Radiosensitization Effect of Resveratrol on Breast Cancer Cells

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Objective

Radiotherapy is an important and effective treatment strategy for breast cancer although it is sometimes limited by radio-resistance and radiotoxicity. Thus, the identification of a radiosensitizer with a high benefit to risk ratio is needed. One of these is resveratrol (RSV). Resveratrol is a polyphenol produced by several plants, including grapes, red wine, berries and peanuts and is a phytoestrogen that possesses anti-oxidant, anti-inflammatory, cardioprotective, and anti-cancer properties. Studies with this substance show its ability to inhibit the expression of cancer-specific genes, induce changes in the cell cycle, and activate apoptosis.

The aim of the study is to investigate the *in vitro* effects of RSV on cellular radiosensitivity and to analyze the expression changes of apoptosis-related gene BCL-2.

Methods

- **Cell culture**. MCF7 and MDA-MB-231 breast cancer cell lines were purchased from CLS Cell Line Service (Germany). Cells were grown in DMEM medium supplemented with 10% FBS, 1% glutamine and 100 IU/ml penicillin-streptomycin solution at 37^{0}C, humidified and filled with 5% CO2air conditions.
- **MTT assay**. The anti-proliferative effect of resveratrol against breast cancer cells was determined using the colorimetric MTT assay. The cells were seeded on 96-well culture plates with DMEM medium at a density of 4x10^3 cells per well. Following incubation for 24 h at 37˚C, the cells were treated with different concentrations of RSV (0, 10, 25, 40, 50, 80, 100, 150 and 200 μM) for 24, 48 and 72 h. Subsequently, 10 μl MTT was separately added to each well, and the cells were cultured at 37˚C for an additional 3 h. Finally, 100 μl SDS (10%) was separately added to each well and the optical density (OD) was determined at 570 nm using a microplate reader (*Thermo Scientific Multiskan Sky*).
- **Irradiation (IR)**. Cells were seeded into culture plates, incubated overnight, treated with resveratrol as described above. After 24 h the media was removed and replaced with fresh resveratrol-free culture medium. Cells were irradiated with 2 or 10 Gy using high energy X-rays, which were generated using an X-rays instrument *Clinac 2100C/D*.
- **Apoptosis analysis.** Cells (2×10^5 cells/well) in 6-well plates were treated as described above, trypsinized, stained according to the instructions of the apoptosis kit and were analyzed using a *Guava Muse Cell Analyzer*.
- **RT-PCR analysis.** The gene expression of BCl-2 was quantitatively determined by real-time PCR. Total RNA was extracted using RNA isolation kit and one microgram of total RNA was reverse transcribed to cDNA using first strand cDNA synthesis kit.
- **Statistical analyses.** All data are represented as the means ± SD. Statistical significance was determined using Student's t-tests. P<0.05 was considered to indicate a statistically significant result.

Results

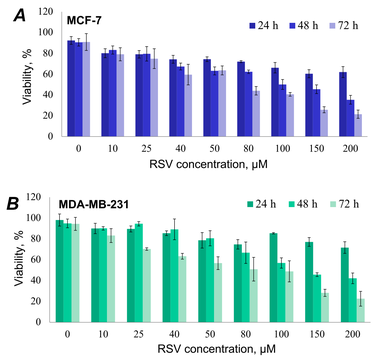

Figure 1. The effect of resveratrol on viability of two breast cancer cell lines: MCF-7 (A) and MDA-MB-231 (B).

Our study suggests that, the inhibition of cell viability was significantly increased in both cell lines in response to resveratrol in a dose-and time-dependent manner compared with the control group (0 μM RSV) (P<0.05). Inhibitory potency did not differ substantially between cell lines (Fig. 1).

The combination of resveratrol and radiation (RSV+IR) treatment produced significantly greater antitumor effects on the breast cancer cells than either treatment alone (Fig. 2).

To determine whether the RSV-induced inhibition of breast cancer cells growth was due to cell apoptosis, MDA-MB-231 and MCF-7 cells were stained with annexin V. Flow cytometry analysis showed that the apoptotic cell population increased in a dose-dependent manner in both cell lines.

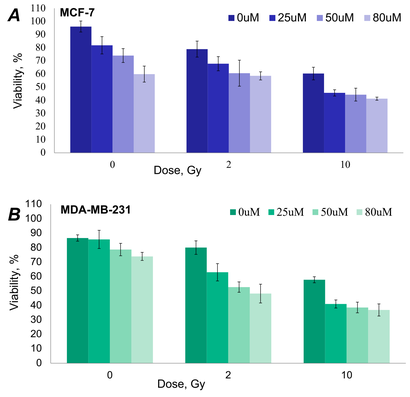

Figure 2. The radiosensitizing effect of resveratrol at different concentrations (25, 50, 80 μM) in 24 h cell cultures irradiated at 0, 2, and 10 Gy. MCF-7 (A) and MDA-MB-231 (B).

However the combination of RSV+IR therapy activates cell apoptosis only in MDA-MB-231 cells. The percentage of cells undergoing apoptosis increased with radiation dose and resveratrol concentration (Fig. 3).

RT-PCR analysis showed that only in MDA-MB-231 cells combination of RSV+IR treatment showed significantly reduced BCL-2 gene expression than either treatment alone.

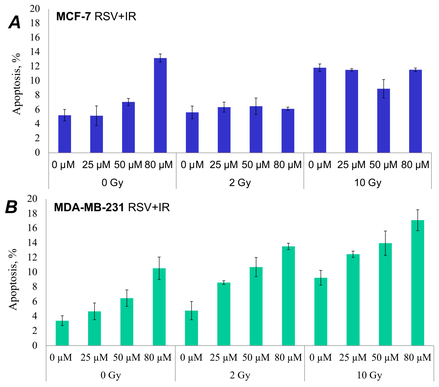

Figure 3. The influence of combination of RSV+IR therapy on apoptosis induction. MCF-7 (A) and MDA-MB-231 (B).

Conclusions

Our study results revealed that resveratrol is a potential radiosensitizer of breast cancer cells. We also demonstrated that the inhibitory effect of combination of RSV+IR on MDA-MB-231 cell growth was related to the induction of apoptosis and the expression reduction of apoptosis-related gene BCL-2.

Key words

Resveratrol, breast cance, radiotherapy, radiosensitivity