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ORIGINAL ARTICLE

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p53 Status does not affect photodynamic cell killing induced by hypericin

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Abstract *Purpose*: Given that p53 is a tumor suppressor that plays a central role in the cellular response to DNA damage and that more than 50% of all cancers have mutated p53, the wider utility of photodynamic therapy (PDT) in the treatment of cancer will depend on an understanding of whether p53 status modulates response to PDT. In this study, we investigated the photosensitivity of isogenic cell lines that differ only in their p53 status to PDT using hypericin as the photosensitizer. *Methods*: Acute (MTT) and chronic (clonogenic) cytotoxic assays were performed on two osteosarcoma cell-lines (U2OS and U2OS + p53DD) that are isogenic except that the latter expresses dominant negative p53. The inducible expression of p53 was determined on western blots. Uptake of hypericin, cell cycle profile analysis, measurement of membrane phosphatidylserine externalization and changes in mitochondrial membrane potential were investigated using flow cytometry. *Results*: Hypericin uptake was observed to be equivalent in U2OS and U2OS + p53DD cells. There were no significant differences in cell killing between these cell-lines in both the MTT and clonogenic assays (IC_{50} of 0.4 μg/ml from MTT assay). p53 expression did not increase up to 24 h after PDT treatment in both cell lines. There were also no significant differences in the cell-cycle arrest profiles and timing of onset of apoptosis. *Conclusions*: Taken together, these results suggest that the status of p53 may not be important in PDT-mediated cell killing or induction of apoptosis. By extension, these results imply that PDT may be used with equal efficacy for the treatment of p53-positive and -negative tumors.

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Introduction

Photodynamic therapy (PDT) is a relatively new cancer treatment that uses the combined effects of light and light-activated toxic drugs (photosensitizers) to target tumor cells [1]. When irradiated by light, the photosensitizers initiate a series of reactions that lead to the generation of reactive oxygen species (ROS) such as singlet oxygen particles and free radicals. These ROS can cause the death of tumor cells by inflicting a variety of cellular damages, including damage to DNA [2].

The tumor suppressor gene p53 has commonly been described as the 'guardian of the genome' by virtue of its central role in the cellular responses and defense against DNA damage [3]. p53 is activated by cellular stresses such as growth factor deprivation, oncogene expression or DNA damage. More than 50% of all cancers are reported to carry a mutant p53 gene and many studies describe altered cellular sensitivity to chemotherapy and radiation therapy when p53 is mutated [4, 5]. In the field of PDT however the dependence of cell-killing on p53 is still much debated, with some investigators finding reliance [6–8] and others no correlation [9]. There is also evidence to show that induction of apoptosis may somehow be influenced by p53 even if total cell death is similar between the wild type and mutant phenotype [10]. These studies have largely been limited because the cancer cell-lines employed by various researchers differ not only in their p53 status but are also likely to carry other genetic differences in part because they are derived from different cell types or different carcinoma samples.

The aim of our study is to investigate the influence of p53 status on photodynamic cell-killing by investigating the photosensitivity of two isogenic cell lines that differ only in their p53 status. These cell-lines, namely U2OS and U2OS + p53DD are derived from osteosarcoma. U2OS + p53DD is a clone of U2OS that has inactive p53

by virtue of ectopic overexpression of p53DD, a dominant negative p53 mutant [11]. In our study, we have used hypericin, which is a commercially available photosensitizer that is currently being investigated for PDT [12]. Hypericin uptake in U2OS and U2OS + p53DD was first measured to determine differences in photosensitizer accumulation. The cell-killing profile upon PDT was then determined using a short term 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) as well as a long term clonogenic survival assay. We investigated the extent to which cellular responses to PDT treatment are modulated by p53 status by measuring the induction of p53 expression, the changes in cell cycle profile and the timing of onset of apoptosis.

Materials and methods

Cell culture

The isogenic osteosarcoma cell lines U2OS and U2OS + p53DD were gifts from Kenneth Raj (Swiss Institute for Experimental Cancer Research) and were grown in Dulbecco's Modified Eagle's Medium supplemented with 5% FBS.

Materials

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise stated. Annexin V conjugated to FITC was purchased from Pharmingen (San Diego, CA, USA) and used according to the manufacturer's protocol. The cationic fluorochrome JC-1 was purchased from Molecular Probes (Eugene, OR, USA) and prepared as a stock solution of 1 mg/ml in DMSO. Hypericin was reconstituted to 1 mg/ml in DMSO. The protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) was prepared as a 20 mM stock solution in absolute ethanol.

Hypericin uptake

U2OS and U2OS + p53DD were seeded at 5×10^4 and 1×10^5 per dish on 40 mm^2 dishes and grown overnight. The cells were then incubated with media containing hypericin at various concentrations for 2 h in the dark. The hypericin containing media was then removed and the cells were washed once in PBS and trypsinized. Cells were resuspended in PBS supplemented with 2% FBS for analysis of hypericin content on a flow cytometer (Beckton Dickinson Facscalibur). The flow cytometer was equipped with a 488 nm excitation laser and hypericin fluorescence emission was measured at 650 nm and above (FL3). For each concentration of hypericin used, 10,000 cells were analyzed and average fluorescence per cell was determined. The protein content per cell was calculated separately using the Bradford assay (Pierce, Rockford, USA). Hypericin uptake was then corrected for protein content and expressed as relative fluorescence units (RFU)/ng protein.

Photodynamic treatment

Cells in tissue culture dishes or 96 well flat-bottomed microplates were incubated in phenol-red free medium containing 5% FBS with or without hypericin and irradiated with the required amount of light energy. Cells were irradiated from the bottom with broadband illumination from a 300 W tungsten halogen lamp that was filtered through an 8 cm column of water to remove heat generating infrared radiation. Energy fluence rate was measured at 16 mW/cm^2 on a Quantel power meter. Exposure times of 3, 5, 10 and 15 min resulted in an incident energy fluence of 2.9, 4.8, 9.6 and 14.4 J/cm^2.

MTT assay

Approximately $1–2\times10^4$ cells/well were seeded into 96-well plates one day prior to PDT treatment. The medium was replaced with phenol-red free medium containing hypericin at doses ranging from 0.06 to 2 μg/ml and incubated for 2 h. The plate was then irradiated with 2.9–14.4 J/cm^2 of light. Twenty-four hours after irradiation, 20 μl of MTT solution (5 mg/ml) was added to all the wells and incubated for a further 4 h. The water-insoluble MTT-formazan crystal was dissolved in DMSO and the absorbance at 570 nm was recorded on an Opsys MR microplate reader. Viability was determined relative to untreated controls. The experiments were performed in triplicate and the results averaged.

Clonogenic assay

Cells were seeded into 40 mm^2 Petri dishes 1 day prior to PDT treatment. The medium was replaced with phenol-red free medium containing hypericin at doses ranging from 0.06 to 2 μg/ml and incubated for 2 h. The plate was then irradiated with 9.6 J/cm^2 of light. 24 h after irradiation, the cells were trypsinized and seeded at low densities (200 and 300 cells per plate for U2OS + p53DD; 150 and 200 cells per plate for U2OS) in 40 mm^2 tissue culture Petri dishes and then returned to the incubator for 10 days to allow for colony formation. Untreated cells served as control. The cells were fixed with 0.2% crystal violet in 10% formalin in PBS and the number of grossly visible colonies was counted. The plating efficiencies of U2OS and U2OS + p53DD are greater than 70 and 30%, respectively.

Western blotting

Cells were washed with PBS, trypsinized and collected. After centrifugation, cell pellets were resuspended in lysis buffer (0.1 M NaCl, 0.01 M Tris base pH 7.6, 1 mM EDTA, 0.2 μg/ml aprotinin, 0.1 mM PMSF, 5 mM DTT and 1% Triton X-100) and incubated on ice for 30 min. The samples were centrifuged at 13,200 rpm for 10 min. Supernatants were collected and protein concentrations measured by the Bradford assay (Pierce, Rockford, USA). Protein samples of 50 μg were size-separated on a 12% polyacrylamide gel and transferred to nitrocellulose membrane and analyzed with antibodies against p53 (DO-1, Santa Cruz, CA, USA), actin (MAB1501, Chemicon, Temecula, CA, USA) and PARP (4C10-5, BD Pharmingen, San Diego, CA, USA).

Cell cycle analysis

Approximately 5×10^5 cells were plated in 60 mm^2 culture dishes and grown overnight. They were then incubated with 0.4 μg/ml hypericin and incubated for 2 h. The plates were then irradiated with 9.6 J/cm^2 of light. Cells were trypsinized at various time points after light irradiation and washed twice with cold PBS. The cells were then fixed in 70% cold ethanol (v/v in PBS) overnight. The fixative was removed by centrifugation and the resulting cell pellet washed twice in cold PBS. The pellet was then resuspended in a PBS solution containing 20 μg/ml RNase A and 5 μg/ml propidium iodide for 30 min to an hour. The cells were analyzed on a flow cytometer. The DNA-PI fluorescence of 10,000 cells was collected and the resulting DNA histogram was analyzed using CellQuest software. The proportion of cells in the sub-G_0/G_1 (apoptotic), G_0/G_1, S and G_2/M phases of the cell cycle were calculated directly in CellQuest by placing appropriate boundary markers on the histogram.

Induction of apoptosis

Flow cytometry was used to measure two markers of apoptotic cell death. Cells were treated as described above. At various time points after irradiation cells were trypsinized and allowed to recover in DMEM supplemented with 5% FBS for 15 min. The cell suspension was then divided equally into two tubes for analysis of membrane phosphatidylserine (PS) externalization and mitochondrial membrane depolarization.

Annexin V-FITC assay

Upon recovery from trypsinization, the cells were centrifuged at 700 *g* for 5 min and resuspended in binding buffer (10 mM Hepes/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) to a concentration of approximately 1×10^5 cells/ml. One hundred microliter of cell suspension was then incubated together with 5 μl of Annexin V-FITC and 5 μl of PI (200 μg/ml in PBS) in a round bottom polystyrene tube (12×75 mm^2, BD Falcon) for a further 10 min. The cells were then analyzed on a flow cytometer and the data acquired on CellQuest software. The cell populations were displayed as a dot plot divided into four quadrants with AnnexinV-FITC fluorescence on the *X*-axis and PI fluorescence on the *Y*-axis.

Mitochondrial membrane depolarization assay

After the cells were equilibrated, they were resuspended in PBS supplemented with 2% FBS to approximately 1×10^5 cells/ml and stained with JC-1 (1 mg/ml) for 10 min. Cells were analyzed by flow cytometry using CellQuest and results were displayed as a dot plot with FL1 fluorescence (monomers) on the *X*-axis and FL2 fluorescence (J-aggregates) on the *Y*-axis. Untreated cells with intact mitochondrial membrane potential, and cells treated with 100 nM of CCCP to achieve complete membrane depolarization, were used to adjust the PMT setting and compensation levels for this experiment.

Results and discussion

The p53DD protein is a C-terminal fragment of p53 that retains the multimerization domain but lacks the transcriptional transactivation domain of p53. When overexpressed in cells, p53DD forms transcriptionally inactive multimers with endogenous wild-type p53 protein. As a result, the level of wild-type p53 increases greatly in cells expressing p53DD because the latter prevents p53 interaction with MDM2 which normally ubiquitinates p53 and targets it for proteasomal degradation. The p53 status of the two osteosarcoma cell-lines was confirmed by checking the level of p53 expression of untreated cells. As expected, U2OS + p53DD cells express a high level of p53 compared to U2OS cells (Fig. 4).

Hypericin uptake

One important factor that may influence cellular susceptibility to PDT is the accumulation of photosensitizer in the cell. Therefore, in comparing photosensitivity of U2OS versus U2OS + p53DD, it was important to first measure hypericin accumulation by these two cell lines. This was achieved by measuring average fluorescence emission of each cell population on flow cytometry after incubation with hypericin in the dark. To correct for size and mass differences between the cell lines, the average fluorescence per cell was corrected to cellular protein content determined by Bradford assay. Hypericin content was then expressed as RFU/ng protein. A similar strategy was previously used by Rainbow and coworkers [13] to quantify photofrin accumulation in human

fibroblast cell lines. In our study, U2OS and U2OS + p53DD accumulated similar levels of hypericin in a 2 h incubation period for all concentrations of hypericin used (Fig. 1). This is to be expected since the U2OS + p53DD cell-line is a sub-clone of U2OS cells. Consequently, any differences in cellular photosensitivity in U2OS and U2OS + p53DD to PDT using hypericin are not due to variations in photosensitizer uptake but a result of p53 abrogation.

Photo-cytotoxicity of hypericin against isogenic cell lines

First, the ability of hypericin to kill the two cell lines in a drug and light dose dependent fashion was studied. Figure 2b–e shows that the percentage of cell survival decreases with increasing concentrations of hypericin and that this was dependent on light fluence. At 1 μg/ml of hypericin concentration, virtually all cells were killed when irradiated with 9.6 J/cm^2 of light. At 0.5 μg/ml however, maximum cell-killing is only observed if cells are irradiated with 14.4 J/cm^2 of light. Therefore, depending on the irradiation time chosen, a certain concentration of hypericin is sufficient to achieve maximum efficacy. Non-irradiated hypericin (Fig. 2a) did not affect cell viability at any concentration used indicating the absence of inherent cytotoxicity. Significantly, the same cell-killing profile is observed in U2OS and U2OS + p53DD cell lines. For instance, both U2OS and U2OS + p53DD exhibit equivalent photosensitivity with IC_{50} of approximately 0.4 μg/ml when irradiated with 9.6 J/cm^2 of light energy. This indicates that p53 status did not alter PDT sensitivity in these cells. Interestingly, other researchers comparing non-isogenic cell-lines have

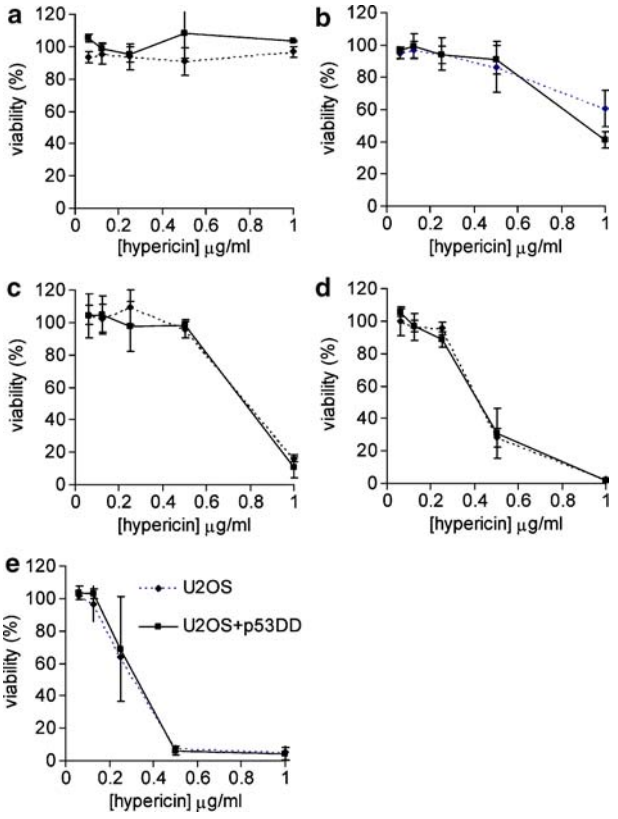

Fig. 2 Cell killing profile for U2OS and U2OS + p53DD cells exposed to increasing amounts of hypericin and durations of light irradiation. Cells were incubated with 0.0625, 0.125, 0.25, 0.5 or 1 μg/ml of hypericin for 2 h prior to light irradiation for 0 (**a**–Dark control), 3 (**b**), 5 (**c**), 10 (**d**) or 15 (**e**) min. MTT cell viability assay was performed 24 h after treatment. The percentage of viable cells exposed to various irradiation times was normalized against that of untreated cells irradiated for the same duration. Data are the mean from three experiments

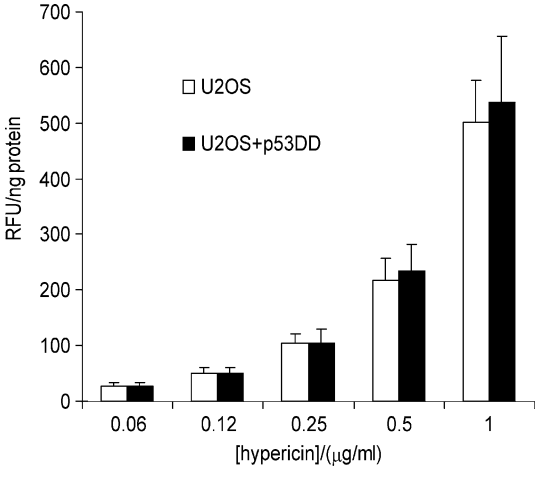

Fig. 1 Hypericin uptake by U2OS and U2OS + p53DD was measured by flow cytometry. The average hypericin fluorescence per cell was determined after a 2 h incubation period. The protein content per cell for U2OS and U2OS + p53DD was calculated at 1.11 ± 0.38 and 1.12 ± 0.16 ng protein/cell, respectively. Hypericin content expressed as RFU/ng protein was found to be similar for both cell lines at all concentrations used. Data are the mean from three experiments

reported a correlation between p53 status and photosensitivity. Fisher et al. [6] demonstrated that a human colon carcinoma cell line with a wild-type p53 phenotype (LS513) exhibits increased PDT sensitivity when compared with a mutated p53 colon carcinoma cell line (LS1034). Rainbow and co-workers also observed that immortalized Li-Fraumeni syndrome cells that express only mutant p53 were more resistant to PDT compared to normal human fibroblast cells that express wild-type p53 [13]. Perhaps cellular differences unrelated to p53 expression could play a role in the differential photosensitivity observed in theses non isogenic cell-lines.

While short-term tests such as the MTT assay are rapid and easy to perform, they measure mitochondrial activity and therefore only reveal cells that are alive at the time of the assay. Cells that have been damaged by cytostatic agents or those that are metabolically active but unable to proliferate are not discernible by short term assays. Conversely, clonogenic assay measures reproductive integrity and the ability to proliferate and has been argued to give a more accurate assessment of overall survival [14].

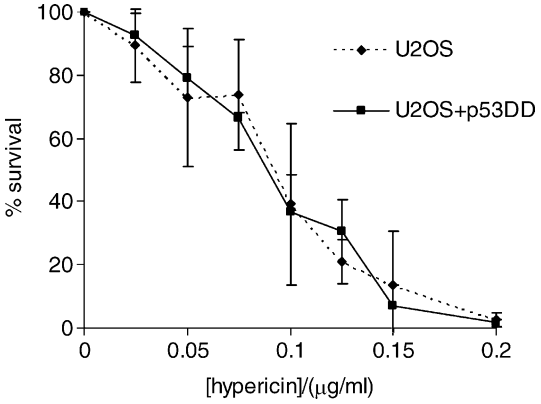

Fig. 3 Dose dependent cell killing of U2OS and U2OS + p53DD cells according to clonogenic assay. Cells were incubated with hypericin for 2 h prior to 9.6 J/cm^2 of light irradiation. The percentage of surviving cells exposed to various concentrations of hypericin was normalized against that of untreated cells. Data are the mean from three experiments. Cells exposed to just hypericin without irradiation did not show any decrease in cell viability (data not shown)

The chronic sensitivity of U2OS and U2OS + p53DD cells to PDT was tested using the clonogenic assay. Figure 3 shows that U2OS and U2OS + p53DD exhibit equivalent photosensitivity. This indicates that p53 status did not alter PDT sensitivity in these cells even when assessed using a long term assay. It is noteworthy that the IC_{50} here is significantly lower that that in the MTT assay, indicating a lower dosage of drug is necessary to cause chronic photodamage in these cells. Perhaps cells are metabolically active as detected in the MTT assay, but they have nonetheless sustained irreparable damage and unable to proliferate further. Fisher et. al. [9] also noted equivalent photosensitivity between parental and p53-abrogated colon (LS513) and breast (MCF-7) carcinoma cells when measured using clonogenic assay.

p53 expression of isogenic cell-lines

Previous studies have shown that PDT treatment using photofrin [13] and also hypericin [15] could induce p53 expression in cultured cells with a p53 wild type phenotype. This in turn, suggested a role for p53 mediated apoptosis and cell cycle alterations observed in those studies. To further study the effect of PDT on these isogenic cells, we investigated the expression of p53 protein in these cells upon PDT. Whole cell lysates were harvested at various times after light irradiation and subjected to Western blot analysis. Figure 4a shows that the level of p53 protein expressed did not increase up to 24 h in both cell-lines when compared to the untreated control. The slight decrease in p53 protein level at 16 and 24 h could be a result of unspecific protein degradation in line with onset of apoptosis. The same cell-lines were also subjected to cytotoxic treatment with cisplatin (Fig. 4b) as a positive control for induction of p53 expression. Here, an increase in p53 expression was clearly observed in U2OS from 8 h onwards. U2OS + p53DD cells did not display a similar obvious increase in p53 level probably because any increment would be dwarfed by the already high background level of p53DD. The data from these two immunoblots indicates that PDT does not activate the expression of p53.

Cell cycle alterations of isogenic cell-lines

The cell cycle profile of U2OS and U2OS + p53DD was examined in a time course experiment where cells were harvested from 2 to 38 h after light irradiation (Fig. 5, Table 1). There is a transient block in the G_2/M phase of the cell cycle that extended from 16 to 24 h. The percentage cell population in G_2/M increased from less than 30% in control cultures to more than 50% at 16 h for both cell lines. After 24 h, there was some recovery and

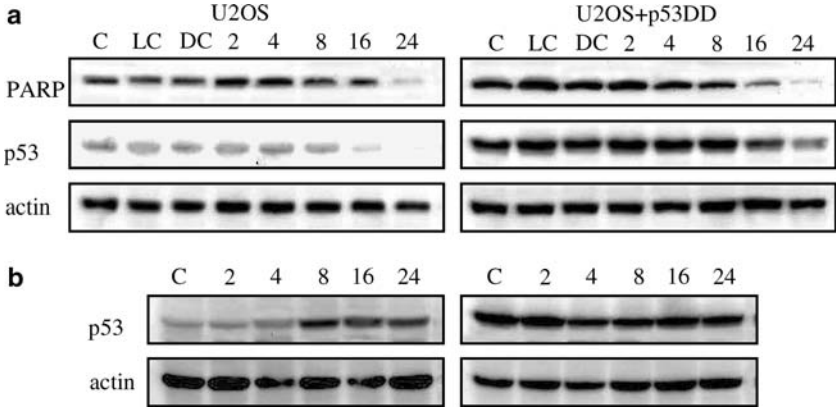

Fig. 4 Western blot analyses for PARP and p53 in U2OS and U2OS + p53DD. **a** PDT samples were incubated with 0.4 μg/ml hypericin for 2 h prior to 9.6 J/cm^2 of light irradiation. **b** Cisplatin treatment (5 μg/ml) serves as a positive control for p53 induction. Western immunoblots are shown for untreated controls (*C*), no drug controls (*LC*), no light controls (*DC*) and for samples harvested at various times after treatment. The blots were each reprobed for actin protein levels as an indicator of protein loading

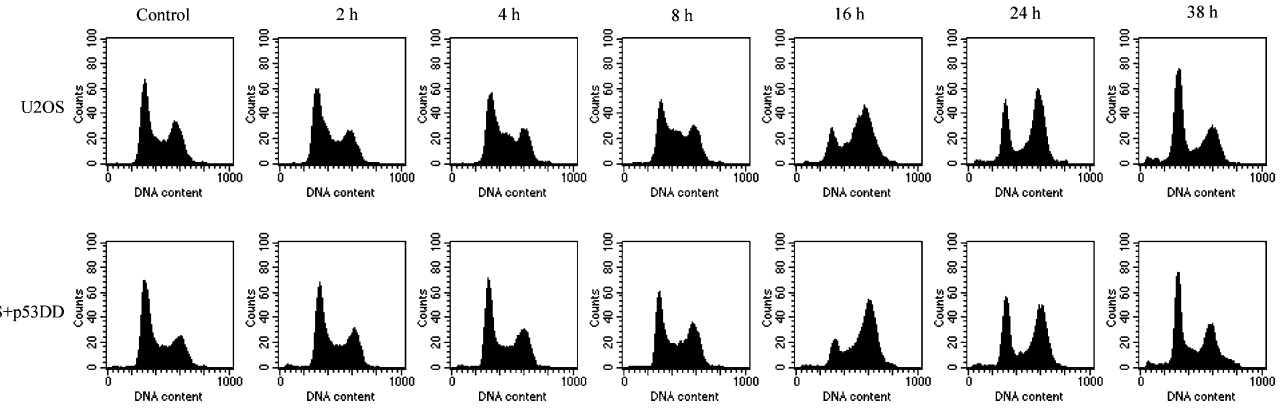

Fig. 5 Effects of hypericin PDT on the cell cycle. U2OS and U2OS + p53DD cells were treated with 0.4 μg/ml of hypericin for 2 h prior to 9.6 J/cm^2 of light irradiation. FACS analysis was performed on untreated controls (*C*) and samples harvested at various times after treatment

Table 1 Cell cycle analysis of U2OS and U2OS + p53DD cell lines upon PDT using hypericin

Treatment	Time (h)	U2OS				U2OS + p53DD			
		Sub-G_0/G_1	G_0/G_1	S	G_2-M	Sub-G_0/G_1	G_0/G_1	S	G_2-M
Control	0	1.3	52.0	18.0	28.0	1.3	54.0	20.0	24.7
LC	0	2.0	52.0	19.5	26.5	1.7	50.3	18.0	29.7
DC	0	2.5	54.0	15.5	27.5	1.3	52.7	17.3	28.7
Ir	2	0.7	50.3	26.0	22.7	1.3	51.3	21.0	26.0
Ir	4	0.7	47.0	26.3	25.3	1.0	48.3	20.3	30.3
Ir	8	1.0	44.3	29.0	25.7	1.0	42.7	25.3	31.0
Ir	16	3.0	31.3	13.0	51.5	3.4	26.5	18.8	50.8
Ir	24	4.0	30.3	16.2	46.5	4.2	31.5	14.8	48.8
Ir	38	4.7	54.0	10.3	30.0	3.7	51.0	13.3	33.8

Values represent the percentage of cells in each phase of the cell cycle. The mean values of three independent experiments are shown; the standard deviations were less than 10%. Control denotes experiments without light irradiation and no drug addition. *LC* denotes cells irradiated for 9.6 J/cm^2 without drug incubation. *DC* denotes cells incubated with 0.4 μg/ml of hypericin but not irradiated. *Ir* cells incubated with 0.4 μg/ml of hypericin and irradiated with 9.6 J/cm^{2}of light

at 38 h post irradiation, the profile mirrored that of the untreated control population again with approximately 30% of the cells in G_2/M phase. Significantly, this pattern of cell cycle alterations is similar for both U2OS and U2OS + p53DD cell lines. This clearly suggests that p53 does not play a role in the response of U2OS and U2OS + p53DD to PDT hypericin. It can also be inferred from our results that the observed G_2/M block was a consequence of p53-independent mechanisms. A similar G_2/M arrest upon PDT has also been reported by Tong et al. [13].

Induction of apoptosis in isogenic cell-lines

The induction of apoptosis was quantified in flow cytometry experiments measuring the externalization of membrane phosphatidylserine and the drop in mitochondrial membrane potential, two events considered characteristic of cells undergoing apoptosis. In the first experiment, positive staining of U2OS and U2OS + p53DD cells with annexinV-FITC was observed as early as 30 min after irradiation (Fig. 6). In both cell-lines, the initial apoptotic population of 40% remained quite constant until 8 h and increased further to over 80% at 38 h. In the second experiment measuring the mitochondrial membrane potential (Ψ_m), both U2OS

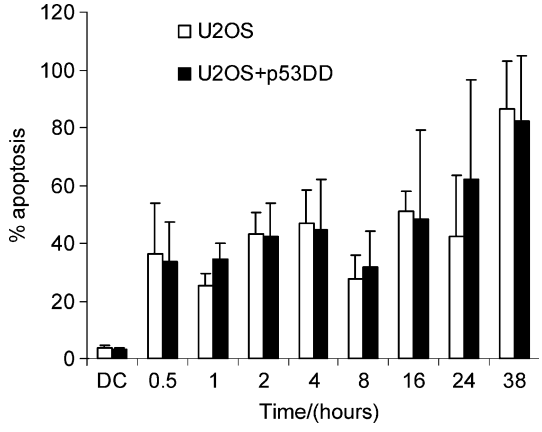

Fig. 6 Flow cytometric quantification of apoptotic cells using Annexin V-FITC. U2OS and U2OS + p53DD cells were incubated with 0.4 μg/ml of hypericin for 2 h prior to 9.6 J/cm^2 of light irradiation and then harvested at the stated time points for flow cytometry. *DC* denotes cells incubated with hypericin but not irradiated. Data are the mean from three experiments

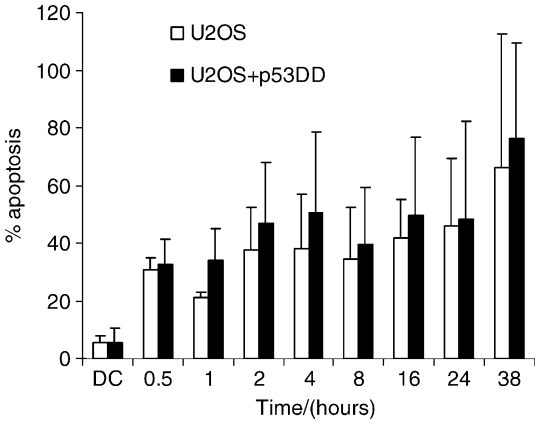

Fig. 7 Flow cytometric quantification of apoptotic cells exhibiting a reduced mitochondrial membrane potential using a cationic mitochondrial dye JC-1. U2OS and U2OS + p53DD cells were incubated with 0.4 μg/ml of hypericin for 2 h prior to 9.6 J/cm^2 of light irradiation and then harvested at the stated time points. Cells were stained with 1 μg/ml of JC-1 for flow cytometry. *DC* denotes cells incubated with hypericin but not irradiated. Data are the mean from three independent experiments

and U2OS + p53DD cells again exhibited similar trends in the onset and progression of apoptosis and the numbers compared closely with those in the annexinV-FITC staining (Fig. 7). The flow cytometry experiments show that apoptosis can happen as early as 30 min post PDT using hypericin and the similar results between U2OS and U2OS + p53DD suggest that it is independent of p53 status. In line with our finding, hypericin has been shown to precipitate a decrease in mitochondrial membrane potential as early as 1 h after irradiation in a process that is mediated by ROS [16].

Additionally, PARP processing by caspase-3 was qualitatively determined as a marker for apoptosis over a similar time course. Western blot analysis of whole cell extracts of untreated controls and cells exposed to hypericin or light alone (Fig. 4a, lane 1–3) showed an intact PARP band. Upon PDT using hypericin, a reduction in PARP level was observed from 16 h and PARP was completely cleaved by 24 h in both cell lines (Fig. 4a, lane 4–7). This delayed caspase-3 activity coincided with the second increase in the apoptotic population in flow cytometry experiments above. This result suggests that PDT using hypericin is capable of inducing caspase-3 activity from about 8 h post-treatment and that apoptosis when measured by caspase-3 activity is independent of p53 function.

The results of this study support the findings of Fisher et al. that p53 status did not influence the photosensitivity of isogenic cell lines to PDT mediated oxidative stress [9]. However, other studies also using isogenic cell lines reported different photosensitivity to PDT according to p53 status [7, 8, 13]. The contradicting findings could be due to the different methods of deriving isogenic cell-lines in the respective experiments. Both Fisher et al. and we utilized isogenic cells generated by abrogating p53 function in a wild type p53 background. We employed a double dominant negative mutant of p53 while Fischer et al. used the viral oncoprotein HPV16 E6. In contrary, the other studies that observed increased photosensitivity restored the p53 function by exogenous expression of p53 protein in a null or mutant background.

For ionizing radiation and some anticancer drugs, Brown and Wouters [17] observed that one of the reasons for inconsistent correlation between p53 status and overall cell killing reported by different groups may be the choice of assay used to determine cell death. Short term assays such as MTT have led to the conclusion that mutations in p53 confer resistance to genotoxic agents while p53 appears to play little or no role in the sensitivity of these cells to killing when long term clonogenic assay is used. Whether this same reasoning applies to PDT agents is unclear. Zhang et al. [7] found that restoring p53 function increased cellular sensitivity to PDT while Heinzelmann-Schwarz [10] and our results here show no correlation even though a short term MTT assay is used in all instances. This is perhaps not surprising since photosensitizers used in PDT do not localize to the nucleus [18] and should therefore not be expected to behave the same way as genotoxic drugs.

In conclusion, PDT using hypericin is equally effective in photo-killing cells regardless of p53 status. Selective abrogation of p53 expression by over-expression of double dominant protein in an osteosarcoma cell-line did not affect the acute or clonogenic survival of cells exposed to hypericin PDT. Moreover, cellular responses to PDT treatment do not appear to be dependent on p53 status. Taken together, the results from this study suggest that the status of p53 does not affect the photosensitivity of tumor cells nor influence their ability to undergo apoptosis. By extension, PDT could be used with equivalent efficiency on both p53-positive and -negative cancers. Moreover, these data suggest that activation of p53 from damage to DNA may not be an important pathway in mediating cell death from hypericin-mediated PDT.

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