

Caspase-1 Enhances the Apoptotic Response of Prostate Cancer Cells to Ionizing Radiation

RACHEL N. WINTER¹, JUONG G. RHEE² and NATASHA KYPRIANOU³

¹Department of Biochemistry and Molecular Biology and Division of Urology and

²Department of Radiation Oncology, University of Maryland School of Medicine, Baltimore, MD;

³Division of Urology, University of Kentucky, College of Medicine, Lexington, KY, U.S.A.

Abstract. *Background:* The significance of caspase-1 in prostate cancer has recently been documented (Cancer Res 61: 1227-1232, 2001). In this study, we investigated the role of caspase-1 in radiation-induced apoptosis in order to identify the significance of this apoptotic initiator in radiation resistance. *Materials and Methods:* Caspase-1 was over-expressed in DU-145 prostate cancer cells (which have weak endogenous expression of caspase-1), via transfection-mediated gene transfer. Stable transfectants were cloned and expression of caspase-1 was established at the mRNA and protein levels by RT-PCR and Western blot, respectively. Caspase-1 overexpressing clones were characterized for their apoptotic response to ionizing irradiation (0-9 Gy) on the basis of cell viability and Hoechst staining assays and profiling of expression of key apoptosis regulators, such as caspase -3 and -9. *Results:* Caspase-1 transfectants exhibited a greater sensitivity in response to ionizing radiation than the neomycin control transfectants, as demonstrated by a dramatic loss in cell viability, that temporally correlated with apoptosis induction. Furthermore, caspase-1 overexpression resulted in a significant decrease in clonogenic survival following treatment with ionizing radiation, while the caspase-1 inhibitor, Z-YVAD.fmk, suppressed apoptosis induction in caspase-1 transfectants ($p < 0.008$). The apoptotic effect was associated with increased expression of the pro-enzyme form of caspase-3 in both the caspase-1 transfectants and neo controls cells, with the activated caspase-3 being detected in caspase-1 transfectants only. While this activation of caspase-3 was paralleled by an elevated caspase-9 expression at 9h post-irradiation, there

was no major induction in Apaf-1 or cytochrome c release. *Conclusion:* The present study provides an initial mechanistic insight into the functional involvement of caspase-1 in changing the apoptotic threshold of prostate cancer cells to radiation. These findings will enhance the understanding of the molecular basis of prostate tumor radioresistance and may have significant clinical relevance in improving the therapeutic index of radiotherapy in prostate cancer patients.

Prostate cancer is a major contributor to cancer mortality in American males of all ages (1). This mortality results from metastasis to the bone and lymph nodes and progression from androgen-dependent to androgen-independent prostatic growth (2). Androgen independent cells can still however, undergo apoptosis in response to non-androgen ablative means such as radiation (3, 4). Radiation therapy has been one of the effective treatments for prostate cancer, especially for those patients unsuitable for surgical intervention (5). Ionizing radiation-induced cell death recruits the activation of the apoptotic pathway as well as disruption of the cell cycle. Identification of cellular or biochemical means that will augment the activity of tumor radiosensitivity will enable the optimization of radiation therapy for the treatment of prostate cancer by enhancing the response to radiation (4, 6, 7).

Tissue homeostasis in the normal prostate gland is maintained by the balance between the rate of cell proliferation and the rate of apoptotic cell death (8). Recent studies on the dynamics of prostate growth suggest that disruption of the mechanisms that regulate apoptosis and cell proliferation is responsible for the abnormal growth of the gland during tumorigenic development (2, 9). Key regulators of apoptosis are members of the *bcl-2* family, whose main functions are to either inhibit or promote apoptosis (9). Expression of these apoptotic regulators (*bcl-2* and *bax*) dictates the therapeutic response to radiation in prostate cancer patients (10-12).

Correspondence to: Dr Natasha Kyprianou, Division of Urology, Department of Surgery, MS 283, University of Kentucky Medical Center, 800, Rose Street, Lexington, KY 40536-0298, U.S.A. Tel: (859)-323-9812, Fax: (859)-323-1944, e-mail: nkypr2@uky.edu

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Repair of a defective apoptotic machinery in cancer cells offers the promise of novel effective anti-cancer modalities (13). Considerable efforts have consequently been directed towards the identification and targeting of cellular modifiers of apoptosis for implementing effective strategies for advanced prostate cancer (9). The mechanism of apoptosis is remarkably conserved across species, executed with the activation of initiator and effector caspases (14), a family of cysteine proteases that are synthesized as inactive proenzymes. Caspases selectively cleave substrates after an aspartate residue to produce the active enzyme (15, 16). Once activated, the effector caspases cleave a broad range of cellular targets and ultimately cause apoptosis in cancer cells (17, 18).

Caspase-1 (known as ICE-interleukin-1 β converting enzyme), is an initiator caspase that was originally characterized as cleaving inactive prointerleukin-1 β to generate the active proinflammatory cytokine interleukin-1 β (15, 19). Overexpression of caspase-1 has been shown to induce apoptosis in mammalian (20, 21) and insect cells (22), while silencing of caspase-1 also confers a growth advantage in the formation of renal cell tumors (23). Recent evidence from this laboratory demonstrated the functional significance of caspase-1 activation in TGF- β -mediated prostate apoptosis. TGF- β induces prostate cancer cell apoptosis *via* up-regulation of caspase-1 expression (24). Caspase-1 has also been implicated in Fas-mediated apoptosis by facilitating caspase-8 activation (25).

Understanding the role of caspases in the mechanism of apoptosis induction in radioresistant prostate tumors may provide a valuable insight into the identification of novel effective treatment modalities that will lead to a successful clinical outcome of prostate cancer patients. In previous studies we demonstrated that the human androgen-independent prostate cancer cells DU-145, exhibit extremely low caspase-1 expression (26). In the present study we hypothesize that loss/reduction of this apoptosis initiator could confer apoptotic resistance in prostate cancer cells. Our findings indicate that overexpression of caspase-1 in DU-145 cells resulted in a considerable enhancement of the radiosensitivity of these cells *via* elevation of their apoptotic threshold.

Materials and Methods

Cell culture and transfection. The human prostate cancer cells DU-145 were obtained from the ATCC (Rockville, MD, USA) and maintained in RPMI-1640 (GIBCO, Gaithersburg, MD, USA) containing 10% FCS and 1 μ g/ml of penicillin/streptomycin in 5% CO₂/95% air at 37°C. Cells were transfected with the β -actin M10Z plasmid, which contains p β actSTNeo B encoded with murine caspase-1, (murine ICE cDNA lacZ fusion) generously provided by Dr. M. Miura, Osaka, Japan (21). The calcium phosphate precipitation procedure was used as previously

described (27) and neomycin selection was performed at 48h post-transfection in medium containing 500 μ g/ml G418 (Geneticin, Life Technologies, Inc.). Selected G418-resistant colonies were cloned and expanded into cell lines.

Characterization of cloned caspase-1 transfectants. Expression of caspase-1 mRNA and protein in isolated cloned transfectants was examined by semi-quantitative RT-PCR and Western blotting, respectively. RT-PCR was performed using 2 μ g of total cellular RNA, and the Invitrogen superscript 1st strand synthesis kit in a Perkin Elmer Amplification Cycler (Wellesley, MA, USA). Primers and conditions used for human ICE-1 β were as described previously (26). The integrity of the RNA used for RT-PCR was confirmed using the GAPDH synthesis as a positive control reaction as previously described (24). The amplified RT-PCR products were electrophoretically analyzed through 1% agarose gels, visualized by ethidium bromide staining and photographed under UV illumination.

For Western analysis, SDS-polyacrylamide gel electrophoresis was applied. Protein samples (40 μ g) were subjected to electrophoretic analysis (12.5%), and proteins were subsequently transferred to a nitrocellulose membrane (1h at 4°C). Following blocking in 5% blocking solution (in TBST) for 1h, membranes were incubated overnight using specific primary antibodies as follows: caspase-1 and Apaf-1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), caspase-1 active fragment (Upstate Biotechnology, NY, USA), caspase-3 and cytochrome c (Pharmingen, San Diego, CA, USA), caspase-9 (New England Biolabs, Beverly, MA, USA), and α -actin (Oncogene, Boston, MA, USA). Following incubation with the respective primary antibody, membranes were washed in TBST and subsequently incubated with the appropriate horseradish-peroxidase secondary antibodies (1h at room temperature). Protein expression was detected using the ECL detection kit (Amersham Int., Arlington Heights, IL, USA). Densitometric analysis was performed using the Scion Image program (Scion Corporation, Frederick, MD, USA), and all bands were normalized to α -actin expression and shown as fold-change of the control non-treated samples.

Analysis of *in vitro* growth of caspase-1 transfectants in response to radiation. *In vitro* growth rates for DU-145 neo transfectants and DU-145 caspase-1 clones expressing high levels of caspase-1 were determined by plating in duplicate, 100,000 cells per well in 6 well-plates. Upon reaching 60% confluency, cells were exposed to single doses of radiation (1-9Gy). Radiation treatment was performed at room temperature using a Seifert 250 KV/15 mA irradiator unit with a 0.5 mm CA/1.00 mm A1 added open field filter. Cell death was assessed at 24h post-irradiation on the basis of loss of cell viability as determined by Trypan blue exclusion assay. Each experiment was performed three times independently. For a time course assay cells were exposed to 3Gy of ionizing radiation and cell viability was assessed at 0, 9, 24, 48 and 72 h post-irradiation.

Detection of apoptosis. Cells were plated in duplicate with 100,000 cells/well in 6 well-plates and at 60% confluency, were exposed to varying radiation doses. Twenty-four h post-irradiation, cells were fixed in paraformaldehyde, solubilized in 10% (v/v) Triton X, and changes in the nuclear chromatin of cells undergoing apoptosis were stained with the DNA binding fluorochrome bis-benzimide (Hoechst 33258, Sigma, St Louis, MO, USA). Cells were visualized using a Zeiss Axiocvert 10 fluorescent microscope with a UV filter

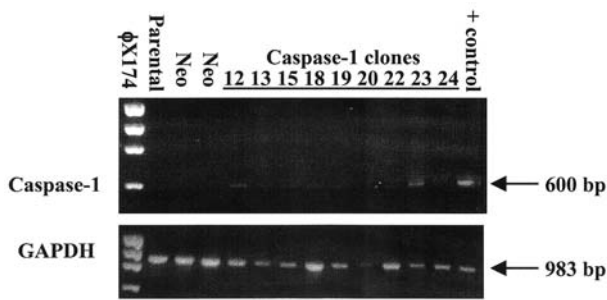


Figure 1A. *Caspase-1* mRNA expression in cloned DU-145 transfectants. RT-PCR analysis of mRNA expression of *caspase-1* in the DU-145 parental cells, DU-145 neo transfectants, and nine DU-145 *caspase-1* cloned transfectants. Lane 1, ϕ X174 molecular weight marker; lane 2, DU-145 parental; lanes 3 and 4, DU-145 neo transfectants; lanes 5-13, DU-145 *caspase-1* clones; lane 14, positive control (normal prostate). GAPDH mRNA expression was analyzed as a normalizing control. The sizes of the individual bands are indicated on the right. RT-PCR was performed using primers for *caspase-1* and GAPDH as described in "Materials and Methods".

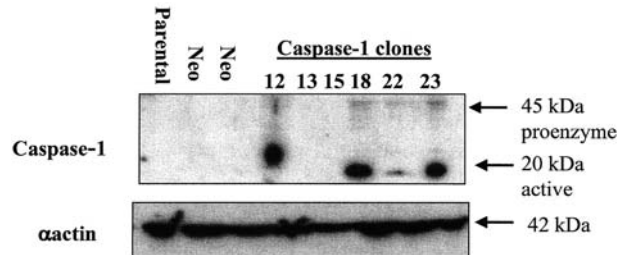


Figure 1B. *Caspase-1* protein expression profile in DU-145 *caspase-1* transfectants. Western blot analysis of *caspase-1* protein expression in the DU-145 cells. Cell lysates were prepared and subjected to PAGE (12.5%) as described in "Materials and Methods". Lane 1, parental DU-145; lanes 2-3, DU-145 neo control transfectants; lanes 4-9, *caspase-1* clones. The molecular weights are indicated on the right. Expression of α -actin was analyzed as a normalizing control.

(365nm) and cells containing fragmented nuclei were designated as apoptotic at 32X magnification. Three random fields were counted per well in duplicate wells (under fluorescence) from triplicate experiments.

A time course of apoptosis induction following ionizing irradiation was also performed under the same conditions. DU-145 parental, neo control and *caspase-1* overexpressing transfectants were exposed to radiation (3Gy) and apoptosis was determined at 0, 9, 24, 48 and 72 h post-irradiation.

Clonogenic assay. DU-145 neo-transfectants and DU-145 *caspase-1* transfectants were plated in 6-well plates at a density of 1000 cells/well at 24h post-irradiation. After 7 days of incubation (37°C), colonies (> 30 cells) were stained with crystal violet (6). Colony forming efficiency was defined as the ratio of the number of colonies to the number of plated cells.

Caspase activity evaluation

A) Caspase inhibitors assay. DU-145 neo transfectants and DU-145 *caspase-1* transfectants were plated at a density of 100,000 cells per well in 6-well plates. At 60% confluency, cells were treated with DMSO (vehicle), the *caspase-1* inhibitor, z-YVAD, FMK (FK-013), or the *caspase-3* inhibitor, z-DEVD.FMK, (FK-034) (Enzyme Systems, Livermore, CA, USA). After addition of the inhibitor or DMSO, cells were irradiated with 3 Gy of ionizing radiation. At 24h post-irradiation, apoptotic cells were evaluated by Hoechst's staining as described above. Experiments were performed in duplicate.

B) Caspase-1 activity assay. The *caspase-1* activity assay was performed using the *Caspase-1* Colorimetric Assay according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). Cells were plated at a density of 500,000 cells/T-75 flask and treated with radiation (3Gy). Cell lysates (200mg of protein) were

collected after 3, 9, 24, 48 and 72h post-irradiation and incubated with the *caspase-1* colorimetric substrate WEHD-pNA for 3.5h at 37°C. Following incubation, the absorbance was measured at 405 nm and *caspase-1* activity was determined by comparison to the non-irradiated cells.

Statistical analysis. Statistical analysis was conducted using the *t*-test for analysis of significance between the different values. Values were expressed as the mean values \pm SEM. Statistical significance was established at values of $p < 0.05$.

Results

The effect of overexpression of *caspase-1* on the cellular response to ionizing radiation was evaluated *in vitro* using the androgen-independent, human prostate cancer cell line, DU-145. Following transfection, semi-quantitative RT-PCR analysis and Western blotting in cloned *caspase-1* transfectants established the expression profile of *caspase-1* at the mRNA and protein levels, respectively. As shown in Figure 1A, the exogenously introduced *caspase-1* is expressed at high levels in three of the 9 transfectant clones analyzed (C-12, C-18 and C-23). Western analysis documented the presence of the pro-enzyme form of *caspase-1* in C-12, C-18 and C-23 indicating the overexpression of *caspase-1* as well as the active fragment of *caspase-1* in the three clones (p20 band).

We subsequently examined whether *caspase-1* overexpression had an effect on cell growth and basal cell death rate. In comparison to the DU-145 parental cells and neo controls, *caspase-1* transfectants exhibited a decrease in the rate of DNA synthesis (as demonstrated

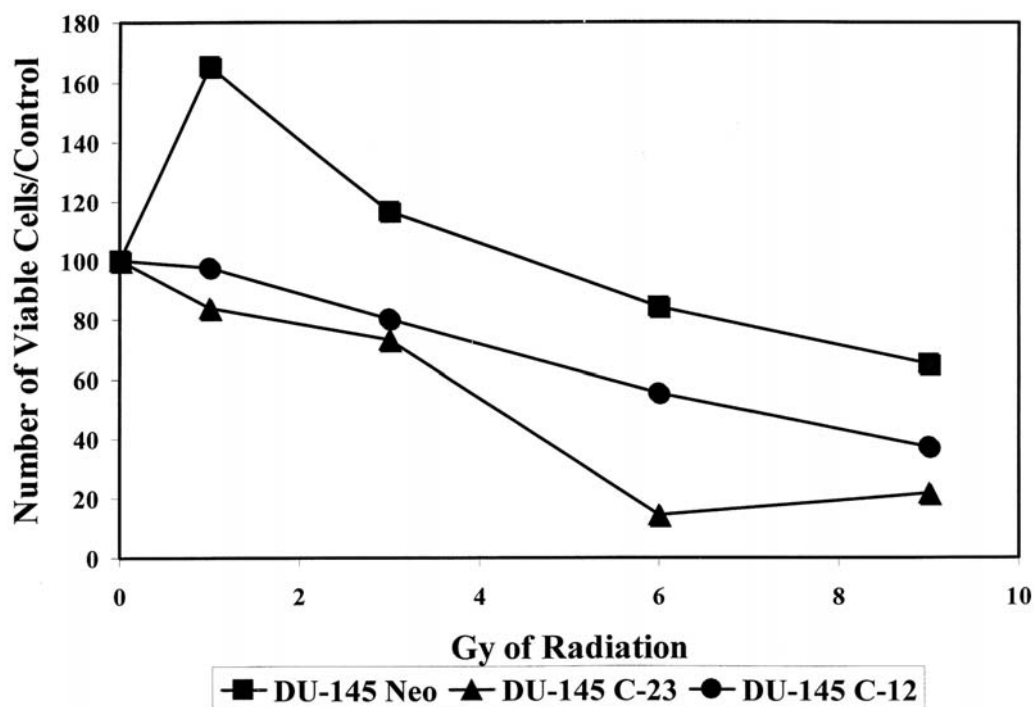


Figure 2A. Dose response of cell death of DU-145 caspase-1 transfectants in response to radiation. DU-145 neo and DU-145 caspase-1 C-12 and C-23 cloned transfectants were exposed to increasing doses of radiation (1-9Gy) and loss of cell viability was determined at 24h post-irradiation using the Trypan blue exclusion assay. Data is expressed as the number of irradiated cells relative to untreated cells (average values from three independent experiments).

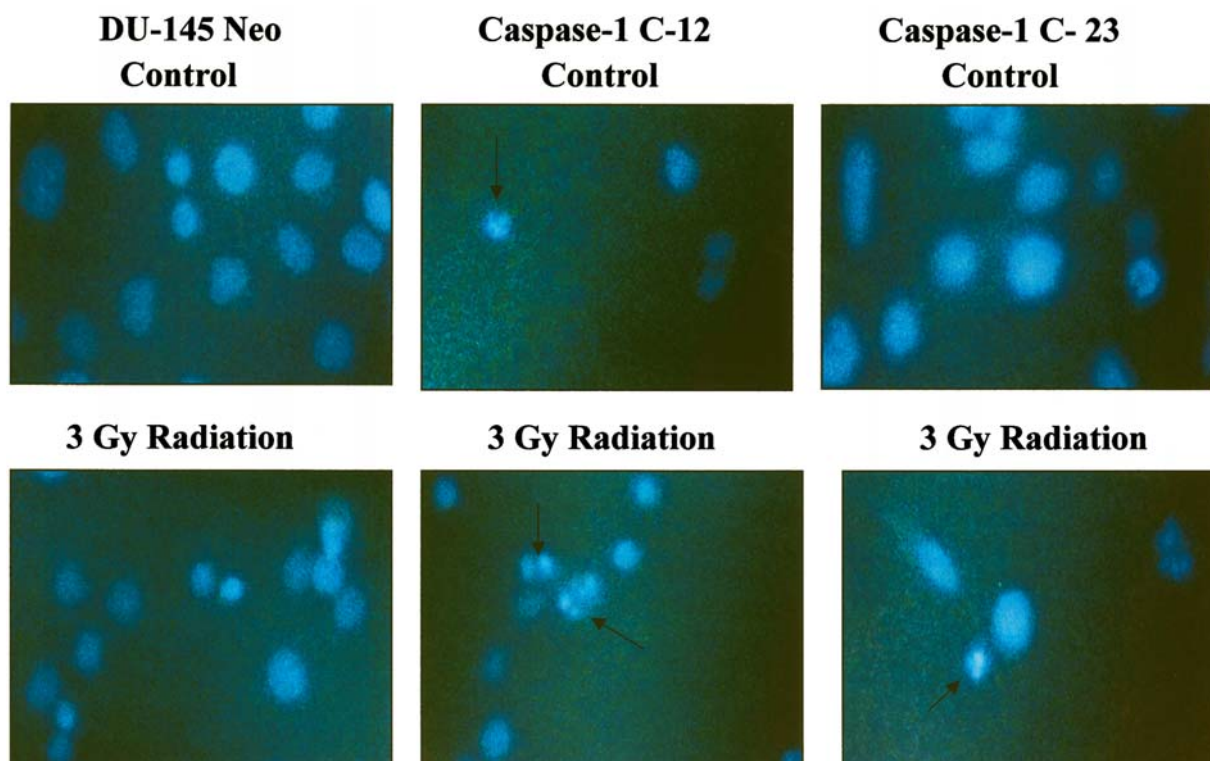


Figure 2B. Detection of apoptosis in irradiated prostate cancer cells. DU-145 neo transfectants and caspase-1 C-12 and C-23 clones were exposed to ionizing irradiation (3Gy) and apoptosis was evaluated at 24h post-irradiation as described in "Materials and Methods".

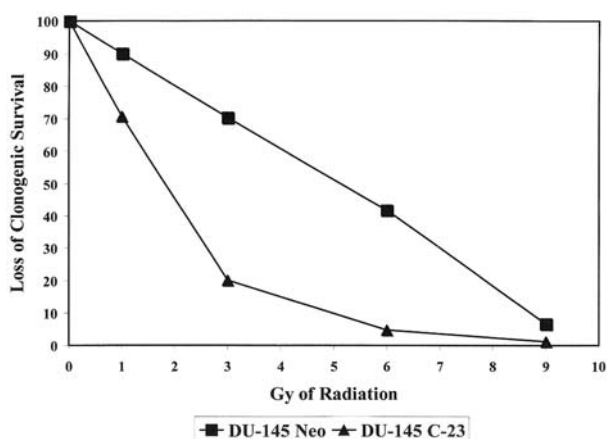


Figure 3. Effect of caspase-1 overexpression on clonogenic survival of DU-145 transfectants following radiation treatment. Neo control and caspase-1 transfectant cells were plated and at 7 days post-irradiation, the clonogenic ability was evaluated as described in "Materials and Methods". Cells were counted and values represent total number of surviving colonies.

Table I. Radiation dose response of apoptotic induction in caspase-1 overexpressing prostate cancer cells.

Radiation dose (Gy)	DU-145 neo	DU-145 C-23 caspase-1
0	0.5±0.2	1.3±0.5
1	0.4±0.3	3.5±0.9*
3	1.7±0.9	4.2±1.1*
6	1.8±0.8	6.7±0.8*
9	2.4±0.5	10.8±1.0*

DU-145 neo and DU-145 caspase-1 C-23 transfectant clones were exposed to increasing doses of radiation and the incidence of apoptosis was determined at 24h post- irradiation using the Hoechst's stain. Data is expressed as the number of apoptotic cells over the total number of cells/section. Values represent mean±SE (mean values from triplicate assessments from three independent experiments).

by ³H-thymidine assay), a higher apoptotic capacity (as demonstrated by Hoechst's staining), and a decrease in cell viability over time (as demonstrated by trypan blue exclusion assay) (data not shown). DU-145 neo transfectants and DU-145 caspase-1 C-12 and C-23 transfectants were exposed to increasing doses of radiation (1-9Gy) and cell viability and the incidence of apoptosis was determined 24 h post-irradiation. Figure 2A demonstrates a loss of cell viability in the caspase-1

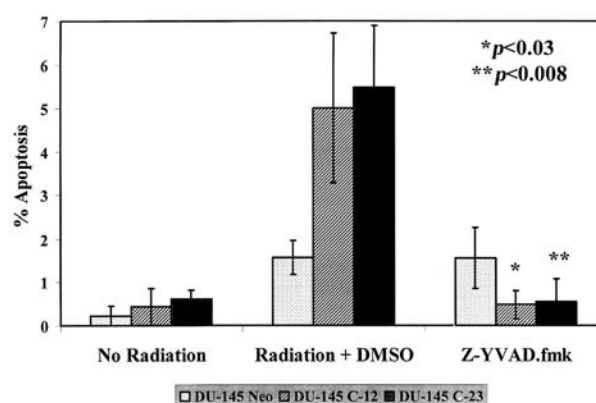


Figure 4A. Effect of the caspase-1 inhibitor on radiation-induced apoptosis in DU-145 caspase-1 clones. Cells were treated with the caspase-1 inhibitor (z-YVAD.fmk) and were subsequently exposed to radiation (3Gy). At 24h post-irradiation, the incidence of apoptosis was determined using Hoechst's stain. Control cells were treated with DMSO (as a vehicle control) z-YVAD.fmk decreased apoptosis induction in the caspase-1 transfectants ($p < 0.03$ for C-12; $p < 0.008$ for C-23) compared to the neo controls. Values represent the mean of three experiments performed in duplicate (\pm S.E.M).

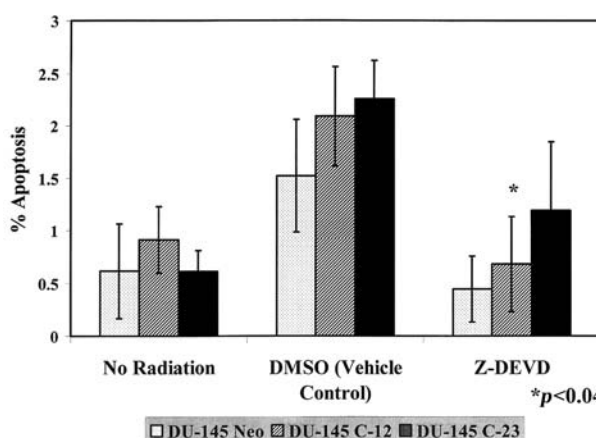


Figure 4B. Effect of the caspase-3 inhibitor on radiation-induced apoptosis in DU-145 caspase-1 clones. Cells were treated with the caspase-3 inhibitor (z-DEVD.fmk) and were subsequently exposed to ionizing radiation (3Gy). At 24h post-irradiation, apoptosis induction was determined as described in the legend for Fig. 5A. Values represent the mean of three experiments performed in duplicate (\pm S.E.M.). * p value = 0.04.

clones compared to the neo controls. A greater sensitivity of the caspase-1 clones in response to radiation is reflected by a decrease in the number of viable cells. At 3Gy of radiation a 30% decrease in cell viability was detected in both caspase-1 clones, C-12 and C-23 compared to the neo transfectants. A further loss in (50%) in cell viability was observed at 6Gy for the caspase-1 transfectants, compared to the modest 20% decrease in the neo control cells.

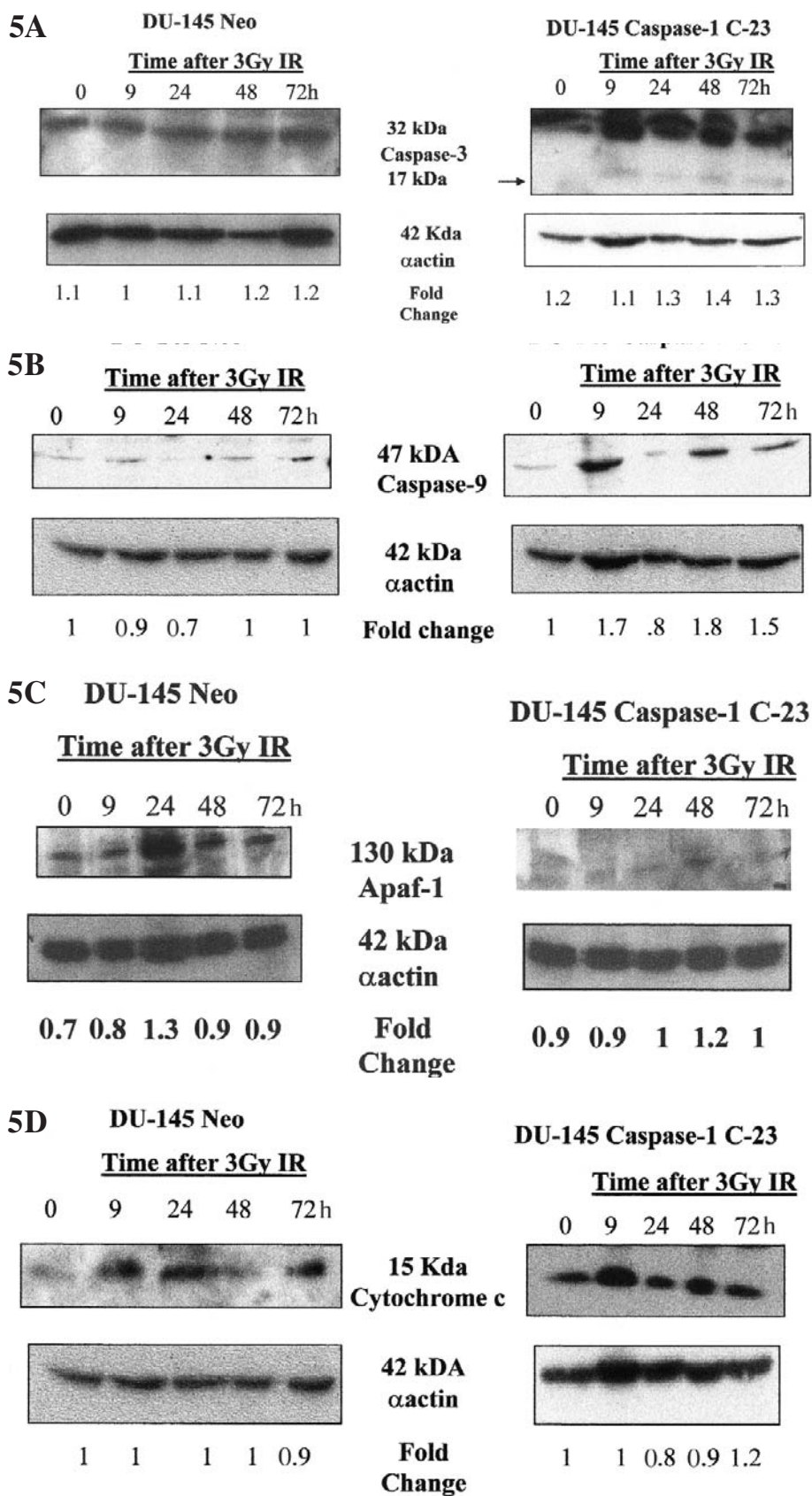


Figure 5.

The sensitivity of the caspase-1 transfectants to ionizing radiation was also established on the basis of Hoechst's apoptosis staining. Induction of apoptosis was based on the number of cells exhibiting fragmented morphology, as identified using Hoechst's staining, and visualized under fluorescence microscopy. The incidence of apoptotic nuclei, after exposure to 3Gy of ionizing irradiation, is more evident in the caspase-1 transfectants (C-12 and C-23) than the neo control transfectants (Figure 2B). Table I shows the results of the quantitative analysis of the apoptotic evaluation in response to increasing radiation doses. A dose-dependent increase in the number of apoptotic cells was significantly higher in C-23 caspase-1 transfectant compared to the neo control cells ($p < 0.05$).

The effect of overexpression of caspase-1 on radiation-induced loss of clonogenic survival was subsequently analyzed. Figure 3 reveals data indicating that overexpression of caspase-1 affects the clonogenic ability of DU-145 prostate cancer cells in response to ionizing radiation. Caspase-1 transfectants exhibited a significant decrease in clonogenic survival compared to the neo controls, that was dose-dependent. At 1Gy of radiation, there was a 30% loss of clonogenic survival of C-23 clone compared to a 10% loss of survival in the neo clone and at higher radiation dose (3Gy) a greater loss of clonogenic survival was detected (80%). With increasing radiation doses however, there was no significant difference between the caspase-1 transfectants and the neo controls.

A time course of apoptosis induction (at 3Gy radiation dose) revealed a 2-fold increase in the number of apoptotic cells for the C-23 caspase-1 clone compared to the neo controls. This increase was statistically significant ($p < 0.03$), and paralleled with an overall loss of cell viability to a single radiation dose (3 Gy) in the caspase-1 transfectants (data not shown). Both the induction of apoptosis and the loss of cell viability were observed at earlier time points (24 h) than the neo controls.

Peptide inhibitors are designed to mimic known sequences of caspase substrates and can suppress or alter caspase-dependent effects in a number of cells (28). The effect of the caspase inhibitors is shown in Figure 4. Cells were treated the caspase-1 inhibitor (z-YVAD.fmk) or caspase-3 inhibitor (z-DEVD.fmk), and were subsequently exposed to 3Gy of radiation. As shown in Figure 4A, Z-YVAD.fmk decreased the incidence of apoptosis in the caspase-1 overexpressing cells as compared to the control cells (treated with DMSO). Suppression of apoptosis by the

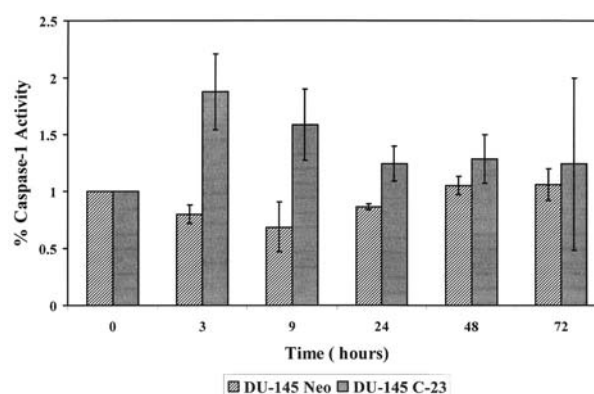


Figure 6. Caspase-1 activity in DU-145 cloned transfectants. Cell lysates (200mg of protein) from DU-145 neo and DU-145 caspase-1 C-23 transfectants were incubated with the caspase-1 colorimetric substrate WEHD-pNA for 3.5 h at 37°C. The absorbance was subsequently measured at 405 nm and caspase-1 activity was determined by comparing with the non-irradiated cells. Values represent the average from two independent experiments performed in duplicate (mean \pm SEM).

caspase-1 inhibitor was significantly different for the caspase-1 clones ($p < 0.03$ for C-12 and $p < 0.008$ for C-23), while the caspase-1 inhibitor had no effect on the neo controls. In the presence of the caspase-3 inhibitor, there was a statistically significant decrease in the number of apoptotic cells in both the neo control and the caspase-1 transfectants (Figure 4B) ($p < 0.04$).

To investigate whether the enhanced radiosensitivity of the DU-145 caspase-1 transfectants involved the mitochondrial pathway of apoptosis execution, Western blot analysis was used to examine the effect of caspase-1 overexpression on the expression/activation profile of key apoptotic regulators, *i.e.* caspase-3, caspase -9, *Apaf-1* and cytochrome c, in response to ionizing radiation (3Gy). As shown in Figure 5A, temporal analysis of the apoptotic process following radiation revealed constitutive expression of the pro-enzyme form of caspase-3 in both the caspase-1 transfectants and neo controls, with the activated form of caspase-3 after 9h of radiation in the caspase-1 transfectants. This activation of caspase-3 was paralleled by a transient increase in pro-caspase-9 levels (Figure 5B) at 9 h post-irradiation. In contrast, no changes were detected in either *Apaf-1* induction (Figure 5C), or in cytochrome c release (Figure 5D), during the first 24 h post-irradiation.

Figure 5. Effect of overexpression of caspase-1 on caspase-9, -3, *Apaf-1* and cytochrome c expression: Western blot analysis of caspase-3 (Panel 6A), caspase-9 (Panel 6B), *Apaf-1* (Panel 6C), and cytochrome c (Panel, 6D). Activation of caspase-3 was observed 9 h post-irradiation in C-23. This activation was paralleled with an induction of caspase-9. No significant difference was observed for *Apaf-1* or cytochrome c between C-23 and the neo control. α -actin was used as a normalizing loading control as described in "Materials and Methods".

To determine whether the overexpression of caspase-1 in prostate cancer cells correlated with caspase-1 activation in response to irradiation, caspase-1 activity was determined after exposure to ionizing radiation. As shown in Figure 6, overexpression of caspase-1 in DU-145 cells results in an increased caspase-1 activity compared to the neo control cells. This activity was noticeably detected within the first 3h after exposure which correlates with expression of other apoptotic members (Figure 5A and Figure 5B).

Discussion

The realization that identification of molecules within the apoptotic pathway that will potentiate tumor radiosensitivity will enable optimization of radiotherapy for the treatment of prostate cancer, by enhancing the therapeutic response to radiation, intensified efforts for the development of novel modulators of the apoptotic threshold of prostate cancer cells. The present study documents that gene-transfer mediated caspase-1 overexpression radiosensitizes androgen-independent prostate cancer cells by lowering their apoptotic threshold to the killing effects of radiation, thus linking caspase-1 to the apoptotic radiosensitivity of human prostate cancer cells. Our results indicate that the caspase-1 overexpressing DU-145 prostate cancer cells had an enhanced sensitivity to radiation, as demonstrated by the significant elevation in apoptosis at lower radiation doses. The suppression of radiation-induced apoptosis by the caspase-1 inhibitor indicates the functional requirement for the overexpressed caspase-1 in the apoptotic response of prostate cancer cells. Consistent with these observations is data indicating that the Fas ligand/TNF- α induce radiation sensitization in the hormone-sensitive prostate cancer cells LNCaP *via* modulation of the caspase cascade (29).

A recent study by Ueki *et al.*, (23), which documented that silencing caspase-1 activity provides a growth advantage in the formation of solid tumors, directly supports our findings. These investigators reported that overexpression of caspase-1 increased the rate of apoptosis *in vitro* and *in vivo* in renal carcinoma cell lines (23). Furthermore adenovirus-mediated transfer of caspase-1 inhibited the growth of experimental prostate cancer *via* induction of apoptosis (30). Caspase-1 has also been shown to be involved in the UVB irradiation of human keratinocytes (31). In sharp contrast to the present findings, a recent study (32) implicated an anti-apoptotic function for caspase-1 in pancreatic carcinoma. A potential anti-death function for this caspase may be a result of alternative splicing of caspase-1, since caspase-1 has four known isoforms, at least two of which may have an antagonistic activity against apoptosis (20). Alternatively one may argue about the possibility of tissue and cell type specificity.

Temporal analysis of the apoptotic process following radiation revealed activation of caspase-3 in the caspase-1 overexpressing prostate cancer cells. This was paralleled by a significant induction of caspase-9 expression, while no changes in the activity of either caspase were observed in the neo control transfectants following irradiation. Since caspase-1 is an initiator caspase, while caspase-9 is a major executioner, activation of both caspases as early events point to potential cross-talk between the receptor-mediated apoptotic pathway and the mitochondrial pathway. Interestingly enough, there were no significant differences in the induction profile of *Apaf-1* and cytochrome c in the caspase-1 overexpressing prostate cancer cells and neo controls following radiation. Indirect support for the concept of targeting caspases to change the apoptotic threshold of cancer cells, by potentially interfering with caspase-1 activity, stems from recent evidence demonstrating that caspase-1 is directly regulated by interferon- γ in certain tumor cell types (33).

In search of the mechanistic component of this effect of caspase-1 on apoptotic threshold of cancer cells, one has to consider that caspase-1 has been identified as a transcriptional target of *p53* because it has a site in its promoter required for the transactivation by *p53* (34). The *p53* gene, the most commonly mutated gene in human cancer, exhibits cell cycle inhibitory effects through induction of p21, a cyclin-dependent kinase inhibitor (35). While p21^{WAF-1} traditionally inhibits the activity of cyclin/cdk complexes thus blocking cell cycle progression, and controlling proliferation, recent evidence suggests that in response to DNA damaging agents such as ionizing irradiation, it acts as a tumor suppressor gene by initiating G1 arrest in cancer cells (36). It is thus of paramount significance that antisense therapy against cell cycle regulators such as p21 has been recently identified to radiosensitize cancer cells including prostate cancer, by converting growth arrest to apoptosis (37). Another critical cell cycle regulator, functional disruption of which is involved in prostate tumorigenesis, is the *Rb* gene (38). The human prostate cell line DU-145, lacks a functional *Rb* (39). The possibility emerges that it is such a loss in *Rb* that may contribute to the relative radiation resistance that characterizes these prostate tumor cells. One could however argue, considering the present results, that overexpression of caspase-1 could potentially override the effects of a *Rb* null cell line (DU-145) to allow for enhanced sensitivity to the apoptotic effects of radiation.

The molecular basis for the heavy involvement of the apoptotic status in predicting clinical radioresistance stems from a rapidly expanding body of evidence indicating that *bcl-2*, *bax* and *p53* expression in prostate cancer was associated with treatment failure to radiation

therapy (11, 12, 40). While further studies are required to define a similar predictive role for caspase-1 in clinical prostate cancer, ongoing studies are focused on the impact of caspase-1 overexpression on the *in vivo* anti-tumor effect of radiation against prostate cancer xenografts. Interfering with the molecular regulation of apoptosis will enable the development of apoptosis-based therapeutic strategies that will reduce prostate cancer radioresistance. Chemical-induced dimerization (CID) of transfected caspase pro-forms may also prove an effective therapeutic strategy for androgen-independent prostatic tumors as was suggested by the elegant studies of Shariat *et al.* (30).

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