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

I. LEON-GALICIA[1*], J. DIAZ-CHAVEZ[2*], M.E. ALBINO-SANCHEZ[3], E. GARCIA-VILLA[3], R. BERMUDEZ-CRUZ[3], J. GARCIA-MENA[3], L.A. HERRERA[2], A. GARCÍA-CARRANCÁ[1] and P. GARIGLIO[3]

[1]Biomedical Unit for Cancer Research, Virus and Cancer Laboratory, Department of Basic Research, Instituto de Investigaciones Biomédicas, UNAM/Instituto Nacional de Cancerologia (INCan); [2]Biomedical Unit for Cancer Research, Carcinogenesis Laboratory, Department of Basic Research, Instituto de Investigaciones Biomédicas, UNAM/Instituto Nacional de Cancerologia (INCan), 14080 Mexico City; [3]Department of Genetics and Molecular Biology, Center of Research and Advanced Studies (Cinvestav-IPN), 07360 Mexico City, Mexico

Received August 4, 2017; Accepted January 12, 2018

DOI: 10.3892/or.2018.6336

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-IC_{40}) during 5 passages, with the IC_{50} value increasing ~3-fold. Using an MTT assay, we estimated the changes in IC_{50} 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 IC_{50} 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.

Correspondence to: Dr Patricio Gariglio, Department of Genetics and Molecular Biology, Center of Research and Advanced Studies (Cinvestav-IPN), Av. IPN 2508, Col. San Pedro Zacatenco, 07360 Mexico City, Mexico
E-mail: vidal@cinvestav.mx

*Contributed equally

Key words: resveratrol, double-strand breaks, homologous recombination, Rad51 inhibition, DNA repair genes, anticancer drugs

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

Table I. Primer sequences for quantitative reverse-transcription polymerase chain reaction.

Gene title	Forward primer 5'-3'	Reverse primer 5'-3'
b2-Microglobulin (B2M)	AACCCCACTGAAAAAGATGAGT	ATGATGCTGCTTACATGTCTCG
Meiotic recombination 11 homolog A (*Saccharomyces cerevisiae*) (MRE11A)	ACTATCAAGATGGCAACCTCAACA	CCACAGACATTGAACGTCCAA
Nbs1 (NBN)	TCCAAGAAAAATCAAGCCTGTTG	AACTGAACGGAGGGATGGAA
RAD50 homolog (*S. cerevisiae*) (Rad50)	TCGCTCACAGCAGCGTAACT	CTAACACTGCATTTCACAATCTCTGA
RAD51 recombinase (HRAD51)	ACGGTTAGAGCAGTGTGGCATA	CTCCTTCTTTGGCGCATAGG

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% CO_2 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 in dimethyl sulfoxide (DMSO) and diluted in culture medium.

MTT proliferation assay. Cells were seeded at a density of $2x10^5$ cells/dish in p60 cell culture dishes 24 h before the assay. The cells were treated with different concentrations of RES (0-250 μM in 0.3% ethanol) and/or CIS and cultured for 24, 48 and 72 h. At the end of each treatment period, the cells were incubated in MTT (0.5 mg/ml in DMEM) at 37˚C for 30 min. The medium was removed, and the formazan dye crystals were 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 IC_{50} 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 $2^{-\Delta\Delta Cq}$ 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 = $2^{-\Delta\Delta Cq}$. 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)

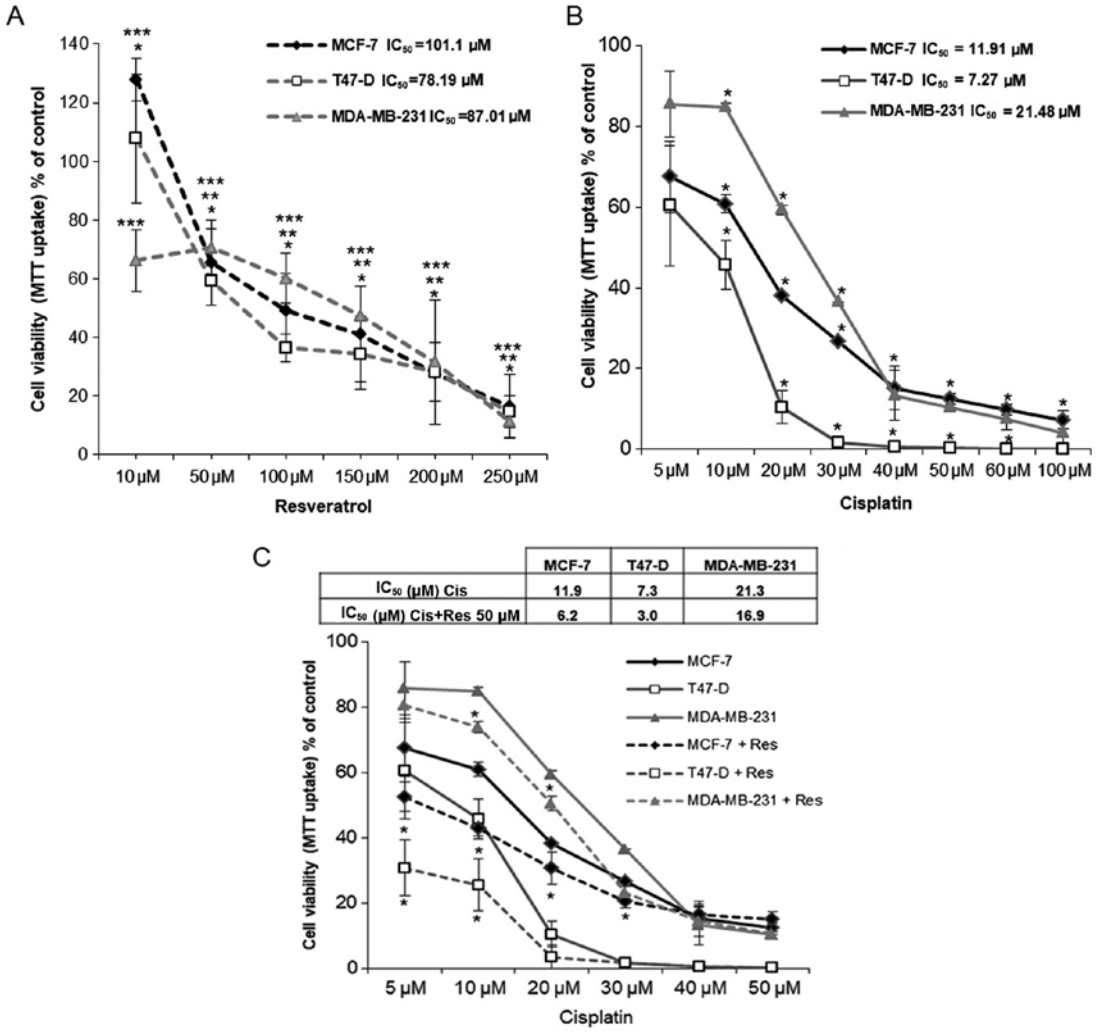

Figure 1. Resveratrol enhances the sensitivity of breast cancer cell lines to cisplatin. MCF-7, T47-D and MDA-MB-231 cells, grown as described in Materials and methods. (A) The three breast cancer cell lines were treated for 48 h with different concentrations of RES. The results are mean ± SD of three independent experiments, each performed in triplicate (MCF-7 $^{*}P<0.05$; T47D $^{**}P<0.05$ and MDA-MB-231 $^{***}P<0.001$). (B) MCF-7, T47-D and MDA-MB-231 cells were treated for 48 h with different concentrations of CIS. The results are mean ± SD of three independent experiments, each performed in triplicate (P<0.001). The IC_{50} value for RES and CIS was calculated by the GraphPad Prism 5 programme. MTT assays were performed as indicated in Materials and methods. (C) MCF-7, T47-D and MDA-MB-231 cells were treated with different CIS concentrations with or without RES for 48 h. A decrease in the IC_{50} 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).

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 Software 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 IC_{50} of RES and CIS on breast cancer cell lines (MCF-7, T47-D and MDA-MB-231) using the MTT assay. The cells were treated for 48 h with different

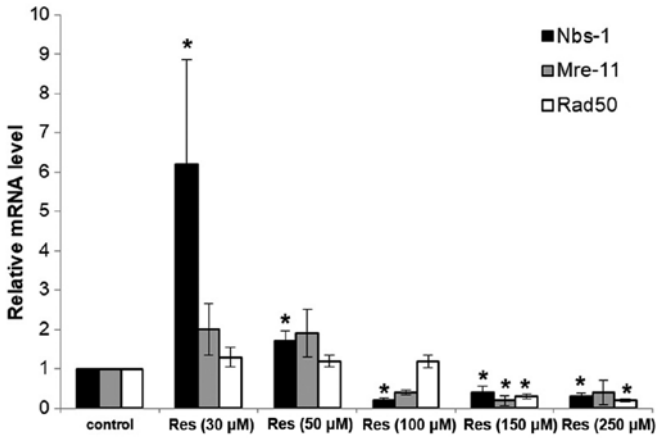

Figure 2. Relative mRNA levels of homologous recombination initiation complex components in resveratrol-treated MCF-7 cells. RT-qPCR analysis of differentially expressed HR initiation complex genes (*Nbs-1*, *Mre-11* and *Rad50*) from MCF-7 cells treated for 48 h with 30, 50, 100, 150 and 250 μM of RES vs. the control data set was carried out. The results are mean ± SD of three independent experiments, each performed in triplicate (*P<0.005). RT-qPCR, reverse transcription-quantitative PCR.

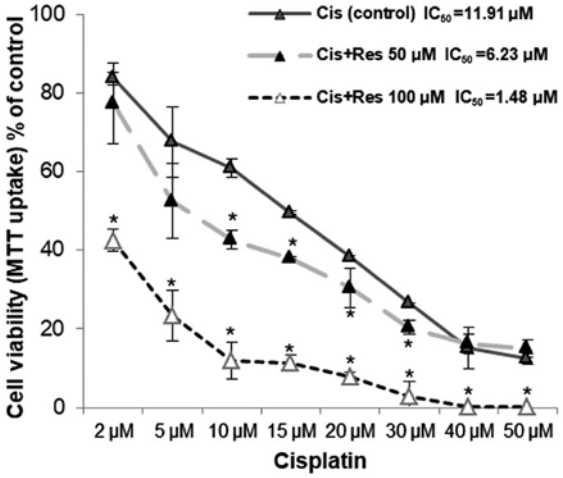

Figure 3. The IC_{50} value for cisplatin on MCF-7 cells is highly reduced by resveratrol at a concentration in which HR genes are reduced. MCF-7 cells were treated for 48 h with different CIS concentrations alone or together with 50 or 100 μM RES. The decrease in the IC_{50} value for CIS due to RES 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).

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 MDA-MB-231 (oestrogen receptor-negative cells) decreased at this concentration (Fig. 1A). At higher concentrations of RES, the decrease in viability was similar for all the cell lines tested, with MCF-7 presenting the highest IC_{50} 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 IC_{50} (7.27 μM), and MDA-MB-231 cells were the most resistant to CIS (IC_{50} = 21.48 μM CIS). We then investigated the antiproliferative effect of RES combined with CIS in MCF-7, T47-D and MDA-MB-231 cells. As shown in Fig. 1C, in all the cell lines, the IC_{50} values for CIS decreased significantly after 48 h of 50 μM RES treatment (P<0.005). In the T47-D cell line, the IC_{50} obtained for CIS decreased >50% (from 7.3 to 3.0 μM) in the presence of 50 μM RES, and the MCF-7 and MDA-MB-231 cell lines also showed a marked reduction (from 11.9 to 6.2 μM and from 21.3 to 16.9 μM, respectively). The IC_{50} value for RES and CIS was calculated as indicated in Materials and methods.

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.

Notably, lower doses of RES near the IC_{25} 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 IC_{50} concentration (100 μM) and 150-250 μM (Fig. 2) as previously reported (20). These results suggested that at doses similar or greater than the IC_{50} 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 IC_{50} in MCF-7 cells by negatively regulating HR initiation complex components.

IC_{50} 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 IC_{50} 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 IC_{50} 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 recombinase involved

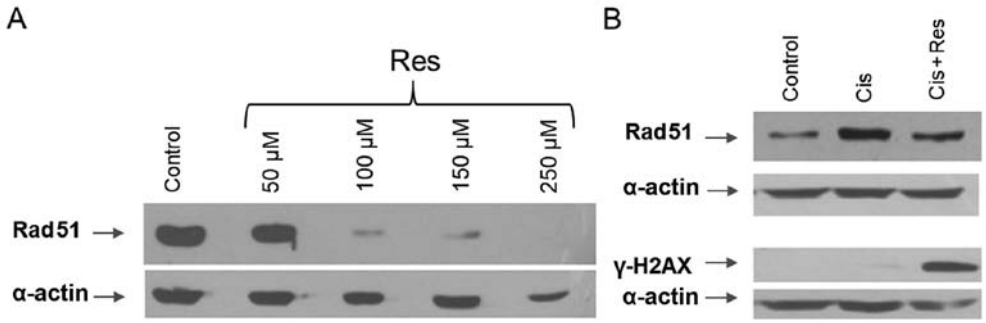

Figure 4. Rad51 and H2AX [pSer^{139}] expression levels in resveratrol- and cisplatin-treated MCF-7 breast cancer cells. Western blot analysis for Rad51 of protein extracts from MCF-7 cells treated for 48 h with increasing RES concentrations, as indicated in Materials and methods (A). (B) After 48 h of 20 μM CIS DNA damage Rad51 and H2AX [pSer^{139}] (γ-H2AX) were measured, in the absence or presence of RES. Equal amounts of total cell lysates were blotted and revealed as described in Materials and methods with Rad51 and γ-H2AX-specific antibodies and appropriate secondary antibody. Blots were stripped and reprobed with anti-α-actin as the loading control. These experiments were repeated at least three times with similar results. A representative gel is shown.

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.

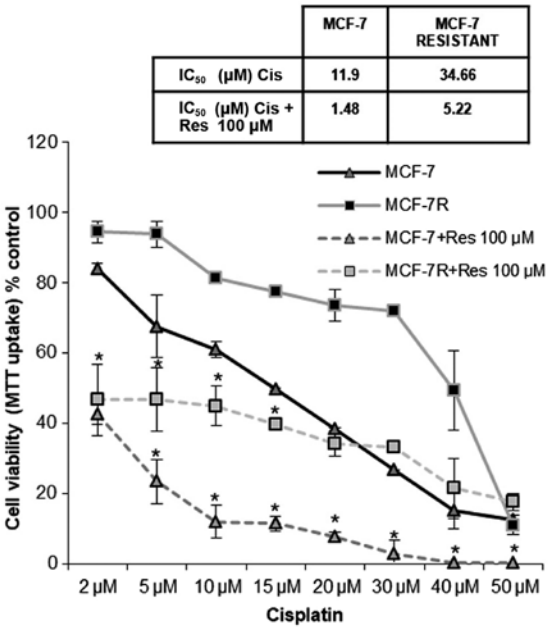

Figure 5. Resveratrol sensitizes MCF-7-resistant cells to cisplatin treatment. MCF-7-resistant (MCF-7R) cells were generated as described in Materials and methods. Both MCF-7 and MCF-7R cells were treated for 48 h with different CIS concentrations alone or together with 100 μM RES. MCF-7 cells were used as a control. The decrease in the IC_{50} 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 MCF-7 cells). The results are mean ± SD of three independent assays, each performed in triplicate (*P<0.001).

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

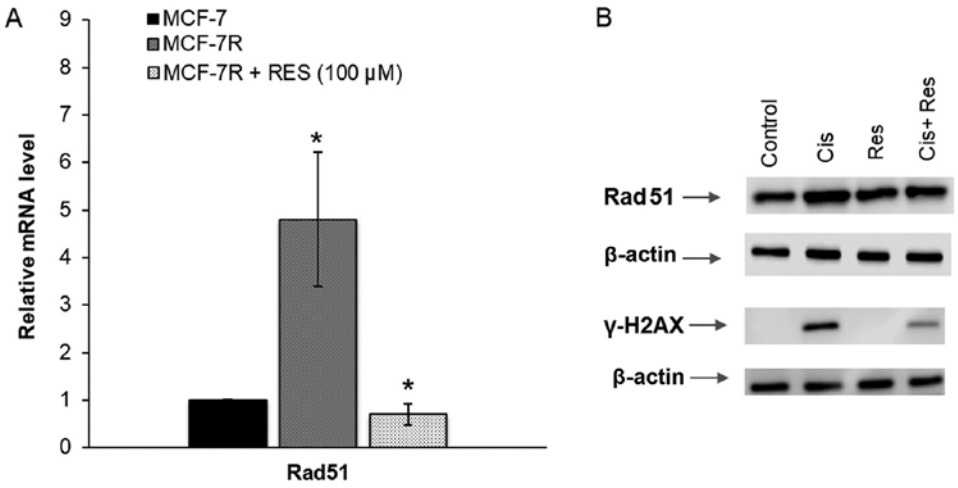

Figure 6. Resveratrol decreases Rad51 mRNA and protein levels and maintained γ-H2AX in MCF-7R cells treated with cisplatin. (A) RT-qPCR analysis of differentially expressed Rad51 generated from MCF-7R cells untreated or treated 48 h with 100 μM RES. MCF-7 cells were used as control. The results are mean ± SD of three independent experiments (*P<0.005). (B) After 48 h of 20 μM CIS DNA damage, Rad51 and H2AX [pSer139] (γ-H2AX) were measured in MCF-7R, in the absence or presence of RES. Equal amounts of total cell lysates were blotted and revealed, as described in Materials and methods with Rad51- and γ-H2AX-specific antibodies and appropriate secondary antibody. Blots were stripped and reprobed with anti-β-actin as the loading control. These experiments were repeated at least three times with similar results. A representative gel is shown.

cells. 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.

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).

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.

Rad51 is an important part of the HR pathway and Rad51 foci formation after DNA damage has been taken as a measurement of HR efficiency (37,38). In fact, Bhattacharyya *et al* generated data indicating that BRCA1 promotes the assembly of subnuclear Rad51 foci following cross-linking damage caused by CIS (37). Using isogenic and mutant mouse embryonic stem (ES) cell lines and clonogenic assays, the researchers of that study showed that BRCA1 mutants (when Rad51 foci is compromised) are 5-fold more sensitive to CIS compared to wild-type cells. Moreover, it has been observed that Rad51 overexpression in cancer cells was associated with augmented chemoresistance (39,40). Other findings have shown that the downregulation of Rad51 by anticancer drugs impairs HR repair, radiosensitizing or chemosensitizing cancer cells. For example, imatinib (Gleevec, c-Abl tyrosine kinase inhibitor) can efficiently reduce the expression of Rad51 in cancer cells, restoring cell radiosensitivity (9,41). Gefitinib, a selective epidermal growth factor receptor and tyrosine kinase inhibitor, also downregulates Rad51 in lung cancer cells sensitizing them

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 downregulating 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 antioxidation 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-tetramethoxystilbene, 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 IC_{50} 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.

Acknowledgements

The authors would like to thank Miriam C. Guido, Rocio Mendez-Martinez (INCan, SS. México), Ricardo Aguilar-Guadarrama (INCMNSZ-SS México) and Rodolfo Ocadiz-Delgado (CINVESTAV-IPN, Mexico) for their technical support. The present study was supported by grants from CONACyT 236767 to PG; CONACyT 253804 to AGC and FOSSIS 261875 to JDC. During this study ILG was the recipient of CONACyT fellowships (agreement nos. 290941 and 291113).

Competing interests

The authors declare that they have no competing interests.

References

1. Hoeijmakers JH: Genome maintenance mechanisms for preventing cancer. Nature 411: 366-374, 2001.
2. Chaney SG and Sancar A: DNA repair: Enzymatic mechanisms and relevance to drug response. J Natl Cancer Inst 88: 1346-1360, 1996.
3. Panasci L, Paiement JP, Christodoulopoulos G, Belenkov A, Malapetsa A and Aloyz R: Chlorambucil drug resistance in chronic lymphocytic leukemia: The emerging role of DNA repair. Clin Cancer Res 7: 454-461, 2001.
4. Monneret C: Platinum anticancer drugs. From serendipity to rational design. Ann Pharm Fr 69: 286-295, 2011.
5. Shen DW, Pouliot LM, Hall MD and Gottesman MM: Cisplatin resistance: A cellular self-defense mechanism resulting from multiple epigenetic and genetic changes. Pharmacol Rev 64: 706-721, 2012.
6. Stewart DJ: Mechanisms of resistance to cisplatin and carboplatin. Crit Rev Oncol Hematol 63: 12-31, 2007.
7. Martin LP, Hamilton TC and Schilder RJ: Platinum resistance: The role of DNA repair pathways. Clin Cancer Res 14: 1291-1295, 2008.
8. Wang ZM, Chen ZP, Xu ZY, Christodoulopoulos G, Bello V, Mohr G, Aloyz R and Panasci LC: In vitro evidence for homologous recombinational repair in resistance to melphalan. J Natl Cancer Inst 93: 1473-1478, 2001.
9. Choudhury A, Zhao H, Jalali F, Al Rashid S, Ran J, Supiot S, Kiltie AE and Bristow RG: Targeting homologous recombination using imatinib results in enhanced tumor cell chemosensitivity and radiosensitivity. Mol Cancer Ther 8: 203-213, 2009.
10. Ko JC, Ciou SC, Cheng CM, Wang LH, Hong JH, Jheng MY, Ling ST, Lin YW, Ko JC, Ciou SC, *et al*: Involvement of Rad51 in cytotoxicity induced by epidermal growth factor receptor inhibitor (gefitinib, IressaR) and chemotherapeutic agents in human lung cancer cells. Carcinogenesis 29: 1448-1458, 2008.
11. Tsai MS, Kuo YH, Chiu YF, Su YC and Lin YW: Down-regulation of Rad51 expression overcomes drug resistance to gemcitabine in human non-small-cell lung cancer cells. J Pharmacol Exp Ther 335: 830-840, 2010.
12. Chen RS, Jhan JY, Su YJ, Lee WT, Cheng CM, Ciou SC, Lin ST, Chuang SM, Ko JC and Lin YW: Emodin enhances gefitinib-induced cytotoxicity via Rad51 downregulation and ERK1/2 inactivation. Exp Cell Res 315: 2658-2672, 2009.
13. Javvadi P, Segan AT, Tuttle SW and Koumenis C: The chemopreventive agent curcumin is a potent radiosensitizer of human cervical tumor cells via increased reactive oxygen species production and overactivation of the mitogen-activated protein kinase pathway. Mol Pharmacol 73: 1491-1501, 2008.
14. Ko JC, Su YJ, Lin ST, Jhan JY, Ciou SC, Cheng CM and Lin YW: Suppression of ERCC1 and Rad51 expression through ERK1/2 inactivation is essential in emodin-mediated cytotoxicity in human non-small cell lung cancer cells. Biochem Pharmacol 79: 655-664, 2010.
15. Fremont L: Biological effects of resveratrol. Life Sci 66: 663-673, 2000.
16. Sanders TH, McMichael RW, Jr and Hendrix KW: Occurrence of resveratrol in edible peanuts. J Agric Food Chem 48: 1243-1246, 2000.
17. Sobolev VS and Cole RJ: Trans-resveratrol content in commercial peanuts and peanut products. J Agric Food Chem 47: 1435-1439, 1999.
18. Bianchini F and Vainio H: Wine and resveratrol: Mechanisms of cancer prevention? Eur J Cancer Prev 12: 417-425, 2003.
19. Borriello A, Bencivenga D, Caldarelli I, Tramontano A, Borgia A, Zappia V and Della Ragione F: Resveratrol: From basic studies to bedside. Cancer Treat Res 159: 167-184, 2014.
20. Leon-Galicia I, Diaz-Chavez J, Garcia-Villa E, Uribe-Figueroa L, Hidalgo-Miranda A, Herrera LA, Alvarez-Rios E, Garcia-Mena J and Gariglio P: Resveratrol induces downregulation of DNA repair genes in MCF-7 human breast cancer cells. Eur J Cancer Prev 22: 11-20, 2013.
21. Yalcin A: Quantification of thioredoxin mRNA expression in the rat hippocampus by real-time PCR following oxidative stress. Acta Biochim Pol 51: 1059-1065, 2004.
22. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. Methods 25: 402-408, 2001.
23. Mao Z, Jiang Y, Liu X, Seluanov A and Gorbunova V: DNA repair by homologous recombination, but not by nonhomologous end joining, is elevated in breast cancer cells. Neoplasia 11: 683-691, 2009.
24. Christodoulopoulos G, Malapetsa A, Schipper H, Golub E, Radding C and Panasci LC: Chlorambucil induction of HsRad51 in B-cell chronic lymphocytic leukemia. Clin Cancer Res 5: 2178-2184, 1999.
25. Hine CM, Seluanov A and Gorbunova V: Use of the Rad51 promoter for targeted anti-cancer therapy. Proc Natl Acad Sci USA 105: 20810-20815, 2008.
26. Maacke H, Hundertmark C, Miska S, Voss M, Kalthoff H and Sturzbecher HW: Autoantibodies in sera of pancreatic cancer patients identify recombination factor Rad51 as a tumour-associated antigen. J Cancer Res Clin Oncol 128: 219-222, 2002.
27. Richardson C: RAD51, genomic stability, and tumorigenesis. Cancer Lett 218: 127-139, 2005.
28. Clingen PH, Wu JY, Miller J, Mistry N, Chin F, Wynne P, Prise KM and Hartley JA: Histone H2AX phosphorylation as a molecular pharmacological marker for DNA interstrand cross-link cancer chemotherapy. Biochem Pharmacol 76: 19-27, 2008.
29. Suwaki N, Klare K and Tarsounas M: RAD51 paralogs: Roles in DNA damage signalling, recombinational repair and tumorigenesis. Semin Cell Dev Biol 22: 898-905, 2011.
30. Hussain SA, Sulaiman AA, Balch C, Chauhan H, Alhadidi QM and Tiwari AK: Natural polyphenols in cancer chemoresistance. Nutr Cancer 68: 879-891, 2016.
31. Mao QQ, Bai Y, Lin YW, Zheng XY, Qin J, Yang K and Xie LP: Resveratrol confers resistance against taxol via induction of cell cycle arrest in human cancer cell lines. Mol Nutr Food Res 54: 1574-1584, 2010.
32. Vanamala J, Reddivari L, Radhakrishnan S and Tarver C: Resveratrol suppresses IGF-1 induced human colon cancer cell proliferation and elevates apoptosis via suppression of IGF-1R/Wnt and activation of p53 signaling pathways. BMC Cancer 10: 238, 2010.
33. Liu PL, Tsai JR, Charles AL, Hwang JJ, Chou SH, Ping YH, Lin FY, Chen YL, Hung CY, Chen WC, *et al*: Resveratrol inhibits human lung adenocarcinoma cell metastasis by suppressing heme oxygenase 1-mediated nuclear factor-kappaB pathway and subsequently downregulating expression of matrix metalloproteinases. Mol Nutr Food Res 2: S196-S204, 2010.
34. Kraft TE, Parisotto D, Schempp C and Efferth T: Fighting cancer with red wine? Molecular mechanisms of resveratrol. Crit Rev Food Sci Nutr 49: 782-799, 2009.
35. Kelley MR, Logsdon D and Fishel ML: Targeting DNA repair pathways for cancer treatment: What's new? Future Oncol 10: 1215-1237, 2014.
36. Bouwman P and Jonkers J: The effects of deregulated DNA damage signalling on cancer chemotherapy response and resistance. Natu Rev Cancer 12: 587-598, 2012.
37. Bhattacharyya A, Ear US, Koller BH, Weichselbaum RR and Bishop DK: The breast cancer susceptibility gene *BRCA1* is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin. J Biol Chem 275: 23899-23903, 2000.
38. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, *et al*: Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434: 917-921, 2005.

39. Hannay JA, Liu J, Zhu QS, Bolshakov SV, Li L, Pisters PW, Lazar AJ, Yu D, Pollock RE and Lev D: Rad51 overexpression contributes to chemoresistance in human soft tissue sarcoma cells: A role for p53/activator protein 2 transcriptional regulation. Mol Cancer Ther 6: 1650-1660, 2007.
40. Slupianek A, Schmutte C, Tombline G, Nieborowska-Skorska M, Hoser G, Nowicki MO, Pierce AJ, Fishel R and Skorski T: BCR/ABL regulates mammalian RecA homologs, resulting in drug resistance. Mol Cell 8: 795-806, 2001.
41. Russell JS, Brady K, Burgan WE, Cerra MA, Oswald KA, Camphausen K and Tofilon PJ: Gleevec-mediated inhibition of Rad51 expression and enhancement of tumor cell radiosensitivity. Cancer Res 63: 7377-7383, 2003.
42. Adimoolam S, Sirisawad M, Chen J, Thiemann P, Ford JM and Buggy JJ: HDAC inhibitor PCI-24781 decreases RAD51 expression and inhibits homologous recombination. Proc Natl Acad Sci USA 104: 19482-19487, 2007.
43. Shirode AB, Kovvuru P, Chittur SV, Henning SM, Heber D and Reliene R: Antiproliferative effects of pomegranate extract in MCF-7 breast cancer cells are associated with reduced DNA repair gene expression and induction of double strand breaks. Mol Carcinog 53: 458-470, 2014.
44. Sharma P, McClees SF and Afaq F: Pomegranate for prevention and treatment of cancer: An update. Molecules 22: E177, 2017.
45. Le Corre L, Fustier P, Chalabi N, Bignon YJ and Bernard-Gallon D: Effects of resveratrol on the expression of a panel of genes interacting with the *BRCA1* oncosuppressor in human breast cell lines. Clin Chim Acta 344: 115-121, 2004.
46. Meng J, Guo F, Xu H, Liang W, Wang C and Yang XD: Combination therapy using Co-encapsulated resveratrol and paclitaxel in liposomes for drug resistance reversal in breast cancer cells in vivo. Sci Rep 6: 22390, 2016.
47. Ruivo J, Francisco C, Oliveira R and Figueiras A: The main potentialities of resveratrol for drug delivery systems. Braz J Pharm Sci 51: 499-513, 2015.
48. Singh G and Pai RS: Trans-resveratrol self-nano-emulsifying drug delivery system (SNEDDS) with enhanced bioavailability potential: Optimization, pharmacokinetics and in situ single pass intestinal perfusion (SPIP) studies. Drug Deliv 22: 522-530, 2015.
49. Smoliga JM and Blanchard O: Enhancing the delivery of resveratrol in humans: If low bioavailability is the problem, what is the solution? Molecules 19: 17154-17172, 2014.
50. Uberti F, Morsanuto V, Aprile S, Ghirlanda S, Stoppa I, Cochis A, Grosa G, Rimondini L and Molinari C: Biological effects of combined resveratrol and vitamin D3 on ovarian tissue. J Ovarian Res 10: 61, 2017.
51. Johnson JJ, Nihal M, Siddiqui IA, Scarlett CO, Bailey HH, Mukhtar H and Ahmad N: Enhancing the bioavailability of resveratrol by combining it with piperine. Mol Nutr Food Res 55: 1169-1176, 2011.
52. Frozza RL, Bernardi A, Paese K, Hoppe JB, da Silva T, Battastini AM, Pohlmann AR, Guterres SS and Salbego C: Characterization of trans-resveratrol-loaded lipid-core nanocapsules and tissue distribution studies in rats. J Biomed Nanotechnol 6: 694-703, 2010.
53. Androutsopoulos VP, Fragiadaki I, Spandidos DA and Tosca A: The resveratrol analogue, 3,4,5,4'trans-tetramethoxystilbene, inhibits the growth of A375 melanoma cells through multiple anticancer modes of action. Int J Oncol 49: 1305-1314, 2016.
54. Androutsopoulos VP, Fragiadaki I and Tosca A: Activation of ERK1/2 is required for the antimitotic activity of the resveratrol analogue 3,4,5,4'-tetramethoxystilbene (DMU-212) in human melanoma cells. Exp Dermatol 24: 632-634, 2015.
55. Androutsopoulos VP, Ruparelia KC, Papakyriakou A, Filippakis H, Tsatsakis AM and Spandidos DA: Anticancer effects of the metabolic products of the resveratrol analogue, DMU-212: Atructural requirements for potency. Eur J Med Chem 46: 2586-2595, 2011.
56. Sale S, Verschoyle RD, Boocock D, Jones DJ, Wilsher N, Ruparelia KC, Potter GA, Farmer PB, Steward WP and Gescher AJ: Pharmacokinetics in mice and growth-inhibitory properties of the putative cancer chemopreventive agent resveratrol and the synthetic analogue trans 3,4,5,4'-tetramethoxystilbene. Br J Cancer 90: 736-744, 2004.
57. Canpolat C, Pearson P and Jaffe N: Cisplatin-associated hemolytic uremic syndrome. Cancer 74: 3059-3062, 1994.
58. Loehrer PJ and Einhorn LH: Drugs five years later. Cisplatin. Ann Intern Med 100: 704-713, 1984.
59. Dasari S and Tchounwou PB: Cisplatin in cancer therapy: Molecular mechanisms of action. Eur J Pharmacol 740: 364-378, 2014.
60. Osman AM, Telity SA, Damanhouri ZA, Al-Harthy SE, Al-Kreathy HM, Ramadan WS, Elshal MF, Khan LM and Kamel F: Chemosensitizing and nephroprotective effect of resveratrol in cisplatin -treated animals. Cancer Cell Int 15: 6, 2015.
61. Valentovic MA, Ball JG, Brown JM, Terneus MV, McQuade E, Van Meter S, Hedrick HM, Roy AA and Williams T: Resveratrol attenuates cisplatin renal cortical cytotoxicity by modifying oxidative stress. Toxicol In Vitro 28: 248-257, 2014.
62. Patel KR, Brown VA, Jones DJ, Britton RG, Hemingway D, Miller AS, West KP, Booth TD, Perloff M, Crowell JA, *et al*: Clinical pharmacology of resveratrol and its metabolites in colorectal cancer patients. Cancer Res 70: 7392-7399, 2010.
63. Helleday T: Homologous recombination in cancer development, treatment and development of drug resistance. Carcinogenesis 31: 955-960, 2010.
64. Quiros S, Roos WP and Kaina B: Rad51 and BRCA2 - New molecular targets for sensitizing glioma cells to alkylating anticancer drugs. PLoS One 6: e27183, 2011.
65. Phuah NH and Nagoor NH: Regulation of microRNAs by natural agents: New strategies in cancer therapies. Biomed Res Int 2014: 804510, 2014.
66. Riffo-Campos AL, Riquelme I and Brebi-Mieville P: Tools for sequence-based miRNA target prediction: What to choose? Int J Mol Sci 17, 2016.
67. Wang Y, Huang JW, Calses P, Kemp CJ and Taniguchi T: MiR-96 downregulates REV1 and RAD51 to promote cellular sensitivity to cisplatin and PARP inhibition. Cancer Res 72: 4037-4046, 2012.