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# Resveratrol promotes apoptosis through the induction of dual specificity phosphatase 1 and sensitizes prostate cancer cells to cisplatin

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ABSTRACT

Resveratrol is a polyphenol with chemopreventive properties against prostate cancer; however, the mechanisms underlying its actions are not completely understood. Previously, we demonstrated that DUSP1 induces apoptosis in prostate cancer cells; therefore in the present study we investigated the role of this phosphatase on resveratrol effects. Moreover, we analysed the efficiency of combined treatment of resveratrol and the chemotherapeutic drug cisplatin on cellular viability and apoptosis and its relation with DUSP1 in prostate cancer cells. We found that resveratrol up-regulates DUSP1 expression in androgen-independent prostate cancer cells, which in turn, is involved in the inhibition of the NF-κB pathway and Cox-2 expression. This phosphatase is required for the induction of apoptosis achieved by resveratrol, but does not regulate the effects of this compound on cell cycle. Furthermore, we show that resveratrol cooperates with cisplatin both in the up-regulation of DUSP1 levels and in the promotion of apoptosis, suggesting that DUSP1 is a major determinant of cisplatin sensitivity to apoptosis. These results reveal a novel molecular mechanism by which resveratrol induces apoptosis in prostate cancer cells, and highlight the importance of DUSP1 in future therapeutic approaches based in the use of this polyphenol and cisplatin.

## 1. Introduction

Prostate cancer is one of the most common malignancies and is the second leading cause of death by cancer in men worldwide (Jemal et al., 2008). Initially, most patients respond to androgen-deprivation therapies, but in many cases, the tumours become androgen independent and progress to a more advanced and aggressive stage, for which there are few treatment options. Among the chemotherapeutic drugs, cisplatin has been used to treat hormone-refractory prostate tumours, but unfortunately, acquired resistance is often observed after long-term treatment with this compound (Dasari and Tchounwou, 2014). Thus, there is a strong demand for combination therapies of cisplatin with other antitumoral agents to treat advanced prostate tumours.

The dietary polyphenol resveratrol is considered as a key element in the prevention of prostate cancer due to its ability to inhibit carcinogenesis as well as progression to a more aggressive stages (Carter et al., 2014). Thus, this compound reduces cell proliferation and increases apoptosis in prostate cancer cells (Kumar et al., 2017; Kundu and Surh, 2008) through different mechanisms, including the modulation of MAPK activation (Harper et al., 2007; Lin et al., 2002) or the inactivation of NF-κB (Benitez et al., 2009; Manna et al., 2000). Despite these data, the entire mechanisms underlying resveratrol effects on cell cycle and apoptosis in prostate cancer remain unclear.

The dual specificity phosphatase DUSP1 plays different roles in the imbalance between cellular proliferation and apoptosis observed in carcinogenesis (Keyse, 2008). Regarding prostate cancer, we and other authors have demonstrated that DUSP1 expression progressively decreases in parallel with prostate tumour progression, being almost completely absent in high-grade prostatic adenocarcinoma (Gil-Araujo et al., 2014). Moreover, we have previously shown that re-expression of DUSP1 has an essential function in prostate cancer, reducing the growth rate and promoting apoptosis through the inhibition of the p38MAPK/NF-κB signalling pathway (Gil-Araujo et al., 2014).

*Abbreviations:* ΔΨm, Mitochondrial membrane potential; Cox-2, Cyclooxygenase-2; DUSP1, Dual specificity phosphatase 1; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; MAPK, Mitogen activated protein kinase; MTT, (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide); NF-κB, Nuclear factor-κB; PARP, Poly (ADP-ribose) polymerase; PI, Propidium iodide; siRNA, Small interfering RNA; TK, Thymidine kinase; TMRM, Tetramethylrhodamine methyl ester; TNFα, Tumour necrosis factor α

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Taking into account that both resveratrol and DUSP1 can independently function as chemopreventive molecules, we aimed to investigate the involvement of DUSP1 on the anti-tumoral effects of resveratrol in prostate cancer cells. Our results demonstrate for the first time that resveratrol up-regulates DUSP1expression in androgen-independent cells. We also show that this phosphatase mediates both the induction of apoptosis and the inhibition of the NF-κB pathway achieved by this phytoestrogen. Moreover, our data also evidence that resveratrol enhances the effectiveness of cisplatin on cellular viability and apoptosis. Our findings demonstrating a new molecular mechanism of resveratrol suggest new opportunities to improve current therapeutic strategies for prostate cancer treatment.

## 2. Materials and methods

### 2.1. Cell lines, compounds, plasmids, siRNA oligos and transfections

Androgen-independent DU145 and PC3 cells, and androgen-dependent LNCaP cells were purchased from the American Tissue Culture Collection, (Rockville, MD, USA), and were cultured as recommended. Resveratrol, cisplatin and dexamethasone were obtained from Sigma Aldrich (Madrid, Spain). Human TNF-α was from PreProTech (Rocky Hill, NJ). In all experiments performed with a single dose of resveratrol, the concentration used was 100 μM. The plasmids pCMV-DUSP1 and 3xNF-κB-TK-Luc were previously described (Lasa et al., 2010). The reporter plasmid containing the human promoter of Cox-2 (P2-1900-Luc; Cox-2-Luc) was previously described (Iniguez et al., 2000). DUSP1 siGENOME (M-003484-02-0010, smart pool) and Silencer™ negative control#1 siRNA were obtained from Dharmacon and Ambion, Inc., respectively. Cells were transfected with the plasmids or with the siRNAs using LipofectAMINE 2000 (Invitrogen), according to manufacturer's protocols.

### 2.2. Cell extracts, antibodies and western blot analysis

Total cell extracts were prepared as previously described (Chiloeches et al., 2008), and Western blot analysis were performed following standard protocols. The antibodies used were anti-DUSP1, and anti-PARP (Santa Cruz Biotechnology, Santa Cruz, CA); anti-tubulin (Sigma Aldrich, Madrid, Spain); and peroxidase-conjugated secondary antibodies (GE Healthcare Europe GMBH, Barcelona, Spain). Quantification of the bands was performed by using the ImageJ software and fold expression changes of DUSP1/tubulin or cleaved PARP/tubulin were related to the control values.

### 2.3. Quantitative RT-PCR

Total RNA isolation, real-time quantitative RT-PCR validations, and quantification of changes in gene expression were performed as previously described (Chiloeches et al., 2008). The DUSP1 gene-specific primers were previously detailed (Gil-Araujo et al., 2014). The primers for GAPDH were: forward (5′-ACA GTC CAT GCC ATC ACT GCC-3′), reverse (5′-GCC TGC TTC ACC ACC TTC TTG-3′).

### 2.4. Luciferase assays and measurement of cell cycle and mitochondrial membrane potential

Luciferase assays were performed as described (Calleros et al., 2006). Measurements of cell cycle and mitochondrial membrane potential were carried out by flow cytometry analysis on a Beckton Dickinson FACScan flow cytometer (BD Biosciences) as previously described (Gil-Araujo et al., 2014).

### 2.5. Statistical analysis

All data were expressed as means ± standard deviation. The student's *t*-test was performed using the SSC-Stat software (V2.18, University of Reading, United Kingdom). The statistical significance of difference between groups was expressed by asterisks (*0.01 < p < 0.05; **0.001 < p < 0.01; ***p < 0.001).

## 3. Results

### 3.1. Resveratrol up-regulates DUSP1 expression in DU145 cells

We previously demonstrated that DUSP1 affects prostate cancer by inhibiting NF-κB activity and inducing apoptosis in prostate cancer cells (Gil-Araujo et al., 2014). On the other hand, it has been shown that resveratrol also inhibits NF-κB activity and induces apoptosis in several prostate cancer cells (Benitez et al., 2009; Manna et al., 2000). Thus, in this paper we have analysed whether the effects of this compound are mediated by the up-regulation of DUSP1. To this purpose, we first examined the DUSP1 mRNA induction in androgen-independent DU145 cells incubated with increasing concentrations of resveratrol, showing that this phytoestrogen significantly induced DUSP1 mRNA in a dose-dependent manner (Fig. 1A). Next, the kinetics of DUSP1 mRNA expression was analysed following stimulation of cells with 100 μM resveratrol, and our data showed that DUSP1 expression was progressively induced up to 15 h of incubation and remained high at least until 30 h (Fig. 1B). Furthermore, we studied whether DUSP1 induction observed by resveratrol was also achieved by the drug dexamethasone, which is used as anti-inflammatory treatment in prostate cancer, suppresses prostate tumour growth (Yano et al., 2006), and has been previously shown to be an inducer of DUSP1 expression in other cellular contexts (Clark and Lasa, 2003). Our results indicated that, in fact, dexamethasone augmented DUSP1 mRNA levels to a similar extend that

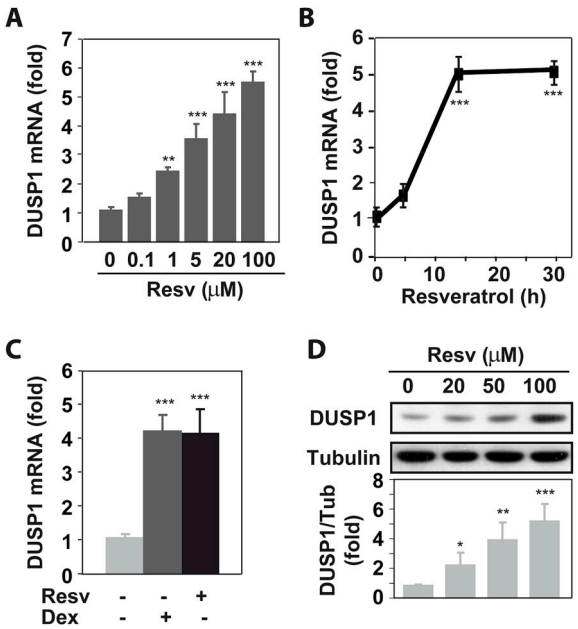


**Fig. 1. Resveratrol up-regulates DUSP1 expression in DU145 cells.** (A) Cells were incubated for 24 h with resveratrol at the indicated doses, total RNA was isolated and the levels of DUSP1 mRNA were monitored by quantitative RT-PCR. DUSP1 mRNA levels were normalized with GAPDH mRNA levels and the results were expressed as the fold change in mRNA expression. (B) Cells were incubated with 100 μM resveratrol for the indicated times, and DUSP1 mRNA levels were determined and normalized as described in (A). (C) Cells were incubated for 24 h in the presence of 100 μM resveratrol or 1 μM dexamethasone, and DUSP1 mRNA levels were determined and normalized as described in (A). (D) Cells were incubated for 24 h with different doses of resveratrol, and total cellular protein lysates were analysed by western blotting with antibodies against DUSP1 and tubulin, to assess equal protein loading. The bar graph shows the mean ± SD of fold expression changes of DUSP1/Tub related to the control from three independent experiments. For RT-PCR results, data are shown as the mean ± SD of three independent experiments.

this phytoestrogen did (Fig. 1C), suggesting that both compounds share common mechanisms to exert their anti-inflammatory effects. Finally, we also measured the levels of DUSP1 protein in resveratrol-treated cells. Consistently with the mRNA data, DUSP1 protein levels were significantly induced by treatment with resveratrol in a dose-dependent manner (Fig. 1D).

These observations indicate that resveratrol induces the expression of DUSP1 in DU145 cells, and are consistent with a possible role of this phosphatase in the effects mediated by this phytoestrogen.

### 3.2. DUSP1 mediates the effects of resveratrol on NF-κB activity and Cox-2 expression

The transcription factor NF-κB is considered a main regulator of inflammation (Karin, 2009), and its activity can be inhibited in prostate cancer cells either by resveratrol or by DUSP1. Taking into account that resveratrol up-regulates DUSP1 expression in our cells, we next tested whether this phosphatase was involved in the inhibition of NF-κB caused by this compound. To this aim, we analysed NF-κB activity in DU145 cells, in which the expression of DUSP1 was abrogated by the transfection of specific siRNA (siDUSP1 cells). These cells, or the corresponding siControl cells, were then incubated in the presence or absence of the NF-κB inductor, TNF-α. As control, the levels of DUSP1 mRNA were monitored, observing that the mRNA expression of this phosphatase was significantly attenuated in all conditions assayed (Fig. 2A). Interestingly, our results showed that, in siControl cells, resveratrol significantly reduced NF-κB transcriptional activity, both basal and induced by TNF-α. By contrast, in siDUSP1 cells, this inhibitory effect of resveratrol on NF-κB activity was abolished (Fig. 2B).

To rule out cell-type specific involvement of DUSP1 on resveratrol effects, we studied the role of this phosphatase in another androgen-independent cell line, PC3, and in the androgen-dependent LNCaP cells. Our data indicated that resveratrol treatment significantly induced DUSP1 mRNA expression in PC3 cells, but not in LNCaP cells (Supporting information Fig. S1A). Moreover, the stimulatory effect of resveratrol on DUSP1 expression in PC3 cells was confirmed by measuring the protein levels of this phosphatase upon treatment of the cells with the phytosterol (Supporting information Fig. S1B). Next, we carried out similar experiments to those performed in DU145 cells to investigate the role of DUSP1 on resveratrol-mediated effects on NF-κB activity in PC3 cells. In agreement to what observed in DU145 cells, resveratrol reduced NF-κB activation in siControl-transfected cells and DUSP1 knock-down impaired this effect (Supporting information Fig. S1C). All these data demonstrate that DUSP1 mediates the anti-inflammatory effect of resveratrol, not only in DU145 cells, but also in PC3 cells.

To further study the role of DUSP1 on the effects of resveratrol as an anti-inflammatory molecule, we next examined whether DUSP1 knock-down was also able to affect resveratrol-induced modulation of cyclooxygenase-2 (Cox-2) expression, which is a NF-κB target gene that plays an important role in inflammation and carcinogenesis in prostate cancer (Sobolewski et al., 2010). To this purpose, DU145 cells were transfected with a Cox-2-Luc reporter construct together with either a siControl (siControl cells) or a siDUSP1 (siDUSP1 cells), and incubated with resveratrol. In concordance with the effects of DUSP1 on resveratrol-mediated regulation of NF-κB activity, our data indicated that this phytoestrogen inhibited Cox-2 expression in siControl cells, while it was unable to reduce it in siDUSP1 cells (Fig. 2C). Since we demonstrated that resveratrol exerted its effects on NF-κB signalling and Cox-2 expression through the induction of DUSP1 (Fig. 2B and C) and we previously described that over-expression of this phosphatase significantly reduces NF-κB activity (Gil-Araujo et al., 2014), we next tested whether DUSP1 over-expression also affected Cox-2 expression. As shown in Fig. 2D, the ectopic expression of DUSP1 significantly reduced Cox-2 expression, reaching to about 60% inhibition.

All these data indicate that DUSP1 mediates the effect of resveratrol on both NF-κB activity and Cox-2 expression, and suggest that both the phytoestrogen and the phosphatase can be considered as anti-inflammatory molecules in prostate cancer cells.

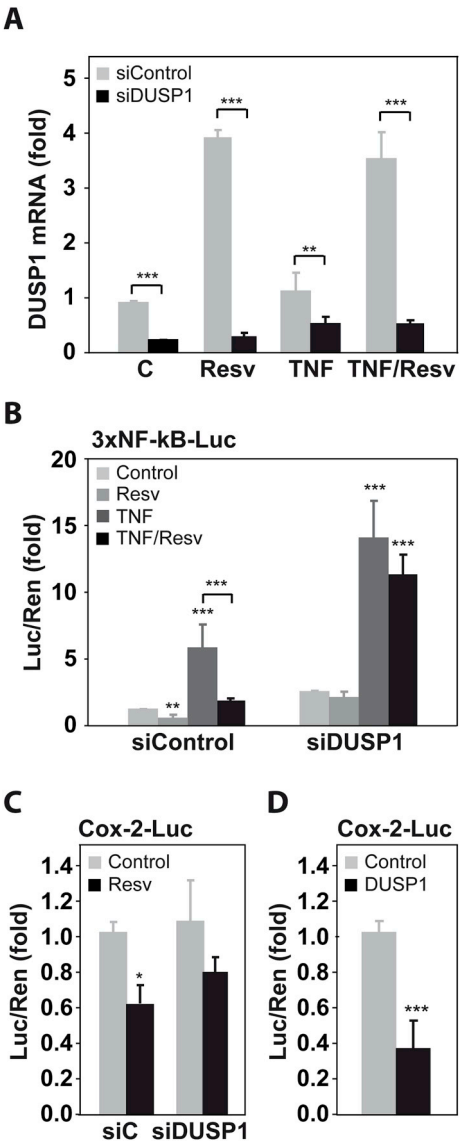


**Fig. 2. DUSP1 mediates the effects of resveratrol on NF-κB activity and Cox-2 expression in DU145 cells** (A) Cells were transfected with the control siRNA (siControl) or the DUSP1 siRNA (siDUSP1) and incubated with 100 μM resveratrol for 24 h in the absence or presence of TNF-α (10 ng/ml). Measurement and normalization of DUSP1 mRNA levels was performed as in Fig. 1 (B) Cells were cotransfected with the siControl or the siDUSP1, together with the 3xNF-κB-Luc plasmid, and incubated with 100 μM resveratrol for 24 h in the absence or presence of TNF-α (10 ng/ml). Cell extracts were prepared and assayed for luciferase activity. The luciferase levels were normalized to those of renilla, and were expressed as the induction over the controls. (C) Cells were cotransfected with the siControl (siC) or the siDUSP1, together with the Cox-2 Luc plasmid, and incubated with 100 μM resveratrol for 24 h. Measurement and normalization of luciferase activity was performed as in (B). (D) Cells were transfected for 24 h with a vector encoding DUSP1 together with the Cox-2-Luc plasmid, and then incubated and assayed for luciferase activity as described in (C). For all the results, data are shown as the mean ± SD of three independent experiments.

### 3.3. DUSP1 is required for the effects of resveratrol on apoptosis, but not on cell cycle

Different reports have demonstrated that resveratrol regulates proliferation and viability of prostate cancer cells by blocking of cell cycle

and by induction of apoptosis (Kumar et al., 2017; Kundu and Surh, 2008). Given that resveratrol up-regulates DUSP1 expression, we further studied the role of this phosphatase on the effects of resveratrol on both processes. Our results showed that the incubation of siControl-transfected cells with resveratrol significantly reduced the percentage of them in G2/M phase and DUSP1 knock-down did not significantly modify this effect of resveratrol on the cell cycle progression (Fig. 3A). Thus, the distribution of siControl-transfected cells in G2/M phase was about 15%, and this percentage decreased to 7% in resveratrol-treated cells. In cells lacking DUSP expression, resveratrol induced a similar decrease of the percentage of cells in G2/M phase (from about 12% to 8%) (Fig. 3A). Moreover, the lack of effect of DUSP1 on resveratrol-induced cell arrest was corroborated by analysing the cell cycle phases upon the ectopic expression of the phosphatase, where no significant effects were observed (Supporting information Fig. S2). Next, we examined the role of DUSP1 on resveratrol-induced apoptosis. To this aim, we first analysed by TMRM staining the percentage of cells undergoing a decrease in mitochondrial membrane potential (ΔΨm), which is a characteristic parameter of active apoptosis. In this case, differently to what observed on cell cycle regulation, resveratrol significantly increased the cell population with low ΔΨm in siControl-transfected cells, and the lack of DUSP1 expression by specific siRNA abrogated this effect (Fig. 3B). Moreover, since PARP is one of the best known caspase substrates and its inactivation by cleavage is considered another apoptosis hallmark, we analysed the role of DUSP1 on the effects of resveratrol on the caspase-mediated PARP cleavage. Consistently with the mitochondrial membrane potential data, resveratrol also induced PARP cleavage in siControl-transfected cells and DUSP1 knock-down significantly reduced this induction (Fig. 3C). We confirmed all these data performing the same experiments in PC3 cells, where we obtained similar results (Supporting information Fig. S3).

Altogether, these results show that DUSP1 mediates the effects of resveratrol on apoptosis, without affecting the regulatory action of this compound on the cell cycle.

### 3.4. Resveratrol sensitizes prostate cancer cells to cisplatin

Cisplatin is a chemotherapeutic agent which has been commonly used in the treatment of hormone-refractory prostate cancer patients (Dasari and Tchounwou, 2014), although many of them frequently develop resistance. Considering that we and others have demonstrated that resveratrol is cytotoxic in prostate cancer cells, we next tested its ability to enhance the effectiveness of cisplatin on cell viability and apoptosis. First, to examine the effects of the combination of both compounds on cell viability, we incubated DU145 cells with different concentrations of cisplatin alone or with resveratrol. As shown in Fig. 4A, these cells were sensitive not only to resveratrol, but also to this chemotherapeutic drug since a reduction on cell viability was already observed at the dose of 5 μM cisplatin. Moreover, the decrease in cell viability was similar after treatment of cells with either resveratrol or 20 μM cisplatin. In addition, the combination of both compounds significantly provoked a higher reduction than individual treatments (Fig. 4A). To reinforce these results, we extended our study performing similar experiments in PC3 cells (Supporting information Fig. S4). These cells were less sensitive to cisplatin than DU145 cells, but we observed similar effects regarding the combination of this chemotherapeutic drug with resveratrol. Thus, cisplatin induced a significant decrease in cell viability in a dose-dependent manner and resveratrol sensitized these cells to the cytotoxic effect of cisplatin (Supporting information Fig. S4A).

To evaluate the effect of the combination of resveratrol and cisplatin on apoptosis, we next measured PARP cleavage and the loss of mitochondrial membrane potential under the same conditions in both cell lines. As expected, comparable levels of both the induction of PARP cleavage and the loss of mitochondrial membrane potential were achieved by incubation of DU145 cells in the presence of either resveratrol or 20 μM cisplatin (Fig. 4B and C). Besides that, DU145 cells incubated in the presence of the combined treatment of resveratrol plus 20 μM cisplatin became more sensitive to apoptosis than cells incubated with either cisplatin or resveratrol alone, indicating that this

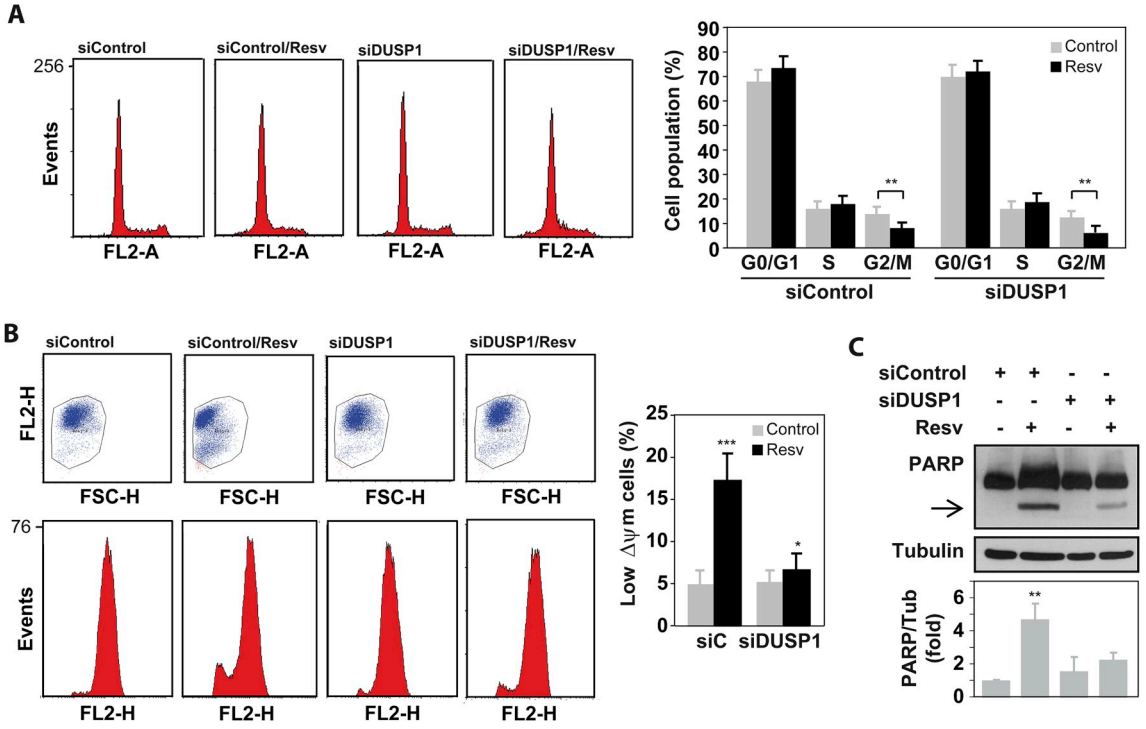


**Fig. 3. DUSP1 is required for the effects of resveratrol on apoptosis, but not on cell cycle in DU145 cells.** Cells were transfected with the control siRNA (siControl) or the DUSP1 siRNA (siDUSP1) and incubated with 100 μM resveratrol for 24 h. (A) Quantification of percentages in cell cycle phases was performed by PI staining. (B) Apoptosis determined by the TMRM assay, as detected by flow cytometry. (C) Western blotting performed to analyse PARP cleavage expression, ensuring equal protein loading with tubulin. The arrow indicates cleaved PARP. The bar graph shows the mean ± SD of fold expression changes of cleaved PARP/Tub related to the control from three independent experiments. For cell cycle and TMRM results, data are shown as the mean ± SD of three independent experiments.

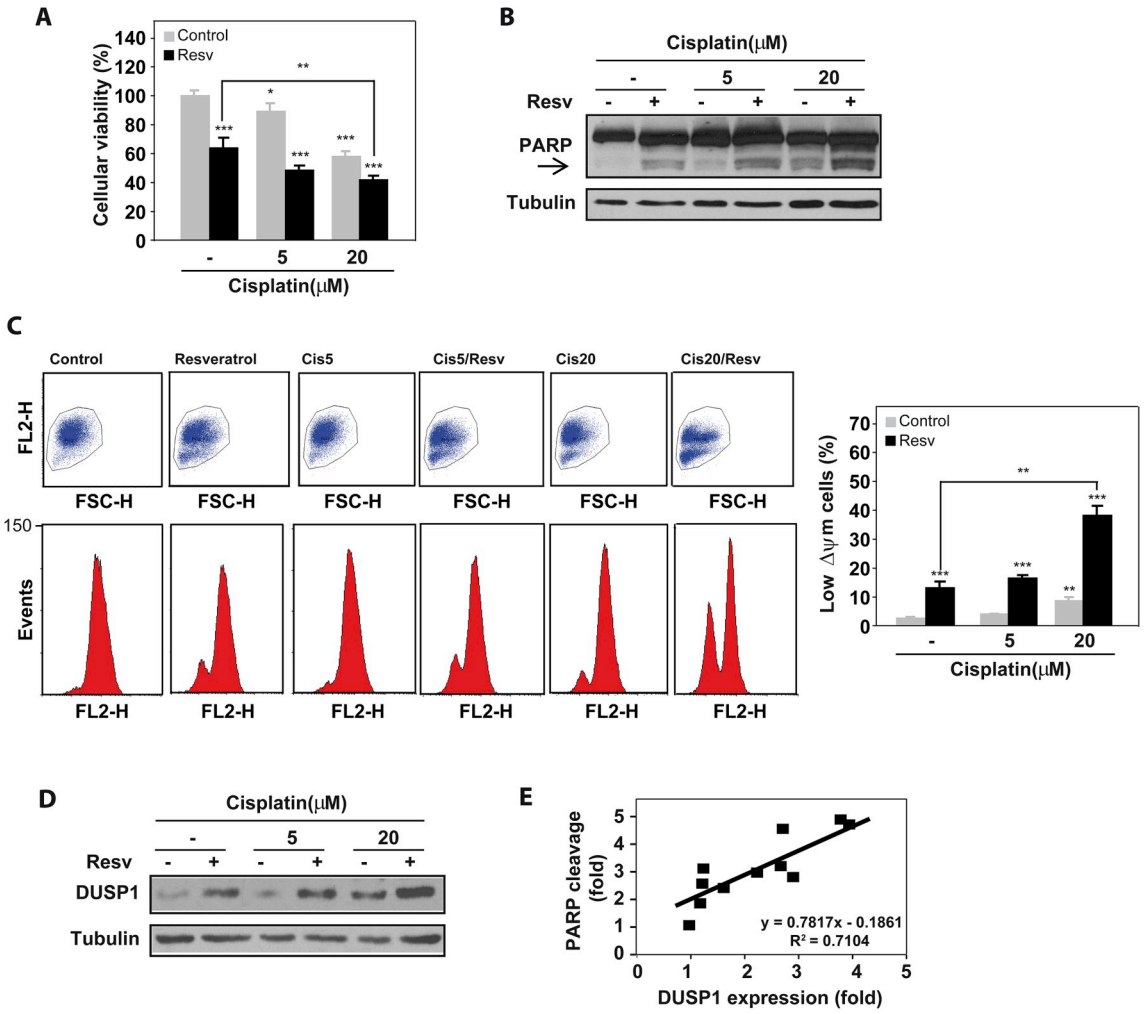


**Fig. 4. Resveratrol sensitizes DU145 prostate cancer cells to cisplatin.** Cells were treated with increasing concentrations of cisplatin for 48 h, and incubated for the last 24 h in the absence or presence of 100 μM resveratrol. (A) Cellular viability was measured by MTT assay. (B) Total cellular protein lysates were analysed by western blotting with antibodies against PARP and tubulin, to assess equal protein loading. The arrow indicates cleaved PARP. (C) Apoptosis was determined by the TMRM assay, as detected by flow cytometry. (D) Total cellular protein lysates were analysed by western blotting with antibodies against DUSP1 and tubulin, to assess equal protein loading. (E) Correlation between PARP cleavage and DUSP1 levels. Results are single values of two experiments performed as in (B) and (D). For MTT and TMRM assays, data are expressed as the mean ± SD of four independent experiments. For western blotting results, one representative image of three independent experiments is shown.

polyphenol cooperated with this chemotherapeutic drug to induce apoptosis (Fig. 4B and C). Moreover, this cooperation of resveratrol and cisplatin on apoptosis was also observed in PC3 cells, in spite of the higher resistance of these cells to cisplatin, compared to DU145 cells (Supporting information Figs. S4B and C).

Finally, in order to study the role of DUSP1 on the effect of the combined treatment on apoptosis, the expression levels of this phosphatase were determined under the same conditions in DU145 cells (Fig. 4D). As shown, both resveratrol and cisplatin induced DUSP1 expression and, similarly to what we observed in the apoptosis experiments, the combined treatment significantly enhanced the induction of DUSP1 expression achieved by the independent treatments (Fig. 4D). To confirm that DUSP1 played a role in the sensitization of cells to the apoptosis induced by resveratrol and cisplatin, we correlated PARP cleavage values *vs.* DUSP1 expression levels obtained after resveratrol, cisplatin and the combined treatment, finding a high linear correlation between them (Fig. 4E).

Together, these results suggest that combination of resveratrol and cisplatin is more effective to decrease cellular viability and to increase cellular apoptosis than individual treatments in prostate cancer cells, and that DUSP1 expression seems to play a role on the sensitivity of them to these treatments.

## 4. Discussion

We have previously demonstrated that the phosphatase DUSP1 plays an important role in prostate cancer because it reduces cellular viability and induces apoptosis through a mechanism involving NF-κB signalling pathway (Gil-Araujo et al., 2014). These results are in concordance with a recent study showing that DUSP1 overexpression remarkably suppresses PC3 cell proliferation, and that its silencing reverses this effect (Zhang et al., 2018). For this reason, agents that induce DUSP1 expression could have potential for prevention/therapy of this disease. On the other hand, resveratrol has emerged as a therapeutic approach for prostate cancer, so here we have studied the role of DUSP1 on the effects of resveratrol on cellular viability, apoptosis and inflammation of prostate cancer cells.

First, in this study, we demonstrate the existence of a functional cross talk between resveratrol, DUSP1 and inflammation in prostate cancer cells. On one hand, we show that this polyphenol inhibits NF-κB activity and down-regulates the expression of Cox-2, a NF-κB target gene involved in cancer-linked inflammation (Sobolewski et al., 2010) and considered an independent predictor of malignancy (Cohen et al., 2006). These data suggest that resveratrol could help to reduce the inflammation associated to this type of cancer. Secondly, our data

demonstrate for the first time that resveratrol induces the expression of DUSP1 in androgen-independent prostate cancer cells to the same extent than dexamethasone, which is used as anti-inflammatory treatment in prostate cancer and it suppresses prostate tumour growth (Yano et al., 2006). Interestingly, we also demonstrate that DUSP1 is involved in the effects of resveratrol on NF-κB activation and Cox-2 expression in androgen-independent prostate cancer cells. These results are consistent with previous reports showing that DUSP1 also mediates the anti-inflammatory effects exerted by dexamethasone in other cellular contexts (Clark and Lasa, 2003). Moreover, our data are consequent with other reports showing that DUSP10, which belongs to the same family of proteins than DUSP1, mediates the anti-inflammatory effects of resveratrol (Nonn et al., 2007) and other natural compounds with chemopreventive properties, such as curcumin and vitamin D in prostatic cells (Nonn et al., 2006, 2007). These data and our results suggest that different members of the DUSP family could be involved in the anti-inflammatory effects of several natural chemopreventive agents, and open new perspectives in the study of the mechanisms of action of these compounds in prostate cancer.

Our study also adds new valuable information about the role of DUSP1 in resveratrol effects on proliferation and apoptosis of prostate cancer cells. On one side, we demonstrate that this compound reduces the cell percentage in G2/M cell cycle phase through a DUSP1-independent mechanism. This is apparently in discordance with a report showing that DUSP1 causes cell cycle arrest in hepatocellular carcinoma cells by activating p53 (Hao et al., 2015). However, it is not surprising that DUSP1 does not mediate the cell cycle arrest induced by resveratrol in DU145 cells, since these cells contain two point mutations in the TP53 gene, producing a nonfunctional protein (Chappell et al., 2012). On the other hand, we show that resveratrol induces apoptosis in androgen-independent prostate cancer cells by a DUSP1-dependent mechanism. This observation is in agreement with a recent report that suggests that the apoptosis triggered by resveratrol in combination with the chemotherapeutic drug docetaxel in prostate cancer cells can be independent on p53 (Singh et al., 2017). For these reasons, the different functions of DUSP1 on apoptosis according to cell p53 status in prostate cancer require further investigation.

Another particularly important point raised from this study is the fact that resveratrol cooperates with cisplatin to decrease cellular viability and to increase apoptosis in prostate cancer cells. Cisplatin has been used to treat hormone-refractory prostate cancer patients, but its prolonged use results in drug resistance. Hence, new therapies based in the combination of this drug with natural compounds are emerging to improve the effectiveness of this chemotherapeutic agent. Similarly to our results, it has been shown that resveratrol exerts an additive effect with cisplatin as pro-apoptotic in human non-small cell lung cancer (Ma et al., 2015) and in malignant mesothelioma cells (Lee et al., 2016). However, regarding prostate cancer, this is the first report showing that the combined treatment of resveratrol and cisplatin cooperate in the inhibition of cellular viability and in the induction of apoptosis. Thus, our data are consistent with other results showing that resveratrol treatment with other chemotherapeutic agents, such as docetaxel, enhances apoptosis in prostate cancer cells (Singh et al., 2017). Interestingly, we show that DUSP1 induction plays an important role in this cooperative cytotoxic effect, since its expression levels achieved by either resveratrol, cisplatin or the combination of them directly correlate with cleaved PARP levels. These data suggest that combinatorial therapies inducing DUSP1 expression can show better effectiveness in treating advanced prostate cancer with cisplatin. In this sense, it has been described that DUSP1 upregulation is a critical event in the associated sensitivity to cisplatin in lung cancer cells (Cimas et al., 2015). Moreover, it seems that this mechanism is not exclusive for DUSP1, since it has been demonstrated that another phosphatase of the same family, DUSP6, also enhances the sensitivity of ovarian cancer cells to cisplatin (Chan et al., 2008). By contrast, DUSP1 may also play a role in the chemo-resistance to cisplatin in other cancer cells (Shen et al., 2016). For example, it has been shown that overexpressing DUSP1 results in a lower apoptotic rate in cisplatin-resistant gallbladder cancer cells (Fang et al., 2018). These results altogether suggest that DUSP1 can exert antagonistic roles regarding the sensitivity to cisplatin depending on the cell context. Despite all these data, other mechanisms should be considered to fully explain the role of DUSP1 in the cellular response to cisplatin in the presence of resveratrol. For example, it is well known that NF-κB inhibition mediates chemosensitivity (Li et al., 2005) and here we show that this pathway is also targeted by resveratrol. Considering our previous observation showing that the activation of NF-κB and DUSP1 expression levels inversely correlate in prostate cancer (Gil-Araujo et al., 2014), we can speculate that NF-κB inhibition by combination of resveratrol and cisplatin may contribute to increase the susceptibility of prostate cancer cells. On the other hand, autophagy has been related to cell death in different types of tumours (Eisenberg-Lerner et al., 2009) and DUSP1, resveratrol and cisplatin have also been involved in this process (Lee et al., 2016; Wang et al., 2016). However, we have not observed that resveratrol affects autophagy in our cells, since it does not modify the levels of the autophagy markers LC3 and p62 (data not shown). In fact, our data are in agreement with those demonstrated by Ouyang et al., who show that the autophagy pathway is genetically impaired in DU145 cells due to the lack of expression of ATG5, a known protein involved in the formation of autophagosomes (Ouyang et al., 2013).

In summary, considering that persistence of inflammation, induction of cell proliferation and evasion of apoptosis are among the processes by which prostate cancer progresses, this study provides new insights about the molecular mechanisms underlying chemopreventive effects of resveratrol in prostate cancer (Fig. 5). In conclusion, our experiments show that resveratrol: (i) up-regulates DUSP1 in prostate

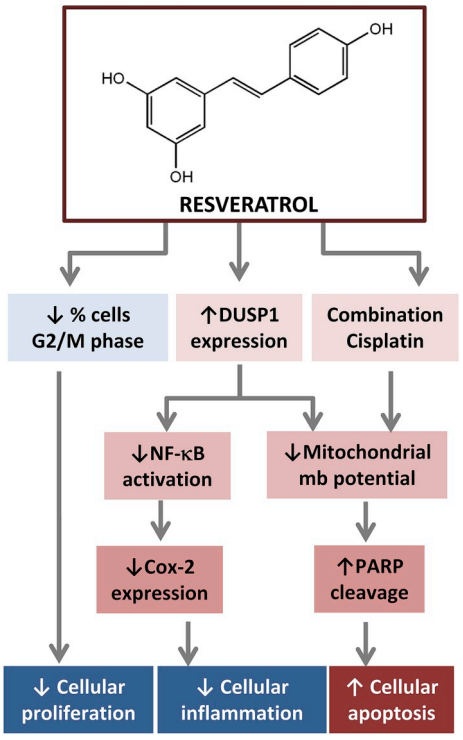


**Fig. 5. Resveratrol regulates cell cycle and apoptosis by both DUSP1-independent and DUSP1-dependent mechanisms.** The effects of resveratrol in prostate cancer cells shown in this study are: (1) up-regulation of DUSP1, which, in turn, acts as an anti-inflammatory molecule by inhibiting both the NF-κB pathway and the expression of the pro-inflammatory determinant, Cox-2; (2) regulation of cell cycle by a mechanism which is independent on DUSP1 induction; (3) promotion of cellular apoptosis by a DUSP1-dependent mechanism; and (4) sensitization to the pro-apoptotic action of cisplatin.

cancer cells, which, in turn, acts as an anti-inflammatory molecule inhibiting the NF-κB pathway and the expression of the pro-inflammatory enzyme, Cox-2; (ii) regulates cell cycle by a mechanism independent of DUSP1 induction; (iii) induces cellular apoptosis by a DUSP1-dependent mechanism; and (iv) sensitizes prostate cancer cells to the pro-apoptotic action of cisplatin. Based on all these data, we believe that the use of resveratrol as a therapeutic agent would be beneficial to patients with advanced prostate cancer.

## Conflicts of interest

The authors declare that there are no conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2018.12.014.

## Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2018.12.014.

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