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ORIGINAL PAPER

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Studies on the influence of DNA repair on radiosensitivity in prostate cell lines

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Abstract The relationship between radiosensitivity and DNA repair was investigated in six human prostate cell lines, 1542-NPTX, BPH-1, 1542-CP_3TX, 1532-CP_2TX, 1535-CP_1TX and LNCaP. Except for LNCaP, these cell lines are new and were derived from primary prostate tumours and normal non-tumourigenic prostate tissue. Cell survival was assessed by clonogenic assay. DNA damage was determined in non-synchronised cells by constant-field gel electrophoresis, and expressed as the fraction of DNA released. For initial damage, cells were embedded in agarose and irradiated on ice with 0–100 Gy ^{60}Co γ-irradiation. Residual DNA damage was measured after 2 h and 20 h of repair. Radiosensitivity, given as the mean inactivation dose, was found to vary between 1.62 and 2.77 Gy. We found that radiosensitivity significantly correlates with the 2 h DNA repair component, giving a correlation coefficient of 0.92 ($P = 0.009$). In the cell lines examined here the 2 h repair component emerges as an indicator of radiosensitivity.

Keywords DNA damage repair · Radiosensitivity · Prostate cell lines

Introduction

Improvements in surgical techniques have succeeded in reducing the side effects of radical prostatectomy [7]. However, there is still considerable controversy regarding the optimal management of localised prostatic cancer [31]. Most authors agree that in elderly patients radical surgery should be avoided [7]. In such cases radiotherapy is an attractive alternative [11]. The success or failure of radiotherapy depends upon a variety of factors. Recent experimental data show that inherent cellular radioresistance may be a major obstacle in achieving a good clinical response [30]. The cell biological approach to this problem has been complicated by the fact that the number of available prostate cell lines is small and that they have been derived from metastatic tissue and not from primary tumours. Only one representative cell line, LNCaP, is androgen dependent whereas DU145 and PC-3 are androgen independent. The metastatic origin and androgen independence manifest in most established prostate cell lines are major limiting factors, and the search for truly representative cell lines is continuing.

The recent advent of prostate cell lines from normal, benign, and primary tumour epithelium [4, 12] prompted us to examine radiosensitivity and DNA repair kinetics in 1542T, 1542N, 1532T, 1535T, BPH-1 and LNCaP cell lines to provide clinically applicable data for new approaches to prostate tumour control by irradiation.

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Materials and methods

Cell culture

The benign prostatic hyperplasia-1 cell line, BPH-1, grows in RPMI 1640 medium supplemented with 10% heat-inactivated foetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 μg/ml) (Highveld Biological, South Africa). The culture was obtained from S.W. Hayward (Department of Urology, University of California).

The malignant 1535-CP_1TX, 1532-CP_2TX and 1542-CP_3TX (1535T, 1532T and 1542T), and the normal 1542-NPTX (1542N) prostate cell lines were grown in WAJC 404 medium (Gibco BRL, Scotland) containing HEPES, sodium hydrogen carbonate, zinc stabilised insulin, cholera toxin, dexamethazone, epidermal growth factor (Sigma–Aldrich, Germany), penicillin/streptomycin and 0.5% heat-inactivated FBS, as described by Bayne et al. [3]. The cultures were provided by J.R.W. Masters (Prostate Research Centre, University College, London) and J.A. Macoska (Department of Urology, University of Michigan Comprehensive Cancer Center).

The LNCaP cell line, obtained from J.H. Visser (Department of Urology, University of Stellenbosch Medical Faculty), was grown in RPMI 1640 medium supplemented with 5% heat-inactivated FBS, penicillin (100 U/ml) and streptomycin (100 μg/ml).

All cell lines were grown at 37°C in a humidified incubator with 5% CO_2.

Irradiation procedure

For the clonogenic assay, cultures were irradiated at room temperature (22°C) at ^{60}Co γ-irradiation doses of 0–10 Gy. The dose rate was 1.0 Gy/min. In the case of DNA repair experiments, prepared gel plugs (Biorad, USA) containing cells were irradiated in ice-cold culture medium over a dose range of 0–100 Gy on ice, as described elsewhere [29]. The dose rate was 2.92 Gy/min.

Radiosensitivity measurements

Cell survival was determined by the clonogenic assay, as previously described [1]. Appropriate numbers of cells (400–20,000) were seeded and allowed to attach for 6 h (48 h in the case of LNCaP), following which they were irradiated with graded doses of ^{60}Co γ-irradiation. After 10–14 days of incubation, colonies were stained, counted and the surviving fractions calculated. Colony numbers ranged from 80–300. The mean plating efficiencies of BPH-1, 1542T, 1542N, 1532T, 1535T, and LNCaP were 30%, 8%, 13%, 12%, 15% and 6%, respectively.

Cell survival curves were fitted to the linear-quadratic survival equation: $(S) = e^{-(\alpha D + \beta D2)}$, where S is the survival ratio, α and β are inactivation constants and D is the dose in Gy. Mean surviving fractions from three independent experiments were plotted on a linear-linear scale, and the mean inactivation dose, $\bar{D}$, (the area under survival-dose response curve) [9] was used as the measure of radioresistance.

DNA dsb repair assay

The amount of DNA double-strand break damage was determined by constant-field gel electrophoresis (CFGE) as previously described [32]. Confluent cultures were used to avoid S-phase variations between cell lines [8]. Cells were encapsulated in agarose during irradiation and repair. This procedure was optimised according to Kysela et al. [15] in order to minimise non-specific DNA damage. Briefly, 1532T, 1535T, 1542T, 1542N, BPH-1 and LNCaP cells were harvested by trypsinisation and re-suspended in a 0.5% low melting point agarose solution. Aliquots of 30 μl, containing $\sim 1\times10^5$ cells were placed into each well of a disposable plug mold (Biorad), and allowed to solidify at 4°C for 45 min. Plugs were irradiated in ice-cold DMEM containing 2% HEPES and 7.5% (w/v) $NaHCO_3$, over a dose range of 0–100 Gy ^{60}Co γ-irradiation on ice. Samples for the determination of initial damage were immediately submitted to subsequent lysing and washing steps. Samples for determining residual damage were incubated at 37°C in growth medium for periods of 2 h and 20 h prior to lysing and washing.

For both protocols (initial and residual damage), plugs were submersed in an ice-cold lysing solution containing 50 mM EDTA, 1% sodium dodecyl sulphate and 1 mg/ml proteinase K. Incubation of 1 h at 4°C was followed by lysing at 37°C for 20 h. Agarose plugs were then washed five times and stored in 2 ml of 50 mM EDTA solution.

The washed agarose plugs were then loaded into a 20×20 cm 0.6% agarose gel and run in 0.5×TBE buffer for 24 h at a constant field strength of 1.2 V/cm. Gels were stained with ethidium bromide (0.5 μg/ml in 0.5×TBE) and subjected to fluorometric analysis with a Gene Snap (VacuTec) image analysis system. Three independent experiments were performed for each cell line. The fraction of DNA released from the plug (F_{rel}) was obtained from the equation: $F_{rel} = fl_{rel}/(fl_{plug} + fl_{rel})$, where fl_{rel} and fl_{plug} are the fluorescence measured in the lane (DNA released) and in the plug, respectively. Untreated control samples were used for each sample subset to subtract background fluorescence caused by non-specific DNA degradation.

Dose response curves were obtained by plotting dose (Gy) vs the fraction of DNA released (F_{rel}) as calculated above, representing initial damage (0 h), residual damage (2 h) and residual damage (20 h). Since data could not be fitted by linear regression, data points were connected and the area under the curve (AUC) was calculated for each curve using GraphPad Prism (GraphPad software, San Diego, USA). DNA dsb rejoining capacity after 2 h or 20 h incubation was then determined as the ratio AUC_0/AUC_2 or AUC_0/AUC_{20}, where AUC_0 is the area under the curve at 0 h.

Data evaluation

Data are presented as the mean ± SD of three independent experiments (Figs 1, 2) and each experiment was repeated three times. Statistical analysis and data fitting were performed by means of GraphPad Prism.

Results

Radiosensitivity

Cell survival data for the six human prostate cell lines were fitted to the linear-quadratic model and are presented in Fig. 1. Cellular radiosensitivity is expressed by the mean inactivation dose, $\bar{D}$, which is defined as the area under the survival curve when plotted on a linear-linear scale [9]. $\bar{D}$-values for the irradiated panel of cell lines vary from 1.62–2.77 Gy (Table 1). The LNCaP and 1542T cell lines are the most radiosensitive, with $\bar{D}$-values of 1.62 and 1.84 Gy, respectively. The 1532T and BPH-1 cell lines are the most radioresistant, with $\bar{D}$-

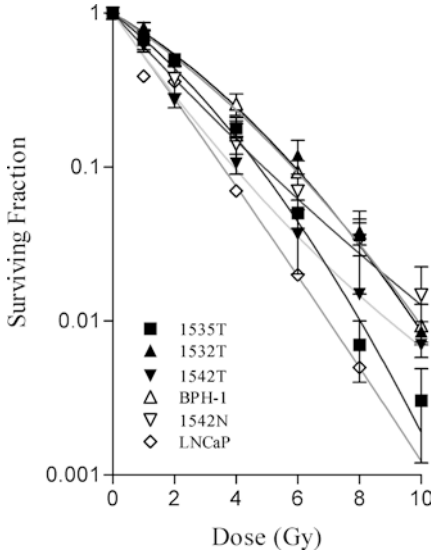

Fig. 1 Survival curves for 1542T, 1542N, 1535T, 1532T, BPH-1 and LNCaP human prostate cell lines following ^{60}Co γ-irradiation. Cell survival was measured by colony assay, and data were fitted to the linear-quadratic equation. For determination of radiosensitivity in terms of mean inactivation doses (area under survival curves) data were plotted on a linear-linear scale

Table 1 Areas under the dose-response curves (AUC) calculated from fractions of DNA released, against irradiation dose, in the range 0–100 Gy for 0 h, 2 h and 20 h after irradiation (Fig. 2), and radiosensitivity measured by the mean inactivation dose, $\bar{D}$ (area under survival dose response curve plotted on a linear–linear scale) for 6 human prostate cancer cell lines

	1532T	1535T	1542 N	1542T	BPH-1	LNCaP
Initial DNA damage	33.94	39.67	30.66	30.72	24.38	33.41
2 h repair	8.86	14.33	10.62	16.61	8.07	19.70
20 h repair	7.26	10.51	7.05	6.97	3.79	2.78
$\bar{D}$	2.72	2.40	2.28	1.84	2.77	1.62

values of 2.72 and 2.77 Gy, respectively, while the 1542N and 1535T cell lines assume intermediate positions with $\bar{D}$-values of 2.28 and 2.40 Gy, respectively.

DNA dsb repair capacity

The dose response curves for initial (0 h) and residual (after 2 h and 20 h of repair) DNA damage are presented as a plot of the fractions of DNA released against irradiation dose (Fig. 2). The areas under the initial damage curves (no repair) range between 24.38 and 39.67 (Table 1). The extent to which DNA double strand breaks have been repaired is expressed as the ratio of the area under the initial damage curve (0 h) to the area under the residual damage curves (after 2 h and 20 h incubation) and is plotted against radiosensitivity (Fig. 3). After 2 h of repair, the cell lines show repair ratios ranging from 1.70 to 3.83. The 20 h repair ratios range from 3.77 to 12.02 (Table 1).

After 2 h DNA repair, the more radioresistant cell lines show higher levels of DNA dsb rejoining than the more radiosensitive cell lines (Fig. 3). The 2 h repair and radiosensitivity, measured in terms of $\bar{D}$, were significantly correlated, giving a regression coefficient of $r = 0.92$ ($P = 0.009$) (Fig. 3A). No relationship was apparent between the 20 h DNA repair capacity and radiosensitivity (Fig. 3B). This indicates that, in terms of clonogenic survival, radiosensitivity in these prostate cell lines is related to the fast (2 h) DNA dsb repair component, and not to the slow (20 h) DNA repair

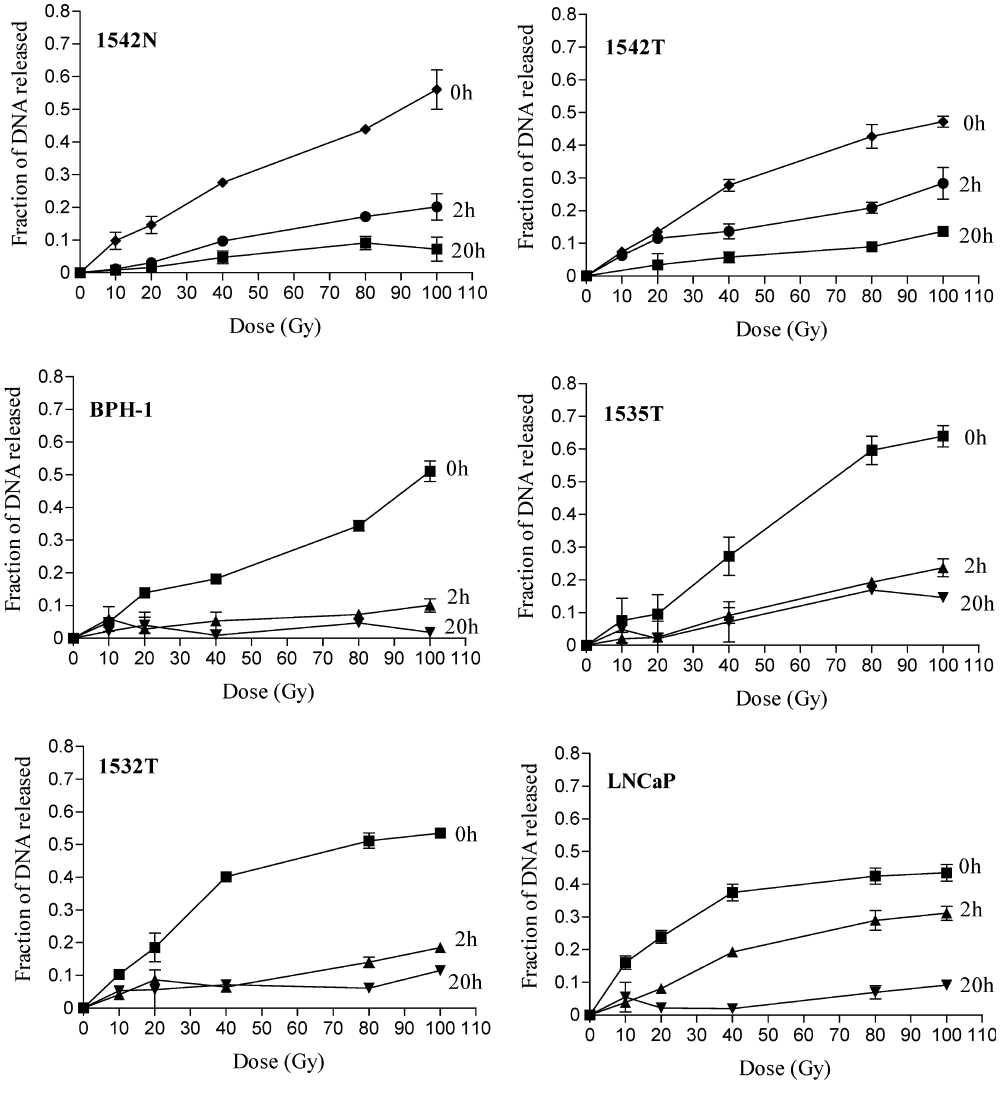

Fig. 2 CFGE data showing fractions of DNA released after exposure to 0–100 Gy in 1542T, 1542N, BPH-1, LNCaP, 1535T and 1532T human prostate cell lines, as determined by fluorescent densitometry of ethidium bromide stained gels. Fractions of DNA released (F_{rel}) were plotted against dose, and the area under each curve was calculated to compare initial DNA dsb to the residual unrepaired dsb after 2 h and 20 h of repair

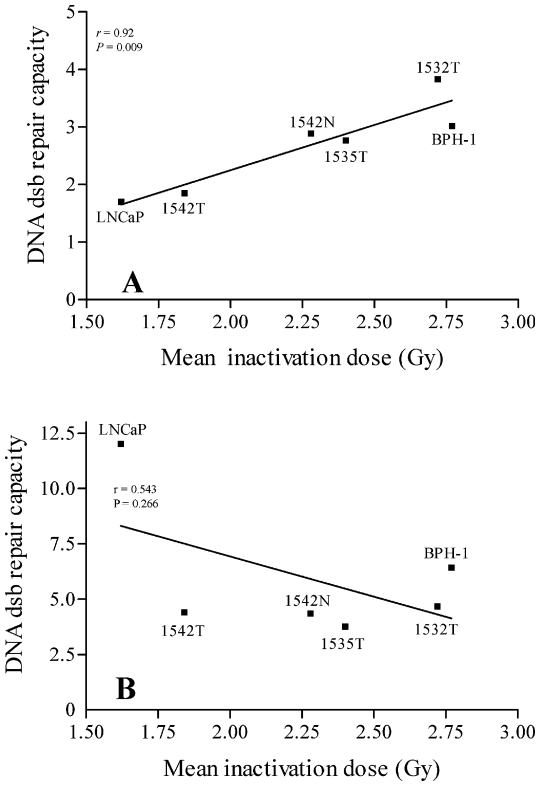

Fig. 3 Plot of DNA repair capacity as a function of radiosensitivity. Repair capacity is defined as the ratio of the area under the initial repair curve to the area under the residual curve at: **A** 2 h and **B** 20 h of repair time. Radiosensitivity is expressed as the mean inactivation dose, which is defined as the area under the cell survival dose response curve plotted on a linear-linear scale

component. The 20 h repair component has been found to be diagnostic of radiosensitivity in a wide variety of tumour cell lines of different origin.

Discussion

The treatment of localised prostate cancer by radiotherapy is an attractive alternative to surgery because it leads to fewer complications. However, no therapy has yet demonstrated a clear superiority in terms of long-term survival [7]. Knowledge of the radiosensitivity of prostate tumours could help to provide a rational basis for a radiotherapy approach and help to optimise therapy. In our cell lines, radiosensitivity varied from a mean inactivation dose of 1.62–2.77 Gy, and thus covered a wide range.

Ionising radiation induces various types of DNA damage, such as double strand breaks (dsb), single strand breaks (ssb), base damage and DNA protein crosslinks [2]. The major factors determining radiosensitivity in human tumour cell lines are DNA dsb induction and repair [14, 20, 23]. Using six prostate cancer cell lines, five of which are derived from primary tumours, we show that the initial level of DNA damage per unit dose is cell line dependent. This is consistent with other data showing that the induction of DNA dsb varies widely between cell lines [23, 24, 28].

Some studies have suggested that no correlation exists between radiosensitivity and repair of double strand breaks [21, 28]. High dose work (0–100 Gy) on AT2BE, NF, R1H, CHO, CHO K1, xrs1 and xrs5 human and rodent cell lines has demonstrated a relationship between radiosensitivity and the number of unrepaired dsb [8, 28]. At low dose (0–10 Gy), radiosensitivity correlates with the initial DNA damage [23]. The absence of unrepaired DNA dsb measured by the CFGE assay gives no information about the quality of the repaired DNA because mutations and misrepair are not detected and will feature as repaired DNA (although loss of clonogenicity may ensue later). We demonstrate here, for six prostate cell lines, that a good correlation exists between the 2 h DNA repair and radiosensitivity, and that the more radioresistant cell lines show better repair competence (Fig. 3A). No dependence of radiosensitivity on 20 h DNA repair could be demonstrated (Fig. 3B). DNA repair consists of a fast component (2–3 h) during which the bulk of the DNA is repaired, and a slow component which is completed 16–24 h after irradiation [8], therefore 2 h and 20 h time points were chosen to examine the correlation between DNA repair and radiosensitivity. Our findings are at variance with results on a wide variety of rodent and human cell lines which have shown that radiosensitivity is correlated with 20 h DNA repair [8, 29]. Within the limitations of a cell biological model, this highlights the different behaviour of prostate tumour cells in terms of recovery from DNA damage. That recovery from DNA damage can be successfully exploited by drugs has been demonstrated for DU145, BM1604 and LNCaP cells which show a markedly enhanced sensitivity to cisplatin, vinblastine and etoposide when the drugs are given under conditions of G2 block abrogation [27]. This staging of irradiation to produce a G2 block followed by cytostatic drugs given with a non-toxic G2 block abrogator (Pentoxifylline) forces cells into mitosis and hence to change repair mode.

DNA damage induces the p53 tumour suppressor protein [16]. When wild-type p53 cells are damaged by irradiation the increase of p53 protein causes a G1 arrest and cells enter repair or apoptosis [16]. P53 mutant cells, present in the majority of tumours, show a G2 cell cycle arrest which facilitates DNA repair and prevents the propagation of the defective genome to daughter cells [17]. The downstream effects of p53 have led to contradictory hypotheses regarding the influence of p53 on radiosensitivity [5]. In p53 wild-type cells, p53 induction could initiate apoptosis and cell death, alternatively cells which arrest in G1 can undergo repair, leading to cell survival. P53-dependent apoptosis and G2 arrest, followed by DNA repair and cellular recovery, appear to be controlled by different pathways which operate differently between cell types [19]. Except for the p53 wild-type LNCaP cells [13], the cell lines used here were immortalised with the large T gene of the simian virus 40

(BPH-1) [12], and with the E6 and E7 genes of the human papilloma virus 16 (1532T, 1535T, 1542T and 1542N) [4] and thus are p53 dysfunctional.

Our results are in agreement with the general notion that cell death mainly results from DNA double strand breaks [10]. Interestingly, however, we found for this range of prostate cell lines that cellular radiosensitivity correlates with the number of dsb measured within 2 h of irradiation. Results on head and neck squamous carcinoma tumour cells, HNSC, SCC, JSQ, and SQ also show this correlation [25]. The fact that the 2 h repair period can separate radiosensitive from radioresistant cells in two groups of human tumour cell lines has implications for therapy, and would be consistent with the clinical observation that prostate tumours can be successfully controlled by LDR-brachytherapy [22].

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