factors leading to radioresistance in CNE1. Since DNA-PK belongs to phosphatidylinositol-3-kinase (PI_3K) family as well as ATM, it is highly possible that DNA-PK be one of the factors affecting radiosensitivity of NPC cell lines CNE1 and CNE2. Therefore, we undertook the present study to investigate the relationship between DNA-PK expression/activity and the known radiosensitivity of NPC cell lines.

1 MATERIALS AND METHODS

1.1 *Cell lines and culture conditions*

CNE1 and CNE2 were provided by the Cancer Center of Sun Yat-sen University. All cells were routinely cultured in RPMI 1640 media supplemented with 10% fetal calf serum (FCS) and antibiotics including 100 U of penicillin per mL and 100 μg of streptomycin per mL. All incubations were performed at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

1.2 *Methods of irradiation*

Exponentially growing cells were treated with 0.25% trypsin to get cell suspension. Then the cells were irradiated with X ionizing radiation of 4 Gy, and with irradiation rate of 104.93 cGy/min at 210 kV and 12 mA. The source-to-target distance was 40 cm and the irradiation field was 10 cm×15 cm with 0.25 Cu filter and 0.52 Cu half values layer. Cells were harvested to be used for laser scanning confocal microscope (LSCM), Western blot analysis and DNA-PK activity assay 0, 15 min, 1 h, 6 h, 12 h and 24 h after irradiation of 4 Gy X-ray.

1.3 *Clonogenic survival assay*

Cells were grown in culture flasks to mid-logarithmic phase with about 60% confluence and fed fresh medium on the day before the experiment. Cells were treated with 0.25% trypsin, single-cell suspensions were prepared, and cell number was counted using a hemocytometer. Cells were then seeded in varying amounts onto 60-mm culture dishes along with nutrient medium. The latter were respectively irradiated with different single dose ranging from 0 to 800

cGy. This was done in triplicate at each dose including 0, 0.5, 1, 2, 4, 6 and 8 Gy. Immediately after irradiation, the treated cells were incubated under the above-described growing condition for 9-14 d until colonies appear. The surviving clones were fixed with methanol and stained with crystal violet. Clones only having a minimum of 50 viable cells were scored. The experiments were repeated at least three times to get an average value. All survival data were analyzed using survival curve program on a microcomputer with Sigma-Plot software in the Department of Radiation Oncology at Sun Yat-sen University Cancer Center. The linear quadratic model was applied to analyze the survival curve data and then the parameters plating efficiency (PE), α, β, survival fraction of 2 Gy (SF2), mean inactivation dose (MID) were calculated.

1.4 *Immunofluorescence*

Cells were seeded (5×10^5 cells) in 40-mm dishes with coverslips (Fisher Scientific), cultured in RPMI 1640 media with 10% FCS at 37 °C overnight. Then cells were fixed in pre-cold 10% paraformaldehyde for 30 min at designed measuring time (0, 15 min, 1 h, 6 h, 12 h and 24 h) after irradiation of 4 Gy ionizing radiation, permeabilized in 0.5% Triton X-100/PBS for 15 min. After an additional wash in PBS, the coverslips crawled with cells were blocked in 10% goat serum in PBS for 30 min, and incubated with anti-Ku70, anti-Ku80 or anti-DNA-PKcs antibodies (BD Biosciences) diluted in 10% goat serum/PBS (1:100 dilution) overnight at 4 °C. After rinsed in PBS for 5 min, coverslips were incubated with FITC-conjugated goat anti-mouse IgG (DAKO) for 1 h in dark and wet box, and incubated with 0.5 μg/mL DAPI (Sigma) to stained the nuclei for 2 min, rinsed in ddH_2O for 5 min, mounted with 80% glycerol/PBS. Confocal images were obtained using a confocal laser microscopy system (Zeiss). HeLa cell was the positive control, and the group with PBS replacing the primary antibody during incubation was regarded as negative control.

1.5 *Western blot analysis*

Sample preparation: the control cells and 4 Gy-irradiated cells were scraped from the dishes in PBS at 0, 15 min, 1 h, 6 h, 12 h and 24 h after irradiation. The collected cells were centrifuged at 4 °C and then the cell pellets were lysed in pre-cooled radio immunoprecipitation (RIPA) buffer (Upstate, Ltd.). The swollen cells were disrupted by incubation on ice for 30 min. The resulting suspension was centrifuged at 15 000 r/min for 10 min at 4 °C, and the clear supernatants were the whole extracts of the cells, stored at -80 °C before use. Protein concentrations were determined with BCA protein assay kit (Piearce, Ltd.). Nuclear-Cytosol Extraction Kit was used to prepare nuclear and cytoplasmic extracts of the cells.

Electrophoresis and transference of the protein: the protein extracts (10 μg of total cell extracts, 15 μg of nuclear or cytoplasmic extracts) were separated on 10% polyacrylamide gel and transferred to polyvinylidene difluoride membranes (Roche Corporation). The membranes were blocked with 5% degreased milk for 1 h at room temperature or overnight at 4 °C. Then, the membranes were incubated with the primary antibodies (all were from Santa Cruz Biotechnology, CA) diluted in Blocking buffer for 3 h at room temperature at the following dilutions: 1:1 000 for Ku70 and Ku80, 1:10 000 for GAPDH, respectively. After that, the membranes were washed and proteins were detected by horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, CA) (1: 2 000) and enhanced chemiluminescence (Amersham Pharmacia Biotech). Equal loading of extracts was confirmed by measuring GAPDH expression. The bands were quantified by densitometric analysis using an apparatus JVC ky-F3OB 3-CCD (Japan) with automatic image analysis system. We introduced the integral optical density (IOD), which was produced by the area of the band multiply the mean light densitometer as a last index.

The experiments of Western blot, which was designed to detect the expression of Kus of CNE1 and CNE2 before and after irradiation, were done in triplicate. The bands normalized to the expression level of HSP70 were measured for its IOD and the results were described with means±SEM. The mean value between two groups was compared using the Student *t*-test.

1.6 *DNA-PK activity assay*

Kinase assays were performed as described previously[23-25] using the Signa TECT DNA-PK assay kit according to the manufacturer's protocol (Promega Inc.), with the following modification: Cell extracts were prepared with ice-cold low salt (LS) buffer (50 mmol/L NaF, 20 mmol/L HEPES/NaOH at pH 7.6, 450 mmol/L NaCl, 25% glycerol, 0.2 mmol/L EDTA, 0.5 mmol/L DTT, 0.5 mmol/L PMSF, 1 μg/mL aprotinin, 1 mg/mL trypsin inhibitor, 1 μg/mL leupeptin, 0.5% NP-40). In order to remove endogenous DNA, the aliquot cell extracts (include 100 μg protein) were mixed with 20 μg dsDNA-cellulose (Sigma Co.) in a total volume of 100 μL Buffer Z (25 mmol/L HEPES/KOH at pH 7.6, 12.5 mmol/L $MgCl_2$, 20% glycerol, 0.1% NP-40, 1 mmol/L DTT and 50 mmol/L KCl). The mixture was incubated at 4 °C for at least 20 h with continuous shaking. And kinase assays were conducted in the following steps.

Prepare the enzyme dilution buffer for the experimental group including DNA-PK biotinylated peptide substrate and for the control group without DNA-PK biotinylated peptide substrate, and djust the final reaction volume to 25 μL with deionized water. After incubating at 30 °C for 5 min, add 15.0 μL enzyme dilution buffer to each sample (25.0 μg protein of each) which involved 0.05 μL radioactivity isotope [γ-^{32}P]ATP (7 000 Ci/mmol; ICN Radiochemical) to activate the reaction. Terminate it by adding 12.5 μL of terminate buffer to each reaction and mix well. Spot 10.0 μL of each terminated reaction onto a SAM^2 biotin capture membrane. After all samples have been spotted, wash the SAM^2 membrane, and the excess free [γ-^{32}P]ATP and nonbiotinylated proteins were removed via a simple washing procedure. After washing, the radioactivity was determined in a liquid scintillation counter (Beckman LS6000). Calculation of DNA-PK activity by using the formula offered in the kit.

1.7 *Statistical method*

Data were expressed as the means±SEM. Results were analyzed for significant differences using SPSS procedures (version 10.0) and Student's *t* tests (Sigma Plot 6.0). Results were considered significant at $P<0.05$.

2 RESULTS

2.1 Radiation dose-survival curve and radiobiological parameters

Radiation dose-survival curve and radiobiological parameters were shown in Fig.1 and Table 1. Obviously, the survival curve of CNE1 was smoother than that of CNE2, indicating higher survival (or more resistance). Also, α value in linear portion of CNE1 was smaller than that of CNE2 and its MID was almost twice of that of CNE2. The data suggested that the CNE2 is intrinsically much more radiosensitive than CNE1.

2.2 DNA-PK activity

The DNA-PK activity was measured by an assay kit. As summarized in Fig.2, before irradiation the DNA-PK activity of CNE1 was higher than that of CNE2 (4.14±0.69 *versus* 0.41±0.26, $P<0.05$). The DNA-PK activity of both

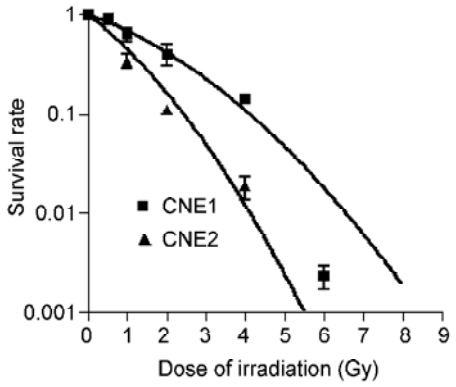


Fig.1. Clonogenic cell survival curves for CNE1 and CNE2 cell lines were generated after exposure to 0, 0.5, 1, 2, 4, 6 and 8 Gy of ionizing radiation.

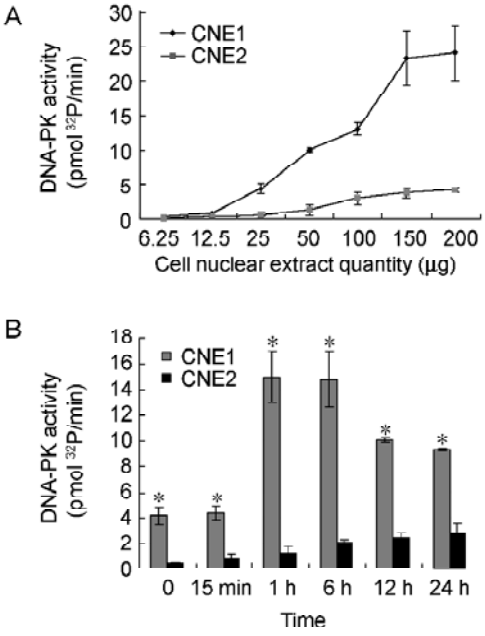


Fig.2. Changes of DNA-PK activity in CNE1/CNE2 cells before and after irradiation. *A*: Relationship between the DNA-PK activity and quantity of cell extracts in CNE1/CNE2 cells. Within 12.5-150 μg of cell extracts, the DNA-PK activity linear increased with the increase of protein quantity. The cumulative means±SEM were shown (n=3). *B*: Changes of DNA-PK activity in CNE1/CNE2 cells before and after 4 Gy X-ray exposure at different time points. The results shown for DNA-PK activity were the means±SEM of three independent experiments. $^*P<0.05$ *vs* CNE2.

Table 1. Dose-survival curve parameters of CNE1 and CNE2

Cell line	PE (%)	$SF2_{read}$ (%)	SEM (%)	$SF2_{est}$ (%)	α (Gy^{-1})	β (Gy^{-1})	MID (Gy)
CNE1	92	37	2.83	54	0.106	0.110	2.345
CNE2	90	16	1.41	17	0.810	0.085	1.111

PE, plating effciency; SF2, survival fraction of 2 Gy; MID, mean inactivation dose.

cell lines increased 1 h after irradiation, while the increase in DNA-PK activity in CNE1 was drastically higher than that of CNE2 ($P<0.05$). Similar results were obtained at all time points examined (Fig.2*B*).

2.3 Protein expressions of Ku70 and Ku80

The protein levels of Ku70 and Ku80 were detected by Western blot in both CNE1 and CNE2 cell lines before and after 4 Gy irradiation (Fig.3). The semi-quantified assay of the protein bands showed that the basic expressions of Ku70 and Ku80 in the two cell lines before irradiation were not significantly different. With 4 Gy irradiation, the Ku protein expression of the total extracts in the two cell lines remained stable, but the levels of Ku of the nuclei extracts were obviously higher than that of pre-irradiation. Notably, the expression of Ku70 in the cytoplasm after irradiation for over 6 h appeared to be consistently lower in CNE1

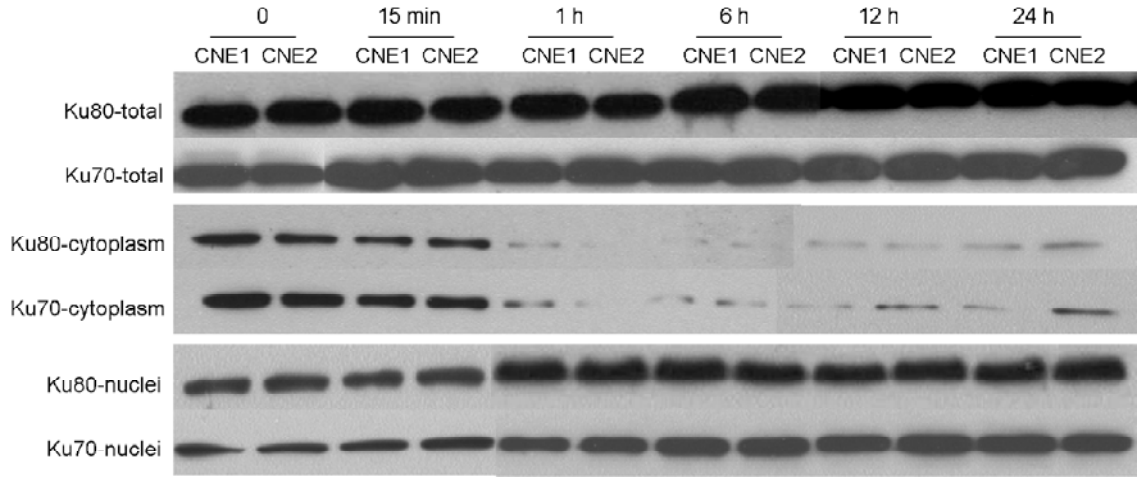
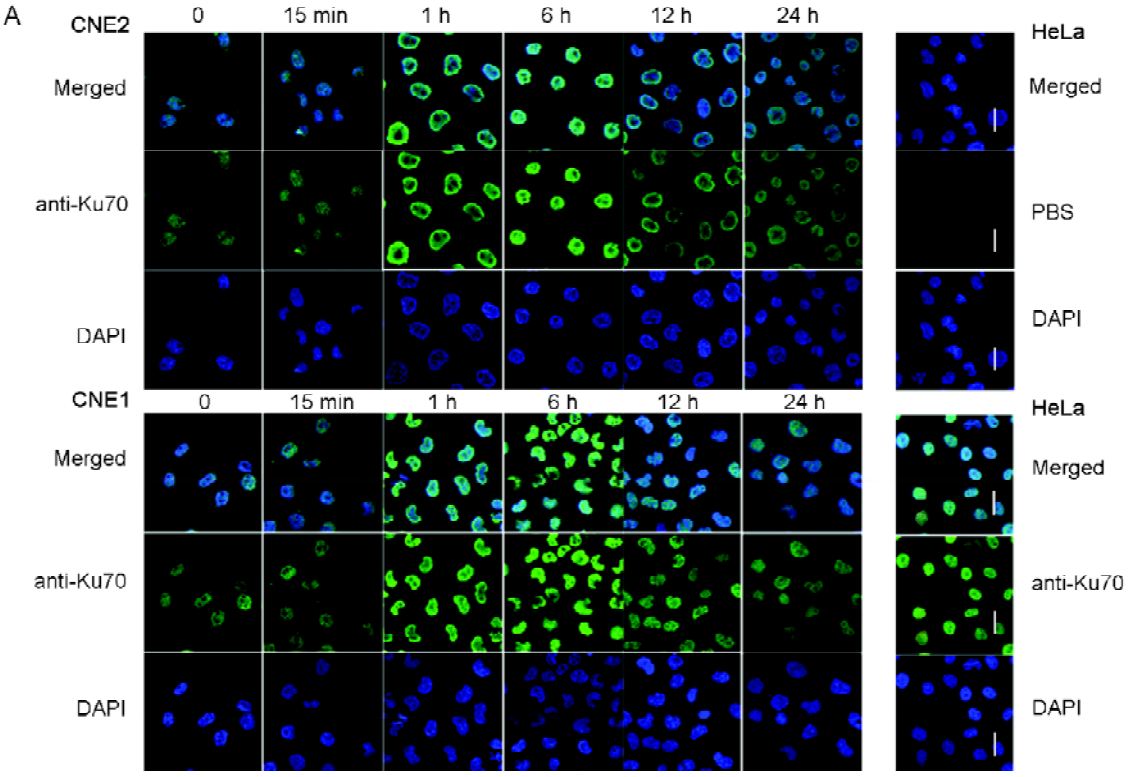


Fig.3. Expression of Ku70 and Ku80 proteins before and after irradiation by Western blot.



(Continued on next page)

(Continued from previous page)

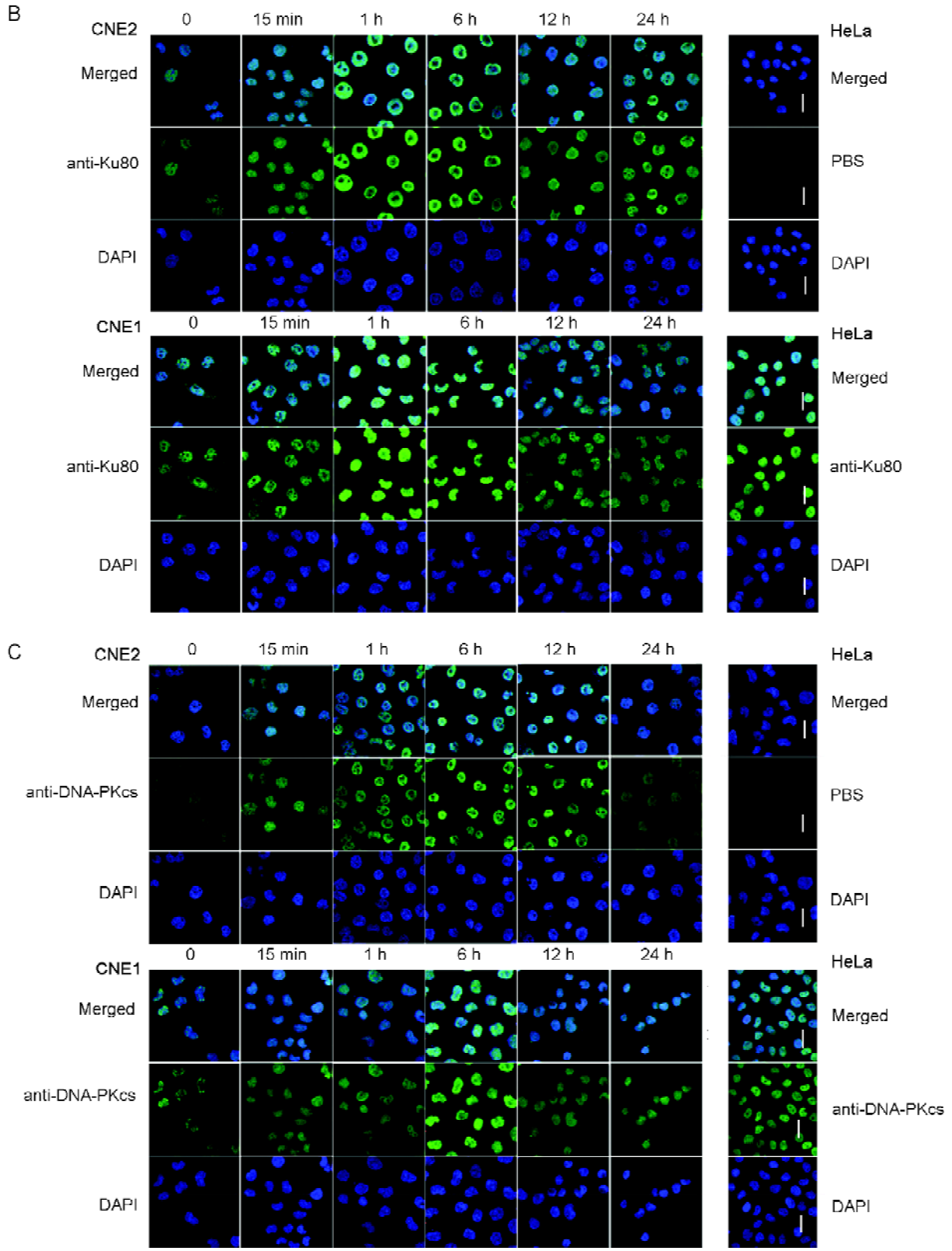


Fig.4. Comparison of time-dependent nuclear translocation of DNA-PK subunits after irradiation between CNE1 and CNE2 by immunofluorescent staining. *A*: Immunofluorescence of Ku70 in CNE1 and CNE2. Scale bar, 20 μm. *B*: Immunofluorescence of Ku80 in CNE1 and CNE2. Scale bar, 20 μm. *C*: Immunofluorescence of DNA-PKcs in CNE1 and CNE2. Scale bar, 20 μm. HeLa cells were used as the positive control, and the PBS replacing the primary antibody during incubation was regarded as negative control.

than that in CNE2 although the difference between the two nuclei fractions in these cells were not obvious.

2.4 Subcellular localization of DNA-PK subunits

Subcellular localization of DNA-PK subunits was examined by immunofluorescence using confocal laser microscopy. All DNA-PK subunits, including Ku70, Ku80 and DNA-PKcs, were localized to both nuclei and cytoplasm before irradiation in both CNE cell lines, which were stained negative in the controls. After irradiation, the intensity of Ku/DNA-PKcs nuclear staining was increasing with irradiation time, especially from 1 to 6 h after irradiation, with stronger intensity of nuclear staining for all subunits observed in CNE1 as compared to that in CNE2 6 h after irradiation (Fig.4).

3 DISCUSSION

Although CNE1 and CNE2 cell lines derived from squamous NPC cells, they show different radiosensitivity[19-21]. In our present data from clonogenic survival assay, MID was 1.111 for CNE2 and 2.345 for CNE1 (Table 1), which also confirmed that CNE2 was more sensitive to ionizing radiation than CNE1. Several reports have proposed that the differences in morphological parameters, such as the nuclear perimeter or ratio of nucleus to cytoplasm, and degree of differentiation may be responsible for the observed difference in radiosensitivity between these cells[23-27]. However, we are interested in whether this difference is determined by intrinsic molecular factor(s), especially those related to DNA repair. DNA-PK is known to play an important role in the repair of DNA double-strand breaks induced by ionizing radiation. While DNA-PK has been proposed as a predictor for radiation therapeutic sensitivity[28-32]; the use of its complex subunits, including Ku proteins, for this purpose remain controversial[15,16]. A recent study has reported that the radiosensitization effect of roscovitine is associated with an inhibition of the DNA-PK activity caused by a marked decrease in Ku-DNA binding instead of changes in expressions of Kus and DNA-PKcs[33]. It was suggested that DNA-PK activity and Ku-DNA binding in the nucleus could really determine the radiosensitivity.

In order to explore possible relationship between DNA-PK activity and the radiosensitivity of CNE1 and CNE2, we measured DNA-PK activity in these cell lines in the present study, and found that the DNA-PK activity in CNE1 was nearly 9 folds of that of CNE2 before and after irradiation, indicating that the lower radiosensitivity of CNE1 compared with that of CNE2 may be related to a higher DNA-PK activity in CNE1. We further compared the expression of Ku proteins between the two cell lines and no significant difference in the total Ku protein level between the two cell lines was found before or after irradiation. Therefore, it seems that the higher DNA-PK activity in CNE1 compared with that in CNE2 may not be directly related to the level of Ku expression, with or without irradiation, which is consistent with other findings from several oral squamous cell carcinoma lines with different radiosensitivities[34,35].

Earlier studies have suggested that nuclear translocation of Ku70 and Ku80 is important for the fast repair process[36], and therefore, the nuclear expression of Ku proteins could be a more accurate measure of radiosensitivity. Indeed, our Western blot analysis showed that the amount of Ku70 and Ku80 proteins in the nuclear fractions of both cell lines had remarkably increased after irradiation. Immunostaining of Ku70/Ku80/DNA-PKcs of the two cell lines confirmed the same results, showing drastic increase in the Kus and DNA-PKcs immunofluorescence intensities in the nuclei 1-6 h after irradiation. These results suggest that the nuclear translocation of DNA-PK subunits upon irradiation is an important event necessary for DNA repair. Interestingly, while Western blot did not seem to show obvious difference in the nuclear Ku levels between CNE1 and CNE2, the Ku70 content in the cytoplasm of CNE1 was detectably lower than that of CNE2 at longer time points after irradiation, suggesting possibly more nuclear translocation of Ku70 in this cell line with lower radiosensitivity. More prominent difference in nuclear translocation of DNA-PK subunits between CNE1 and CNE2 was demonstrated by the immunostaining, showing consistently higher nuclear immunostaining intensities in CNE1 as compared that in CNE2 for all the subunits examined, especially at 6 h after irradiation (Fig.4). The difference in nuclear translocation of DNA-PK subunits between the two cell lines may result in the observed difference in DNA-PK activity that underlines the difference in radiosensitivity of these two cell lines. Indeed, it has been proposed that Ku-DNA binding which takes place in the nucleus is closely related to DNA-PK activity and radiosensitivity[37-39]. An increase in DNA-PK activity in the nucleus leads to increased formation of the DNA end-binding protein complexes containing DNA-PK which is essential for repair of DNA double-strand breaks, and further results in radioresistance of the cells[39-41]. It should be noted, however, that the differences of the Kus in the nuclear fractions between CNE1 and CNE2 were difficult to detect by Western blot and yet remarkable increases in DNA-PK activity with observed difference between CNE1 and CNE2 cells after irradiation could be

obtained. These results suggest that DNA-PK activity is a more sensitive measure, as compared to its complex subunits, for prediction of radiosensitivity.

* * *

ACKNOWLEDGEMENTS: We are grateful to Professor LI Jin-Tian (Cancer Center, Sun Yat-Sen University) for instruction. And we thank Dr. GAO Jin-Hui *et al*. of State Medical Nuclear Physics Laboratory (Medical College, Sun Yat-Sen University) for offering ^{32}P Laboratory in DNA-PK activity experiment. The authors thank Prof. CHAN Hsiao-Chang for critical reading and revision of the manuscript.

REFERENCES

1 Xia YF, Qian JY, Zhang EP. eds. Practical Radiotherapy for Nasopharyngeal Carcinoma. Beijing: Peking University Medical Press, 2003, 211-214.

2 Gottlieb TM, Jackson SP. The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. Cell 1993; 72: 131-142.

3 Burma S, Chen DJ. Role of DNA-PK in the cellular response to DNA double-strand breaks. DNA Repair (Amserdamt) 2004; 3: 909-918.

4 Chen BP, Chan DW, Kobayashi J, Burma S, Asaithamby A, Morotomi-Yano K, Botvinick E, Qin J, Chen DJ. Cell cycle dependence of DNA-dependent protein kinase phosphorylation in response to DNA double strand breaks. J Biol Chem 2005; 280(15): 14709-14715.

5 Lees-Miller SP, Godbout R, Chan DW, Weinfeld M, Day RS, Barron GM, Allalunis-Turner J. Absence of p350 subunit of DNA-activated protein kinase from a radiosensitive human cell line. Science 1995; 267(5201): 1183-1185.

6 Finnie NJ, Gottlieb TM, Blunt T, Jeggo PA, Jackson SP. DNA-dependent protein kinase activity is absent in xrs-6 cells: implications for site-specific recombination and DNA double-strand break repair. Proc Natl Acad Sci USA 1995; 92(1): 320-324.

7 Blunt T, Finnie NJ, Taccioli GE, Smith GC, Demengeot J, Gottlieb TM, Mizuta R, Varghese AJ, Alt FW, Jeggo PA. Defective DNA-dependent protein kinase activity is linked to V(D)J recombination and DNA repair defects associated with the murine scid mutation. Cell 1995; 80(5): 813-823.

8 Virsik-Kopp P, Rave-Frank M, Hofman-Huther H, Schmidberger H. Role of DNA-dependent protein kinase in the process of radiation-induced aberration formation. Int J Radiat Biol 2004; 80(2): 125-133.

9 Belenkov AI, Paiement JP, Panasci LC, Monia BP, Chow TY. An antisense oligonucleotide targeted to human Ku86 messenger RNA sensitizes M059K malignant glioma cells to ionizing radiation, bleomycin, and etoposide but not DNA cross-linking agents. Cancer Res 2002; 62: 5888-5896.

10 Sak A, Stuschke M, Wurm R, Schroeder G, Sinn B, Wolf G, Budach V. Selective inactivation of DNA-dependent protein kinase with antisense oligodeoxynucleotides: consequences for the rejoining of radiation-induced DNA double-strand breaks and radiosensitivity of human cancer cell lines. Cancer Res 2002; 62 (22): 6621-6624.

11 Collis SJ, Swartz MJ, Nelson WG, DeWeese TL. Enhanced radiation and chemotherapy-mediated cell killing of human cancer cells by small inhibitory RNA silencing of DNA repair factors. Cancer Res 2003; (7): 1550-1554.

12 Komuro Y, Watanable T, Hosoi Y, Matsumoto Y, Nakagawa K, Tsuno N, Kazama S, Kitayama J, Suzuki N, Nagawa H. The expression pattern of Ku correlates with radiosensitivity and disease free survival in patients with rectal carcinoma. Cancer 2002; 95: 1199-1205.

13 Sakata K, Matsumoto Y, Satoh M, Oouchi A, Nagakura H, Koito K, Hosoi Y, Hareyama M, Suzuki N. Clinical studies of immunohistochemical staining of DNA-dependent protein kinase in oropharyngeal and hypopharyngeal carcinomas. Radiat Med 2001; 19: 93-97.

14 Wilson CR, Davidson SE, Margison GP, Jackson SP, Hendry JH, West CM. Expression of Ku70 correlates with survival in carcinoma of the cervix. Br J Cancer 2000; 83: 1702-1706.

15 Noguchi T, Shibata T, Fumoto S, Uchida Y, Mueller W, Takeno S Uchida Y, Mueller W, Takeno S. DNA-PKcs expression in esophageal cancer as a predictor for chemoradiation therapeutic sensitivity. Ann Surg Oncol 2002; 9(10): 1017-1022.

16 Lee SW, Cho KJ, Park JH, Kim SY, Nam SY, Lee BJ, Kim SB, Choi SH, Kim JH, Ahn SD, Shin SS, Choi EK, Yu E. Expressions of Ku70 and DNA-PKcs as prognostic indicators of local control in nasopharyngeal carcinoma. Int J Radiat Oncol Biol Phys 2005; 62: 1451-1457.

17 Virus Laboratory of Cancer Institute of Chinese Medical Academy of Science, Pathogenetic Laboratory of Cancer Institute of Sun Yat-Sen Medical University. Establishemnt of an epitheloid cell line and a fusiform cell line from a patient with nasopharyngeal carcinoma. Sci Sin 1978; 1: 113-118.

18 Gu SY, Zhao WP, Zeng Y. Epithelium cell lines of human nasopharyngeal carcinoma establish from poorly-differntiated nasopharyngeal carcinoma. Chin J Cancer (癌症杂志)1983; 2: 70-72 (Chinese, English abstract).

19 Li ZQ, Fang Y, Zhong NQ, He YX, Xia YF. Detection for radiosensitivity parameter of the nasopharyngeal carcinoma cell lines CNE1. J Prac Med 2001; 17: 802-803.

20 Xia YF (夏云飞), Huang B, Dong XY, Qian JY, Zhong QR. Experiment for radiosensitivity characteristic of the nasopharyngeal carcinoma cell lines CNE2. Chin J Cancer (癌症杂志) 1999;

18: 86-87 (Chinese, English abstract).

21 Xia YF (夏云飞), Li MZ, Huang B, Chen JJ, Li ZQ, Wang HM, William AB. Cellular radiobiological characteristics of human nasopharyngeal carcinoma cell lines. Chin J Cancer (癌症杂志) 2001; 20 (7): 683-687 (Chinese, English abstract).

22 Wang HM (王宏梅), Wu XY, Xia YF, Qian JY. The expression of ATM protein in the nasopharyngeal carcinoma cell lines different from radiosensitivity. Chin J Cancer (癌症杂志) 2003; 22 (6): 579-581 (Chinese, English abstract).

23 Sak A, Stuschke M, Wurm R, Schroeder G, Sinn B, Wolf G, Budach V. Selective inactivation of DNA-dependent protein kinase with antisense oligodeoxynucleotides. Cancer Res 2002; 62: 6621-6624.

24 Anderson CW, Lees-Miller SP. The nuclear serine/threonine protein kinase DNA-PK. Crit Rev Eukaryot Gene Expr 1992; 2 (4): 283-314.

25 Lees-Miller SP, Sakaguchi K, Ullrich SJ. Appella E, Anderson CW. Human DNA-activated protein kinase phosphorylates serines 15 and 37 in the amino-terminal transactivation domain of human p53. Mol Cell Biol 1992; 12(11): 5041-5049.

26 Xiong H (熊晖), Sun N, Yao YH. Relationship between cell proliferation, differentiation and radiosensitivity in nasopharyngeal carcinoma. J Guangdong Med Coll (广东医学院学报) 2004; 22(2): 112-114 (Chinese, English abstract).

27 Jiang JH, Wang J, Yu ZH. Radiobiologic study on progenys from the irradiated NPC subline possessing the potentiality of lymphoid metastasis. Prac J Cancer 2002; 17(6): 566-568.

28 Friesland S, Kanter-Lewensohn L, Tell R, Munck-Wikland E, Lewensohn R, Nilsson A. Expression of Ku86 confers favorable outcome of tonsillar carcinoma treated with radiotherapy. Head Neck 2003; 25: 313-321.

29 Wilson CR, Davidson SE, Margison GP, Jackson SP, Hendry JH, West CM. Expression of Ku70 correlates with survival in carcinoma of the cervix. Br J Cancer 2000; 83: 1702-1706.

30 Yoko H, Satoshi S, Yoshitaka M, Kin K, Ishihara H, Imamura M, Sougawa M, Shikata N, OhnishiT. Expression of Ku80 in cervical cancer correlates with response to radiotherapy and survival. Am J Clin Oncol 2003; 26: e80-e85.

31 He F, Li L, Kim D, Wen B, Deng X, Gutin PH, Ling CC, Li GC. Adenovirus-mediated expression of a dominant negative Ku70 fragment radiosensitize human tumor cells under aerobic and hypoxic conditions. Cancer Res 2007; 67(2): 634-642.

32 Tai YT, Teoh G, Lin B, Davies FE, Chauhan D, Treon SP, Raje N, Hideshima T, Shima Y, Podar K, Anderson KC. Ku86 variant expression and function in multiple myeloma cells is associated with increased sensitivity to DNA damage. J Immunol 2000; 165(11): 6347-6355.

33 Maggiorella L, Deutsch E, Frascogna V, Chavaudra N, Jeanson L, Milliat F, Eschwege F, Bourhis J. Enhancement of radiation response by roscovitine in human breast carcinoma *in vitro* and *in vivo*. Cancer Res 2003; 63(10): 2513-2517.

34 Lee SE, Mitchell RA, Cheng A, Hendrickson EA. Evidence for DNA-PK-dependent and -independent DNA double-strand break repair pathways in mammalian cell as a function of the cell cycle. Mol Cell Biol 1997; 3: 1425-1433.

35 Shintani S, Mihara M, Li C, Nakahara Y, Hino S, Nakashiro K, Hamakawa H. Up-regulation of DNA-dependent protein kinase correlates with radiation resistance in oral squamous cell carcinoma. Cancer Sci 2003; 94: 894-900.

36 Bjork-Eriksson T, West C, Nilsson A, Magnusson B, Svensson M, Karlsson E, Slevin N, Lewensohn R, Mercke C. The immunohistochemical expression of DNA-PKcs and Ku(p70/80) in head and neck cancer: relationships with radiosensitivity. Int J Radiat Oncol Biol Phys 1999; 45: 1005-1010.

37 Endoh D, Okui T, Kon Y, Hayashi M. Hayashi M. Hypertonic treatment inhibits radiation-induced nuclear translacation of the Ku protein G22p1 (Ku70) and Xrcc5 (Ku80) in rat fibroblasts. Radiat Res 2001; 155: 320-327.

38 Cosaceanu D, Budiu RA, Carapancea M, Castro J, Lewensohn R, Dricu A. Ionizing radiation activates IGF-1R triggering a cytoprotective signaling by interfering with Ku-DNA binding and by modulating Ku86 expression via a p38 kinase-dependent mechanism. Oncogen 2007; 26(17): 2423-2434.

39 Pawelczak KS, Andrews BJ, Turchi JJ. Differential activation of DNA-PK based on DNA strand orientation and sequence bias. Nucleic Acids Res 2005; 33(1): 152-161.

40 Dittmann K, Mayer C, Fehrenbacher B. Radiation-induced epidermal growth factor receptor nuclear import is linked to activation of DNA-dependent protein kinase. J Biol Chem 2005; 280 (35): 31182-31189.

41 Brown KD, Lataxes TA, Shangary S, Mannino JL, Giardina JF, Chen J, Baskaran R. Ionizing radiation exposure results in up-regulation of Ku70 via a p53/Ataxia-telangiectasia-mutated protein-dependent mechanism. J Biol Chem 2000; 275: 6651-6656.

42 Liu XF, Xia YF, Li MZ, Zheng ML, Yang HL, Huang WL. The effect of p21 antisense oligodeoxynucleotides on the radiosensitivity of nasopharyngeal carcinoma cells with normal P53 function. Cell Biol Inter 2006; 30: 283-287.

43 Koike M. Dimerization, translocation and localization of Ku70 and Ku80 proteins. J Radiat Res (Tokyo) 2002; 43(3): 223-236.

44 Muller C, Paupert J, Monferran S, Salles B. The double life of the Ku protein: facing the DNA breaks and the extracellular environment. Cell Cycle 2005; 4(3): 438-441.

* * * * *

XIA Yun-Fei, M.D.

Yun-Fei Xia M.D. is a Professor in Cancer Center Department of Radiation Oncology of Sun Yat-Sen University and general secretary of National Society of Neuro-Oncology, CACA. As the principal investigator of a National 863 Program Project (2006AA02Z4B4, 2006-2009) titled "New Regimen of Individual Treatment for Nasopharyngeal Carcinoma Based on Biological Behavior and Molecular Character", he is responsible for facilitating and coordinating 9 research projects and 4 core components. His research interests are focused on radiotherapy-related typing in clinical treatment of nasopharyngeal carcinoma and basic research on diagnostic markers and molecular mechanism of radiosensitivity of malignant tumor. His clinical investigations involve assessing the efficacy of biologically founded multidisplinary treatment regiment in the management of nasopharyngeal carcinoma, malignant lymphoma, and malignant glioma. His basic research focuses on 2 major themes: identification of the spectrosocopic parameters and molecular markers that are used to predict the radiosensitivity of normal tissue and tumor in patients receiving radiotherapy or in combination with chemotherapy, and establishment of different radiosensitive and metastatic cell lines cultured from individual tumor tissues from patients with nasopharyngeal carcinoma.