Pergamon

*European Journal of Cancer*, Vol. 35, No. 1, pp. 111–116, 1999

Printed in Great Britain
0959-8049/99/$—see front matter

PII: S0959-8049(98)00289-5

## Original Paper

# DNA-dependent Protein Kinase Content and Activity in Lung Carcinoma Cell Lines: Correlation with Intrinsic Radiosensitivity

F. Sirzén,[1,4] A. Nilsson,[2] B. Zhivotovsky[3] and R. Lewensohn[1,2,5]

[1]Department of Oncology, Radiumhemmet; [2]Department of Medical Radiobiology; [3]Division of Toxicology, Institute of Environmental Medicine, Karolinska Institute, S-171 76 Stockholm; [4]Department of Respiratory Diseases, Huddinge University Hospital; and [5]Swedish Radiation Protection Institute, Sweden

**Intrinsic radiosensitivity and rejoining of radiation-induced DNA double-strand breaks (DNA-dsb) were analysed in five lung carcinoma cell lines: U-1285, U-1906, H-69, H-82 and U-1810. RS correlated with both the initial phase of DNA-dsb rejoining, at 15 min ($r^2 = 0.818$) and the late phase, at 120 min postirradiation ($r^2 = 0.774$), the most sensitive cell line (U-1285) showing least dsb rejoining and the most resistant (U-1810) showing most dsb rejoining of all five cell lines studied. As DNA-PK has been recognised as an important molecular component involved in DNA-dsb repair, we analysed content and activity of this kinase. We found that DNA-PK content and activity correlated with RS ($r^2 = 0.941$ and $r^2 = 0.944$, respectively). The lowest DNA-dependent content/activity was found in the most radiosensitive cells, U-1285 and H-69, whilst the highest content/activity was found in the most radioresistant cells U-1810. These results suggest a correlation between RS and DNA-PK content/activity in lung carcinoma cell lines.** 

**Key words: lung carcinoma cells, radiosensitivity, DNA-PK, DNA-dsb**
*Eur J Cancer*, Vol. 35, No. 1, pp. 111–116, 1999

## INTRODUCTION

A SUBSTANTIAL amount of data indicate that the cytocidal effect of γ-irradiation is a consequence of genomic DNA damage. DNA double-strand breaks (DNA-dsb) represent a critical DNA lesion and therefore it is likely that DNA-dsb repair is an important determinant of cellular radiosensitivity [1]. Several studies have focused on the relationship between cellular radiosensitivity and the level of unrejoined dsb [2], rate of dsb rejoining [3] and fidelity of dsb rejoining [4].

DNA-dependent protein kinase (DNA-PK), a serine/threonine protein kinase has been recognised as an important component in the process of DNA-dsb repair. The evidence for this first appeared in studies on severe combined immunodeficient (scid) mice and hamster xrs cells exhibiting increased RS and impaired V(D)J recombination [5, 6]. The mutations known to induce these defects are localised to genes coding for a 465 kDa protein, the DNA-PK catalytic subunit (DNA-PKcs) and for a 86 kDa DNA binding protein Ku86 [7]. DNA-PKcs is, for its activity, strictly dependent on the DNA-binding Ku heterodimer Ku86 and Ku70 [8]. DNA-PKcs together with Ku proteins form an active complex designated DNA-PK, binding mainly to linear double-stranded DNA ends but also to other DNA constructs [9]. Although the *in vivo* substrates have not yet been identified several transcription factors including RNApol II, c-Myc, c-Fos, c-Jun, RPA and p53 are phosphorylated by DNA-PK *in vitro* [10, 11]. These substrates suggest a role for DNA-PK in regulation of transcription and DNA repair.

Few human cell lines with radiosensitivity similar to the xrs and scid cells have been identified. A radiosensitive human glioma cell line M059J lacking DNA-PKcs has been characterised, being 30-fold more radiosensitive than the radioresistant M059K cell line isolated from the same glioma biopsy specimen [12]. To our knowledge DNA-PK has not been studied in lung carcinomas. In this report we addressed the question of whether DNA-PKcs content and DNA-PK activity may relate to intrinsic radiosensitivity in lung carcinoma cell lines.

Correspondence to R. Lewensohn, e-mail: rolf.lewesohn@onkpat.ki.se
Department of Medical Radiobiology, Karolinska Institute, S-171 76 Stockholm, Sweden.
Received 25 Nov. 1997; revised 5 Jun. 1998; accepted 29 Jun. 1998.

## MATERIALS AND METHODS

*Cell lines*

Five human lung cancer cell lines were used, four corresponding to small cell lung carcinoma (SCLC) (U-1285, U-1906, H-69 and H-82) and one corresponding to an undifferentiated non-small cell lung carcinoma (NSCLC) (U-1810). U-1285, U-1906 and U-1810 have been previously characterised [13, 14]. H-69 and H-82 were obtained from the American Tissue Culture Collection (ATCC); Rockville, Maryland, U.S.A. Cells were grown in $CO_2$-independent medium (Gibco BRL, U.K.) supplemented with 10% fetal calf serum, 2 mM L-glutamine and (Penicillin 100 IU and Streptomycin 100 ng/ml) and incubated at 37°C. The viability was evaluated using trypan blue exclusion. We used early passages of all cell lines and different batches for different experiments.

Radiosensitivity has previously been studied in U-1285, U-1906, U-1810 [15], H-69 and H-82 [16]. The survival fractions after 2 Gy determined by clonogenic assays and extrapolation method were as follows:

$$SF2_{U\text{-}1285} = 0.25,\ SF2_{H\text{-}69} = 0.25,\ SF2_{H\text{-}82} = 0.61,$$
$$SF2_{U\text{-}1906} = 0.45 \text{ and } SF2_{U\text{-}1810} = 0.88.$$

*Irradiation procedure*

For the *in vitro* radiosensitivity assay, cells were X-irradiated (250 kV, 15 mA, 0.5 mm Cu filter, SSD 50 cm, 1.33 Gy/min) in culture medium at ambient room temperature. The dosimetry was based on lithium fluoride dosimetry and was applied using a Simplex Universal dosimeter. Irradiation was performed 24 h after plating the cells in culture dishes with fresh medium. The cell density was kept at levels allowing exponential growth at the moment of irradiation. These levels were determined for each cell line from growth curves of untreated cells during 72 h. Mock irradiation was used in controls.

For DNA dsb experiments, where pulsed field gel electrophoresis was used, cells were labelled for 18 h with $^3$H-Tdr (25 Ci/mmol). After a 2 h chase in non-radioactive medium, the cells were harvested, washed and embedded into a final concentration of 0.5% low melting point agarose (Bethesda Research Laboratories, Maryland, U.S.A.). The density of cells was approximately $3\times10^5$ cells per 100 µl blocks. The blocks were cooled and irradiated on ice with 250 keV X-rays at a dose rate of 1.3 Gy per min. DNA-dsb rejoining was followed at 37°C for 0, 15, 30, 60 and 120 min in cells exposed to 40 Gy and controls.

In vitro *radiosensitivity assay*

Cells were X-irradiated with 2, 4 and 8 Gy. Untreated and treated cells were then counted and tested for viability (trypan blue exclusion) every 24 h for 7 days (168 h). In all cell lines there was an exponential growth in controls up to 120 h postirradiation. The fraction of viable cells in irradiated samples did not increase or decrease significantly between 120 and 168 h. Growth inhibition after 2 Gy (GI2) values were used as surrogates for survival fractions after X-irradiation with 2 Gy and were calculated as follows: GI2 = amount viable cells (irradiated 2 Gy) at 120 h/amount viable cells (untreated) at 120 h.

*DNA preparation*

After irradiation and repair, cells in the agarose blocks were lysed and digested with 2% sarcosyl and proteinase K (Boehringer Mannheim, Germany), 1 mg/ml, in 0.5 M $Na_2EDTA$, pH 8.0, at 50°C for 16 h. The blocks were then treated with 1 mg RNAse/block and washed four times in TE buffer, pH 8.0 (10 mM Tris HCl, 1 mM EDTA). All chemicals were from Sigma Co., Germany, except where stated.

*Pulsed field gel electrophoresis*

Blocks were loaded into the wells of the separation gel. The gel, 0.9% agarose (SeaKem GTG, FMC) in 0.5×TBE (90 mM tris-base, 90 mM boric acid, 2.5 mM $Na_2EDTA$) was cast to a size of 15×15 cm and had a thickness of 10 mm. The electrophoretic equipment was a CHEF-DR II (BioRad Laboratories, California, U.S.A.). Electrophoresis was run for a total of 96 h at 13–14°C with the following protocol:

| | | | |
|---|---|---|---|
| Run time (h) | 24 | 48 | 24 |
| Voltage (V) | 35 | 50 | 60 |
| Pulse time (min) | 90⟶45 | ⟵30⟶ | |

After complete separation the gel was stained overnight with ethidium bromide. The gel was placed on an ultraviolet (u.v.) transilluminator and the red fluorescence photographed with Polaroid film type positive 667. The separate gel lanes were then dissected and radioactivity was determined by liquid scintillation counting after dissolving and hydrolysing the agarose pieces in 0.5 ml hot 1 M HCl. DNA rejoining was followed by counting the radioactivity fractions released in the gel (FAR values) at 0, 15, 30, 60 and 120 min postirradiation. Relative FAR values were counted as a ratio between FAR values at 0 min (100%) and FAR values at 15, 30, 60 and 120 min postirradiation. Using FAR data, the possibility of a linear correlation with SF2 and GI2 values was tested.

*Cell extracts*

Cell pellets were washed with tris-base saline pH 7.3 and lysed in a volume equivalent to three times the cell pellet volume with low salt buffer (10 mM Hepes pH 7.6, 25 mM KCl, 10 mM NaCl, 1.1 mM Mg2Cl, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 1 µg/ml aprotinin, 1 µg/ml leupeptin, 10 µg/ml trypsin inhibitor, 10 µg/ml TPCK) by incubation on ice for 20 min. After centrifugation, supernatants were collected and stored at − 80°C.

*Immunoblots*

Extracts adjusted to an equivalent amount of protein (60 µg) were subjected to 4–15% linear gradient SDS-PAGE and then transferred to a nitrocellulose membrane (Amersham, Buckinghamshire, U.K.) using a semidry transfer cell (Bio-Rad). After blocking with 5% milk the membranes were probed with anti-DNA-PKcs (AHP 318, Serotec, U.K.), anti-Ku86 and anti-Ku70 antibodies. Detection was performed with ECL (Amersham). DNA-PKcs, Ku86 and Ku70 contents were measured by optic densitometry (OD) (Bio-Rad). Results were tested for the possibility of linear correlation with SF2 and GI2 values. Two tailed *t* tests were used for testing statistical significance.

*Determination of kinase activity*

The DNA-PK kinase activity was determined by a DNA-PK assay. Briefly, samples containing 20 µg protein were mixed with 35 µg specific substrate peptide (Promega, Wisconsin, U.S.A.) with or without double-stranded DNA

(dsDNA) in a reaction buffer containing 10 mM Hepes, 25 mM KCl, 10 mM NaCl, 1.1 mM $MgCl_2$, 0.1 mM EDTA pH 7.2, 1 mM DTT, 5 mM ATP and 0.3 μCi[$^{33}$P]ATP. The reaction mixtures were incubated at 37°C for 20 min. The reaction in each sample was stopped with 2 μl stopbuffer (0.32 M EDTA pH 7 and 0.1 M ATP) and the mixture was spotted on to Whatman P81 paper. After washing, radioactivity was determined by liquid scintillation counting. The DNA-PK activity was defined as counts per minute (cpm) $^{33}$P incorporated in the presence of dsDNA minus cpm $^{33}$P incorporated in the absence of dsDNA. Relative DNA-PK activity values were used in the diagrams by relating the cpm counts in different cell lines to the lowest cpm count found in the cell panel (1 in H-69 cells). Results were tested for the possibility of linear correlation with both SF2 and GI2 values. Two tailed *t*-tests were used for testing statistical significance.

## RESULTS

In vitro *radiosensitivity assay*

Survival after X-irradiation in U-1285, U-1906, U-1810, H-69 and H-82 cells is described in Figure 1(a). The GI2 values for these cell lines (mean values of three or four experiments) were as follows:

$GI2_{U\text{-}1285} = 0.30$, $GI2_{H\text{-}69} = 0.32$, $GI2_{H\text{-}82} = 0.48$, $GI2_{U\text{-}1906} = 0.59$ and $GI2_{U\text{-}1810} = 0.8$.

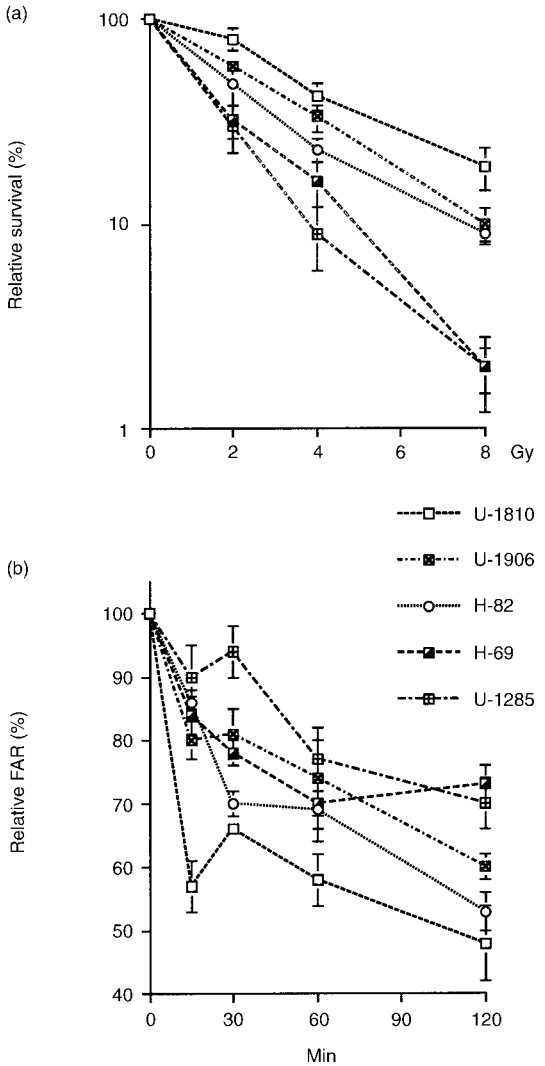


**Figure 1. (a) Relative survival of U-1285, U-1906, U1810, H-82, and H-69 cells after X-irradiation (*y*-axis) as a function of dose (Gy). (b) Relative FAR values in the same cell lines after X-irradiation with 40 Gy (*y*-axis) as a function of repair time (min).**

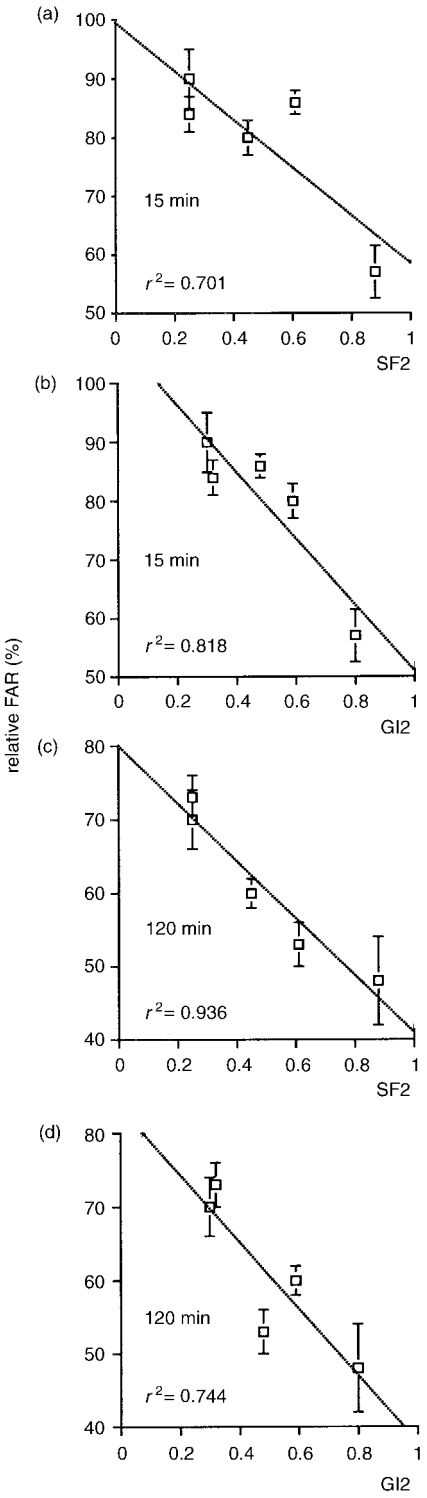


**Figure 2. (a) Correlation between SF2 values and initial DNA-dsb rejoining expressed as relative FAR at 15 min. (b) Correlation between GI2 values and relative FAR at 15 min. (c) Correlation between SF2 values and unrejoined DNA-dsb expressed as relative FAR at 120 min. (d) Correlation between GI2 values and relative FAR at 120 min.**

Thus the SCLC lines U-1285 and H-69 were the most radiosensitive, SCLC lines U-1906 and H-82 were intermediate sensitive and the NSCLC line U-1810, the most radioresistant. These results are similar to previously published SF2 values for these lines [15, 16].

*Rejoining of radiation-induced DNA-dsbs*

There were no significant differences between induction levels for FAR values for the different cell lines: U-1285 (range 57–74%), H-69 (range 59–63%), H-82 (range 60–64%), U-1906 (range 57–67%), U-1810 (range 56–82%). Relative rejoining of DNA-dsbs after 40 Gy X-irradiation of the five cell lines is shown in Figure 1(b) as relative FAR values which were obtained at 15, 30, 60 and 120 min post-irradiation. In radiosensitive U-1285 cells, less than 10% of dsb were initially rejoined (9% at 15 min) compared with radioresistant U-1810 cells, in which more than 30% of dsb were initially rejoined (34% at 15 min). DNA-dsb rejoining at 15 min correlated with both SF2 ($r^2 = 0.701$) and GI2 ($r^2 = 0.818$) values for the five cell lines (Figure 2). A similar correlation between DNA-dsb rejoining and SF2 ($r^2 = 0.936$)/GI2 ($r^2 = 0.774$) values was found at 120 min postirradiation (Figure 2).

*Content of DNA-PKcs, Ku86 and Ku70*

The content of the components of the DNA-PK complex were analysed by Western blotting. There was a variability of the DNA-PKcs content whilst Ku86 and Ku70 were detected at similar levels in all five cell lines. Figure 3 shows an example of an immunoblot of DNA-PKcs, Ku86 and Ku70. Figure 4(a) shows the content of DNA-PKcs expressed as relative OD units in the five cell lines. The content of DNA-PKcs correlated with the SF2 values (Figure 4b) and GI2 values (Figure 4c). Using *t*-test (five experiments) a significant difference between the DNA-PKcs content of radiosensitive (U-1285, H-69) and intermediate sensitive cells (U-1906, H-82) was found ($P = 0.002$). Comparison of intermediate sensitive (U-1906, H-82) and radioresistant cells (U-1810) also showed a significant difference ($P = 0.04$).

*DNA-PK activity*

The relative DNA-PK activity in the five cell lines could be grouped according to three different activity levels. The radioresistant U-1810 cells showed the highest kinase activity. Intermediate sensitive U-1906 and H-82 cells showed intermediate kinase activity and radiosensitive U-1285 and H-69 showed the lowest DNA-PK activity, less than 30% of the activity in U-1810 cells (Figure 5a). DNA-PK activity correlated with both SF2 (Figure 5b) and GI2 values (Figure 5c). A significant difference between the DNA-PK activity of radiosensitive (U-1285, H-69) and intermediate sensitive cells (U-1906, H-82) was found ($P = 0.0067$). Comparison of the DNA-PK activity in intermediate sensitive (U-1906, H-82) and radioresistant cells (U-1810) also showed a significant difference ($P = 0.03$).

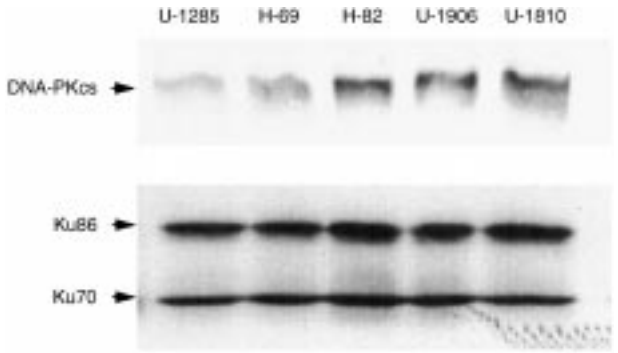


**Figure 3. An example of a Western blot, detecting DNA-PKcs, Ku86 and Ku70. Ku86 and Ku70 were detected with fairly uniform intensity. In contrast, DNA-PKcs showed variable intensity in the five cell lines.**

## DISCUSSION

Studies on the molecular background of cellular radiosensitivity may have relevance for radiotherapy. A correlation

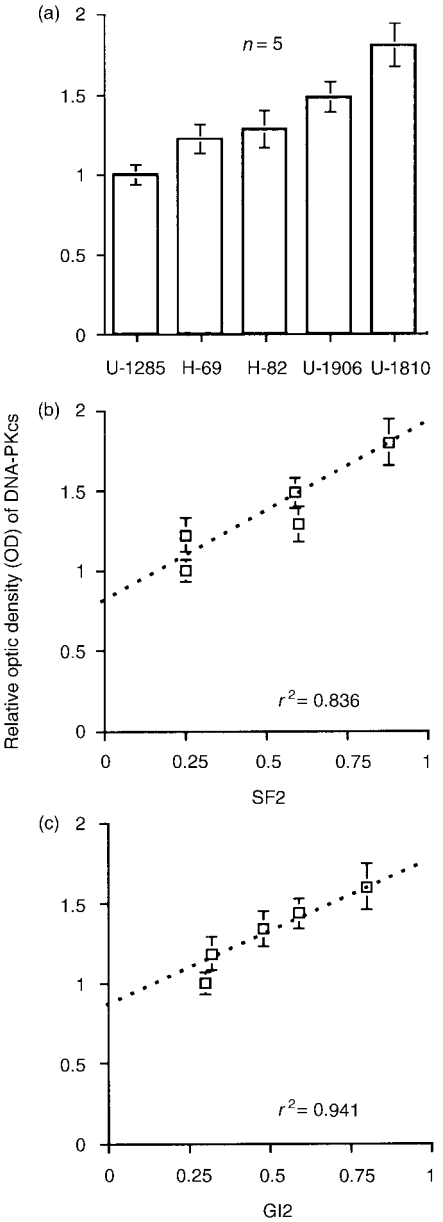


**Figure 4. (a) Relative DNA-PKcs content in the five cell lines expressed as relative optic density (OD) (*y*-axis). The DNA-PKcs contents were related to the lowest content (1.0) in U-1285 cells. Standard errors of the mean (SEM) of five experiments are shown. (b) Correlation between SF2 values (*x*-axis) and relative OD values (*y*-axis). (c) Correlation between GI2 values (*x*-axis) and relative OD values (*y*-axis).**

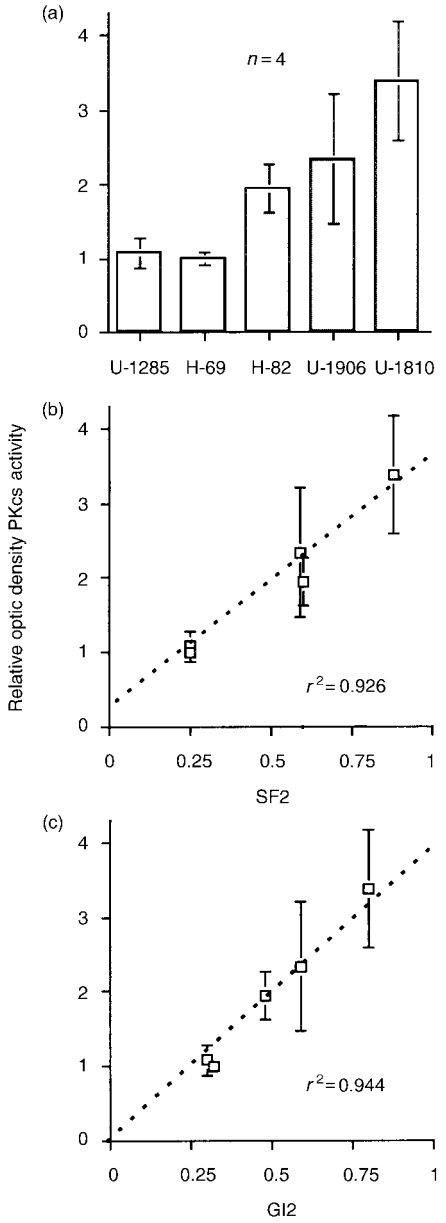


**Figure 5. (a) Relative DNA-PK activity (*y*-axis) in the five cell lines. The DNA-PK activities were related to the lowest activity (1.0) in H-69 cells. Standard errors of the mean (SEM) of four experiments are shown. (b) Correlation between SF2 values (*x*-axis) and relative DNA-PK activity (*y*-axis). (c) Correlation between GI2 values (*x*-axis) and relative DNA-PK activity (*y*-axis).**

between clinical response and *in vitro* radiosensitivity has been found in many tumours [17, 18]. Low SF2 values generally correlate with greater clinical sensitivity. For instance SF2 values of SCLC cell lines are in the range of 0.016–0.53 while NSCLC cell lines show SF2 values in the range of 0.18–0.90 [18]. Treatment with 50 Gy given in 2 Gy fractions in SCLC results in locoregional failure of 40% compared with 70% of NSCLC cases irradiated with the same dose and fractions [19, 20]. For a given tumour it is not possible to predict radiosensitivity in the clinical situation. Clonogenic assays performed on fresh cell cultures from tumour samples are time-consuming and difficult to perform. Predictive tests based on identification of specific molecular changes may provide means to evaluate radiosensitivity. Such tests may be performed directly on tumour specimens.

In this study we focused mainly on SCLC cell lines with different radiosensitivity but selected a NSCLC cell line to illustrate high grade radioresistance. As the purpose of this investigation was not to determine absolute numbers of DNA-dsb but to detect a relative difference in the kinetics of dsb rejoining, the evaluation was performed by using FAR values as estimates of the number of DNA-dsb rejoined at different repair times. The relative FAR values for U-1285 and U-1810 at 15, 30, 60 and 120 min postirradiation obtained in the present study are consistent with previously determined kinetics for DNA-dsb rejoining in these cells using both the Q-method and FAR values [21], although the relative number of unrejoined dsb at 120 min was higher in the present study. A correlation between RS, the rate of unrejoined dsb [2] and the rate of dsb rejoining [3] has previously been shown. We found a correlation between radiosensitivity and both unrejoined dsb at 120 min and initial dsb rejoining, confirming our previous results with lung carcinoma cells [21].

The main purpose of this study was to investigate the link between radiosensitivity and DNA-PK content/activity in lung carcinoma cell lines. We found a good correlation between radiosensitivity and constitutive DNA-PK content/activity in this cell system. However, a generalisation of these findings should be viewed with caution owing to the limitation of the cell system used in this study since we used cell lines and not tumour biopsies. Another study, also using tumour cell lines, was not able to show a correlation between DNA-PK activity and radiosensitivity [22].

Another limitation of our analysis was the use of only one molecular parameter, the DNA-PK complex, in relation to DNA-dsb rejoining and radiosensitivity. The molecular basis of DNA-dsb processing is multifactorial, with other proteins such as the RAD 52 epistasis group being involved [23]. Moreover, important regulatory interactions between DNA-PK and other proteins that may be relevant for radiosensitivity, such as p53 and mdm-2, have been described [24, 25]. However, no cell line in our panel has wtp53 [26, 27].

In conclusion, this is the first report showing a correlation between SF2 and DNA-PK content/activity in human tumour cells and a larger screening, using both cell lines and primary cell cultures from viable tumours, is warranted. If the correlation between SF2 and DNA-PKcs content can be validated in a larger study, it should offer a simple predictive test for radiosensitivity and, possibly, a target for biomodulation of radiosensitivity.

1. Iliakis G. The role of DNA double-strand breaks in ionizing radiation induced killing of eukaryotic cells. *Bio Essays* 1991, **13**, 641–648.
2. Wurm R, Burnet NG, Duggal N, Yarnold JR, Peacock JH. Cellular radiosensitivity and DNA damage in primary human fibroblasts. *Int J Radiat Oncol Biol Phys* 1995, **30**, 625–633.
3. Whitaker SJ, Ung YC, McMillan TJ. DNA double-strand break induction and rejoining as determinants of human tumour cell radiosensitivity. A pulsed-field gel electrophoresis study. *Int J Radiat Biol* 1995, **67**, 7–18.
4. Powell SN, McMillan TJ. The repair fidelity of restriction enzyme-induced double strand breaks in plasmid DNA correlates with radioresistance in human tumour cell lines. *Int J Radiat Oncol Biol Phys* 1994, **29**, 1035–1040.
5. Blunt T, Finnie NJ, Taccioli GE, *et al.* 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**, 813–823.
6. Taccioli GE, Gottlieb TM, Blunt T, *et al.* Ku 80: the product of the XRCC5 gene and its role in DNA repair and V(D)J recombination. *Science* 1994, **265**, 1442–1445.
7. Sipley JD, Menninger JC, Hartley KO, Ward DC, Jackson SP, Anderson CW. The gene for the catalytic subunit of the human DNA-activated protein kinase maps to the site of the XRCC7 gene on chromosome 8. *Proc Natl Acad Sci USA* 1995, **92**, 7515–7519.
8. Gottlieb TM, Jackson SP. The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. *Cell* 1993, **72**, 131–142.
9. Morozov VE, Falzon M, Anderson CW, Kuff EL. DNA-dependent protein kinase is activated by nicks and larger single-stranded gaps. *J Biol Chem* 1994, **269**, 16684–16688.
10. Anderson CW, Lees-Miller SP. The nuclear serine/threonine protein kinase DNA-PK. *Crit Rev Eukaryotic Gene Expression* 1992, **2**, 283–314.
11. Iljima S, Teraoka H, Date T, Tsukada K. DNA-activated protein kinase in Raji Burkitt's lymphoma cells. *Eur J Biochem* 1992, **206**, 595–603.
12. Allalunis-Turner MJ, Zia PKY, Barron GM, Mirzayans R, Day RS. Radiation-induced DNA damage and repair in cells of a radiosensitive human malignant glioma cell line. *Radiation Res* 1996, **144**, 244–293.
13. Bergh J, Larsson E, Zech L, Nilsson K. Establishment and characterization of two neoplastic cell lines (U-1285 and U-1568) derived from small cell carcinoma of the lung. *Acta Path Microbiol Immunol Scand (SecA)* 1982, **90**, 149–158.
14. Bergh J, Nilsson K, Ekman R, Giovanella B. Establishment and characterization of cell lines from human small cell and large cell carcinomas of the lung. *Acta Path Microbiol Immunol Scand (SecA)* 1985, **93**, 133–147.
15. Brodin O, Lennartsson L, Nilsson S. Single-dose and fractionated irradiation of four human lung cancer cell lines *in vitro*. *Acta Oncol* 1991, **30**, 967–974.
16. Carney DN, Mitchell JB, Kinsella TJ. *In vitro* radiation and chemotherapy sensitivity of established cell lines of human small cell lung cancer and its large cell morphological variants. *Cancer Res* 1983, **43**, 2806–2811.
17. Fertil B, Malaise EP. Inherent cellular radiosensitivity as a basic concept for human tumor radiotherapy. *Int J Radiat Oncol Biol Phys* 1981, 7, 621–629.
18. Fertil B, Malaise EP. Intrinsic radiosensitivity of human cell lines is correlated with radioresponsiveness of human tumors: analysis of 101 published survival curves. *Int J Radiat Oncol Biol Phys* 1985, **11**, 1699–1707.
19. Turrisi AT. Current perspectives in the treatment of small cell lung cancer. *Lung Cancer* 1993, **9**, 109–117.
20. Perez CA, Stanley K, Rubin P, *et al.* A prospective randomized study of various irradiation doses and fractionation schedules in the treatment of inoperable non-oat cell carcinoma of the lung: preliminary report by the Radiation Therapy Oncology Group. *Cancer* 1980, **45**, 2744–2753.
21. Cedervall B, Sirzea F, Brodin O, Lewensohn R. Less initial repair of X-ray induced DNA double-strand breaks in a small cell (U-1285) as compared to a large cell (U-1810) lung carcinoma cell line. *Radiation Res* 1994, **139**, 34–39.
22. Allalunis-Turner MJ, Lintott LG, Barron GM, Day III RS, Lees-Miller SP. Lack of correlation between DNA-dependent protein kinase activity and tumor cell radiosensitivity. *Cancer Res* 1995, **55**, 5200–5202.
23. Park MS. Expression of human RAD52 confers resistance to ionizing radiation in mammalian cells. *J Biol Chem* 1995, **270**, 15467–15470.
24. Lees-Miller SP, Sakaguchi K, Ullrich SJ, Apella E, Andersson CW. Human DNA-activated protein kinase phosphorylates serines 15 and 37 at the amino terminal transactivation domain of human p53. *Mol Cell Biol* 1992, **12**, 5041–5049.
25. Mayo LD, Turchi JJ, Berberich SJ. Mdm-2 phosphorylation by DNA-dependent protein kinase prevents interaction with p53. *Cancer Res* 1997, **57**, 5013–5016.
26. Sirzén F, Zhivotovsky B, Nilsson A, Bergh J, Lewensohn R. Spontaneous and radiation induced apoptosis in lung carcinoma cells with different intrinsic radiosensitivities. *Anticancer Res* 1998, **18**, 695–700.
27. D'Amico D, Carbone D, Mitsudomi T, *et al.* High frequency of somatically acquired p53 mutations in small cell lung cancer cell lines and tumors. *Oncogene* 1992, **7**, 339–346.

**Acknowledgements**—M. Langéen, U.-S. Kronquist and A.-L. Borg are greatly acknowledged for their technical skill. This project was supported by the Stockholm Cancer Society (project 96:153), the Swedish Cancer Society (Cancerfonden, Project No. 3829-B96-01XAB) and the Funds of the Karolinska Institute.