Resveratrol decreases Rad51 expression and sensitizes cisplatin-resistant MCF-7 breast cancer cells


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Abstract. Resveratrol (RES), a polyphenol compound with anti-proliferative properties, has been previously evaluated for its beneficial effects against a variety of tumour cells. The current study elucidated the means by which RES enhances the anti-proliferative effects of cisplatin (CIS) on MCF-7 cells, focusing on the inhibitory effects on DNA repair of double-strand breaks (DSBs). Chemoresistant MCF-7 cells (MCF-7R) were generated by continuous exposure to low concentrations of CIS (10 µM CIS-IC40) during 5 passages, with the IC50 value increasing ~3-fold. Using an MTT assay, we estimated the changes in IC50 for CIS in MCF-7, T47-D, MDA-MB-231 and MCF-7R cells in the presence of RES. The relative transcript level of Nbs-1, Mre-11 and Rad-50 genes was assessed using RT-qPCR analysis. Rad51 and H2AX [pSer139] protein expression was determined by western blot analysis. RES at 50 and 100 µM significantly enhanced the anti-proliferative effects of CIS in both MCF-7 and MCF-7R cells, decreasing the IC50 values for CIS to one-tenth and one-sixth, respectively. A total of 100 µM RES decreased the relative transcript levels of homologous recombination (HR) initiation complex components and the Rad51 protein level in MCF-7 and MCF-7R cells. After 48 h of CIS DNA damage, the levels of Rad51 protein increased, but this effect was inhibited by 100 µM RES. RES also maintained serine 139 phosphorylation of histone H2AX, suggesting that RES prevents the repair of DSBs. It was observed that RES exerts an antagonistic effect over CIS on the activation of Rad51 and sustained phosphorylation of H2AX. The results suggest that RES in combination with DNA damage-based therapy has potential as a strategy to overcome resistance and provide much safer and more effective treatment for breast cancer.

Introduction

The resistance to chemotherapeutic compounds is a major obstacle to the successful treatment of various human cancers. Therefore, elucidation of the mechanisms involved in drug resistance and the development of new strategies to re-sensitize cancer-resistant cells are key elements in the generation of improved therapies. Upregulation of DNA repair mechanisms necessary for the maintenance of the genetic stability of the cell (1) has been associated with resistance to alkylating agents, platinum-based drugs and radiation (2,3). CIS is commonly used in various types of solid cancers (4). However, the acquired resistance associated with the agent’s toxicity can limit the effectiveness of these drugs in the clinic (5). Accordingly, strategies to restore cancer cell sensitivity to platinum agents are of high clinical importance. CIS can induce both intrastrand and interstrand crosslinks in living cells, with the former accounting for more than 90% of the total DNA damage. Intrastrand crosslinks, the most abundant lesion, can be removed through the nucleotide excision repair (NER) pathway, while interstrand crosslinks are removed through the co-operation of several DNA repair pathways, including NER and HR (reviewed in ref. 3). Since CIS induction of apoptosis is partially achieved through the induction of DNA damage, enhanced DNA repair is believed to be one of the major mechanisms of CIS resistance (reviewed in ref. 6) by enabling tumour cells to overcome CIS toxicity. Therefore, the relationship between DNA repair efficiency of cancer cells and CIS resistance has been extensively studied (reviewed in ref. 7). Moreover, it has been demonstrated that increased HR, which is related to the increase of Rad51 nuclear foci density, correlates with CIS drug resistance in a variety of human tumour cell lines (8). In addition, previous studies showed that the...
downregulation of Rad51 by some anticancer drugs restores cancer cell radiosensitivity and chemosensitivity by impairing HR repair (9-11). Natural compounds, such as RES, have also been found to confer radiosensitivity and chemosensitivity on cancer cells (12-14). RES is a polyphenol present in a wide variety of fruits and vegetables, such as grapes, berries, peanuts, pines and various herbs (15-17). There is evidence that the RES present in red wine may contribute to the cancer-preventive effects of this beverage (18). Earlier studies have reported growth inhibitory, proapoptotic, and anti-invasive properties of RES in different cancer cell lines, including human oral squamous carcinoma, promyelocytic leukaemia, breast, lung, prostate, rhabdomyosarcoma, and colon cancer cells (19). Moreover, we previously reported the inhibition of DNA repair genes by RES (20) suggesting that this polyphenol may help to overcome drug resistance and cooperate with other therapeutic agents such as CIS. In the present study, we demonstrated that CIS co-treatment with RES in both chemoresistant and chemosensitive MCF-7 cells effectively reduced the concentration of CIS needed for the equivalent effect at higher doses, correlating with downregulation of Rad51 and impairment of the repair of DSBs. Our findings thus identified a new biological activity of RES, enhancing chemosensitivity of breast cancer cells to CIS by the downregulation of essential proteins in the HR repair pathway.

Materials and methods

Cell lines and reagents. MCF-7, T47-D and MDA-MB-231 human breast cancer cells [American Type Culture Collection (ATCC) Manassas, VA, USA] were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS), in a 5% CO2 incubator at 37°C. RES and CIS were obtained from Sigma Chemical Company (St. Louis, MO, USA). RES stock solution was solubilized in absolute ethanol and diluted in DMEM. CIS stock solution was solubilized with 500 µl of acid isopropanol. Absorbance was measured by a colorimetric assay at 540 nm wavelength (Bio-Rad Laboratories, Hercules, CA, USA). The growth percentage was calculated using the initial number of control cells as 100% at 0 h. The IC50 values for RES and CIS were calculated using the GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA).

RNA isolation. Total RNA was extracted using TRIzol (Invitrogen) as described elsewhere and purified using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA), according to the manufacturer's protocol. RNA was recovered in 30 µl of nuclease-free water and either used immediately or stored at -80°C until further analysis.

Reverse transcriptase-quantitative PCR (RT-qPCR). cDNA synthesis was performed with a First Strand kit as previously described (21). Each sample was tested in triplicate, and relative gene expression levels were calculated using the mRNA ratios relative to the β2-microglobulin house-keeping. The primer sequences were designed using Primer Express Software (Table I). SYBR-Green reaction was conducted using a Quantitect™ SYBR-Green PCR Reagents kit (Qiagen) following the manufacturer's recommendations. Before performing the RT-qPCR, a reaction optimization was performed for each gene-specific pair of primers to confirm the specificity of the amplification signal. Changes in fluorescence were recorded as the temperature was increased from 65-95°C at a rate of 0.2°C/sec to obtain a DNA melting curve.

Data analysis using the 2-ΔΔCq method. The data were analysed using the equation described by Livak and Schmittgen (22). Briefly, we used the average ΔCq from RES-untreated MCF-7 or MCF-7R cells as the calibrator for each gene tested to obtain the amount of target = 2−ΔΔCq. Validation of the method was performed as previously reported (21). Data are presented as the mean ± standard deviation (SD). Statistical evaluation of significant differences was performed using Student's t-test. Differences of P<0.05 were considered statistically significant.

Western blot analyses. MCF-7 and MCF-7R cells were treated for 48 h with the proper vehicle, RES and/or CIS. Briefly, the cells were lysed with RIPA lysis buffer, and 30 µg protein was loaded on an SDS–10% polyacrylamide gel. The proteins were transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA), blocked with 5% (w/v)
non-fat milk and washed with Tris-buffered saline-Tween solution (TBST). The membrane was probed overnight at 4°C with a specific primary antibody [anti-Rad51, 1:1,000; rabbit polyclonal; cat. no. SC-8349; Santa Cruz Biotechnology, Santa Cruz, CA, USA; anti-H2AX (pSer139), 1:1,000; rabbit polyclonal; cat. no. H5912; Sigma-Aldrich Co. LLC]. The blots were developed using chemiluminescent detection reagents (Immobilon™ Western; Millipore). After stripping, the blots were re-probed with anti-α-actin (1:200; mouse monoclonal; prepared in the laboratory of PhD José M. Hernandez Hernandez, Department of Cell Biology, Cinvestav-IPN, Mexico City, Mexico) or anti-β-actin (1:20,000; mouse monoclonal; cat. no. A3854; Sigma-Aldrich, Co. LLC).

Statistical analysis. Data were evaluated in triplicate against the untreated control cells and collected from three independent experiments. The RT-qPCR results were evaluated by the Student's paired t-test. Two-tailed P-values <0.05 were considered statistically significant. Data from CIS and RES treatments were graphed and analysed by GraphPad Prism 5.0 using a two-way ANOVA, with post hoc Tukey HSD. P<0.005 was considered statistically significant. The data are presented as the mean ± standard deviation.

Results
Resveratrol enhances the sensitivity of breast cancer cell lines to cisplatin. In a previous study, we observed that RES decreased the level of HR proteins in MCF-7 breast cancer cells, suggesting that this polyphenol may enhance the efficacy of DNA damage agents (20). We investigated the antiproliferative effect of RES combined with CIS in MCF-7, T47-D and MDA-MB-231 cells using MTT assays. First, we determined the IC50 of RES and CIS on breast cancer cell lines (MCF-7, T47-D and MDA-MB-231) using MTT assays. The cells were treated for 48 h with different concentrations of CIS. A decrease in the IC50 value for CIS was calculated by the GraphPad Prism 5 programme. MTT assays were performed as indicated in Materials and methods. Three independent assays were compared with their controls (100%, untreated cells). The results are mean ± SD of three independent experiments, each performed in triplicate (P<0.001).
concentrations of RES (Fig. 1A) or CIS (Fig. 1B). At 10 µM RES, the cell viability of MCF-7 and T47-D (both oestrogen receptor-positive cells) increased. By contrast, the viability of RES, the cell viability of MCF-7 and T47-D (both oestrogen receptor-positive cells) increased. By contrast, the viability of MCF-7 presenting the highest IC50 value (101.1 µM) and T47-D cell line presenting the lowest (78.19 µM) (Fig. 1A). In Fig. 1B, we can observe a dose-dependent reduction in cell viability for the MCF-7, T47-D and MDA-MB-231 cell lines treated with CIS (P<0.005). T47-D cells presented the lowest IC50 value for cisplatin on MCF-7 cells is highly reduced by resveratrol at a concentration in which HR genes are reduced. MCF-7 cells were treated for 48 h with different CIS concentrations alone or together with 50 or 100 µM RES. The decrease in the IC50 value for CIS due to RES was calculated by the GraphPad Prism 5 programme. MTT assays were performed as indicated in Materials and methods. Three independent assays were compared with their controls (100%, untreated cells). The results are mean ± SD of three independent experiments, each performed in triplicate (P<0.001).

Notably, lower doses of RES near the IC50 value (30 and 50 µM) induced an increase in Nbs-1, Mre-11 and Rad-50 mRNA levels at 48 h. However, these values decreased at RES doses near the IC50 concentration (100 µM) and 150-250 µM (Fig. 2) as previously reported (20). These results suggested that at doses similar or greater than the IC50 value, RES appears to reduce the mRNA level of the HR initiation complex components. Consequently, 100 µM of RES was used to sensitize MCF-7 cells to CIS treatment. Thus, RES may contribute to decreased CIS IC50 in MCF-7 cells by negatively regulating HR initiation complex components. 

**Resveratrol decreases the transcriptional expression of HR initiation complex components in MCF-7 cells.** It has been reported that many of the anticancer properties of RES are dependent on p53, so although we observed that RES increased the effectiveness of CIS in the three different cell lines, we focused on a scenario where the p53 protein was present in order to understand the mechanisms of action of RES. For this reason, experiments were subsequently focused on the MCF-7 cell line. Since the main action of the reported molecular mechanisms of CIS activity is to cause DNA damage, and HR is the pathway related to the DNA damage response to CIS, one interesting possibility for RES activity would be to interfere with the expression of the canonical HR system components. We examined the effects of RES on the mRNA level of the canonical HR initiation complex components (Nbs-1, Mre-11 and Rad-50) in the MCF-7 cells, using RT-qPCR assays.

**IC50 value for cisplatin on MCF-7 cells is highly reduced by resveratrol at a concentration in which HR genes are reduced.** As we observed that 100 µM RES decreases the expression of HR genes, we analysed whether 100 µM RES concentration increased sensibility to CIS in MCF-7 cells. We observed a significant decrease in cell viability when we treated MCF-7 cells with 50 µM RES combined with 10 µM of CIS (P<0.001). In addition, when the MCF-7 cells were treated with 100 µM RES in combination with CIS, the cell viability was significantly decreased after treatment with 2 µM of cisplatin (P<0.001). The IC50 values for CIS decreased ~8-fold (11.91 vs. 1.48 µM) in cells treated for 48 h with the combination of 100 µM RES (Fig. 3) (P<0.001). These data suggested that a large decrease in the IC50 value may be associated with the ability of 100 µM RES to reduce the HR initiation complex mRNA components.

**Resveratrol decreased Rad51 protein expression and maintained serine 139 phosphorylated H2AX in cisplatin-treated MCF-7 cells.** Rad51 is the central recombinate involved...
in the HR repair of DNA DSBs (23) and overexpression of Rad51 has been detected in various cancer cell types (24-27). We investigated whether RES induced changes in Rad51 protein expression in MCF-7 cells. The cells were treated for 48 h with either vehicle, 50, 100, 150 or 250 µM RES, and cellular extracts were evaluated for the presence of Rad51 protein (Fig. 4A). Western blot analysis revealed that Rad51 expression was decreased in the presence (48 h) of 100 µM or higher RES concentrations. Then, we explored whether the reduced level of Rad51 was related to unrepaired damaged DNA by measuring the levels of phosphorylated H2AX [Ser\(^{139}\)] since γ-H2AX formation is used as a marker for DNA damage (notably DNA DSB) (28). The cells were treated for 48 h with vehicle, 20 µM CIS or 20 µM CIS plus 100 µM RES. As shown in Fig. 4A, the MCF-7 cells expressed basal levels of Rad51, and as expected this level was further induced by CIS treatment. Of note, however, this induction was blocked by 100 µM RES (Fig. 4B). Induced levels of γ-H2AX after 48 h of CIS treatment were barely detectable (Fig. 4B, lanes 1 and 2). This finding suggested that with high levels and activity of Rad51, DNA damage is rapidly repaired, and γ-H2AX is no longer needed. However, γ-H2AX is significantly present in cells treated with 20 µM CIS plus 100 µM RES (Fig. 4B, lane 3), suggesting that the reduction of HR activity (due to decreased Rad51, Nbs-1, Mre-11 and Rad50) may affect DNA repair, and high levels of γ-H2AX may be evidence of a defective DNA repair. Therefore, these results strongly indicated that RES suppressed the repair of DNA damage caused by CIS in MCF-7 cells.

**MCF-7-resistant cells becomes sensitive to cisplatin in the presence of resveratrol.** To demonstrate the contribution of RES to enhance CIS sensitivity in CIS-resistant cells, we generated MCF-7 cells resistant to CIS treatment (MCF-7R) by continuous exposure to low concentrations of CIS (10 µM CIS-IC\(_{40}\)). After selection, the IC\(_{50}\) value of these MCF-7R cells increased ~3-fold (from 11.91 to 34.66 µM) (Fig. 5). To investigate whether RES has an impact on cellular sensitivity towards CIS in MCF-7R, the IC\(_{50}\) was determined. According to our previous results with MCF-7 cells (Fig. 3), 100 µM RES was also able to reduce the viability of the MCF-7R cells. We observed a significant decrease in the viability of MCF-7R cells after 2-15 µM of CIS treatment (P<0.001). The CIS IC\(_{50}\) decreased to one-sixth of the original MCF-7R IC\(_{50}\) value when the cells were treated with a combination of 100 µM of RES (from 34.66 to 5.22 µM, Fig. 5). These findings suggested that RES may be a potent adjuvant to recover CIS sensitivity in CIS resistance.

**Resveratrol decreased Rad51 mRNA and protein levels and maintained γ-H2AX in MCF-7R cells treated with cisplatin.** Given that Rad51 is the central recombinase involved in HR (29), we also examined whether RES decreases the Rad51 expression levels and maintains γ-H2AX levels in MCF-7R cells as we previously observed in MCF-7 non-resistant
We then examined the levels of Rad51 mRNA in MCF-7R cells with or without RES treatment. The RT-qPCR assay demonstrated that Rad51 mRNA is overexpressed in MCF-7R (Fig. 6A), but successfully reduced by RES treatment at 100 µM. These results suggested that as with MCF-7 cells, a partial inhibition of DNA repair mechanisms by RES may contribute to CIS sensitivity in MCF-7R cells. To induce DNA damage, we treated MCF-7R cells with a high dose of CIS (20 µM), and observed an increase in Rad51 protein levels and γ-H2AX levels, indicating the presence of DNA damage (Fig. 6B). By contrast, 100 µM of RES did not increase the Rad51 levels (Fig. 6B). In the MCF-7R cells treated with 20 µM CIS plus 100 µM RES we observed that the induction of Rad51 expression by CIS DNA damage was partially inhibited by treatment with RES, indicating that RES was able to suppress Rad51 overexpression in MCF-7R cells (Fig. 6B, upper right panel). We also examined whether RES would promote sustained γ-H2AX after CIS induced DNA damage by affecting Rad51. Although MCF-7R cells were created under CIS conditions, at this time, they had no basal γ-H2AX signal (Fig. 6B, lower panel). As opposed to that identified in Fig. 4B, at the same time (48 h), CIS damage was able to promote significant levels of γ-H2AX highlighting the different nature of the resistant cells compared to the normal cells (Fig. 6B). RES alone had no effect on the level of γ-H2AX of MCF-7R, but γ-H2AX was still present in MCF-7R cells treated with 20 µM CIS plus 100 µM RES (Fig. 6B, lower right panel), further confirming that RES may partially inhibit the repair of DNA damage caused by CIS, even in chemoresistant MCF-7R cells.

Several reports have demonstrated that the natural compound RES has been able to inhibit the growth of a wide variety of human cancer cells, such as breast, skin, lung, prostate and colon cancers (31-34). Many chemotherapy drugs, such as CIS, eliminate cancer cells by inducing damage in the DNA of the cells. However, in 50% of cancer cases, malignant cells survive the treatment by diverse mechanisms, including the upregulation of DNA repair proteins (35). The HR pathway has been increasingly recognized as a DNA repair mechanism related to intrinsic and acquired resistance to platinum-based chemotherapy (36). In a previous study, we observed by DNA microarray analysis in MCF-7 breast cancer cells that the expression of several DNA repair genes involved in DNA repair by HR, such as Rad51, BRCA1 and BRCA2, were downregulated by RES (20). In the same study, we found a decrease of the protein levels of the MRN complex (MRE11-NBS1-RAD50), which is also involved in HR in MCF-7 cells treated with RES.

Discussion

New approaches to sensitize resistant tumour cells to chemotherapy include the use of natural compounds as modulators of chemotherapy to increase the efficiency of the cytostatic agents (30).
to mitomycin C (10) and gemcitabine (11). Phenyl hydroxamic acid PCI-24781, a histone deacetylase inhibitor that has a radiosensitizing effect on cancer cells, also acts by down-regulating Rad51 (42). In agreement with these reports, in the present study, we demonstrated, to the best of our knowledge, for the first time that RES can effectively downregulate Rad51 expression in MCF-7 cancer cells and restore chemosensitivity to CIS in CIS-resistant MCF-7 cells. We also found a decrease in the expression of MRN complex genes by qPCR in MCF-7 cells treated with RES. Consistent with our results, it has been reported that a pomegranate extract, which is a potent antioxidant such as RES, showed cellular and molecular actions beyond antioxidant in MCF-7 cells including evidence of the downregulation of DNA repair genes in MCF-7 cells (43). Earlier studies have also reported that the pomegranate extract is a growth inhibitor, pro-apoptotic, and anti-invasive agent in different cancer cell lines similar to RES, suggesting that inhibition of DNA repair gene expression may be an anticancer mechanism common of natural compounds (44).

In a previous study, it was observed that RES at low concentrations (30 µM) has the capacity to increase the mRNA of Rad51 in different cell lines (45). Notably, in MCF-7 cells these low concentrations have no effect on the levels of Rad51. However, in the present study, we report a decrease of Rad51 protein in concentrations 100 µM or higher. This finding highlights the importance of elucidating the optimal concentration of RES to achieve a particular effect. Both the mentioned reference and our findings support the hypothesis that the effects of RES are concentration-dependent.

In addition, since the main biological effects of RES occurred seemingly due to its ability to be absorbed in cells and tissues, achieving high concentrations of RES remains a challenge for therapy in humans. Researchers have recently attempted to improve RES chemical stability, bioavailability and therapeutic efficacy of RES (46-50). For example, piperine, the active compound found in pepper, increased the levels of RES in blood by a 1,000-fold in rats and delayed the formation of one of its major metabolites (51). However, this effect has not been proven experimentally in humans, although the brain blood level of RES was shown to increase. In addition, nanotechnology has yielded promising results in rat trials, with the use of RES nano-particles in various formulations. Such formulations show increased stability and bioavailability. Nanotechnology also prevented metabolism, thereby increasing tissue availability. Increased tissue concentrations have been observed especially in the liver, brain and kidney of healthy rats (52). This nanotechnology includes lipid-mounted, solid or albumin-mounted nanoparticles. On the other hand, another strategy to improve the pharmacokinetic properties of RES and extend its cancer-protecting activity, is the synthesis of synthetic analogues, and several analogues of RES have been identified in in vitro models. A promising analogue of RES is 3,4,5,4'-trans-tetramethoxyxystilbene, which is a methoxylated analogue of RES that has demonstrated antiproliferative activity in cancer cell lines and animal models (53-56).

However, a serious problem with platinum drugs, such as CIS and oxaliplatin, which are routinely used to treat various types of cancer, including breast cancer, is the side-effects found in patients, including nausea, nephrotoxicity and haemolytic anaemia (57,58). Renal dysfunction associated with CIS is dose-dependent, cumulative and occurs in 33% of patients receiving CIS (59). CIS accumulates in high levels in renal tissue due to active transport along the basolateral membrane by the organic cation and copper transporter (59). In addition to its anticancer activities of RES, it has been reported that RES has renal protective effects against nephrotoxicity induced by CIS in animal models (60). Pharmacokinetic studies have indicated that the liver and kidney have the highest RES levels when compared to other organs, which suggests that RES has a greater potential to induce its effects in these organs (61). In a clinical study, RES reduced tumour cell proliferation in colorectal cancer patients who took 500 or 1,000 mg RES prior to surgery (62). The results further showed that RES accumulated in patient tumours, probably protecting the kidney from nephrotoxicity (62). In agreement with this hypothesis, recently, it was demonstrated in a mouse model that RES increases the cytotoxic activity of CIS and protects against its nephrotoxicity effect. Consequently, we observed that RES treatment significantly decreased the IC50 values for CIS in malignant cells (possibly, by HR inhibition), suggesting that RES at the same time may increase the cytotoxic activity of CIS while reducing its toxic effects.

In addition to the downregulation of Rad51, we observed an increase in the accumulation of DSBs (seen by the γ-H2AX long signal), suggesting that this is a possible mechanism for reduced cancer cell survival following RES treatment. Although the exact mechanism of the downregulation of DNA repair genes by RES is currently unclear, it has been reported that the inhibition of HR amplifies toxic replication-associated DNA lesions that directly result in cell death (9,63). It was also observed that the downregulation of HR genes, BRCA2 and Rad51, by interference RNA, sensitizes cancer cells to chemotherapeutic compounds (64).

Previous findings have shown that natural compounds such as RES, curcumin and genistein, partly exert their antitumour effects through the regulation of one or more miRNAs (65). Therefore, it is possible that RES regulates Rad51 expression through the regulation of miRNAs. To explore this possibility, we used three different bioinformatic algorithms, namely, miRanda, TargetScan and miRTarBase (66) to identify miRNAs predicted or validated to target the mRNA of Rad51, and we found two miRNAs (miR-221 and miR-328) predicted to target Rad51 and one miRNA validated experimentally (miR-96), which were previously reported to be upregulated by RES. For example, it was reported that miR-96 directly targeted the coding region of Rad51, and the overexpression of miR-96 decreased the efficiency of HR and enhanced sensitivity to the poly(ADP-ribose) polymerase (PARP) inhibitor AZD2281 in vitro and to CIS both in vitro and in vivo (67), suggesting that RES may be used as an adjuvant in chemotherapy and treatment with PARP inhibitors.

In summary, co-treatment with RES in both MCF-7 chemoresistant and chemosensitive cells effectively reduced the concentrations of CIS needed for the equivalent effect of higher doses. RES probably acts by downregulating Rad51, a key player in HR repair, leading to impairment of the repair of DSBs. Our findings thus identified an unrecognized biological activity of a common natural compound. The low toxicity of RES makes it a promising candidate to improve cancer chemotherapy and cancer prevention. Thus, the reduction in DNA
damage repair induced by RES may be an excellent adjuvant in therapy, particularly in classic cases of cis resistance.

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Competing interests

The authors declare that they have no competing interests.

References


