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Steroid-mediated inhibition of radiation-induced apoptosis in C4-1 cervical carcinoma cells is p53-dependent

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Abstract

In human papillomavirus (HPV) infected cervical epithelial cells the synthetic steroid dexamethasone inhibits radiation-induced apoptosis and increases the transcription of HPV *E6/E7*, enhancing p53 degradation. The aim of this study was to determine if suppression of apoptosis was mechanistically linked to changes in p53. HPV 16 *E6* or *E6/E7* expression vectors were transiently transfected into C4-1 HPV 18-positive cervical carcinoma cells to mimic the enhanced transcription following steroid treatment. After irradiation, apoptosis was suppressed in these cells comparable to the effect observed after steroid treatment alone. To confirm whether loss of p53 was responsible for the inhibition of apoptosis, residual p53 in C4-1 cells was targeted by stable transfection with a dominant-negative p53 mutant. While radiation-induced apoptosis increased after mutant transfection, inhibition of programmed cell death by steroid treatment was either eliminated or substantially reduced. Steroid-dependent inhibition of radiation-induced apoptosis in carcinoma of the cervix involves E6 modulation of p53 expression and may adversely affect treatment.

Keywords: Apoptosis; Steroids; Dexamethasone; p53; Cervical carcinoma

1. Introduction

Infection with the human papillomavirus (HPV) is a major risk factor in the development of cervical carcinoma (for review, see Refs. [1,2]). The E6 protein of high-risk HPV types can suppress cellular p53 function by sequestering p53, which inhibits its ability to bind DNA [3,4,5] and in association with the E6 Associated Protein (E6AP), E6 can target p53 for ubiquitin-dependent proteolysis [6–8]. Expression of HPV E6 or E6/E7 results in abrogation of p53-dependent cell cycle arrest, increased cellular proliferation and decreased apoptosis [9–12]. Glucocorticoids such as dexamethasone have been shown to activate the transcription of HPV *E6/E7* through glucocorticoid response elements in the transcriptional control region of the HPV genome [13]. We have shown previously that in HPV 18 transformed C4-1 cells, dexamethasone upregulates HPV E6/E7 which corresponds to inhibition of radiation-induced apoptosis concurrent with decreased, but detectable, expression of p53 and p21 [14,15]. Therefore, it appears that despite high-risk HPV infection, C4-1 cells retain functional p53 which allows access to p53-dependent apoptosis following genotoxic stress [16–18]. The protective effect of dexamethasone in this system appears to be positively mediated by HPV E6 or E7 expression since steroid-dependent inhibition of apoptosis was not observed in cells that either lacked HPV or in which HPV E6/E7 expression was repressed by dexamethasone [15]. However, in contrast, cell lines that do not contain HPV may also be protected from clonogenic death following steroid treatment and thus the cytoprotective

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effects of these hormones may operate through a variety of pathways and be cell type-dependent [19,20].

The tumour suppressor protein p53 is the most frequent target for mutation or inactivation in human cancers [21]. Following DNA damage, p53 can transactivate target genes which trigger cell cycle arrest through p21/WAF1, or promoters of apoptotic cell death such as bax and Fas/APO1/CD95 [22,23]. p53 has been shown to facilitate radiation-induced apoptosis and loss of p53 by mutation can result in decreased cellular sensitivity to radiation and chemotherapeutic agents and a worse prognosis for patient outcome [24–26]. However, mutation of p53 does not necessarily restrict access to apoptosis. Increased apoptosis is observed in cells expressing mutant p53, which supports the existence of either a p53-independent pathway to apoptosis, or a gain in function subsequent to mutation [27–29].

The main aim of this study was to elucidate the mechanism by which cervical carcinoma cells exhibit decreased apoptotic potential following dexamethasone treatment.

2. Materials and methods

2.1. Cell culture

The human cervical carcinoma cell line C4-1 and transfectants of this parental line were cultured in Dulbecco's modified eagle medium (DMEM; Sigma, St. Louis, MO, USA) supplemented with 4.5 g/l glucose, 4 mM L-glutamine, 100 U/ml penicillin/streptomycin, and 10% fetal calf serum (Biologos, Naperville, IL, USA). All cultures were maintained in a 5% CO_2 atmosphere at 37 °C. Cells were irradiated at 6 Gy using a dual head Cs-137 Gammacell 40 irradiator (Nordion International, Ontario, Canada). Dexamethasone (Decadron Phosphate, Merck, Sharp and Dohme, West Point, PA, USA) was added to the cultures at a final concentration of 1 μM, 30 min prior to irradiation.

2.2. Stable and transient transfections

2.2.1. *HPV 16* E6 *and* E6/E7

Cells (2×10^5) were plated onto tissue culture compatible coverslips in 24-well plates and allowed to adhere overnight. HPV 16 *E6* or *E6*/*E7* cDNA plasmids were co-transfected into C4-1 cells with the green fluorescent protein (GFP) reporter construct pEGFP-N1 (Clontech), using the GenePorter Reagent (Gene Therapy Systems). After transfection, 20% fetal bovine serum was added alone or supplemented with dexamethasone for a final concentration of 1 μM and cells were subsequently irradiated. Fresh media, with dexamethasone as appropriate, was added 18 h after transfection and cells were collected 48 h following treatment. Apoptosis was determined by analysing the nuclear morphology of the GFP-positive cells by counterstaining with propidium iodide. Transfection was confirmed by reverse transciptase polymerase chain reaction (RT-PCR) to detect the HPV 16 *E6* and *E6*/*E7* genes.

2.2.2. Dominant-negative mutant p53

A vector containing dominant-negative (dn) mutant p53 was the generous gift of Daphne Haas-Kogan (University of California at San Francisco Medical Center). The pc DNA3.1-based vector (Invitrogen) contains the human cytomegalovirus (CMV) promoter/enhancer upstream of p53 containing a point mutation at amino acid 175 from arginine to histidine (R175H), as well as the neomycin resistance gene (*NEO*). C4-1 cells were transfected with either control plasmid (pc) or plasmid containing the mutant p53 (dnp53) with the GenePorter Reagent. Cells were allowed to recover for 48 h at which time selection with 800 μg/ml G418 eliminated the untransfected cells. Following selection, the cell pool was subcloned to select cells that expressed mutant p53 protein. Colonies arising from single cells were rescued by trypsinisation using cloning cylinders and clones were expanded in the presence of G418.

2.3. Analysis of *HPV 16* E6 *and* E6/E7 *cDNA using RT-PCR*

In order to determine whether the transient transfection was successful, the HPV 16 *E6* or *E6*/*E7* genes were amplified from transfected cells using the following primers.

*E*6 (Fwd) 5′-atg cac caa aag aga act gca-3′
*E*6 (Rev) 5′-tta cag ctg ggt ttc tct acg-3′
*E*6/*E*7 (Rev) 5′-tta tgg ttt ctg aga aca gat-3′

RNA was collected by the Trizol method (Gibco) and RT-PCR was performed using 10 ng RNA. A 50 μl PCR mix containing 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM HPV 16 *E6*/*E7* primers, 2U *Taq* DNA polymerase and 2 μl cDNA from the first strand synthesis reaction was made. Cycling was performed in a Perkin Elmer DNA Thermal Cycler 480 as follows: 94 °C 1 min, 58 °C 1 min and 30 s, 72 °C 3 min for 25 cycles. PCR products were subject to gel electrophoresis on a 2% agarose gel and were visualised by ethidium bromide staining. Bands corresponding to HPV 16 *E6* (475 basepairs (bp) and *E6*/*E7* (771 bp) were confirmed by comparison to the 100 bp ladder (New England Biolabs) (Fig. 1).

2.4. Analysis of apoptosis:

2.4.1. Morphology

For morphological analysis of GFP-positive cells, coverslips with adherent cells were washed in posphate-buffered solution (PBS), fixed in 2% paraformaldehyde

and placed onto slides. Cells were then analysed to determine the percentage of GFP-positive cells that exhibited apoptotic characteristics. Apoptotic cells are those with fragmented or condensed nuclei, while viable cells exhibit a round, intact nucleus. At least 200 cells from 3 to 6 independent experiments were analysed without knowledge of the treatment.

2.4.2. DNA fragmentation

DNA was isolated as previously described in Ref. [14]. Briefly, 2×10^6 cells were lysed in 500 μl lysis buffer (5 mM Tris–HCl (pH 7.4), 20 mM ethylene diamine tetraacetic acid (EDTA), 0.5% Triton X-100) at 4 °C for 2 h. Cell debris was removed by centrifugation at 30 000 *g* for 30 min at 4 °C. The supernatant was collected and treated with 0.5 mg/ml RNAse A at 50 °C for 1 h, followed by 0.4 mg/ml proteinase K in 1% sodium dodecyl sulphate (SDS) at 50 °C for 1 h. The DNA was then extracted with chloroform/isoamyl alcohol (24:1) and precipitated overnight in 95% ethanol. The DNA was dissolved in 10 μl TE (10 mM Tris–HCl (pH 8.0), 1 mM EDTA) and subjected to gel electrophoresis in a 1% agarose gel in 1×TAE buffer. Gels were stained with ethidium bromide, visualised by ultraviolet light and photographed.

2.5. p53 and p21 analysis

Protein extraction was performed for 30 min on ice in RIPA buffer containing 250 mM NaCl, 50 mM Tris, 0.1% Nonidet P-40, 0.1% SDS, 0.5% sodium deoxycholate and the protease inhibitors leupeptin and phenyl methylsulphonyl fluoride (PMSF). Protein amounts were determined using the BioRad Detergent Compatible Protein Assay kit. Equal amounts of protein (15–20 μg) were diluted in Laemmli buffer containing β-mercaptoethanol and resolved on a 10% minigel by SDS–PolyAcrylamide Gel Electrophoresis. Proteins were then transferred to a PVDF membrane (BioRad) using the NOVEX system followed by immunoblotting using antibodies to either p53, which recognises both wild-type and mutant p53 (Oncogene Science Ab 6) or p21, (Pharmingen 15091A). The membrane was subsequently developed using Enhanced ChemiLuminescence (ECL, Amersham).

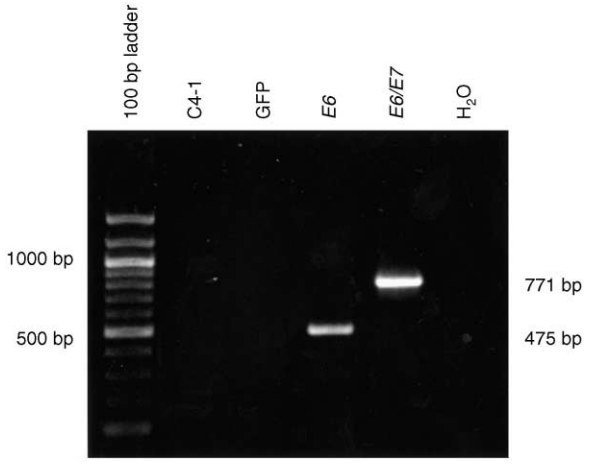

Fig. 1. Detection of human papillomavirus (HPV) 16 *E6* and *E6*/*E7* sequences by polymerase chain reaction (PCR). C4-1 cells (HPV 18-positive) were co-transfected with vectors encoding the green fluorescent protein (GFP) and either HPV 16 *E6* or *E6*/*E7* cDNA. To confirm the transfection of the viral gene sequence, reverse transcriptase (RT)-PCR was performed using primers specific to either HPV 16 *E6* or *E6*/*E7*. Untransfected cells (C4-1) and cells transfected with control vector alone (GFP) did not express the HPV 16 genes. However, cells transfected with *E6* or *E6*/*E7* revealed characteristic bands for either *E6* (475 bp) or *E6*/*E7* (771 bp).

2.6. Clonogenic survival

A single cell suspension of C4-1 cells was plated into 60 mm tissue culture dishes. Cells were either left untreated or treated with 1 μM dexamethasone and/or 2 and 4 Gy γ irradiation. Clones were grown for 10 days at which time cells were fixed in 95% ethanol and stained with 1% Methyl Blue. Colonies of 50 cells or more were counted and considered to have arisen from a single viable cell. Controls for each irradiated sample were untreated or treated with dexamethasone without irradiation and the percent survival following irradiation was calculated relative to the controls. The percent clonogenic survival was obtained by averaging the results of three separate experiments.

3. Results

3.1. Expression of HPV 16 E6/E7 suppresses apoptosis

HPV 16 was utilised for the transfection experiments so that endogenous HPV 18 *E6*/*E7* could be differentiated from the transfected HPV 16 *E6*/*E7* by RT-PCR. The presence of the HPV 16 *E6* (475 bp) and *E6*/*E7* (771 bp) bands confirmed a successful transfection, while no HPV 16 sequences were detected in the C4-1 cells or in control vector-transfected cells (Fig. 1). The percent apoptosis was determined in the transfected cells (which express the GFP) by morphological analysis (Fig. 2). Green fluorescent cells that exhibited condensed or fragmented nuclei were considered apoptotic (Fig. 2a). Control vector-transfected cells exhibited 12.9±1.5% apoptosis 48 h following irradiation, while cells irradiated in the presence of dexamethasone showed a significant ($P=0.002$) decrease, to 5.2±0.5%, comparable to the level of spontaneous apoptosis observed in the untreated cells. Both the *E6*- and *E6*/*E7*-transfected cells exhibited reduced levels of apoptosis following irradiation (6.5±1.2% and 5.9±0.4%, respectively). This was significantly lower ($P<0.05$)

than that observed in the control vector-transfected cells and comparable to cells irradiated in the presence of dexamethasone (Fig. 2b). Addition of dexamethasone to *E6*- or *E6*/*E7*-transfected cells gave no further significant decrease in apoptosis. These results support the hypothesis that access to apoptosis following irradiation is suppressed by dexamethasone due to the over-expression of the viral oncogenes *E6*/*E7*.

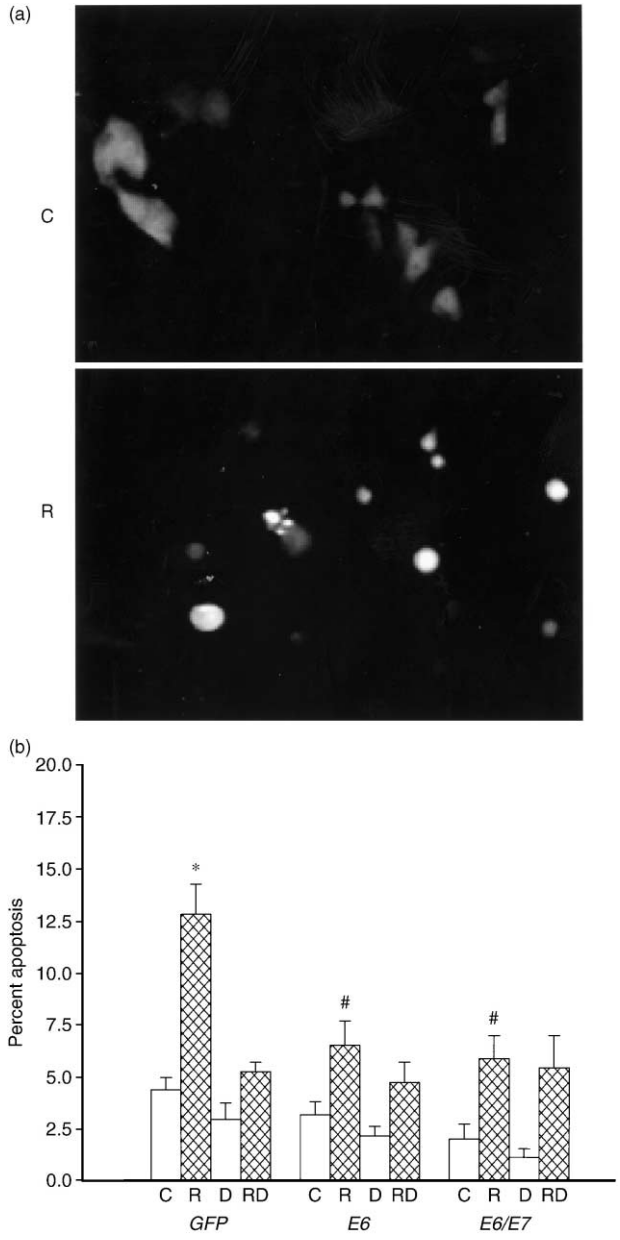

Fig. 2. Human papillomavirus (HPV) 16 *E6* and *E6*/*E7* transfection inhibits radiation-induced apoptosis as determined by morphology. Cells expressing green fluorescent protein (GFP) were analysed for apoptosis 48 h following treatment. (a) Control cells (C) displayed a flat morphology and homogenous green staining, while apoptotic cells observed after irradiation (R) exhibited a rounded morphology and condensed or fragmented nuclei. (b) Cells transfected with either HPV 16 *E6* or *E6*/*E7* showed a significantly lower radiation-induced apoptosis in comparison to control (GFP)-transfected cells and this level was not suppressed by dexamethasone. (*Apoptosis of cells irradiated in the presence of dexamethasone is suppressed compared with radiation alone, $P = 0.002$; #apoptosis of irradiated *E6*- or *E6*/*E7*-containing cells is significantly different from irradiated cells containing the GFP vector only $P < 0.05$; as determined by ANOVA).

3.2. Dexamethasone-mediated inhibition of radiation-induced apoptosis is not observed in C4-1 cells containing dominant-negative p53 (dn p53)

To confirm whether the dexamethasone-dependent inhibition of apoptosis observed in C4-1 cells is due to loss of p53 activity, cells were transfected with a mutant p53 protein to disrupt wild-type p53 function [30]. p53 expression was determined by flow cytometry and Western blot analysis using an antibody that reacts with both wild-type and mutant p53. As determined by flow cytometry, the pooled cells transfected with mutant p53 (dn p53) showed in the majority (80–85%) of cells the expression of wild-type levels of p53. Therefore, individual cells that expressed mutant p53 were subcloned and expanded to obtain a population of cells that highly express the mutant form of p53 (Fig. 3a, Clone D3 shown). As expected, cells transfected with the control vector (pc) behaved similarly to wild-type cells in that p53 levels increased following irradiation, were reduced after dexamethasone treatment and were at an intermediate level when cells were irradiated in the presence of dexamethasone (Fig. 3a) [14,15]. The suppression of normal p53 function in cells transfected with dn p53 was confirmed by the analysis of the p53-dependent increase in p21 after irradiation. In cells transfected with the

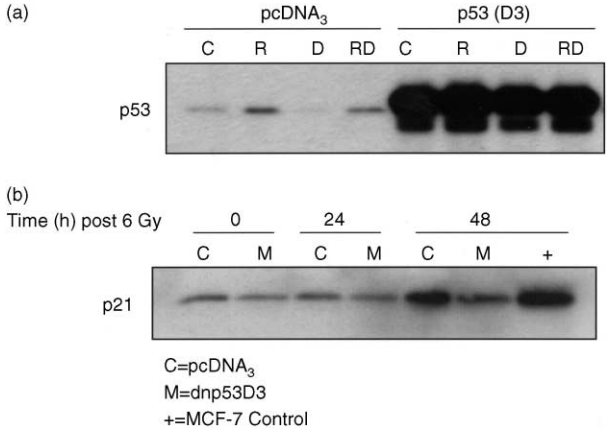

Fig. 3. Transactivation of p21 by p53 is suppressed in cells containing dominant-negative (dn) p53. (a) In the control vector-transfected cells (pc), radiation increased the levels of p53, while dexamethasone decreased p53 levels. Cells irradiated in the presence of dexamethasone show an intermediate level of p53. The dn p53-transfected cells (clone D3) exhibit high levels of p53 due to the mutation at R175H, regardless of treatment, as determined by Western blot. (b) Control vector-expressing cells exhibited an approximately 5-fold increase in p21 in response to irradiation (6 Gy) at 48 h, (c) while cells stably transfected with dn p53 exhibited only a slight elevation in p21 following irradiation (M). Lane marked (+) contains protein from MCF-7, a cell line with a constitutively high expression of p21.

control vector, and subsequently irradiated with 6 Gy, an approximately 5-fold increase in p21 protein was observed, 48 h after 6 Gy irradiation. In cells containing the dn p53 expression system, however, only a slight increase was observed at the same time. These data were interpreted as an elimination of the majority of the p53-dependent activity by dn p53 (Fig. 3b).

Cells were analysed for apoptosis at 3 days following treatment using morphology (data not shown) and DNA fragmentation as endpoints (Fig. 4). Three different passages of the D3 clone were analysed and all demonstrated increased DNA fragmentation following irradiation compared with control cells, supporting an apoptotic mechanism independent of p53 or an effect which may be linked to a p53 gain of function. However, while control vector-transfected cells showed an inhibition of apoptosis when irradiated in the presence of dexamethasone, steroid treatment had little to no effect on the inhibition of radiation-induced apoptosis in clones D3.1 or D3.2. The dn p53 clone D3.3 exhibited a partial inhibition of apoptosis following dexamethasone treatment which may reflect a preservation of wild-type p53 activity in some circumstances. In support of this possibility, western blotting analysis of p21, a transcriptional target of p53, shows that those cells containing the dn p53 vector exhibit a small increase in p21 protein, 48 h following irradiation, indicating that some wild-type p53 function may be preserved (Fig. 3b). The extent of dexamethasone-mediated inhibition of DNA fragmentation was determined and ranked as complete, partial or no inhibition of apoptosis ($n=33$ for control; $n=8$ for dn p53). Analysis of the parental C4-1 cells and control vector-transfected cells revealed that dexamethasone inhibits apoptosis completely in the majority of experiments (27/33). In contrast, dexamethasone treatment either slightly inhibited or had no effect on apoptosis in cells transfected with dn p53 (6/8; Table 1, $P<0.001$, Mann–Whitney test). Thus overall, dexamethasone specifically modulates a p53-dependent apoptotic mechanism, but has only a slight effect on cells in which p53 is suppressed by complex formation with mutant p53.

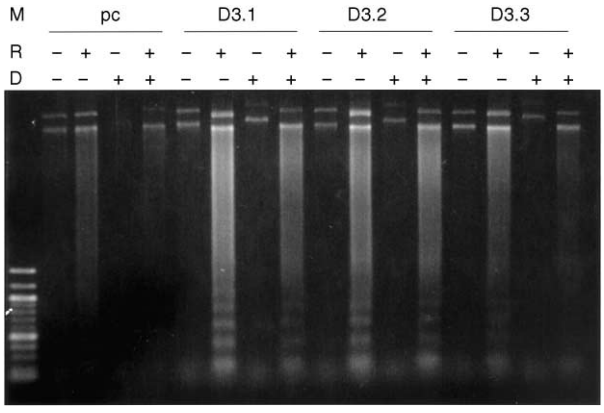

Fig. 4. Dexamethasone inhibits radiation-induced apoptosis in control-transfected cells (pc), but incompletely in dn p53 clones. Apoptosis was determined by DNA fragmentation analysis 3 days following treatment in C4-1 cells transfected with either vector alone (pc) or dn p53 (Clone D3). Dexamethasone treatment completely inhibited apoptosis in the cells transfected with vector alone. Three different experiments are shown with the D3 clone. Each demonstrated increased DNA fragmentation following irradiation alone. Dexamethasone treatment had no effect on the inhibition of radiation-induced apoptosis in clones D3.1 and D3.2 and a partial effect in D3.3. Cells were untreated, treated with 6 Gy irradiation (R) or 1 μM dexamethasone (D) or both radiation and dexamethasone (RD) as indicated; M = marker lane (100 bp ladder).

3.3. Clonogenic survival of control and dn p53-containing cells

Dexamethasone increased clonogenic radiation survival of both control vector-transfected cells and the dn p53 cells (clone D3) (Fig. 5). However, dexamethasone increased the survival of the control cells to a greater

Table 1
C4-1 cells transfected with dominant-negative (dn) p53 are less sensitive to dexamethasone[a]

Apoptosis inhibition	Control/control vector (%)	dn p53 Clones (%)
Complete	82	13
Partial	15	38
No	3	50

[a] The extent of dexamethasone-mediated inhibition of radiation-induced DNA fragmentation was visually ranked as either complete, partial or no inhibition for both C4-1 and control vector-expressing cells ($n=33$) and for the dnp53 clones ($n=8$). Characteristic profiles are shown in Fig. 4. There was a significant difference in the extent of apoptotic inhibition by dexamethasone in control cells versus the dn p53-expressing cells (Mann–Whitney U test, $P=0.001$).

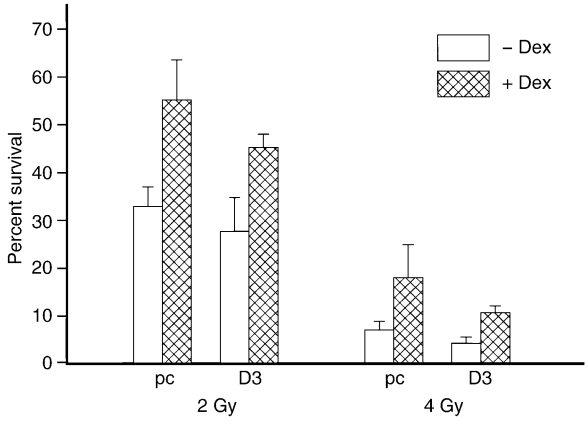

Fig. 5. Dexamethasone (Dex)-mediated changes in clonogenic survival in dn p53 and control cells. Clonogenic survival was determined in control (pc) cells and in C4-1 cells transfected with dn p53 (clone D3). Dexamethasone increased the clonogenic survival of both pc cells and dn p53 (clone D3) cells when irradiated with 2 or 4 Gy. The data represents three plates per treatment group averaged from three independent experiments.

extent than in the dn p53 D3 cells, especially after 4 Gy treatment. These data suggest that either the inactivation of p53 was incomplete, leading to regulation of p53-dependent apoptosis by dexamethasone, or that alternative pathways are present that determine steroid modulation of clonogenic survival. While p53 expression has been linked to changes in clonogenic radiosensitivity, the present literature does not support the generalisation that p53-deficient cells are more resistant to radiation-induced killing than those containing wild-type p53 [31]. Furthermore, the data reported here supports the theory that the alteration in the proportion of cells dying through apoptosis does not accurately predict the proportion of cells which succumb to clonogenic cell death, a phenomenon which has been documented by others [31].

4. Discussion

Glucocorticoids such as dexamethasone have been shown to activate transcription of HPV *E6*/*E7* through glucocorticoid response elements in the transcriptional control region of the HPV genome [13]. This effect has been linked to increases in both cellular proliferation and tumour growth of cervical tumour cells in nude mice [32]. Previous work in our laboratory has identified a steroid-dependent mechanism by which cervical carcinoma cells escape apoptosis [14,15]. Upon addition of dexamethasone, inhibition of radiation-induced apoptosis in C4-1 cervical carcinoma cells is associated with upregulation of HPV *E6*/*E7* and loss of p53 activity, as determined by mRNA expression of its transcriptional target gene, *p21* [15]. Dexamethasone had no effect on apoptosis for cells that either lack the HPV genome (C33-a) or in which HPV *E6*/*E7* transcription is repressed by dexamethasone (SW756) [15]. However, while dexamethasone-mediated inhibition of apoptosis was observed only in cells expressing increased *E6*/*E7* following steroid treatment, it is possible that dexamethasone alters apoptosis by an alternative mechanism, as has been suggested by others [33]. To directly address whether the steroid-mediated inhibition of apoptosis is dependent upon increased expression of viral genes, HPV 16 *E6* and *E6*/*E7* expression vectors were transiently overexpressed in C4-1 cells. Transient expression of *E6* or *E6*/*E7* in C4-1 cells produced a similar inhibition of radiation-induced apoptosis as was observed in cells exposed to dexamethasone. These data support the hypothesis that dexamethasone rescues cells from apoptosis through upregulation of viral *E6* expression, thus modulation of apoptosis is mediated through a p53-dependent mechanism.

Since functionally active wild-type p53 has been observed in HPV-infected cervical epithelial cells, a dominant- negative mutant of p53 (dn p53) was used to directly test the involvement of p53 in the suppression of apoptosis by dexamethasone [16]. Despite the fact that dn p53-containing cells were more susceptible to radiation-induced apoptosis, the addition of dexamethasone did not have a significant suppressive effect in the majority of experiments (Table 1). In some experiments, a slight dexamethasone-mediated inhibition of apoptosis in the dn p53 cells was observed indicating that either some wild-type p53 activity may be present, an interpretation supported by the small elevation in p21 after 6 Gy, or an alternative pathway for steroid suppression of apoptosis is active (Fig. 3b). It is also possible that introduction of the dn p53 used, mutated at R175H, may modulate the p73-dependent apoptosis pathway, as this specific mutant p53 and p73 have been shown to directly associate with each other [34]. In either case, in C4-1 cells, the predominant mechanism by which dexamethasone inhibits apoptosis requires functional p53 protein. Overall, the results from this study are consistent with steroid-mediated inhibition of apoptosis being dependent upon functional p53.

In contrast to the pathway established above linking steroid exposure to apoptosis through HPV *E6*/*E7* and p53, a parallel effect on clonogenic survival is less clear. The assays used are quite different, measuring either cell death or cell survival, thus it is not surprising that manipulation of each by dexamethasone yield different results, a phenomenon which has been documented by others [31]. It has been shown that dexamethasone may increase clonogenic resistance to irradiation in a variety of cell systems that do not contain HPV. Dexamethasone protected hamster V-79 cells from cell killing following irradiation and also afforded radioprotection in neuronal cell lines [19,20]. Furthermore, others have reported dexamethasone-mediated radioresistance in some, but not all, human carcinoma cell lines [35–37]. However, while such studies variably exhibit a dexamethasone-dependent increase in survival, the mechanism by which dexamethasone promotes radioresistance is unknown. Previous studies in our laboratory demonstrated that dexamethasone-mediated clonogenic radioresistance is linked to the upregulation of HPV *E6*/*E7* and loss of functional p53 [14]. However, the effect of dexamethasone on clonogenic survival in HPV-positive C4-1 cervical carcinoma cells may be a composite effect involving both the modulation of p53-dependent routes to apoptosis and other as yet undocumented mechanism(s). Thus, while dexamethasone can induce resistance to irradiation in many cell types, the relative contribution of the mechanism(s) involved is unknown and warrants further investigation.

In summary, the mechanism by which dexamethasone mediates inhibition of programmed cell death in C4-1 cervical carcinoma cells following irradiation is primarily dependent upon the upregulation of HPV *E6*/*E7*

and loss of p53 function. The clinical implication of these data is that for patients with HPV-positive tumours, exposure to artificial or natural steroids may inhibit tumour cell death by apoptosis, potentially limiting the effectiveness of the radiation treatment. Therefore, the use of a hormone antagonist may be beneficial in the treatment of cervical tumours since it may restore wild-type p53 function, improving the cellular response to irradiation [14].

References

1. Walboomers JM, Jacobs MV, Manos MM, *et al*. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999, **189**, 12–19.
2. Lazo PA. The molecular genetics of cervical carcinoma. *Br J Cancer* 1999, **80**, 2008–2018.
3. Werness BA, Levine AJ, Howley PM. Association of HPV types 16 and 18 E6 proteins with p53. *Science* 1990, **248**, 76–79.
4. Lechner MS, Laimins LA. Inhibition of p53 DNA binding by human papillomavirus E6 proteins. *J Virol* 1994, **68**, 4262–4273.
5. Thomas M, Nassimi P, Jenkins J, Banks L, *et al*. HPV-18 E6 mediated inhibition of p53 DNA binding activity is independent of E6 induced degradation. *Oncogene* 1995, **10**, 261–268.
6. Crook T, Tidy JA, Vousden KH. Degradation of p53 can be targeted by HPV E6 sequences distinct from those required for p53 binding and trans-activation. *Cell* 1991, **67**, 547–556.
7. Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by HPV types 16 and 18 promotes the degradation of p53. *Cell* 1990, **63**, 1129–1136.
8. Scheffner M, Munger K, Byrne JC, Howley PM. The state of the p53 and retinoblastoma genes in human cervical carcinoma cell lines. *Proc Natl Acad Sci USA* 1991, **88**, 5523–5527.
9. Pan H, Griep AE. Altered cell cycle regulation in the lens of HPV-16 E6 or E7 transgenic mice: implications for tumour suppressor gene function in development. *Genes Dev* 1994, **7**, 285–1299.
10. Puthenveettil JA, Frederickson SM, Reznikoff CA. Apoptosis in human papillomavirus 16 E7-, but not E6-immortalised human uro-epithelial cells. *Oncogene* 1996, **13**, 1123–1131.
11. Thomas M, Matlashewski G, Pim D, Banks L. Induction of apoptosis by p53 is independent of it oligomeric state and can be abolished by HPV-18 E6 through ubiquitin mediated degradation. *Oncogene* 1996, **10**, 109–115.
12. Nair P, Nair KM, Jayaprakash PG, Pillai MR. Decreased programmed cell death in the uterine cervix associated with high risk human papillomavirus infection. *Pathol Oncol Res* 1999, **5**, 95–103.
13. Gloss B, Bernard HU, Seedorf K, Klock G. The upstream regulatory region of the human papillomavirus contains an E2 protein independent enhancer which is specific for cervical carcinoma and regulated by glucocorticoid hormones. *EMBO* 1991, **6**, 3735–3743.
14. Kamradt MC, Mohideen N, Vaughan ATM. RU486 increases radiosensitivity and restores apoptosis through modulation of HPV E6/E7 and p53 in dexamethasone-treated cervical carcinoma cells. *Gynecol Oncol* 2000, **77**, 177–182.
15. Kamradt MC, Mohideen N, Krueger EA, Walter S, Vaughan ATM. Inhibition of radiation-induced apoptosis is dependent upon upregulation of HPV E6/E7 by dexamethasone in immortalized cervical carcinoma cells. *Brit J Cancer* 2000, **82**, 1709–1716.
16. Butz K, Shahabeddin L, Geisen C, Spitovsky P, Ullmann A, Hoppe-Seyler F. Functional p53 protein in human papillomavirus-positive cancer cells. *Oncogene* 1995, **10**, 927–936.
17. Butz K, Geisen C, Ullmann A, Spitovsky D, Hoppe-Seyler F. Cellular responses of HPV-positive cancer cells to genotoxic anticancer agents, repression of E6/E7-oncogene expression and induction of apoptosis. *Int J Cancer* 1996, **68**, 506–513.
18. Butz K, Whitaker N, Denk C, Ullmann A, Geisen C, Hoppe-Seyler F. Induction of the p53-target gene GADD45 in HPV-positive cancer cells. *Oncogene* 1999, **18**, 2381–2386.
19. Brock WA, Williams M, McNaney D, Milas L, Peters LJ, Weichselbaum RR. Modification by dexamethasone of radiation response of in vitro cultured cells. *Int J Radiat Oncol Biol Phys* 1984, **10**, 2113–2117.
20. Millar BC, Jinks S. The effect of dexamethasone on the radiation survival response and misonidazole-induced hypoxic-cell cytotoxicity in Chinese hamster cells V-79-753B in vitro. *Br J Radiol* 1981, **54**, 505–511.
21. Hollstein M, Soussi T, Thomas G, von Brevern MC, Bartsch 2nd. p53 gene alterations in human tumors: perspectives for cancer control. *Recent Results Cancer Res* 1997, **143**, 369–389.
22. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* 1993, **75**, 805–816.
23. Fuchs EJ, McKenna KA, Bedi A. p53-dependent DNA damage-induced apoptosis requires Fas/APO-1-independent activation of CPP32beta. *Cancer Res* 1997, **57**, 2550–2554.
24. Clark AR, Purdie CA, Harrison DJ, *et al*. Thymocyte dependent apoptosis induced by p53-dependent and independent pathways. *Nature* 1993, **362**, 849–852.
25. Lowe SW, Schmitt EA, Smith SW, Osbourne BA, Jacks T. p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature* 1993, **362**, 847–849.
26. Lowe SW. Cancer therapy and p53. *Curr Opin Oncol* 1995, **7**, 547–553.
27. Kaneuchi M, Yamashita T, Shindoh M, *et al*. Induction of apoptosis by the p53-273L (Arg→Leu) mutant in HSC3 cells without transactivation of p21Waf1/Cip1/Sdi1 and bax. *Mol Carcinog* 1999, **26**, 44–52.
28. Saller E, Tom E, Brunori M, *et al*. Increased apoptosis induction by 121F mutant p53. *EMBO J* 1999, **18**, 4424–4437.
29. Burger H, Nooter K, Boersma AW, *et al*. Distinct p53-independent apoptotic cell death signalling pathways in testicular germ cell tumour cell lines. *Int J Cancer* 1999, **81**, 620–628.
30. Herskowitz I. Functional inactivation of genes by dominant negative mutations. *Nature* 1987, **329**, 219–222.
31. Brown JM, Wouters BG. Apoptosis, p53, and tumor cell sensitivity to anticancer agents. *Cancer Res* 1999, **59**, 1391–1399.
32. Von Knebel Doeberitz M, Ritmuller C, Zur hausen H, Durst M. Inhibition of tumorigenicity of cervical cells in nude mice by HPV E6/E7 anti-sense RNA. *Int. J. Cancer* **55**, 831–834.
33. Naumann U, Durka S, Weller M. Dexamethasone-mediated protection from drug cytotoxicity, association with p21WAF1/CIP1 protein accumulation? *Oncogene* 1998, **7**, 1567–1575.
34. Di Como CJ, Gaiddon C, Prives C. p73 function is inhibited by tumor-derived p53 mutants in mammalian cells. *Mol Cell Biol* 1999, **19**, 1438–1449.
35. Renan MJ, Dowman PI. Increased radioresistance of tumor cells exposed to metallothionein-inducing agents. *Radiat Res* 1989, **120**, 442–455.
36. Rutz HP, Mariotta M, von Knebel Doeberitz M, Mirimanoff RO. Dexamethasone-induced radioresistance occurring independent of human papilloma virus gene expression in cervical carcinoma cells. *Strahlenther Onkol* 1998, **174**, 71–74.
37. Mariotta M, Perewusnyk G, Koechli OR, *et al*. Dexamethasone-induced enhancement of resistance to ionizing radiation and chemotherapeutic agents in human tumor cells. *Strahlenther Onkol* 1999, **175**, 392–396.