Downmodulation of dimethyl transferase activity enhances tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in prostate cancer cells

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Abstract. One of the major obstacles in curing prostate cancer is the development of drug resistance. It is not only imperative to discover the molecular basis of resistance but also to find therapeutic agents that can disrupt the resistant pathways. Tumor necrosis factor TNF-related apoptosis-inducing ligand TRAIL-like ligands or agonist TRAIL-receptor monoclonal antibodies have entered phase I and II clinical trials with a very limited cytotoxic profile when used systemically in a variety of cancers. Therefore, TRAIL-receptor agonists are new proapoptotic pharmaceutical agents with great potential as new cancer therapeutic agents. Although many cancer cells undergo TRAIL-mediated apoptosis, some are resistant to TRAIL. Therefore, we have been investigating mechanisms to overcome TRAIL resistance in cancer cells so that TRAIL-associated compounds can be used effectively in clinical trials. Epigenetic inactivation of proapoptotic genes, or activation of survival signaling, can cause cross-resistance to several anti-tumor therapies and to immune cytotoxic lymphocytes. We hypothesize that 5-aza-2 deoxycytidine aza-dCR, decitabine may render TRAIL-resistant prostate cancer cells sensitive to caspase-8-mediated apoptosis and may, therefore, be therapeutically efficient. We evaluated the antiproliferative effects of decitabine on the following four prostate cancer cell lines: well-differentiated AR positive LnCaP p53$^+$, PTEN$^-$ and 22rv1 p53$^+$ and PTEN$^+$]; poorly-differentiated AR negative PC3 p53$^-$, PTEN$^-$ and DU145 p53 mutant, PTEN$^+$. Here, we provide evidence that treatment with sub-optimal concentrations of decitabine are additive to TRAIL effects in well-differentiated PCa cells whereas the same treatment shows synergistic effects in poorly-differentiated PCa cells through increased caspase-8 expression, down-modulation of Akt activation and through the expression of certain anti-apoptotic molecules including FLIP, PED/PEA-15, survivin and c-IAP-1. Our findings demonstrate that decitabine at relatively low concentrations restores caspase-8 expression and sensitises resistant PCa cells to TRAIL-induced apoptosis leading to important implications in novel therapeutic strategies targeting defective apoptosis pathways in advanced prostate tumors.

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Introduction

Prostate cancer PCa is one of the most common malignancies among men (1) and in organ-confined tumors, treatments such as radical prostatectomy, radiotherapy and chemotherapy are often effective. This tumor begins as an androgen-dependent, non-invasive disease, which is easily treated with surgery and hormone therapy in early stages (2). However PCa frequently develops into a hormone-independent, highly aggressive and invasive disease (3) and multiple drug resistance often develops in patients with advanced disease.

Promoter CpG island hypermethylation is an important carcinogenic event in prostate adenocarcinoma. Regardless of tissue type, human cancers have in common both focal CpG island hypermethylation and global genomic hypomethylation. Transcriptional silencing of different tumor-suppressor genes, associated with aberrant promoter methylation, contributes to tumor initiation and progression and most neoplasms show hypermethylation of one or more genes (4-6).

Lodygin and coworkers (5) as well as Cho and coworkers (6) demonstrated that several CpG island loci were found in prostate cancer cells and tumors to display cancer-related hypermethylation including RASSF1A, GSTP1, RARB, TNFRSF10C, APC, BCL2, MDR1, ASC, TIG1, RBP1, COX2, THBS1, TNFRSF10D, CD44, p16, and RUNX3.

Demethylating agents, such as azacytidine and its derivative deoxycytidine, have been shown to prevent tumorigenesis in the TRAMP mouse model of PCa (10) and, therefore, could be appealing to molecular targeted therapies (7-9). In addition, the expression of cell death receptors, which usually modulates the number of cells present in a given tissue, is often deregulated

by transcriptional silencing of downstream molecules. The modulation of tumor growth has also been associated both to the activity of cytotoxic lymphocytes, that kill target cells by various mechanisms including perforin/granzymes and the TNF-α superfamily that induces apoptosis in tumor cells (11). Tumors that develop anti-apoptotic mechanisms can also develop cross-resistance to cytotoxic immune lymphocytes (12,13). The molecular mechanisms that govern anti-apoptotic resistance in cancer cells are numerous and vary from one type of tumor to another. Recent studies suggest that apoptosis resistance, by blockade of the Fas apoptotic pathway, may play an important role in tumor progression in several malignancies (14-16). The TNF-related apoptosis-inducing ligand TRAIL, newest member of the TNF-α family, selectively induces apoptosis through the DR4 and DR5 death receptors. Therefore, TRAIL has attracted much attention as a promising agent for the development for cancer therapy. Unfortunately, cancer cells develop resistance to TRAIL-induced apoptosis through different pathways, including phosphatidylinositol-3-kinase PI3-K/Akt signaling pathway (17,18). Elevated AKT levels in PTEN negative LNCaP cells have been shown to be a major cause of resistance to TRAIL-induced apoptosis (19-21). Agents that could sensitise resistant cancer cells to TRAIL might be particularly important for developing combination regimens that can increase the overall therapeutic efficacy of TRAIL (22,23).

Multiple genes control the apoptotic pathway in order to restrain inadequate cell proliferation and a defect in the signaling mechanism gives the cancer cells an added survival advantage leading to tumor initiation, progression and even drug resistance. Apoptosis is mediated by a family of cystine proteases called caspases. Caspases exist as latent pro-enzymes and are activated by proteolytic cleavage. Several key genes involved in apoptosis have been shown to be the target of epigenetic changes. The death receptor pathway involves the engagement of the death receptors and the recruitment of the adaptor protein FADD and procaspase-8 to form a complex known as the death-inducing signaling complex. Active caspase-8 can directly activate caspase-3, whose activity is often regulated even by the abundance of apoptosis inhibitors. Caspase-8 has previously been reported to be frequently inactivated by epigenetic silencing in many tumors including PCa (22). Although TRAIL is a potentially excellent selective therapeutic agent with minimal toxic side-effects, PCa cells develop resistance to it (24,25). We hypothesize that 5-aza-2 deoxycytidine aza-dCR, decitabine may render TRAIL-resistant prostate cancer cells sensitive to caspase-8-mediated apoptosis and may, therefore, be therapeutically efficient. Here, we provide evidence that treatment with sub-optimal concentrations of decitabine induces caspase-8 re-expression in poorly-differentiated PCa cells. Consequently, activation of caspase-8 and downstream caspases, upon addition of human recombinant soluble TRAIL, was restored by pretreatment with decitabine.

Materials and methods

Reagents and cells. All tissue culture material was purchased from Hyclone (Cramlington, NE, USA). Plasticware was purchased from Nunc (Roskilde, Denmark). Antibodies, when not otherwise specified, were purchased from Santa Cruz (Santa Cruz, CA, USA). Anti-DNMT1 clone 60 B1220; Biocarta (Hamburg, Germany), -DNMT3a clone 64B 1446; Biocarta, and -DNMT3b clone 52A 1018, Biocarta were used to demonstrate DNMT inhibition. DNA methyltransferase activity was evaluated in nuclear cell extracts using a colorimetric EpiQuik DNA methyltransferase Activity/Inhibitor Assay Kit BioVision (Mountain View, CA, USA). Goat anti-TRAIL-receptor antibodies R&D Systems (Minneapolis, MN). TRAIL *Killer*TRAIL was purchased from Alexis Biochemicals (San Diego, CA, USA). Akt kinase activity was tested using a non-radio-active assay kit purchased from Stressgene Bioreagents (Victoria, BC, Canada) on cell lysates after partial purification on a MonoQ exchange column as recommended by the supplier. The Caspase-9 colorimetric kit assay was purchased from Biomol International (L.P Plymouth Meeting, PA, USA). TiterTACS *in situ* 96-well apoptosis detection kit was purchased from Trevigen (Gaitherburg, MD, USA). 5-Aza-deoxycytidine Aza/dCR, decitabine was purchased from Sigma Aldrich (St. Louis, MO, USA), dissolved in DMSO 100 mmol/l, and aliquots were stored at -20˚C.

Cell lines. We used LnCaP, 22rv1, PC3 and DU145 human PCa cell lines which were obtained from the American Tissue Culture Collection ATCC (Rockville, MD, USA).

Growth assays. Cells were seeded at a density of $2x10^4$ cells per dish in 50-mm Petri dishes. Cells were left to attach and grow in 5% FCS DMEM for 24 h. After this time, cells were maintained in different culture conditions. Morphological controls were performed every day with an inverted phase-contrast photomicroscope Nikon Diaphot, Tokyo, Japan, before cell trypsinisation and counting. Cells trypsinised and resuspended in 20 ml of saline, were counted by a haemocytometer every 24 h (LabRecyclers, Gaithersburg, MD, USA) and 5 independent counts were performed for each dish. All experiments were conducted in triplicate. In order to calculate the inhibitory concentrations at 50% IC_{50} of aza-CR, 2,500 cells were cultured in 96-well plate for 96 h. After adhesion 16 h, cells were grown as described. After 96 h the cells were exposed for 4 h to thyazol blue MTS; Promega (Madison, WI, USA). The 96-well culture plate were then placed on a microplate shaker for 5 min and the absorbance of the converted dye was measured at the wavelength of 490 nm using a Bio-Rad multiscan plate reader Bio-Rad (Richmond, CA, USA). Inhibition curves were drawn by means of values obtained by OD percentages versus a control for each concentration. IC_{50} values were calculated by the GraFit method Erithacus Software Ltd. (Staines, UK) considering the slopes of inhibition curves obtained for each group of tests.

The data obtained from the growth inhibition assay of the co-treatments were analysed using the Combination Index CI that describes the interaction between two mutually non-exclusive drugs i.e. two drugs that have different or independent mechanisms of action. When CI <1 drugs are synergistic, when CI = 1 drugs are additive and when CI >1 drugs are antagonistic. The CI is calculated from the following equation:

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$$CI = \frac{D_1}{D_{X1}} + \frac{D_2}{D_{X2}} + \frac{D_1 D_2}{D_{X1} D_{X2}}$$

where D_{x1} and D_{x2} are the doses of drug 1 and 2 which are required to inhibit growth by x%.

FACScan analysis cell cycle and apoptosis analysis. Cells to be used for DNA demethylation experiments were grown in the presence of 0.1 to 10 μmol/l decitabine for 96 h. Decitabine-containing medium was replaced daily. Cells were treated with TRAIL in complete medium for apoptosis induction. Cell viability was then analyzed by CellTiter 96 assay Promega and adherent cells were trypsinised, pooled with the culture supernatant containing the apoptotic cells already detached from the dish and centrifuged. Cells ($1x10^6$) were washed in PBS and fixed for 30 min by the addition of 1 ml of 70% ethanol. After 30 min, the cells were pelleted by centrifugation 720 g; 5 min, and resuspended in 1 ml of DNA staining solution PBS containing 200 mg/ml RNase A, 20 mg/ml propidium iodide plus 0.1% Triton X-100 and stained by incubation at room temperature for 60 min. All cells were then measured on a FACScan flow cytometer Becton-Dickinson (UK) and analysed using CellQuest software (Becton-Dickinson). Apoptotic cells were detected by a quantifiable peak in sub-G1 phase corresponding to the red fluorescence light emitted by subdiploid nuclei of cells.

Western blot analysis. Cells were washed with cold PBS and immediately lysed with 1 ml of lysis buffer 50 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 1 mM sodium orthovanadate, 30 mM p-nitrophenyl phosphate, 10 mM sodium pyrophosphate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin and 10 μg/ml leupeptin. Lysates were electrophoresed in 7% SDS-PAGE, and separated proteins transferred to nitrocellulose and probed with the appropriate antibodies using the conditions recommended by the antibody suppliers.

Statistical analyses. Data are expressed as the mean + SEM of at least three independent experiments. Statistical analysis was performed using an unpaired Student's t-test.

Results

It has been demonstrated that decitabine is able to reduce DNA methylation after 24 h of treatment. Thus a single 24 h pulse of 0.5-20.0 μM decitabine aza-dCR was administered in two well-differentiated, AR positive LnCaP $p53^+$, $PTEN^-$ and 22rv1 $p53^+$ and $PTEN^+$ and two poorly-differentiated AR negative PC3 $p53^-$, $PTEN^-$ and DU145 p53 mutant, $PTEN^+$ PCa cell lines. To measure the proliferative response to aza-dCR, we chose to analyze mitochondria function by MTT absorbance since it measures both cytostatic and cytotoxic responses and since it provides an accurate overall measure of drug sensitivity. Exposure to decitabine resulted in a dose-dependent growth rate reduction as shown in Fig. 1A. A single 24-h pulse of decitabine, followed by 72 h of culture in drug-free medium, induced 50% growth inhibition in cells at a concentration IC_{50} ranging between 0.5 and 25.0 μM.

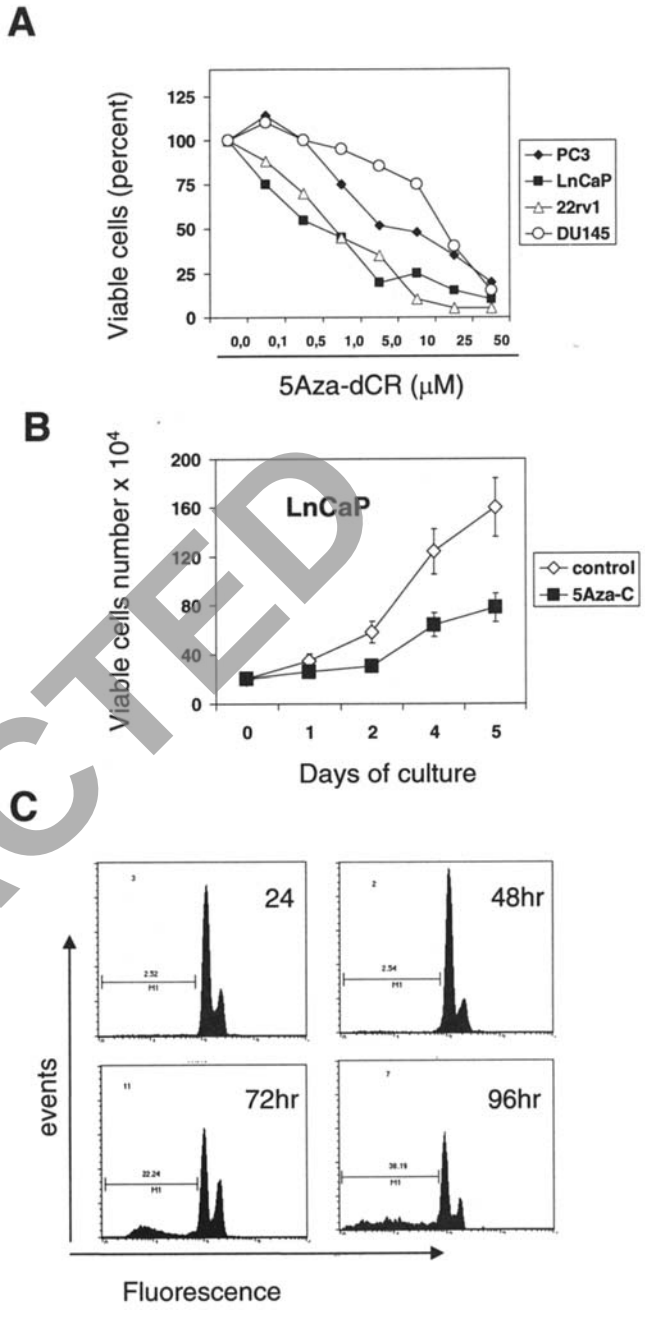


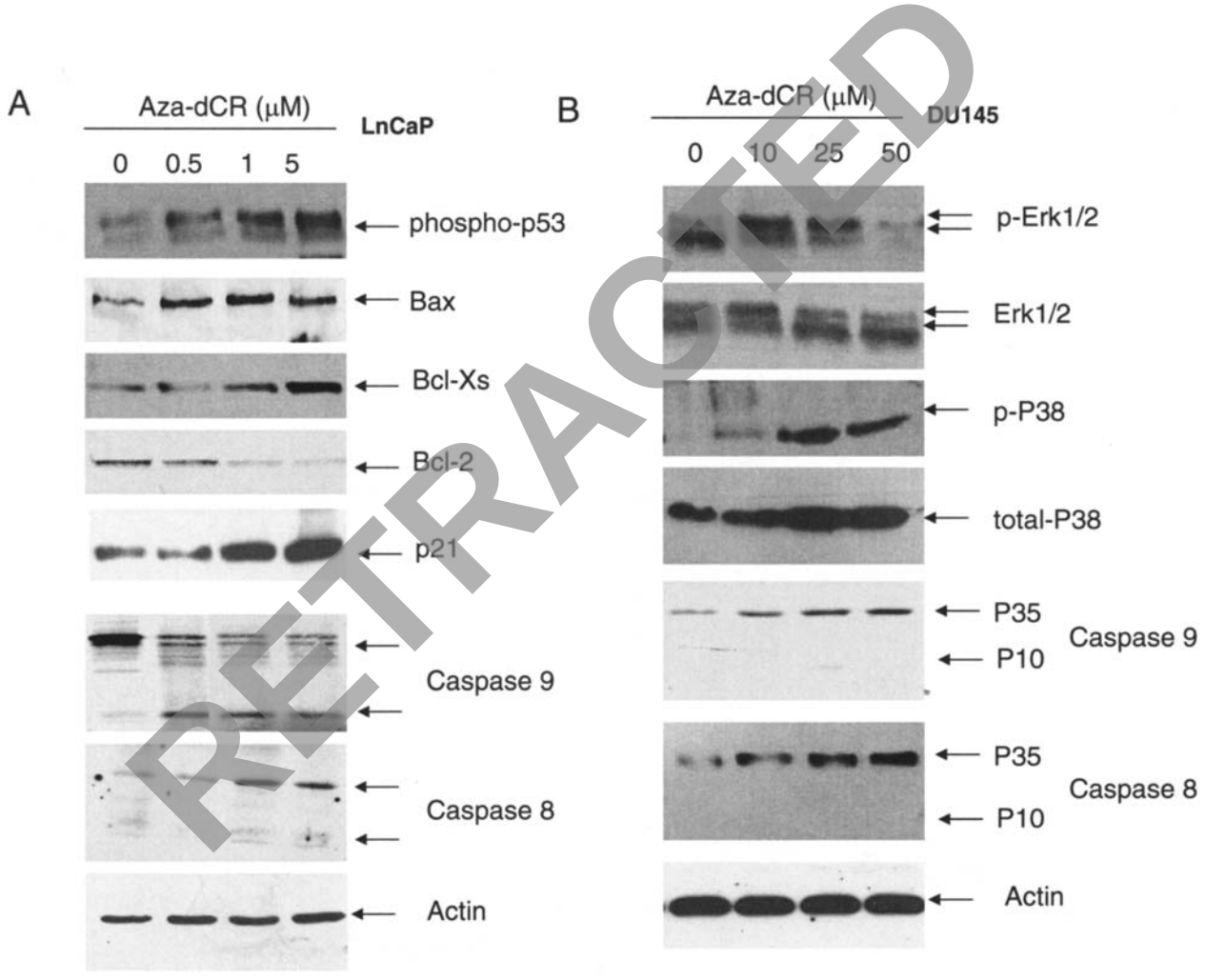
Figure 1. A, Growth response to aza-CR treatment in androgen-sensitive LNCaP and 22rv1 and in androgen insensitive PC3 and DU145 cells. LnCaP and 22rv1 cells show a higher antiproliferative acute effect when compared to undifferentiated PC3 and DU145 cells. B, Growth curves of LnCaP cells treated or not with 1 μM decitabine. C, LnCaP cells treated with 1.0 μM decitabine for 24 h time 0 followed by 24, 48 and 72 h in drug-free medium were induced to trigger apoptotic events in a time-dependent manner.

The effects were higher in 22rv1 IC_{50} = 0.5 μM and LnCaP IC_{50} = 1.0 μM when compared to PC3 IC_{50}=5.0 μM and DU145 IC_{50} = 25 μM cells. Fig. 1B shows the proliferation curve of LnCaP cells cultured in the presence of 1.0 μM decitabine. Cell cycle distribution analysis showed a dose- and time-dependent increment of cell proportion in the G0/G1 phase in highly sensitive LnCaP and 22rv1 cells Table I. Furthermore, these cells showed an evident dose- and time-dependent increase of apoptotic cells. Fig. 1C shows the FCAScan analysis in LnCaP cells at 24, 48, 72 and

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Table I. Cell cycle distribution in 22rv1, LnCaP, PC3 and DU145 PCa cell lines after a 48-h treatment after a 24-h single pulse of decitabine.

	Decitabine (μM)																			
	22rv1					LnCaP					DU145					PC3				
	0	1	5	10	25	0	1	5	10	25	0	1	5	10	25	0	1	5	10	25
Apoptosis	<1	15	27	20	45	<1	7	25	38	45	<1	<1	<1	<1	5	<1	<1	<1	10	20
C0/G1	54	63	76	81	92	64	71	79	78	93	53	55	52	52	47	64	64	55	55	50
S	25	22	13	13	7	16	19	11	11	2	27	25	15	7	<1	24	22	25	20	10
G2/M	20	15	11	6	<1	20	10	10	6	5	20	20	33	41	53	12	14	20	25	40



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Figure 2. A, Molecular events involved in decitabine-mediated LnCaP apoptosis. Western blot analysis on the expression of pro-apoptotic and anti-apoptotic elements in LnCaP cells treated with 0.5, 1.0 and 5.0 μM decitabine corresponding to IC_{20}, IC_{50} and IC_{80}, respectively. B, Modulation of MAPK pathways in DU145 treated with 10, 25 and 50 μM decitabine, corresponding to IC_{20}, IC_{50} and IC_{80}, respectively. Each lane was loaded with 40 μg of proteins. This experiment is representative of three individual experiments.

96 h after the single pulse of 24 h time 0 followed by 24, 48 and 72 h in drug-free medium showing the time-dependent apoptosis induced by decitabine. In PC3 and DU145 cells, the treatment with decitabine determined very little PC3 or no apoptosis DU145 at all, with a dose- and time-dependent cell cycle increment of cells in G2/M phase (Table I). We analyzed the molecular mediators of the apoptotic process in LnCaP and 22rv1 cells which are p53 wt and decitabine responders. We observed reduced Bcl-2 levels with increased levels of phosphorylated p53. This was associated with increased Bax, Bcl-Xs and p21 levels. In parallel, caspase-9 and -8 were cleaved. Fig. 2A shows these effects in LnCaP cells at low doses 0.5, 1.0 and 5.0 μM of decitabine, corresponding to IC_{20}, IC_{50} and IC_{80} values for this cell line. Similar dose-

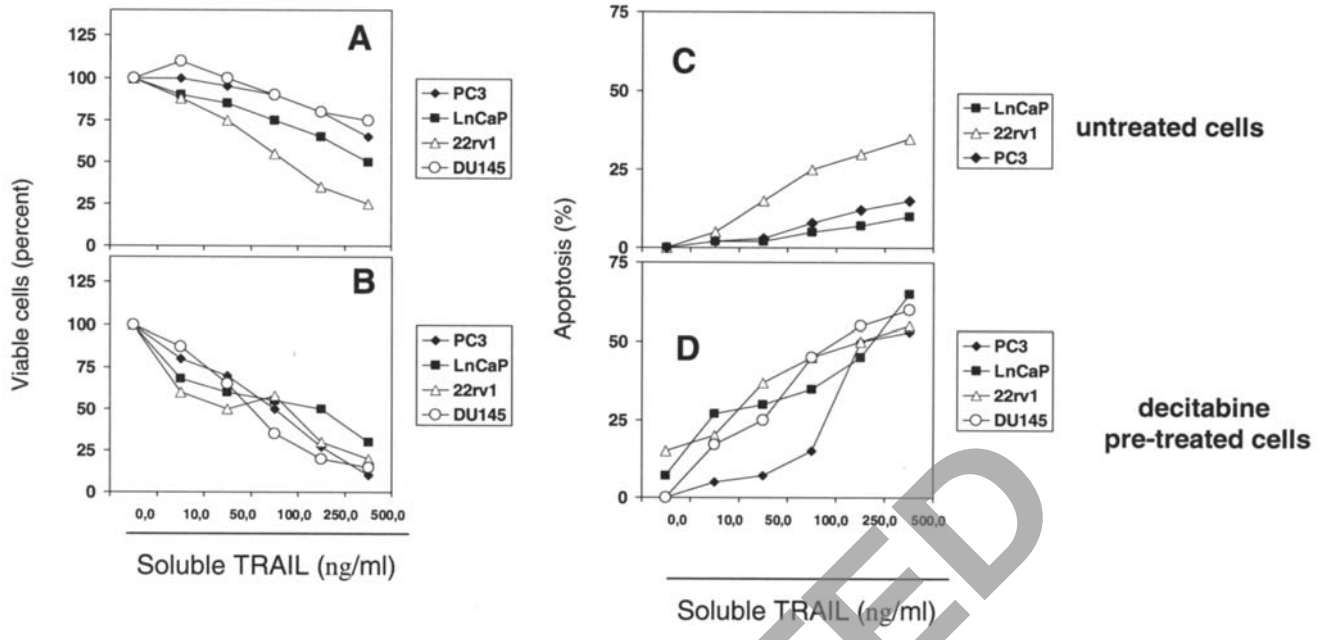


Figure 3. A and B, Comparisons amongst antiproliferative effects to TRAIL with (B) or without (A) 1.0 μM decitabine. C and D, Comparisons of apoptotic effects mediated by TRAIL with (A) or without (B) 1.0 μM decitabine.

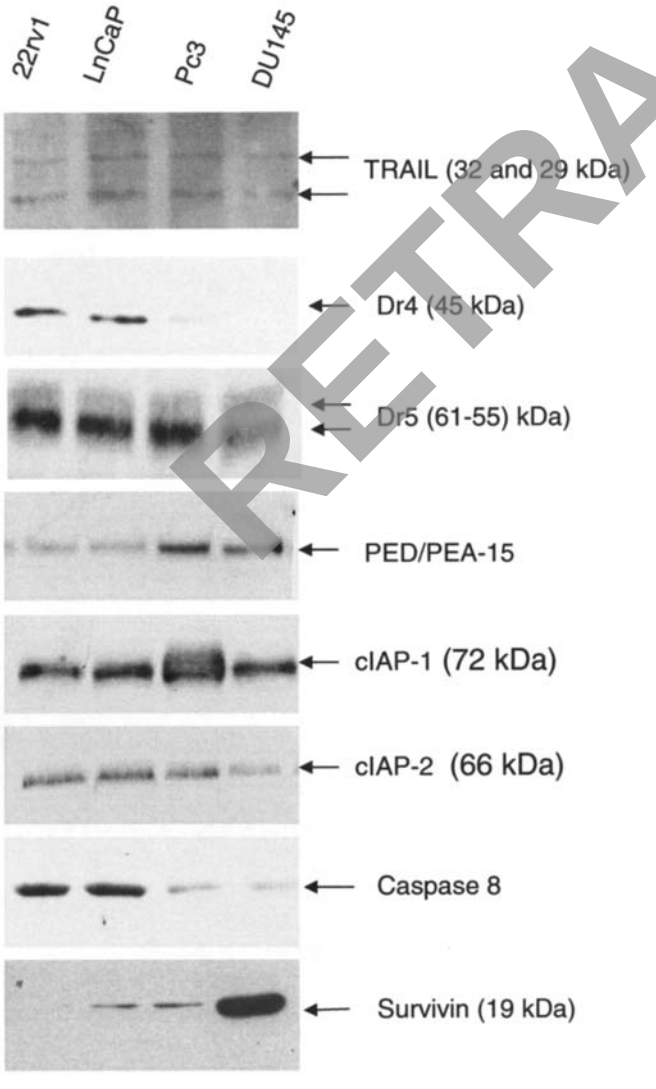


Figure 4. Molecular arrangements involved in TRAIL-dependent cell death in four PCa cells lines having different TRAIL effectiveness.

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dependent results were observed for 22rv1 cells in which even 0.1 μM decitabine shows a significant modification in the analyzed molecular mediators of apoptosis. Analysis of p53-mediated p21 induction and Bcl-2 reduction also revealed that tumor cells differing in p53 status show corresponding differences in sensitivity to decitabine toxicity. In fact, decitabine-induced cell-arrest/apoptosis was higher in p53 wt LnCaP and 22rv1 cells than in p53 negative PC3 and p53 mutant inactive DU145 cells. In addition, we observed that in PC3 and DU145 cells, caspase-8 and -9 expression levels were very low in basal conditions and that these increased after decitabine treatment. However, this caspase induction was not able to produce appreciable cell death without p21 induction or Bcl2 downmodulation (data not shown). We observed that these cells were induced to modulate MAPK pathways. Indeed, Erk activity was reduced and p38 MAPK activity was induced. Fig. 2B shows the molecular variations in DU145 cells treated with high doses of decitabine 10, 25 and 50 μM corresponding to IC_{20}, IC_{50} and IC_{80} values, respectively. The increased activity of p38 MAPK could be required for the induction of cell cycle arrest in the G2 phase. The induction of both caspase-8 and -9 also suggests that the apoptotic process can be modulated by the activation of death receptors which are present but not activated in well- and poorly-differentiated PCa cells. Therefore, we investigated the possible role of decitabine in restoring cell sensitivity to the tumor necrosis factor-related apoptosis-inducing ligand TRAIL. As expected, human recombinant TRAIL 10-500 ng/ml induced a modest anti-proliferative effect in PC3 and DU145 cell lines (Fig. 3A) and these did not undergo apoptosis even when exposed to high doses of TRAIL (Fig. 3C); on the contrary, the more differentiated cells 22rv1 particularly and LnCaP less evidently were sensitive to TRAIL-induced cell growth inhibition (Fig. 3A) and apoptosis (Fig. 3C). However, a single 24-h pulse of decitabine, used at relative IC_{50} values, followed by 72 h of

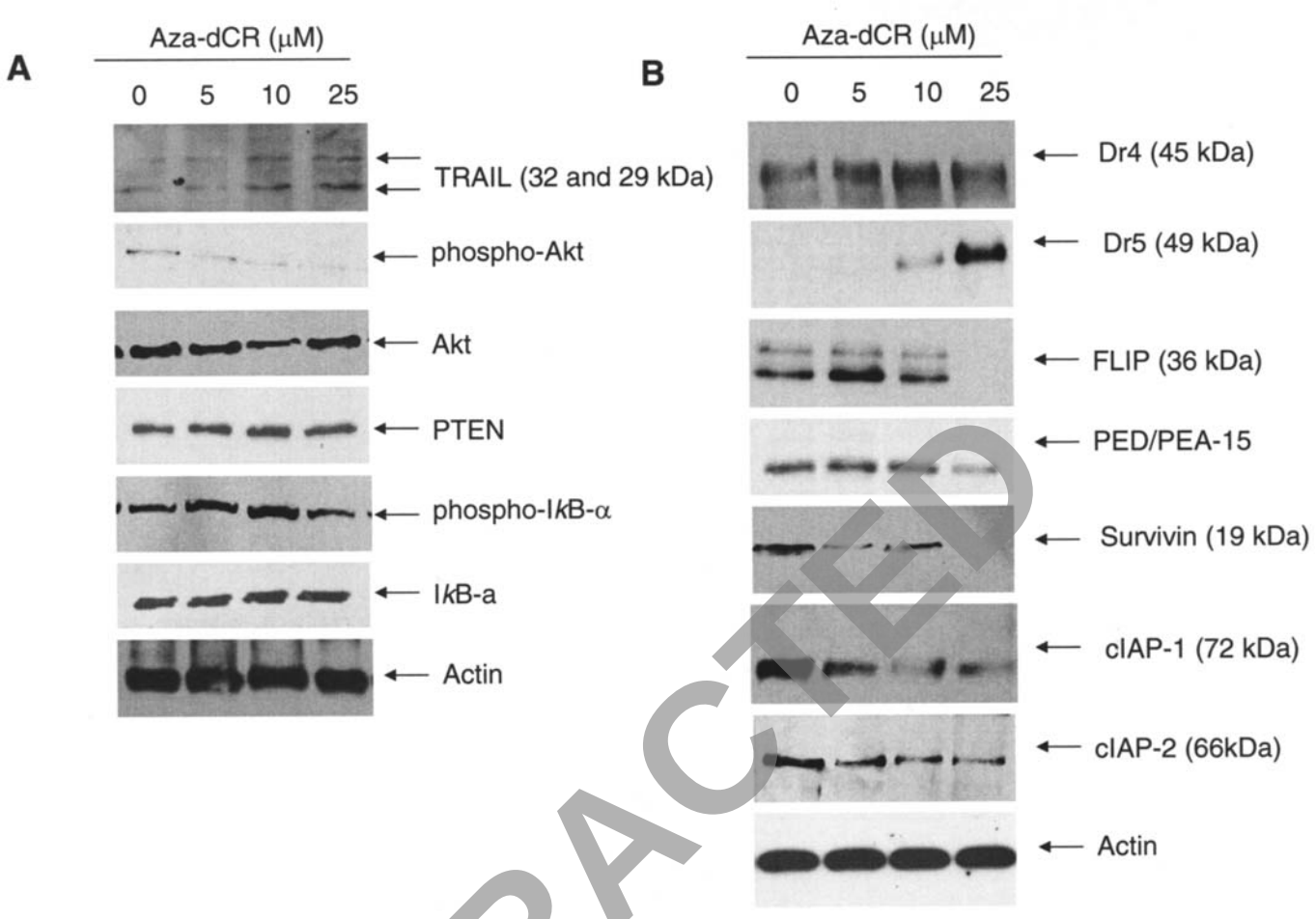


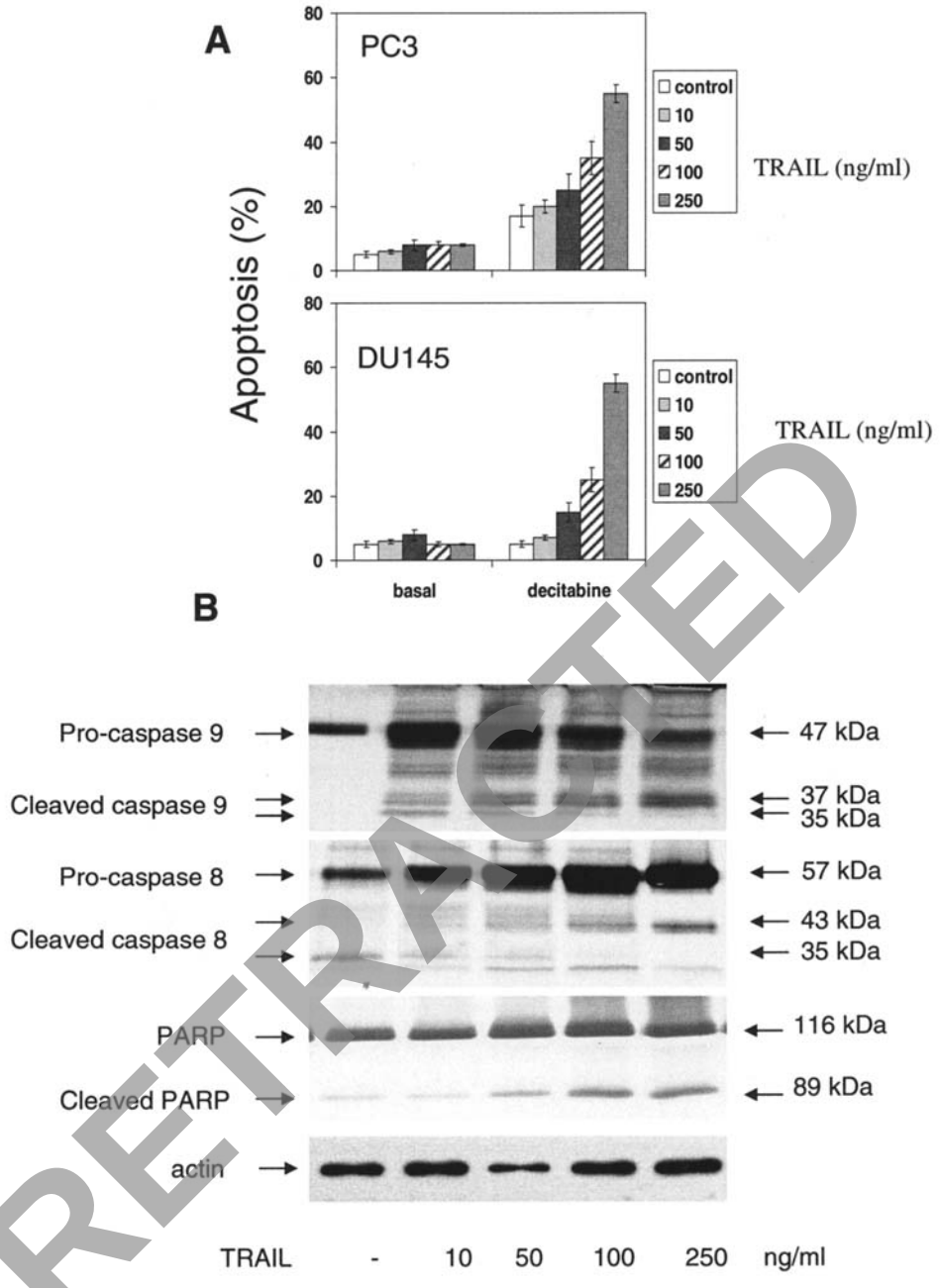
Figure 5. Molecular events involved in TRAIL-depedent cell death after decitabine pretreatment in PC3 cells. Role of the active form of Akt in aza-CR-induced TRAIL in DU145 cells. A, Effects of aza-CR treatment on the levels of phosphorylated Akt, total Akt, PTEN, phosphorylated IκBα phospho-IIκBα and total IκBα were determined by Western blotting. ß-actin was used as a loading control. B, Effects of aza-CR treatment on the levels of death receptors Dr4 and Dr5, anti-apoptotic protein FLICE-inhibitory protein FLIP, caspase-8 inhibitory protein PED/PEA-15, Survivin, cIAPs 1 and 2.

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culture in drug-free medium, resulted in a considerable dose-dependent growth reduction (Fig. 3B) and apoptosis (Fig. 3D) after human recombinant TRAIL was added to all cell models used in the study. However, the effects were higher in undifferentiated PC3 and DU145 cells when compared to differentiated LnCaP and 22rv1 cells. The comparison of combination indices revealed that decitabine was additive with TRAIL in 22rv1 CI = 0.80 and LnCaP CI = 0.91 cells and was super-additive synergistically in PC3 CI = 0.35 and DU145 CI = 0.27 cells.

To explore the mechanisms responsible for TRAIL resistance, we investigated whether an altered expression of key elements of the proximal TRAIL pathway could be involved in impaired transmission of the apoptotic signal. We first analyzed the expression levels of TRAIL and TRAIL receptors death receptors DR4, TRAIL-R1 and DR5, TRAIL-R2 in our cell system. We observed that all cells secreted appreciable amounts of TRAIL whereas DR4 and DR5 levels were very low and very high, respectively, in all the cells analyzed. Fig. 4 shows that caspase-8 inhibitory protein PED/PEA15 expression was expressed at similar levels in 22rv1 and LnCaP cells whereas these were higher in PC3 and DU145 cells. Moreover, the levels of cIAP-1 was similar and low in 22rv1, LnCaP and DU145 cells and considerably higher in PC3 cells. The levels of cIAP-2 was similar and low in 22rv1, LnCaP and PC3 cells and considerably lower in DU145 cells. Survivin was absent in 22rv1, low in LnCaP and PC3 and very high in DU145 cells. Levels of caspase-8 were very low in PC3 and DU145 cells, when compared to 22rv1 and LnCaP cells.

We observed that decitabine treatment was not able to increase the levels of secreted TRAIL in our cell line panel. Moreover, decitabine treatment was able to reduce akt activation. Fig. 5A shows the effects of decitabine treatment in DU145 cells. Death receptor expression analysis of DR4 and DR5 revealed that aza-CR induced these proteins (Fig. 5B) in DU145 cells. Similar results were obtained in PC3 and to a lesser extent in LnCaP and 22rv1 cells. Contemporaneously, protective molecules against TRAIL-mediated cell apoptosis like FLIP, PEA15, survivin and cIAP-1, but not cIAP-2 were significantly reduced. Fig. 5B shows the effects of decitabine treatment on FLIP, PEA15 and c-IAPs expression in DU145 cells. When we combined decitabine and TRAIL we observed that decitabine was able to synergize with TRAIL inducting a strong cell death in TRAIL-resistant PC3 and DU145 cells, in a dose-dependent manner, by activating caspase-8 and -9 and by cleaving PARP (Fig. 6).



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Figure 6. A, TRAIL-depedent cell death after decitabine pretreatment in PC3 and DU145 cells cells. B, Caspase activation and PARP cleavage in decitabine pretreated PC3 cells by TRAIL.

Discussion

Abnormal DNA methylation patterns observed in cancer cells usually correspond to overall hypomethylation of the genome accompanied by regional hypermethylation. Treatment of PCa cell lines with decitabine caused p53 induction and a dose- and time-dependent inhibition of cell proliferation. We found that wild-type p53-expressing LnCaP cells are more sensitive to decitabine-induced toxicity than p53 defective PC3 and DU145 cell lines. Therefore, we examined whether cellular toxicity and p53 activation after decitabine treatment was a consequence of specific DNA methyltransferase inhibition. PC3 and DU145 cells p53 null were induced to a G2/M cell cycle arrest with increased activation of p38 MAPK without downmodulation of Akt activity and induction of Bad and p21 activity which are triggered in a normal program of cell death apoptosis. Although tumor necrosis factor-related apoptosis-inducing ligand TRAIL is potentially an excellent and selective therapeutic agent with minimal toxic side-effects, PCa cells develop resistance to TRAIL. We showed that PCa cells are completely refractory to TRAIL-mediated apoptosis. However, treatment with decitabine restored TRAIL responsiveness through caspase-8 and TRAIL-R1 up-regulation and through PED/PEA-15 down-regulation. We observed that high levels of the anti-apoptotic protein PED/PEA-15 correlated with increased resistance to TRAIL. The balance between the expression of caspase-8 and its inhibitor PED/PEA-15 seems extremely relevant for determining susceptibility to TRAIL-mediated apoptosis. DNA methylation inhibition in PCa cells resulted in a

modification of this balance in favour of caspase-8, thus increasing apoptosis susceptibility. Although methyltransferase-mediated caspase-8 silencing occurs in other cancers, there is a lack of agreement concerning the identification of the promoter responsible for epigenetic regulation of caspase-8 expression. Therefore, the significance of direct caspase-8 promoter demethylation or transacting factors acting on this promoter in decitabine-induced caspase-8 up-regulation remains to be determined. We also found that DNA demethylation resulted in increased TRAIL-R1 expression. Decitabine-induced TRAIL-R1 expression could represent a possible sensitization strategy to treat these types of cancer. For instance, low levels of TRAIL-R1 expression seem to be associated with TRAIL resistance in non-small cell lung carcinoma cells. A combined treatment with decitabine and IFN-γ was reported to increase both TRAIL-R1 levels and apoptosis sensitivity of these cells, indicating that epigenetic control of TRAIL-R1 transcription might occur in some other cancers (22). Decitabine has been used in humans for the treatment of myelodysplastic syndromes, leukemia, and solid tumors. Phase I and II trials showed that decitabine is well tolerated and moderately effective in some types of cancer (26-29). To date, no data are available concerning clinical toxicity of TRAIL and agonist TRAIL receptor antibodies, currently undergoing phase I and II studies. However, our experimental data are very promising with respect to anti-tumor activity high and toxicity low. However, intense efforts are essential in order to assess the possible clinical use of decitabine and TRAIL combinations for the treatment of advanced forms of PCa given the high malignancy of these tumors and the short life expectancy in these patients.

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