REVIEW

Synergistic Effect of Resveratrol and Radiotherapy in Control of Cancers

Lakhan Kma

Abstract

Cancers will continue to be a threat to health unless they can be controlled by combinations of treatment modalities. In this review, evaluate the role of resveratrol (RSV) as a radiosensitizing agent was evaluated and underlying mechanisms holistically explored in different cancer models focusing on therapeutic possibilities. The ability of RSV to modify the effect of radiation exposure in normal and cancer cells has indeed been shown quite convincingly, the combination of RSV and IR exhibiting synergistic effects on different cancer cells. This is relevant since controlled exposure to IR is one of the most frequently applied treatments in cancer patients. However, radiotherapy (XRT) treatment regimes are very often not effective in clinical practice as observed in patients with glioma, prostate cancer (PCa), melanoma, for example, largely due to tumour radioresistant properties. Sensitization of IR-induced apoptosis by natural products such as RSV is likely to be relevant in cancer control and treatment. However, all cancers do not respond to RSV+IR in a similar manner. Therefore, for those such as the radioresistant PCa or melanoma cells, the RSV+IR regime has to be very carefully chosen in order to achieve effective and desirable outcomes with minimum toxicity to normal cells. They are reports that the highest concentration of 100 μM RSV and highest dose of 5 Gy IR are sufficient to kill cells by induction of apoptosis, indicating that RSV is effective in radiosensitizing otherwise radioresistant cells. In general, it has been shown in different cancer cells that RSV+XRT effectively act by enhancing expression of anti-proliferative and pro-apoptotic molecules, and inhibiting pro-proliferative and anti-apoptotic molecules, leading to induction of apoptosis through various pathways, and cell death. If RSV+XRT can suppress the signature of cancer stemness, enhance the radiosensitivity by either targeting the mitochondrial functionality or modulating the tumour necrosis factor-mediated or Fas-FasL-mediated pathways of apoptosis in different cancers, particularly *in vivo*, its therapeutic use in the control of cancers holds promise in the near future.

Keywords: Radiotherapy - resveratrol - radiosensitivity - apoptosis - tumour

Asian Pac J Cancer Prev, **14 (11)**, 6197-6208

Introduction

The cytotoxic effects of ionizing radiation (IR) are known to mediate through diverse mechanisms, which include induction of DNA damage, followed by activation of DNA damage-induced signaling pathways (Elmore et al., 2006; Sowa et al., 2006; Suit et al., 2007). These pathways results in cell cycle arrest and/or induction of cell death by apoptosis, necrosis, autophagy or mitotic catastrophe, depending on the total dose and dose rate (Debatin and Krammer, 2004; Okada and Mak, 2004; Elmore et al., 2006; Sowa et al., 2006; Wang et al., 2006; Suit et al., 2007). Exploiting these and other known damaging effects of IR, radiotherapy (XRT) is targeted to kill cancer cells and, continues to be one of the main treatment modality for different type of cancers. It has been extensively used in breast cancer (Collins et al., 2005; Moon et al., 2009), prostate cancer (Zietman et al., 2010), cervical tumour (Bisht et al., 2003), lung carcinoma (Machida et al., 2003), medulloblastoma (Habrand and De Crevoisier, 2001), melanoma (Ivanov et al., 2007), etc. However, the effectiveness of XRT is limited by radioresistance displayed by cancer cells (Brown, 1999; Ruan et al., 2009; Kelley, 2012). For example, prostate cancer is known to be highly resistant to IR (Forman et al., 1993; Crook et al., 1995; Hagan et al., 2000). Conventional radiotherapy doses up to 70 Gy have demonstrated biochemical failure rates of 30% or more in localized disease, leading to a need for XRT dose escalation, which can result into impotence, rectal and bladder toxicity (Leith, 1994; Zietman et al., 2010). It has also been reported that increased radiation dose leads to incidence of skin toxicity in patients undergoing adjuvant XRT (Weiss, et al., 2003). These effects adds up to some of the other side effects of XRT such as pituitary hormone dysfunction and behaviour problems (Hoppe-Hirsch et al., 1995; Dennis et al., 1996), resulting into diminished therapeutic outcome and poor quality of life among survivors.

Since an alternative to XRT to treat patients with

*Cancer and Radiation Countermeasures Unit, Department of Biochemistry, North-Eastern Hill University, Meghalaya, India *For correspondence: lakhonkma@gmail.com*

cancer is far from reality, it is important to address the dose-limiting normal tissue toxicity and radioresistant tumours, which can result into radiation treatment failure (Kabakov et al., 2010). Currently, a lot of attention is being drawn into augmentation of the effect of radiation on tumours. It has been suggested that combining XRT with targeted tumour therapeutics could enhance the therapeutic efficacy (Camphausen and Tofilon, 2004). Since the control and suppression of cancer development is very vital, it has also been suggested to combine XRT with specific inhibitors of the cell survival pathways (Ivanov et al., 2007). Therefore, research directed to find effective radiosensitizers, which lower the radiation dose-response threshold for cancer cells, with minimal radiation-induced side-effects on normal cells (Moeller et al., 2005; Kabakov et al., 2010) deserved to be accorded utmost priority. In this quest, the polyphenol resveratrol (RSV; 3, 5, 4'-trihydroxy-trans-stilbene) has deservingly received a lot of attention. It is a phytoalexin found in a wide variety of dietary sources including grapes, peanuts and wines, especially red wines (Pervaiz, 2003; Delmas et al., 2005; de la Lastra and Villegas, 2007). RSV has important antioxidant properties, possibly by its direct scavenging effect and/or activation of pathways such as those that up-regulate natural antioxidant defences (Baur and Sinclair, 2006; Bastianetto and Quirion, 2010). It has been reported to work both as a chelating agent and as a radical scavenger, and takes part in inflammation by inhibiting the production of IL-8 and blocking of nuclear factor-kappa-B (NF-κB) activation (Benitez et al., 2009; Oh et al., 2009). The anti-inflammatory activity of RSV was supported by the observation that it induced relaxing effect on vessels that cause an improvement in skin microcirculation in irritant dermatitis (Filomeni et al., 2007; Reagan-Shaw et al., 2008; Kao et al., 2009). Many other studies have shown that RSV can prevent or slow the progression of a wide variety of illnesses, including cardiovascular diseases and cancer (Fremont, 2000; Pervaiz, 2004; Ungvari et al., 2009).

The *in vivo* anticancer efficiency of RSV has been evaluated in a xenograft model of human lung cancer using A549 cell line (Yin et al., 2013). Here, nude mice were implanted by subcutaneously injection at the left axillary space with 0.1 ml of cell suspension containing 4-6×10^6 A549 cells. The mice, whose tumour reached a tumour volume of 100 mm^3, generally after 7-8 days of implantation, were selected for treatments with RSV intravenously, at an equivalent dose of 15, 30, and 60 mg/kg body weight (BW). Cytotoxicity of RSV against A549 cells was monitored at different doses incubated for 48 h, and exhibited dose- and time-dependent cytotoxicity at a dose from 4-64 μM. The IC_{50} value (concentration that result at 50% cell death) of RSV against A549 cells was 8.9±1.3 μM. In this study, it was also shown that RSV significantly activated caspase-3, the crucial mediators of apoptosis (Mcllwain et al., 2013; Zhou et al., 2013), in a dose-dependent manner. It also exhibited a dose-dependent tumour growth inhibition effect. It was observed that all the three doses of RSV used in this experiment significantly inhibited the growth of lung cancer in mice. The high dose of RSV (60 mg/kg) showed the strongest antitumour effect in this case.

In another study on the effect of RSV on lung carcinoma (Busquets et al., 2007), it was shown that mice treated for 15 days before sacrifice, a daily intraperitoneal (i.p.) dose of RSV either 5 mg/kg BW or 25 mg/kg BW, after an inoculum of 5×10^5 of Lewis lung carcinoma, had significantly less number of the secondary tumour nodules compared to untreated control mice. Similar decreases were observed for both the doses i.e. 36 and 40%, respectively. Even in the actual weight of the metastases, there was significant decrease in treated mice in compared to control, although the higher dose had a much more marked effect than the lower one (74 and 45%, respectively). It was suggested through this study that RSV act as anti-metastatic agent, probably by decreasing angiogenesis in the secondary cancer nodules.

The radioprotective effects RSV *in vivo*, was probably demonstrate for the first time in a study on mice where it was shown that RSV in combination with IR resulted in a statistically significant reduction in the mean total chromosome aberration frequency in mouse bone marrow cells (Carsten et al., 2008), in comparison to untreated group, RSV and IR alone groups. In this case, mice were administered with RSV at a dose of 100 mg/kg BW daily, initiated 2 days prior to 3 Gy (at a dose rate of 1.18 Gy/min) whole-body irradiation and analyzed 1 and 30 days after irradiation. It was apparent from this observation that RSV possesses potential radiosensitizing property. Therefore, its role as an effective radiosensitizing agent and the underlying mechanisms deserved to be explored holistically in different cancer models in light of its therapeutic possibilities. The current review on radiosensitive effect of RSV in some cancers is an attempt in that direction.

Examples of Cancers and Radiosensitizing Effects of RSV

Leukemia

The potential of RSV as a radiosensitizer has been studied in human leukemia cell line, EOL-1, derived from a patient with eosinophilic leukemia (Baatout et al., 2005). Here it was shown that RSV+IR exhibited dose-dependent reduction in cell proliferation and enhanced radiation-induced apoptosis in these cells during 1, 2 or 3 days of culture. It was observed that IR exposure (0, 2, 4, 6 or 8 Gy) alone induced a dose-related reduction in cell proliferation and the appearance of polyploid cells in EOL-1 cells. It was also reported that EOL-1 cells underwent a dose-related increase of apoptosis which, from the second day on, was accompanied by a dose-related increase of necrosis. When cells were exposed to RSV (50 μM) alone, a decrease in cell proliferation was observed, while an increase in the percentage of apoptotic cells was noted from RSV (100 μM) in EOL-1 cells, after only one day of culture. Simultaneous exposure to IR and RSV resulted in a synergistic decrease of cell proliferation as well as in a synergistic increase of apoptosis and necrosis. This result supported an earlier report on the potential of RSV as a radiosensitizer in leukemia cells (human leukemia cell lines 32Dp210, HL-

60 (promyelocytic) and U937 (monocytic), and mouse leukemia cell line L1210 (lymphocytic)) where it was shown that normal hematopoietic progenitor cells were less sensitive to the growth inhibitory activity of RSV (10-80 μM) compared to these leukemia cells in a dose-related manner (Gautam et al., 2000). Here, the analysis on the dose-response effect of RSV on leukemia and bone marrow (BM) progenitors indicated that RSV inhibited the proliferation of all four leukemic cell lines tested (32Dp210, HL-60, U937 and L1210) in a dose-dependent manner. The maximum suppression (98%) was seen at 80 μM RSV. There was roughly 70-80% suppression of colony development by leukemic cell lines at 40 μM RSV. Low concentrations of RSV were not as inhibitory as the high concentration. The IC_{50} of RSV for 32Dp210, HL-60, U937 and L1210 cells was found to be 18, 20, 26 and 34 μM, respectively. Although RSV also reduced the clonal expansion of normal hematopoietic progenitor cells (55-60%), progenitor cells were less sensitive to the inhibitory effect of RSV than leukemia cells. At the highest concentration (80 μM), RSV inhibited the clonal growth of leukemia cells by more than 98%, the inhibition of normal progenitor cell growth was 55-60%. The IC_{50} of RSV for normal BM progenitor cells was substantially higher (59 μM) than that for leukemia cell lines. It was further shown in this study that these leukemia cells, Assessment of DNA fragmentation demonstrated lack of apoptosis in untreated leukemia cells compared to ones treated with 40 μM RSV for 6 h. However, treatment with RSV induced DNA fragmentation (apoptosis) in all these leukemic cell lines tested. Normal BM cells treated with 80 μM RSV for 20 h also showed DNA fragmentation. However, in contrast to the absence of DNA fragmentation in leukemia cells, bone marrow cells cultured for 20 h in the absence of RSV showed spontaneous fragmentation of DNA. DNA prepared from bone marrow cells within 1 h of their removal from the mouse showed very little fragmentation. Overall, leukemia cells exhibited greater sensitivity to RSV compared to BM progenitors in this study. Combining these observations with the previously mentioned radiosensitizing effect of RSV, the synergistic effect of RSV+IR appears promising in the treatment of leukemia. It is further supported by the observation that RSV-treated hematopoietic progenitor cells maintain their ability to engraft, which was established by comparing the engraftment of untreated and RSV (80 μM)-treated normal bone marrow cells in lethally irradiated mice (10 Gy) (Gautam et al., 2000). Here, it was shown convincingly that treatment of bone marrow cells with RSV does not impede the engraftment of bone marrow.

Prostate cancer

The role of RSV in potentiating the radiation response of human prostate cancer (PCa) cell line DU145, which are known radioresistant cancer cells, has also been investigated (Scarlatti et al., 2007). In this investigation, cells were irradiated (0.5-2.0 Gy/day) for three consecutive days at dose rate of 0.63 Gy/min. The IC_{50} values of ionizing radiation and RSV in DU145 cells were estimated to be 2.17 Gy and 2.15 μM, respectively. The combined treatment with RSV (0.5-32 μM, for 72 h) and radiation (0.5-2.0 Gy every day) dramatically reduced cell survival and number of viable cells compared to radiation treatment alone. The IC_{50} value for ionizing radiation was reduced to 1.0 Gy in RSV and IR combined group. The dose reduction index (DRI) values indicated that the synergic effect resulted in 2.2-7.8-fold reduction of the IR dose and 4.0-40.1-fold reduction of RSV dose. Similarly, the values of the combination index (CI) suggest that the combination of IR and RSV was additive at 50% and 60% inhibition, synergistic at 75% and 90%, and strongly synergistic at 95%. Using the diacylglycerol kinase assay and the corresponding cell viability, it was also shown that endogenous ceramide increased 2.3-fold in DU145 cells after combined treatment compared to untreated cells and 1.6- and 2.0-fold, respectively, during separate radiation (1.0 Gy every day for three days) and RSV (16 μM). At the same time, it was also shown that cell viability decreased to 10% after combined treatment in comparison to untreated cells, and 83% and 22% respectively, in radiation and RSV treatments alone group. It was suggested that the increase of ceramide observed in cells treated with the both agents was correlated to RSV-induced sensitivity of DU145 cells. This was further supported by incubation of cells with myriocin, an inhibitor of serine palmitoyl transferase, the rate-limiting enzyme of de novo ceramide biosynthesis. It was shown that myriocin was able to significantly reverses ceramide generation after both RSV alone treatment and combined treatments, concurrent with a very significant reduction in cell death.

In another study, polyphenol RSV role in modulating both the radioresistance-mediating serine-threonine kinase (Akt) and the tumour suppressor AMP-activated protein kinase (AMPK) pathways in human PCa (PC3, 22RV1) and normal prostate epithelial (PNT1A) cell lines were evaluated (Rashid et al., 2011). In this case, cells were pre-treated with RSV (10 μM) 1 h prior to 2-8 Gy IR. It was then incubated for 1 h following IR exposure. For cell cycle and clonogenic assays, cells were exposed to RSV throughout the experiments. It was shown that RSV inhibited survival of both PC3 and 22RV1 PCa cells with the IC_{50} values of 10 μM and 2.5 μM for PC3 and 22RV1 cells, respectively. It was also reported that 2.5 μM significantly reduced survival in 22RV-1 cells to 40±3.06% of control. However, PNT1A normal epithelial cells exhibited 5 and 10% inhibition of survival at 2.5 and 5 μM, respectively. It was also shown that only the higher dose of 10 μM resulted in significantly reduced number of cells in PNT1A (to 60±12.26% of control). It was also reported that PC3 cells showed greater resistance to IR alone compared to 22RV1 cells (surviving fractions after 2 Gy (SF2) of 60±5.30% *vs* 40±3.53%, respectively, which was comparable to that of PNT1A cells (SF2 of 62.9±2.26%. The use of standard therapeutic IR dose of 2 Gy and not high doses of IR in clonogenic assays was justified for clinical relevance, and higher doses (i.e. 6-8 Gy) was resulted in very high toxicity to PCa cells. Combination of RSV and IR (RSV+IR) enhanced the IR-induced inhibition of survival in both PC3 and 22RV1 PCa cells. RSV (2.5 and 5 μM) reduced survival of IR-treated PC3 cells by (13.8±0.09% and 20.4±0.9%). RSV (10 μM)

caused a significant 2.5-fold inhibition in SF2 in 22RV1 cells but 5 μM showed non-significant reduction of SF2. RSV (5 μM) pre-treatment did not decrease further SF2 of PNT1A prostate epithelial cells but did so with 10 μM RSV. Using the clonogenic assay, it was also estimated in this study that RSV (5 μM) may be able to reduce the dose of radiotherapy needed to eliminate 22RV1 type (hormone-sensitive) PCa tumours (from about 47-40 Gy) and may be able to eliminate PC3 type (hormone-insensitive) PCa tumours (with about 54 Gy) that would otherwise be incurable with doses even higher than 80 Gy.

It was further shown in this investigation that IR induced an arrest of cells at the G2-M interphase (Control: 3.4% *vs* IR: 31.7%) but pre-treatment with RSV (5 μM) prevented this event (RSV+IR: 8.3% *vs* 31.7% for IR). RSV caused accumulation of radiated cells into the G1-S phases of the cycle (IR: 58% *vs* RSV+IR: 70.7%) and increased the population of cells in the sub-G1 region by 2-fold compared to those treated with IR alone (IR: 10.3% *vs* RSV+IR: 21.0%), indicating induction of apoptosis. RSV enhanced the IR-induced expression of p53 and the cyclin-dependent kinase inhibitors (CDKI) $p21^{cip1}$ and $p27^{kip1}$ in 22RV1 cells. It was also shown similar change in expression occurred for the two CDKIs in p53-null PC3 cells. It was demonstrated by immunoblotting with a cleaved caspase-3-specific antibody that pre-treatment with RSV (2.5 and 5 μM) enhanced the IR-induced cleaved caspase-3 levels in a dose-dependent fashion within 1 hour of IR exposure in both PC3 and 22RV1 cells. Morphological change was also evaluated microscopically after staining the cells with Hoechst 33258. Counting of cells with nuclear aberrations such as fragmentation, micro-nuclei and multi-nucleation (polysomy), the markers predictive of apoptosis and/or mitotic cell death (mitotic catastrophe), revealed that RSV significantly enhanced the IR-induced nuclear aberrations to 70±3.11% (an increase of 27%) of total cell counts and reduced overall cellular viability. However, IR alone induced slightly low yet, significant nuclear aberrations (43±2.18%), while RSV alone (5 μM) increased nuclear abnormalities by 6%.

In this study, examination of the effects of RSV on the well-described radioresistance pathway of Akt-mTOR (Mammalian target of rapamycin) indicated that IR alone induced a significant time-dependent increase in the levels of phosphorylated-Akt (p-Akt) (both S473 and T308 sites) in PC3 cells, with negligible effects on total levels of the protein. Highest levels of p-Akt were reached 30 minutes to 1 hour after IR exposure. Akt phosphorylation was associated with increased activity of this enzyme indicated by the IR-induced phosphorylation of mTOR, a key effector of Akt. It was shown that treatment of PC3 and 22RV1 cells with RSV reduced basal levels of p-Akt. Pre-treatment with RSV led to significant inhibition of IR-induced p-Akt with no effects on total levels of the protein. Although IR exposure led to an increase in p-Akt, RSV pre-treatment (2.5 and 5 μM) significantly reduced and/or prevented IR-induced phosphorylation of Akt. It was also reported that IR induced a very significant phosphorylation of AMPK on Thr172 of the catalytic α-subunit that was detectable within 15 min. Highest levels of phosphorylated AMPK (p-AMPK) were detected 1 hour after IR exposure and returned to almost basal levels 4 hours after radiation. This was associated with activation of AMPK highlighted by the detected phosphorylation of the AMPK substrate Acetyl CoA Carboxylase (ACC). RSV induced a very significant activation of AMPK in both PC3 and 22RV1 cells at both 2.5 and 5 μM. It was also reported further that RSV pre-treatment increased significantly the IR-induced AMPK phosphorylation in both cells. Involvement of ataxia telangiectasia mutated (ATM) in Akt activation by IR was analyzed by using the ATM-specific inhibitor KU55933 in PCa cells. It was shown that pre-incubation with KU55933 prevented IR-induced ATM and AMPK phosphorylation, and phosphorylation of Akt at S473 and activation of its kinase activity as indicated by reduced phosphorylation of mTOR. A very significant phosphorylation of ATM and AMPK as well as induction of $p21^{cip1}$ in PC3 PCa cells in response to IR was also reported. It was also shown that siRNA knockdown of both α1 and α2 subunits of AMPK blocked p53 and $p21^{cip1}$ induction by IR. RSV pre-treatment enhanced IR-induced phosphorylation of ATM and of its substrate histone H2Ax (gH2Ax), as well as phosphorylation of AMPK and induction of $p21^{cip1}$.

In another study involving PC-3 cells derived from human PCa, it was shown that RSV enhanced radiation sensitivity on cell proliferation and apoptosis pathway (Fang et al., 2012). In this study, PC-3 cells at 70-80% confluence, were treated with different concentrations of RSV (0-50 μM) for 24 h, followed by IR exposure at 2, 4, or 8 Gy (dose, 280 cGy/min), or sham treatment. Clonogenic survival assay carried out after 24 h of radiation exposure revealed that the combined effect of RSV and IR (RSV+IR) decreased cell proliferation in a dose-dependent manner. It was observed that the percentage of colonies of PC-3 after IR and RSV (50 μM) treatment decreased to 15±4% (2 Gy), 8±3% (4 Gy) and 5±2% (8 Gy). However, the percentage of colonies of PC-3 after IR at the dosage of 2 Gy was similar to that of controls without IR. At 4 Gy and 8 Gy, more than half and one-third of PC-3 cells, respectively, survived after IR exposure, which indicated the radioresistance of PC-3 cells. In the expected trend it was also observed that in the absence of RSV, the percentage of colonies of PC-3 proliferating after IR exposure was 91±4% (2 Gy), 51±4% (4 Gy) and 34±5% (8 Gy). There was slight reduction in survival when PC-3 cells were treated with RSV alone, the percentage of colonies of PC-3 were 77±8% (2 μM), 62±5% (10 μM), and 41±6% (50 μM). These results suggested that RSV sensitized PC-3 to IR. This observation was further supported by immunohistochemistry (IHC) staining for PCNA. It was shown that 92±4% PC-3 cells were PCN^{A+} in controls, 52±4% with RSV alone (50 μM) and 70±8% with IR alone (8 Gy). The combine effect of IR (8 Gy) +RSV (50 μM) resulted in drastic reduction in PCN^{A+} PC-3 cells, which was only 8±4%. Similarly, synergistic effect of RSV+IR on cell survival resulted in ~90% decrease in optical density in RSV+IR in comparison to control, reaffirming that RSV+IR inhibited cell proliferation and decrease the survival of PC-3 cells.

RT-PCR analysis on the effect of RSV (50 μM)+IR

(8Gy) under identical treatment conditions as mentioned earlier, on the mRNA expression of pro- and anti-proliferative molecules in PC-3 cells showed that there was significant increase in the mRNA expression of p21 and p53, compared to little effect on mRNA expression of these antiproliferative molecules in IR or RSV alone, suggesting their potent combined effect on the upregulation of p21 and p53. The level of p15 was also significantly higher, and the mRNA expression of the pro-proliferative molecules cyclin B and cdk2 was significantly lower in cells treated with RSV+IR than in controls, again suggesting their synergistic effect on mRNA expression of these molecules. The low level of expression of p27 was similar in IR and RSV+IR group compared to controls, attributed largely to IR rather than RSV. It was also reported in this study that XRT resulted in an increase in the mRNA expression of antiproliferative molecule p18 in IR group in comparison to control and strangely, there was decreased expression in RSV+IR compared to cells treated with IR alone. Unexpected result was also obtained in case of the mRNA expression of pro-proliferative molecule cyclin D, which was increased in cells treated with RSV or IR alone, whereas significant reduction in its expression was found in RSV+IR group. The changes in p21 and p53 expression was supported by relative immunostaining intensity for p21 and p53, which was similar in cells treated with RSV or IR alone in comparison to controls in this report. On the other hand, the relative immunostaining intensity of these two molecules was stronger after RSV+IR than that of controls or IR alone cells. Similarly, immunostaining intensity for p27 in different groups was consistent with its mRNA expression patterns as mentioned earlier. Even the Western blot for p21, p27, and p53 confirmed the pattern of changes as observed in mRNA and immunostaining analysis of these molecules. It was concluded through this study that synergistic effect of RSV with IR resulted in increased expression of antiproliferative molecules p15, p21, and p53 and decreased expression of pro-proliferative molecules cyclin B, cyclin D, and cdk2 leading to inhibition in cell proliferation and decrease survival of PC-3 cells.

It was also shown in this study that the synergistic effect of combined RSV+IR enhanced apoptosis and inhibition of survival of PC-3 cells which was evaluated using terminal deoxynucleotidyl transferase-mediated d-UTP nick end labelling (TUNEL) staining 72 h after exposure to IR. It was observed that there were only few $TUNEL^+$ cells in the IR exposure group, compared to significantly higher number of 67±5% PC-3 cells in case of the RSV+IR group. Even the caspase-3 activity of PC-3 cells was in conformity with this observation. It was found that the synergistic effect of RSV with IR resulted in significantly higher mRNA expression of the pro-apoptotic molecules Fas and TRAILR1in comparison to cells treated with IR alone. However, the mRNA expression of Bcl-2 was increase in RSV+IR group compared to cells with IR alone contrary to expected observation. However, in this study, IHC analysis for p-Akt could not established the relationship between the expression level of p-Akt and the synergistic effect of RSV+IR in inhibition of cell proliferation and decreased survival of PC-3 cells. As mentioned earlier, Akt pathway is important for cell proliferation and survival in many tumours including PCa. Similar to apoptosis, cellular senescence has been shown to function as a potent mechanism to inhibit tumour cell proliferation and survival, and p-H2A.X has been suggested to be a marker for cell senescence. It was also noted through IHC staining of p-H2A.X that the immunostaining intensity for p-H2A.X was much stronger in cells treated with RSV+IR compared to IR alone. Western blot for p-H2A.X confirmed this observation. It was concluded from this study that senescence played a role in the synergistic effect of RSV with XRT to inhibit cell proliferation and decrease survival of PC-3 cells.

Skin cancer

Melanoma, the most aggressive form of skin cancer, is also known to be relatively resistant to conventional radiotherapy and chemotherapy. Evaluation of the role of RSV to increase radiosensitivity of melanoma cells was carried out in human melanoma cell lines (LU1205 and WM35), and mouse melanoma line (SW1) (Johnson et al., 2008). The clonogenic survival assay of these cell lines, 12 days after IR of 1.25-5 Gy revealed that SW1 was the most radioresistant while WM35 and LU1205 cells were less resistant to IR. However, synergistic effects of sequential treatment of melanoma cells by IR and RSV displayed enhanced radiosensitivity. It was found from clonogenic survival assay of melanoma cell line SW1, 12 days after IR exposure of 2.5 and 5 Gy (dose rate, 0.82 Gy/min) and RSV (25-100 μM) that there was very significant reduction in surviving cells in a dose-dependent manner, the most effective being 100 μM and 5 Gy. Clonogenic survival assays also showed substantially decreased survival for WM35 cells after combined treatment with IR and RSV. It was also shown that RSV (50 μM) induced higher levels of apoptosis 16 h after treatment in SW1 than in WM35 and LU1205 cells. IR (5 Gy) resulted in an increase in tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)-R, but did not affect TRAIL surface expression in SW1 cells. It was also reported that sequential treatment of SW1 cells first by IR to increase surface expression of TRAIL-R, and then 16h after irradiation with RSV for an additional 24h to induce endogenous TRAIL translocation to the cell surface, and to suppress anti-apoptotic cFLIP and Bcl-xL, resulted in strong upregulation of RV-induced apoptosis and dramatic downregulation of SW1 clonogenic survival. This observation on the radiosensitizing effects of RSV on apoptosis was supported by another study in mouse melanoma B16F10 cells (Tak et al., 2012). It was found here that RSV (0, 10, 20 μM) enhanced the radiation (5Gy; dose rate of 1Gy/min)-induced apoptotic induction higher than IR or RSV alone under similar conditions. It was also shown that cell viability following exposure to IR after 48 h was significantly reduced by RSV in this cancer cells in comparison to IR or RSV alone group.

The effect of XRT in combination with RSV on radioresistant melanoma lines, SK-Mel-5 and HTB-65, has been studied by assessment of proliferation and apoptosis (Fang et al., 2013). In this study, clonogenic

survival assay of these cells with variable dosage of radiation (0-4 Gy), in the presence or absence of RSV (0, 2, 10, 50 μM), revealed a concentration and dose-dependent reduction in the number of colonies of the SK-Mel-5 and HTB-65 cells. It was shown that the combined effect of RSV/XRT resulted in 70-90% growth inhibition after 2 and 4 Gy of IR in cells pre-treated with RSV (50 μM) in comparison to controls (without XRT or RSV). IHC analysis in SK-Mel-5 cells for proliferating cell nuclear antigen (PCNA)-positive cells treated with or without XRT (4 Gy) in the presence or absence of RSV (50 μM) indicated that there was significantly reduction (~90%) in PCNA in RSV+XRT in comparison to control (without XRT or RSV). Even the optical density value of melanoma cells were significantly reduced in RSV+XRT. These observations were further confirmed by the assessment of the mRNA expression profile of pro- and anti-proliferative molecules using RT-PCR as well as IHC analysis. It was demonstrated that RSV+XRT significantly reduced the expression of p27, p53, cyclin B and D, cdk2 and 4 mRNA and its corresponding protein in compared to control or XRT alone. These were expressed as the mean ratio of pro- and anti-proliferative molecule densitometric units/ glyceraldehyde 3-phosphate dehydrogenase+SEM (×100). In the same study it was also reported that RSV+XRT increases apoptosis of melanoma cells which was revealed by TUNEL staining (60-75%) increase in apoptosis. Even the cellular caspase-3 activity was measured significantly higher in RSV+XRT in comparison to XRT alone. Further, RT-PCR analysis of effect of RSV+XRT on the expression of pro- and anti-apoptotic molecules also revealed significant decrease in Fas, FLIP, Bcl-2 and survivin (an inhibitor of apoptosis protein), in compared to XRT alone. These were expressed as the mean ratio of pro- and anti-apoptotic molecule densitometric units/ glyceraldehyde 3-phosphate dehydrogenase+SEM (×100). This observation was further confirmed by IHC of FLIP and survivin. A significant decrease in the relative staining intensity in the group treated with RSV+XRT in comparison to XRT alone was observed in this case. It was concluded in this study that RSV enhances radiation sensitivity of melanoma cells by inhibiting proliferation and promoting apoptosis. Therefore, it was proposed that RSV may have a potential role as a radiation sensitizer for melanoma treatment.

Brain cancers

Medulloblastoma: Radiosensitizing role of RSV in medulloblastoma (MB), a malignant cerebellum tumour predominantly found in children, (Habrand and De Crevoisier, 2001) have been evaluated in cancer stem-like cells (CSC) isolated from medulloblastoma tissues of patients (Lu et al., 2009). It was shown that the viability of MB-CSCs was reduced by 40-45% when the concentration of RSV was 100 μM, and indicated that 100 μM RSV leads to a significant cytotoxic effect in MB-CSCs. Exposure to IR doses from 0 to 10 Gy (at a dose rate of 1.1 Gy/min) to MB and MB-CSCs cells, confirmed that MB-CSCs showed significant radioresistance than the parental MB cells. It was further shown that the treatment effect of IR (2 Gy) on MB-CSCs was also significantly improved with the addition of 100 μM RSV, indicating the synergistic effect of RSV and IR in MB and MB-CSCs. It was also reported in this study that in comparison to the IR (2 Gy) treatment alone, migration/invasion and tumour colony formation were significantly inhibited in MB-CSCs treated with 100 μM RV alone or 100 μM RV combined with IR in the same dose. It was suggested that RSV treatment could result into enhanced effectiveness and radiosensitivity of radiation treatment of MB-CSCs.

Glioblastoma multiforme: Glioblastoma multiforme (GBM) is the most common primary brain tumour. The role of RSV in its control have been studied in human brain tumour-derived $CD133^-$ positive cells ($CD133^+$)-tumour initiating cells (TIC) that possess cancer stem-like cells (CSC) capabilities such as self-renewal division, multipotent differentiation and radiochemoresistance (Yang et al., 2012). Here it was found that RSV (100 μM) could effectively induce apoptosis in radioresistant $CD133^+$ TIC (Bao et al., 2006; Nguyen and Ravid, 2006; Chiou et al., 2008; Kao et al., 2009) obtained from patients with GBM. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay indicated that the cell viability of GBM-$CD133^+$ treated with RSV combined with both sh-STAT3 (signal transducer and activator of transcription, a transcription factor for cytokine signalling) and IR treatment (2 Gy) was significantly decreased compared to that of $CD133^+$ with no treatment. It was also shown that GBM-$CD133^+$ treated with RSV combined with both sh-STAT3 and IR treatment also significantly increased caspase-3 activity. It was further shown that the migration/invasion ability of GBM-$CD133^+$ treated with RSV was suppressed. It was suggested in this study based on these observations that p-STAT3 was vital for functional properties of GBM-$CD133^+$ that was inhibited by RSV.

Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of interleukin-6 (IL-6) signalling pathway, the upstream activators of STAT3 (Wang et al., 2009), revealed the mechanisms of RSV-induced inhibition of STAT3 in GBM-TICs. It was reported that the transcripts of IL-6 receptor and glycoprotein130 (gp130) in GBM-$CD133^+$ were higher than those in GBM-$CD133^-$ cells. This result was supported by ELISA analysis of IL-6 level, which was higher in GBM-$CD133^+$. Using anti-IL-6 monoclonal antibody (mAB) and RSV treatment in GBM-$CD133^+$, it was shown that spheroid-like bodies (SB) formation ability and p-STAT3 activity of GBM-$CD133^+$ was significantly attenuated with treatment of the IL-6 mAB, and RSV. It was also shown that IL-6 stimulated phosphorylated-STAT3 levels over time in GBM-$CD133^-$. It was also confirmed that RSV treatment suppressed IL-6-induced activation of STAT3 and SB formation, an effect that increased with increasing RV concentration in GBM-$CD133^-$ cells. It was concluded in this study that RSV inhibits STAT3 activity and stemness by inhibiting IL-6.

In the same study, investigation on the role of the STAT3 signalling pathway and the RSV-mediated effect of GBM-CSC *in vivo* was carried out by treating GBM-$CD133^+$ with RSV and injecting 1×10^5 GBM-$CD133^+$-GFP cells into the stratum of severe combined

immunodeficiency (SCID) mice. GBM-CD133$^+$ cells was infected using a lentiviral vector containing the green fluorescent protein gene (GFP), and *in vivo* GFP imaging was used to monitor tumour growth. It was found that the tumour volumes in GBM-CD133$^+$-GFP cells transplanted mice were significantly decreased in mice treated with RSV or sh-STAT3 in combination with IR compared to GBM-CD133$^+$-GFP cells without any treatment. It was also shown that there was no obvious effect on the reduction of tumour volume between different groups of GBMCD133$^+$ mice with anticancer drug-cisplatin or without cisplatin treatment. It was also shown that in comparison to untreated GBM-CD133$^+$ mice, treatment with RSV or sh-STAT3 combined with IR increased the survival of GBM-CD133$^+$ mice bearing intracranial xenografts. This was supported by the fact that $1x10^5$ GBM-CD133$^-$-GFP cells did not form tumours in SCID mice within 6 weeks of xenotransplantation. It was concluded that treatment with RV or sh-STAT3 combined with IR can inhibit the tumourigenicity, enhance radiosensitivity, and improve the survival of the GBM-CD133$^+$-xenotransplanted mice. These result corroborated reports from an earlier study on the possible role of RSV in radiosensitivity of CD133$^-$positive/-negative cells derived from atypical teratoid/rhabdoid tumours (AT/RT-CD133$^{(+/-)}$) (Kao et al., 2009). In this study it was reported that AT/RT-CD133$^+$ displayed enhanced self-renewal and highly co-expressed stem cell genes and drug-resistant genes, in addition to showing significant resistance to XRT as compared with CD133$^-$ cells. After treatment with 200 μM RSV, the *in vitro* proliferation rates and *in vivo* tumour restoration abilities of ATRT-CD133$^+$ were dramatically inhibited. It was also reported that treatment with 150 μM RSV significantly enhance the radiosensitivity (0-10 Gy) and IR-induced apoptosis in RSV-treated AT/RT-CD133$^{(+/-)}$, measured by caspase-3 and TUNEL assays. Even in this study, it was shown that there was significant improvement in mean survival rate of xenotranplanted mice with ATRT-CD133$^+$ that were treated with IR (2 Gy)+RSV (150 μM) as compared to those receiving CD133$^+$ alone or CD133$^+$ with IR.

Colon carcinoma

The radiosensitizing effects of RSV on IR-induced apoptosis have also been investigated in mouse colon carcinoma CT26 cells (Tak et al., 2012). In this study, cells were treated with vehicle (dimethyl sulfoxide, DMSO) or RSV (0, 10, 20 μM) dissolved in DMSO and exposed to 15 Gy IR (dose rate of 1Gy/min). Cell viability following exposure to IR was determined by trypan blue exclusion after 48 h. An increase in cell death and a sensitizing effect of RSV on apoptosis in IR-exposed cell was observed here. It was also observed that IR-induced cleavage of procaspase-3 was more pronounced in cells treated with RSV. It was also noted that cleaved poly (ADP-ribose) polymerase products increased significantly in RSV+IR group in comparison to those in IR or RSV alone groups. It was also shown that the level of the anti-apoptotic protein Bcl-2, reduced significantly in IR and RSV combined treatment groups. Bid, a death agonist member of the Bcl-2/Bcl-xl family, is a specific proximal substrate of caspase-8 in the Fas signaling pathway and, it was observed here that Bid cleavage was increased in irradiated cells treated with RSV compared to that in untreated cells. In the induction of apoptosis, the alteration in mitochondrial integrity and function was assessed by the change in the mitochondrial membrane potential (MMP). Here it was measured by the intensity of fluorescence emitted from the lipophilic cation dye rhodamine 123 and it was found that significantly less rhodamine 123 dye was taken up by the mitochondria of irradiated cells treated with RSV compared to untreated cells. This observation was correlated with alteration in mitochondrial reactive oxygen species (ROS), measured by the levels of peroxides in the mitochondria of cancer cells using fluorescence microscopy with the oxidant-sensitive probe dihydrorhodamine (DHR) 123. It was shown that irradiated cells treated with RSV had significantly higher fluorescence intensity when compared with mitochondria of untreated cells. The cellular redox status was also assessed by measuring the ROS generation using oxidative-sensitive fluorescent probe 2',7'-dichlorofluorescin diacetate (DCFH-DA). DCF fluorescence (excitation, 488 nm; emission, 520 nm) was imaged by fluorescence microscopy. There was an increase in 2',7'-dichlorofluorescein (DCF) fluorescence in irradiated cancer cells which was further significantly enhanced in cells treated with RSV. It was also reported in this investigation that cellular glutathione (GSH), determined with the GSH-sensitive fluorescent dye t-butoxycarbonyl-Leu-Met-7-amino-4-chloromethylcoumarin (CMAC), in irradiated cancer cells treated with RSV decreased significantly compared to those in untreated cells.

Breast cancer

Breast cancer is the leading cause of malignancy among women. In a study in human patients of breast cancer, the effect of dietary supplements based on RSV, lycopene, vitamin C and anthocyanins (Ixor®) in reducing skin toxicity, due to external beam XRT directed to the breast, was evaluated (Di Franco et al., 2012). In this study, all enrolled patients underwent conservative surgery (quadrantectomy), and the patients were subjected to adjuvant radiation treatment with 6 MV photons, with a dose of 50 Gy (2 Gy/fraction) directed to whole breast, and a subsequent additional dose of 10 Gy (2 Gy/fraction) directed to original tumour site. The patients were divided into two different groups. One group, taken as control (CG), comprising of 41 patients, were prescribed with a topical prophylactic treatment based on hyaluronic acids. The treatment lasted for the entire therapy and then for the 4-6 weeks after XRT. The other Ixor-Group (IG), comprising of 30 patients were administered with an oral therapy based on RSV, lycopene, vitamin C and anthocyanins (Ixor®), at a dose of 2 tablets/day, in addition to topic prophylactic treatment. This therapy was started 10 days before XRT and ended 10 days after XRT. Each patient in both the groups were subjected to weekly visits and the recorded the acute dermal toxicity according to established criteria of the Radiation Therapy Oncology Group/European Organization for Research and Treatment

Cancer (RTOG/EORTC). It was reported that when the Planning Target Volume (PTV) was >500 ml, the Control Group (CG) showed a high toxicity (G2+G3; acute dermal toxicity of grade 2 or 3) in 30% of cases in comparison to 25% of cases of Ixor®-Group (IG), with absolute risk reduction (ARR) of 5%, relative risk (RR) of 0.83 and odds ratio (OR) equal to 0.77. When PTV was <500 ml, the CG presented skin toxicity (G2+G3) in 18% of cases, versus 0% of IG, with ARR of 18%, RR of 0.28 and or equal to 0.23. The skin toxicity was then related to dosimetric values of each treatment plan. In was observed that in patients receiving a maximum dose less or equal to 107% of the prescribed, CG presented a toxicity G2+G3 in 12.5% of cases, compared with 0% of IG with ARR 12.5%, RR of 0.77 and OR of 0.73. However, in patients who received a maximum dose higher than 107% and lower than 110% of the prescribed, CG presented a high skin toxicity (G2+G3) in 35% of cases oppose to 21% of the IG, with ARR of 14%, RR of 0.60 and OR of 0.50. It was also seen that in patients receiving a maximum dose higher than 110% of the prescribed dose, the CG and the IG showed the same percentage of toxicity G2+G3 with RR and OR of 1. It was also reported that a protective effect of RSV, lycopene, vitamin C and anthocyanins (Ixor®) in both groups with ARR 25%, RR of 0.22 and OR of 0.17 in patients who were not submitted to chemotherapy, and with ARR 7%, RR of 0.74 and or of 0.68 in patients who underwent chemotherapy with antracyclines and taxanes, the widely used anticancer drugs. Therefore, RSV along with the other photochemical, at the dose and regime used in this case, enhances radiological effect on breast tumour patients.

In another study on the radiosensitizing effect of RSV on breast cancer, estrogen receptor positive human adenocarcinoma (MCF-7) breast cancer (BCa) cells were used (Aravindan et al., 2013). The cells, plated in 100 mm tissue culture plates containing 6 ml of complete growth medium, were allowed to grow up to 70-80% confluence. Then the cells were made quiescent by serum starvation overnight followed by treatment. For growth under hypoxia, the cells were incubated at 37°C in a modular chamber flushed with 2.5% O_2, 5%C O_2 and 92.5% N_2. Here, 100 μM RSV was added to the medium for 1 h before hypoxia. The cells were irradiated with one single high dose of 4 Gy (dose rate of 0.81 Gy/min). Sham irradiated cells were treated identical except that the cells were not subjected to radiation exposure. In all the groups, the cells were harvested 24 h after irradiation. Electrophoretic mobility shift assay (EMSA) showed a significant induction (300.3±71.6%) of NF-κB-DNA binding activity in hypoxic BCa cells. Exposure of hypoxic cells to IR resulted in a very high (813.7±216.5) induction of NFκB DNA-binding activity. However, a very significantly attenuation of this IR-induced DNA-binding activity in case of RSV (36.9±7.7%) pre-treated hypoxic MCF-7 cells. Significant inhibitory potential of RSV was confirmed by the observation that the NF-κB-DNA binding levels were lesser than that of normoxic-sham-IR cells. Western blot analysis from the nuclear extract demonstrated a significant induction of NFκB p50 and p65 levels in hypoxic BCa cells in consistent with previous observation. It was reported that the hypoxia-induced nuclear translocation of p50 and p65 was further increased due to radiation exposure. It was observed that RSV administration reversed the IR-induced p50/p65 nuclear translocation significantly. It was also observed in this study that there was induction of IκBα phosphorylation in BCa cells grown under hypoxic conditions. The induction was much higher in cells exposed to IR than sham-IR group under the same condition. However, inhibition of IκBα phosphorylation in hypoxic cells pre-treated with RSV and exposed to IR was observed.

Transcriptomic analysis further confirms the involvement of RSV in targeting NF-κB signalling in this investigation. For this, MCF-7 cells, cultured under hypoxic conditions were either sham-irradiated or exposed to IR (2 Gy) with or without RSV pre-treatment and harvested after 3 h after IR treatment. Custom quantitative PCR (qPCR) profiling of 88 NF-κB upstream/downstream signal transduction pathway molecules has shown that radiation significantly induced 53 genes and suppressed 35 genes in hypoxic BCa. Moreover, IR significantly increased the transcription of NF-κB family molecules including Rel, RelA, RelB, IKKβ and IKKγ, which served as the positive controls for the study. It was also reported that addition of RSV reverted this IR-induced NF-κB signal transduction in hypoxic cells very significantly. Administration of RSV resulted in total suppression of all 53 IR-induced NF-κB signal transduction genes in these cells under hypoxic conditions. In comparison to sham-IR hypoxic cells, IR exposure significantly induced cell death under hypoxia. However, the IR-induced cell death was enhanced in cells treated with RSV indicating its radiopotentiating effect. It was summarized in this study that in hypoxic breast cancer cells, RSV resulted in the: *i*) complete suppression of IR-induced NF-κB-DNA binding activity; *ii*) attenuation of IR-induced NF-κB signal transduction and target transcriptome; *iii*) mitigation of IR-induced Akt, Nos3, Erk1/2, SOD2, p50, p65, TNF-α, Birc 1, 2 and 5; and *iv*) potentiates IR-induced cell killing. Therefore, it was suggested that the bioactive RSV may play a key role in regulating NF-κB signalling pathway dependent 'hypoxic processes' and may potentiate RT in this scheme.

Hepatoma

The role of RSV in regulating SirT1, a nicotinamide adenine dinucleotide (NAD^+)-dependent protein deacetylase (Mammalian class-III type) that can deacetylate non-histone proteins in the nucleus, cytoplasm or mitochondria, have been studied in hypoxic hepatoma HepG2 cells (Xie et al., 2012). RSV is also widely known to be a potent activator of SirT1 protein. In this study, the IC_{50} value of RSV was found to be 505.5 μM. Here, the HepG2 cells were pre-treated for 0.5 h with 150 μM RSV, followed by continual incubation in a regular normoxic incubator or a hypoxic incubator for 12 h before IR exposure (3 Gy; dose rate of 0.79 Gy/min). It was observed that micronuclei (MN) formation was induced in the irradiated cells, and the addition of RSV markedly suppressed radiation-induced MN formation in hypoxic cells, whereas RSV had no effect on the irradiated

normoxic cells. It was also shown that RSV stimulation had a significant effect on SirT1 protein expression. While IR did not significantly alter the deacetylase activity of SirT1 during hypoxia, RSV not only enhanced the expression of SirT1 protein but also increased the deacetylase activity of SirT1 remarkably. Further, it was revealed by Western blotting experiments that c-Myc proto-oncogene protein level was markedly regulated by RSV in hypoxic HepG2 cells. Stimulation of SirT1 by RSV led to decrease in c-Myc protein expression in the hypoxic HepG2 cells after irradiation. Furthermore, it was shown that the RSV treatment could aggravate the degradation of hypoxia-induced c-Myc. Corelationship between the loss of c-Myc in RSV-treated hypoxic HepG2 cells and protein degradation was carried out by pre-treating HepG2 cells with the proteasome inhibitor MG132, widely used for the inhibition of ubiquitin-mediated proteolysis, in the presence of RSV. It was observed that MG132 prevented the RSV-induced loss of c-Myc in the hypoxic cells, suggesting that the RSV could reduce the expression of c-Myc protein by activating a proteasome dependent degradation process in the hypoxic HepG2 cells.

Conclusions

Cancers will continue to be a threat for a healthy life unless it can be controlled by different possible modalities. RSV in combination with IR appears to be a possible therapeutic scheme if its effectiveness can be tested and proven in various models of cancer, particularly *in vivo*. It is apparent that RSV has several intracellular targets including multiple but interrelated pathways, whose modulation in combination with IR, leads to growth arrest and death as seen in the cancer cases that has been reviewed here. These cellular modulations by RSV+IR have been summarized in Table 1. Exploring the ability of RSV to modify the effect of radiation exposure in normal and cancer cells have indeed shown quite convincingly that the combination of RSV and IR exhibited synergistic effect on different cancer cells. This becomes relevant since controlled exposure to IR is one of the most used treatments in cancer patients. However, XRT treatment regime often not effective in clinical practice, as observed in patients with glioma, PCa, melanoma, etc., largely due to the tumour's radioresistant ability (Leone et al., 2008; Yang et al., 2012). Therefore, the combined treatment protocols employing RSV and IR look promising in control of several cancers.

It is quite significant that treatment with RSV at μM concentrations in combination with clinically relevant doses of IR arrested the proliferative cycle in the critical phases of cell cycle, and resulted in apoptotic death as seen in several human cancer cell lines. It was also observed that in some cancer cell, cell death is mediated through direct modulation of the apoptotic pathway with RSV and XRT combine. In this context, the role of caspases becomes quite vital. Caspases are crucial mediators of apoptosis induced by a range of apoptotic stimuli (Creagh and Martin, 2001; Zhou et al., 2013). Among these molecules, caspase-3 is a frequently activated death protease, catalyzing the specific cleavage of many key cellular proteins (Mcllwain et al., 2013). Not surprising that the synergistic effect of RSV+IR resulted in increase in caspase-3 activity and induction of apoptosis in many cancers (Table 1). However, there are hardly any report on the role of other effector caspases such as caspase-7, -6 in apoptotic induction in RSV+IR combinatorial scheme in cancers, and therefore more research needs to be directed in that field to fill the gaps.

The role of ROS has not been very critically studies in several cancer models with RSV+IR treatment except in colon carcinoma. Its role becomes critical since reduction in radiation-induced ROS generation by plant-derived compound, conferring protection to normal cells have been reported (Lee et al., 2010). More importantly, in the colon carcinoma study (Tak et al., 2012), increase in the ROS have been shown to contribute to decrease in cancer cell viability. Therefore, ROS role warrants further investigation in different models of cancers using combine RSV+IR treatment. Similarly, further evaluation of the role of NF-κB is needed in RSV+XRT in different cancers. In human breast cancer cells (MCF-7) (Aravindan et al., 2013), the inhibition of NF-κB-DNA binding activity by RSV+IR combined treatment, have been shown to trigger a cascade of events leading to suppression of 53 NF-κB signal transduction genes, which ultimately lead to cell death. These significant events by the synergistic effect of RSV+IR plus the known role of radiation in modulation of NF-κB (Linard et al., 2004; Gao et al., 2011; Presser et al., 2013), which in turn regulate several inflammatory response, highlights the need for the comprehensive analysis of the role of NF-κB in different cancer with combine treatment of RSV+IR.

Sensitization of IR-induced apoptosis by natural product RSV is likely to be relevant to cancer treatment. The development of resistance to apoptosis in cancer cells is a major cause of treatment failure during consecutive XRT and hence a radiosensitizing agent can make XRT be very effective in the control of cancers. However, as summarized in Table 1, all cancers do not respond to RSV+IR in similar manner. For those such as the radioresistant PCa or melanoma cells, the RSV+IR regime have to be very carefully chosen in order to achieve effective and desirable outcome with minimum toxicity to normal cells. The highest concentration of 100 μM RSV and highest dose of 5 Gy IR was enough to kill these cells by induction of apoptosis indicated that RSV was effective in radiosensitizing these otherwise radioresistant cells. It only further consolidates the therapeutic potential of RSV+XRT in control of several cancers.

As mentioned previously, RSV+IR exert differential synergistic effect on different mediators of apoptotic pathways in different cancer models (Table 1). Among them, the modulatory role of RSV in the radioresistance-mediating Akt pathway in human PCa cells (PC3 and 22RV1) (Rashid et al., 2011) is quite significant. It was very convincingly shown that 5 μM of RSV in combination with 2 Gy of IR was sufficient to inhibit Akt phosphorylation, enhance expression of antiproliferative molecules, enhance nuclear aberration, etc., resulting in the death of the cells. RSV+XRT might induce similar effects in other radioresistant cancers cells making them

Table 1. Summary of Significant Alterations in the Level of Cellular Molecules and Events by the Combined Effect of Resveratrol (RSV) and Ionizing Radiation (IR) in Different Cancers

Type of cancer/ cancer cell lines	Concentration of resveratrol	Dose of radiation	Anti-	Pro-	Anti-	Pro-	Other relevant molecular events
			proliferative molecules		apoptotic molecules		
Brain cancer: *i*) Cancer stem-like cells (CSC) from medulloblastoma tissue of patients; *ii*) CD133$^+$ TIC* from glioblastoma multiforme (GBM) patients	100 μM	2 Gy					Reduction in cell viability; inhibition of tumour colony formation
	100 μM	2 Gy		Reduction in cyclin D1	Reduction in survivin	Elevation of caspase-3 activity	Inhibition of self-renewal and tumorigenic capacity; Induction of apoptosis; inhibition of IL-6** and STAT3***; Reduction in c-Myc and Cox-2# protein; decrease cancer stemness; decrease in tumour volume and increase survival in GBM-CD133133$^+$ xenotransplanted mice
Human leukemia cell line, EOL-1, derived from patient with eosinophilic leukemia	50 and 100 μM	8 Gy					Decrease cell proliferation; increase apoptosis and necrosis
Prostate cancer: *i*) Human prostate cancer cell line (DU145); *ii*) Human prostate cell line (PC3, 22RV1); *iii*) Human prostate cancer (PC-3 cells)	0.5-32 μM	0.5-2.0 Gy					Induction of endogenous ceramide synthesis; decrease cell viability
	5 μM	2 Gy	-[c]		Induction of caspase-3 cleavage		Reduce cell survival; G1/S arrest; enhance nuclear aberrations; inhibition of Akt$^{\#\#}$ phosphorylation; enhance AMPK$^{\#\#\#}$ phosphorylation; enhance ATM$^{\$\$\$}$ and histone H2Ax phosphorylation
	50 μM	8 Gy		Reduction in cyclin B, cyclin D and cdk2	Increase expression of Bcl-2$^{@}$	Increase caspase-3 activity; increase expression of Fas and TRAIL-R1	Inhibition of cell proliferation and survival; reduction in PCNA$^{+@@}$ cells; increase apoptosis; increase p-H2A.X
Skin cancer: *i*) Human melanoma cell lines (LU1205 and WM35); mouse melanoma line (SW1); *ii*) Mouse melanoma (B16F10 cells); *iii*) Melanoma (SK-Mel-5 and HTB-65 cells)	25-100 µM	2.5-5 Gy			Suppression in the level of cFLIP$^{@@@}$ and Bcl-xL$^{*\#}$	Increase expression of TRAIL-R	Reduction in cell viability in SW1, WM35; enhance apoptosis in SW1;
	0, 10, 20 μM	5 Gy					Decrease cell viability; enhance apoptosis
	50 µM	4 Gy	-[e]	Reduction in expression of cyclin B, cylin D, cdk2 and cdk4 mRNA and the corresponding protein	Decrease in FLIP, Bcl-2, survivin mRNA expression; decrease IHC$^{@\#}$ staining of FLIP and survivin	Increase in Fas mRNA expression; Increase in caspase-3 activity	Inhibition of cell growth; reduction in PCNA; increase apoptosis
Mouse colon carcinoma (CT26 cells)	20 μM	15 Gy			Reduction in Bcl-2 protein; enhance cleavage of procaspase-3	Increase in Bid$^{\#\$}$ cleavage; increase in cleaved poly (ADP-ribose) polymerase products;	Decrease cell viability; decrease GSH$^{\$\%}$; increase in ROS$^{@\$}$
Brain cancer: *i*) Human patients of breast cancer; *ii*) Human adenocarcinoma (MCF-7) breast cancer cells	-[a]	-[b]					Reduction in acute dermal toxicity; improvement in absolute risk reduction, relative risk and odds ratio
	100 µM	4 Gy					Inhibition of NFκB$^{@\#\$}$-DNA binding activity; reverse the p50/p65 nuclear translocation; inhibition of IκBα$^{@\$*}$ phosphorylation; suppression of 53 IR-induced NFκB signal transduction genes; enhance cell death
Hepatoma (HepG2 cells)	150 µM	3 Gy					Suppression of MN$^{\%\#\$}$ formation in hypoxic cells; enhance expression of SirT1$^{\#\$@}$ protein; decrease in c-Myc protein expression

[a]Dietary supplements based on RSV, lycopene, vitamin C and anthocyanins (Ixor®) (2 tablets/day); [b]Adjuvant radiation treatment (50 Gy to the whole breast, plus 10 Gy to original tumor site); [c]Enhance expression of p53, p21$^{cip1\$}$ and p27$^{kip1\$\$}$; [d]Increase mRNA expression of p15, p21 and p53; decrease p18; increase immunostaining of p21 and p53; increase p21 and p53 proteins; [e]Increase expression of p27 and p53 mRNA and the corresponding protein; *TIC: Tumor initiating cells;**IL-6: Interleukin-6; ***STAT3: Signal transducer and activator of transcription; [#]Cox-2: Cytochrome oxidase 2; [##]Akt: Serine-threonine kinase; [###]AMPK: AMP-activated protein kinase; [$]p21^{cip1} and [$$]p27kip1 : Cyclin-dependent kinase inhibitors; [$$$]ATM: ataxia telangiectasia mutated; @Bcl-2: B-cell lymphoma-2; [@@]PCNA: proliferating cell nuclear antigen; [@@@]FLIP: FLICE (FADD-like IL-1beta-converting enzyme)-inhibitory protein; [*#]Bcl-xL: B-cell lymphoma-extra large; [@#]IHC: Immunohistochemistry; [#$]Bid: A death agonist member of the Bcl-2/Bcl-xL

more radiosensitive and possibly can be controlled in a better way. However, this effect on the Akt pathway needs to be evaluated in other cancers with RSV+IR treatment to reach to a common conclusion.

In general, it has been reported in different cancer cells that RSV+XRT effectively acted by enhancing anti-proliferative and pro-apoptotic molecules, inhibiting the pro-proliferative and anti-apoptotic molecules (Table 1), leading to induction of apoptosis through various pathways, and cell death. If RSV+XRT can suppress the signature of cancer stemness, enhance the radiosensitivity by either targeting the mitochondrial functionality or modulating the tumour necrosis factor-mediated or Fas-FasL-mediated pathways of apoptosis in different cancer cells, its therapeutic use in the control of cancers can be a promising modality in near future.

References

Aravindan S, Natarajan M, Herman TS, et al (2013). Molecular basis of 'hypoxic' breast cancer cell radio-sensitization: phytochemicals converge on radiation induced Rel signaling. *Radiat Oncol*, **8**, 46-7.

Baatout S, Derradji H, Jacquet P, et al (2005). Increased radiation sensitivity of an eosinophilic cell line following treatment with epigallocatechin-gallate,resveratrol and curcuma. *Int J Mol Med*, **15**, 337-52.

Bao S, Wu Q, McLendon RE, et al (2006). Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*, **444**, 756-0.

Bastianetto S, Quirion R (2010). Heme oxygenase 1: another possible target to explain the neuroprotective action of resveratrol, a multifaceted nutrient-based molecule. *Exp Neurol*, **225**, 237-9.

Baur JA, Sinclair DA (2006). Therapeutic potential of resveratrol: the *in vivo* evidence. *Nat Rev Drug Discov*, **5**, 493-06.

Benitez DA, Hermoso MA, Pozo-Guisado E, et al (2009). Regulation of cell survival by resveratrol involves inhibition of NF kappa B-regulated gene expression in prostate cancer cells. *Prostate*, **69**, 1045-4.

Bisht KS, Bradbury CM, Mattson D, et al (2003). Geldanamycin and 17-allylamino-17-demethoxygeldanamycin potentiate the in vitro and *in vivo* radiation response of cervical tumor cells via the heat shock protein 90-mediated intracellular signaling and cytotoxicity. *Cancer Res*, **63**, 8984-95.

Brown JM (1999). The hypoxic cell: A target for selective cancer therapy-Eighteenth Bruce F. Cain Memorial Award lecture. *Cancer Res*, **59**, 5863-0.

Busquets S, Ametller E, Fuster G, et al (2007). Resveratrol, a natural diphenol, reduces metastatic growth in an experimental cancer model. *Cancer Lett*, **245**, 144-8.

Camphausen K, Tofilon PJ (2004). Combining radiation and molecular targeting in cancer therapy. *Cancer Biol Ther*, **3**, 247-0.

Carsten RE, Bachand AM, Bailey SM, et al (2008). Resveratrol reduces radiation-induced chromosome aberration frequencies in mouse bone marrow cells. *Radiat Res*, **169**, 633-8.

Chiou SH, Kao CL, Chen YW, et al (2008a). Identification of CD133 positive radioresistant cells in atypical teratoid/rhabdoid tumor. *PLoS ONE*, **3**, 2090.

Collins R, Darby S, Davies C, et al (2005). Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of randomized trials. *Lancet*, **366**, 2087-106.

Creagh, EM, Martin SJ (2001). Caspases: cellular demolition experts. *Biochem Soc Trans*, **29**, 696-2.

Crook JM, Perry GA, Robertson S, et al (1995). Routine prostate biopsies following radiotherapy for prostate cancer: results for 226 patients. *Urology*, **45**, 624-1.

de la Lastra CA, Villegas I (2007). Resveratrol as an antioxidant and pro-oxidant agent: mechanisms and clinical implications. *Biochem Soc Trans*, **35**, 1156-0.

Debatin KM, Krammer PH (2004). Death receptors in chemotherapy and cancer. *Oncogene*, **23**, 2950-66.

Delmas D, Jannin B, Latruffe N (2005). Resveratrol: preventing properties against vascular alterations and ageing. *Mol Nutr Food Res*, **49**, 377-95.

Dennis M, Spiegler BJ, Hetherington CR, et al (1996). Neuropsychological sequelae of the treatment of children with medulloblastoma. *J Neurooncol*, **29**, 91-1.

Di Franco R, Calvanese M, Murino P, et al (2012). Skin toxicity from external beam radiation therapy in breast cancer patients: protective effects of Resveratrol, Lycopene, Vitamin C and anthocianin (Ixor®). *Radiat Oncol*, **7**, 12-8.

Elmore E, Lao XY, Kapadia R, et al (2006). The effect of dose rate on radiation-induced neoplastic transformation in vitro by low doses of low-LET radiation. *Radiat Res*, **166**, 832-8.

Fang Y, Bradley MJ, Cook KM, Herrick EJ, Nicholl MB (2013). A potential role for resveratrol as a radiation sensitizer for melanoma treatment. *J Surg Res*, **183**, 645-3.

Fang Y, DeMarco VG, Nicholl MB (2012). Resveratrol enhances radiation sensitivity in prostate cancer by inhibiting cell proliferation and promoting cell senescence and apoptosis. *Cancer Sci*, **103**, 1090-8.

Filomeni G, Graziani I, Rotilio G, et al (2007). Trans-Resveratrol induces apoptosis in human breast cancer cells MCF-7 by the activation of MAP kinases pathways. *Genes Nutr*, **2**, 295-5.

Forman JD, Oppenheim T, Liu H, et al (1993). Frequency of residual neoplasm in the prostate following three-dimensional conformal radiotherapy. *Prostate*, **23**, 235-3.

Fremont L (2000). Biological effects of resveratrol. *Life Sci*, **66**, 663-3.

Gao HS, Rong X, Peng D, et al (2011). Cross-talk of the related bioactivity mediators in serum after injection of soluble TNF-α receptor on silicosis model of rats. *Toxicol Ind Health*, **27**, 607-16.

Gautam SC, Xu YX, Dumaguin M, et al (2000). Resveratrol selectively inhibits leukemia cells: a prospective agent for ex vivo bone marrow purging. *Bone Marrow Transplant*, **25**, 639-5.

Habrand JL, De Crevoisier R (2001). Radiation therapy in the management of childhood brain tumors. *Childs Nerv Syst*, **17**, 121-33.

Hagan M, Wang L, Hanley JR, et al (2000). Ionizing radiation-induced mitogen-activated protein (MAP) kinase activation in DU145 prostate carcinoma cells: MAP kinase inhibition enhances radiation-induced cell killing and G2/M-phase arrest. *Radiat Res*, **153**, 371-83.

Hoppe-Hirsch E, Brunet L, Laroussinie F, et al (1995). Intellectual outcome in children with malignant tumors of the posterior fossa: influence of the field of irradiation and quality of surgery. *Childs Nerv Syst*, **11**, 340-5.

Ivanov VN, Zhou H, Hei TK (2007). Sequential treatment by ionizing radiation and sodium arsenite dramatically accelerates TRAIL-mediated apoptosis of human melanoma cells. *Cancer Res*, **67**, 5397-7.

Johnson GE, Ivanov VN, Hei TK (2008). Radiosensitization of melanoma cells through combined inhibition of protein regulators of cell survival. *Apoptosis*, **13**, 790-02.

Kabakov AE, Kudryavtsev VA, Gabai VL (2010). Hsp90 inhibitors as promising agents for radiotherapy. *J Mol Med*,

88, 241-7.
Kao CL, Huang PI, Tsai PH, et al (2009). Resveratrol-induced apoptosis and increased radiosensitivity in CD 133 positive cells derived from atypical teratoid/rhabdoid tumor. *Int J Radiat Oncol Biol Phys*, **74**, 219-8.
Kelley MR (2012). DNA Repair in Cancer Therapy: Molecular Targets and Clinical Applications, Academic Press, New York, NY, USA.
Lee JC, Kinniry PA, Arguiri E, et al (2010). Dietary Curcumin increases antioxidant defenses in lung, ameliorates radiation-induced pulmonary fibrosis, and improves survival in mice. *Radiat Res*, **173**, 590-01.
Leith JT (1994). *In vitro* radiation sensitivity of the LNCaP prostate tumor cell line. *Prostate*, **24**, 119-4.
Leone S, Fiore M, Lauro MG, et al (2008). Resveratrol and X rays affect gap junction intercellular communications in human glioblastoma cells. *Mol Carcinog*, **47**, 587-98.
Linard C, Marquette C, Mathieu J, et al (2004). Acute induction of inflammatory cytokine expression after gamma-irradiation in the rat: effect of an NF-κB inhibitor. *Int J Radiat Oncol Biol Phys*, **58**, 427-4.
Lu KH, Chen YW, Tsai PH, et al (2009). Evaluation of radiotherapy effect in resveratrol-treated medulloblastoma cancer stem-like cells. *Childs Nerv Syst*, **25**, 543-0.
Machida H, Matsumoto Y, Shirai M, et al (2003). Geldanamycin, an inhibitor of Hsp90, sensitizes human tumour cells to radiation. *Int J Radiat Biol*, **79**, 9730.
Mcllwain DR, Berger T, Mak TW (2013). Caspase functions in cell death and disease. *Cold Spring Harb Perspect Med*, **3**, 8656.
Moeller BJ, Dreher MR, Rabbani ZN, et al (2005). Pleiotropic effects of HIF-1 blockade on tumor radiosensitivity. *Cancer Cells*, **8**, 99-10.
Moon SH, Shin KH, Kim TH, et al (2009). Dosimetric comparison of four different external beam partial breast irradiation techniques: three-dimensional conformal radiotherapy, intensity-modulated radiotherapy, helical tomotherapy, and proton beam therapy. *Radiother Oncol*, **90**, 66-3.
Nguyen HG, Ravid K (2006). Tetraploidy/aneuploidy and stem cells in cancer promotion: the role of chromosome passenger proteins. *J Cell Physiol*, **208**, 12-2.
Oh YC, Kang OH, Choi JG, et al (2009). Anti-inflammatory effect of resveratrol by inhibition of IL-8 production in LPS-induced THP-1 cells. *Am J Chin Med*, **37**, 1203-14.
Okada H, Mak TW (2004). Pathways of apoptotic and nonapoptotic death in tumour cells. *Nat Rev Cancer*, **4**, 592-3.
Pervaiz S (2003). Resveratrol: from grapevines to mammalian biology. *FASEB J*, **17**, 1975-5.
Pervaiz S (2004). Chemotherapeutic potential of the chemopreventive phytoalexin resveratrol. *Drug Resist Updat*, **7**, 333-44.
Presser LD, McRae S, Waris G (2013). Activation of TGF-b1 promoter by hepatitis C virus-induced AP-1 and Sp1: role of TGF-b1 in hepatic stellate cell activation and invasion. *PLoS ONE*, **8**, 56367.
Rashid A, Liu C, Sanli T, et al (2011). Resveratrol enhances prostate cancer cell response to ionizing radiation. Modulation of the AMPK, Akt and mTOR pathways. *Radiat Oncol*, **6**, 144-55.
Reagan-Shaw S, Mukhtar H, Ahmad N (2008) Resveratrol imparts photoprotection of normal cells and enhances the efficacy of radiation therapy in cancer cells. *Photochem Photobiol*, **84**, 415-1.
Ruan K, Song G, Ouyang G (2009). Role of hypoxia in the hallmarks of human cancer. *J Cell Biochem*, **107**, 1053-2.
Scarlatti F, Sala G, Ricci C, et al (2007). Resveratrol sensitization of DU145 prostate cancer cells to ionizing radiation is associated to ceramide increase. *Cancer Lett*, **253**, 124-0.
Sowa M, Arthurs BJ, Estes BJ, et al (2006). Effects of ionizing radiation on cellular structures, induced instability and carcinogenesis. *EXS*, **96**, 293-1.
Suit H, Goldberg S, Niemierko A, et al (2007). Secondary carcinogenesis in patients treated with radiation: a review of data on radiation-induced cancers in human, non-human primate, canine and rodent subjects. *Radiat Res*, **167**, 12-42.
Tak JK, Lee JH, Park JW (2012). Resveratrol and piperine enhance radiosensitivity of tumor cells. *BMB Rep*, **45**, 242-6.
Ungvari Z, Labinskyy N, Mukhopadhyay P, et al (2009). Resveratrol attenuates mitochondrial oxidative stress in coronary arterial endothelial cells. *Am J Physiol Heart Circ Physiol*, **297**, 1876-1.
Wang C, Chen L, Hou X, et al (2006). Interactions between E2F1 and SirT1 regulate apoptotic response to DNA damage. *Nat Cell Biol*, **8**, 1025-1.
Wang H, Lathia JD,Wu Q, et al (2009). Targeting interleukin 6 signaling suppresses glioma stem cell survival and tumor growth. *Stem Cells*, **27**, 2393-04.
Weiss JF, Landauer MR (2003). Protection against ionizing radiation by antioxidant nutrients and phytochemicals. *Toxicology*, **189**, 1-20.
Xie Y, Zhang J, Xu Y, et al (2012). SirT1 confers hypoxia-induced radioresistance via the modulation of c-Myc stabilization on hepatoma cells. *J Radiat Res*, **53**, 44-0.
Yang YP, Chang YL, Huang PI, et al (2012). Resveratrol suppresses tumorigenicity and enhances radiosensitivity in primary glioblastoma tumor initiating cells by inhibiting the STAT3 axis. *J Cell Physiol*, **227**, 976-93.
Yin HT, Tian QZ, Guan L, et al (2013). In vitro and *in vivo* evaluation of the antitumor efficiency of resveratrol against lung cancer. *Asian Pac J Cancer Prev*, **14**, 1703-6.
Zhou Y, Peng Y, Mao QQ, et al (2013). Casticin induces caspase-mediated apoptosis via activation of mitochondrial pathway and upregulation of DR5 in human lung cancer cells. *Asian Pac J Trop Med*, **6**, 372-8.
Zietman AL, Bae K, Slater JD, et al (2010). Randomized trial comparing conventional-dose with high-dose conformal radiation therapy in early-stage adenocarcinoma of the prostate: long-term results from proton radiation oncology group/american college of radiology 95-09. *J Clin Oncol*, **28**, 1106-1.