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ANALYSIS OF RESVERATROL AND RADIATION EFFECTS IN LUNG CANCER CELLS BY MICRONUCLEUS ASSAY

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ABSTRACT

Mucoepidermoid lung carcinoma is frequently manifested by obstructive trachea symptoms. Radiation and drugs combinations are commonly used in the lung cancer treatment. Currently there is a strong tendency to develop therapeutic strategies focused at the administration of high potential compounds to improve the ionizing radiation treatments, so as to increase the radiation effects on tumor cell while minimizing these effects to surrounding normal tissues. Resveratrol is a polyphenolic phytoalexin compound present in wines and several plants. This compound has a broad spectrum of biological activities such as antioxidant, anticarcinogenic, and induction of cell cycle arrest effects. Analysis of biological effects of ionizing radiation in the presence of resveratrol in different cell cultures has been the subject of many studies. To verify the genotoxic effects in cells exposed to ionizing radiation many methods have been proposed. The cytokinesis-block micronucleus technique is one of the preferred methods. The main of this study was to detect and quantify radioinduced DNA damage in mucoepidermoid lung carcinoma cells (NCI-H292) by cytokinesis-block micronucleus technique using cytocalasin-B. The cell culture was irradiated at a single fraction from a TrueBeam^{TM} linear accelerator (0, 0.8, 5, and 10 Gy), in the absence or presence of different resveratrol concentrations (0, 15, 30, and 60 μM). The results showed that resveratrol (15 and 60 μM) induced significant increase frequency ($p<0.05$) of micronucleus formation in NCI-H292 cell culture non-irradiated and exposed at 5 Gy dose. Moreover, resveratrol (30 μM) induced micronucleus formation at 0.8 Gy dose.

1. INTRODUCTION

Lung cancer is one of the most lethal malignant tumors. Mucoepidermoid lung carcinoma is frequently manifested by obstructive symptoms and it tends to spread more locally compromising the trachea [1].

Radiation therapy is an effective modality of treatments for cancer. The benefits are associated with the local or regional control of the tumor by exposure to clinically tolerated doses of ionizing radiation [2]. Radiation therapy and chemotherapy combination normally results in major survival rates for oncologic patients [3].

Resveratrol (3,4',5trihydroxystilbene) is a natural phenolic phytoalexin found in several plants such as grapes and consequently in wine as response to injury as fungal infections and exposure to ultraviolet light and chemical agents [4]. Growing evidence suggests that resveratrol exerts radioprotective effect acting as a scavenger of free radical contained in several biologic processes [5]. This property provides a prevention of human pathological processes such cancer prevention and therapy [6]. However, there are also evidences that resveratrol exerts radiosensitive effect in tumor cells acting in cell cycle progression [7].

The objective of this research was to detect and quantify radioinduced DNA damage in mucoepidermoid lung carcinoma cells (NCI-H292) by cytokinesis-block micronucleus technique using cytocalasin-B. Cell culture was exposed to ionizing radiation from True Beam^{TM} linear accelerator.

Therefore, this study supports the *in vitro* future investigation about radiosensitive resveratrol potential in lung cancer cells.

2. METHODOLOGY

The reagents were purchased from Sigma-Aldrich and Merck. Resveratrol was purchased from FarmaNostra, Anápolis, GO, Brasil.

2.1. Cell culture

The micronucleus assay (MN) was performed with human lung carcinoma cell culture NCI-H292, acquired from American Type Culture Collection (ATCC).

NCI-H292 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), and incubated at 37°C and 5% CO_2 atmosphere until cell monolayer formation. For cells dispersion the culture was treated with 0.20% trypsin and 0.02% EDTA solution. The cells were counted in Neubauer counting chamber and the cell suspension was adjusted for the micronucleus assay.

2.2. Resveratrol solutions

A resveratrol stock-solution was prepared at 25 mM concentration in ethanol PA. From this solution, five serial dilutions were made in RPMI 1640 medium with 10% FBS, yielding concentrations of 60 μM, 30 μM and 15 μM. These solutions were sterilized by passing through a 0.22 μm membrane system.

2.3. Petri dishes preparation for irradiation

The MN assay was performed in triplicate. 500 μL of $1.0x10^5$ cells ml^{-1} suspension was placed on coverslip which was inside Petri dish. After the cell adhesion on the coverslip it was added 1.5 mL of RPMI 1640 medium on each plate and the Petri dishes stayed in a CO_2 humidified incubator for 48 h. The culture medium contained in Petri dishes was replaced by 5 ml of resveratrol solutions. The plates that had a concentration of 0 mM of resveratrol received only RPMI 1640. All the plates were incubated for 24 h at 37ºC in a 5% CO_2

humidified atmosphere. Prior to irradiation of the Petri dishes containing cells on the coverslip, the resveratrol solution was replaced by PBS (phosphate buffer solution, pH 7.4).

2.4. Irradiation and cytokinesis-block

Irradiation of cell culture was performed by TrueBeam^{TM} linear accelerator (Varian Medical Systems, Inc., Palo Alto, CA, USA) in Hospital Israelita Albert Einstein, São Paulo, Brazil. The Petri dishes were irradiated at different doses: 0.8, 5, and 10 Gy and 14 Gy/min dose rate, in a single fraction at room temperature. Non-irradiated cells, used as control were kept under light at room temperature during the irradiation process of the others.

After irradiation, PBS was replaced in each Petri dish by RPMI 1640 medium containing cytochalasin B (4 μg mL^{-1}). The plates were then incubated for 48 h at 37°C in a 5% CO_2 humidified atmosphere.

2.5. Fixation and staining

The culture cell were washed once with PBS, treated with isotonic saline solution (0.9% NaCl) for 15 min, fixed with 4% formaldehyde in water for 15 min, and washed three times with PBS. Fixed cells on coverslip were stained with acridine orange (0.003% in PBS) and deposited on histological slide (26x76 mm) for microscopic analysis.

2.6. Microscopic visualization

Visualization of MN was held in fluorescence microscope using appropriate filters and respecting some pre-established criteria for cells with blocked cytokinesis [8, 9, 10]. For analyzes it was considered only binucleated cells with intact cytoplasm and a minimum of 1000 cells per concentration of resveratrol. Moreover, it was also counted the number of mononuclear cells (one nuclei per cell) multinucleated (three or more nuclei per cell) for the statistical calculation.

3. RESULTS AND DISCUSSION

Micronucleus (MN) on binucleated cells (BNC) was identified after acridine orange staining in NCI-H292 cell culture as shown in Fig. 1.

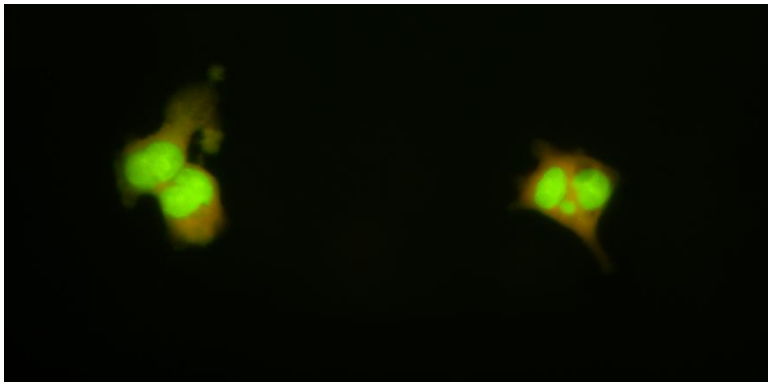

Figure 1: Micronuclei on NCI-H292 cell after acridine orange staining, x20.

Microscopically, nuclei and micronuclei are seen in bright green, and cytoplasm is seen in red color. In this analysis, it was detected just one micronuclei formation per cell.

Fig. 2 show MN scoring of culture cell exposed to different resveratrol concentrations and ionizing radiation doses.

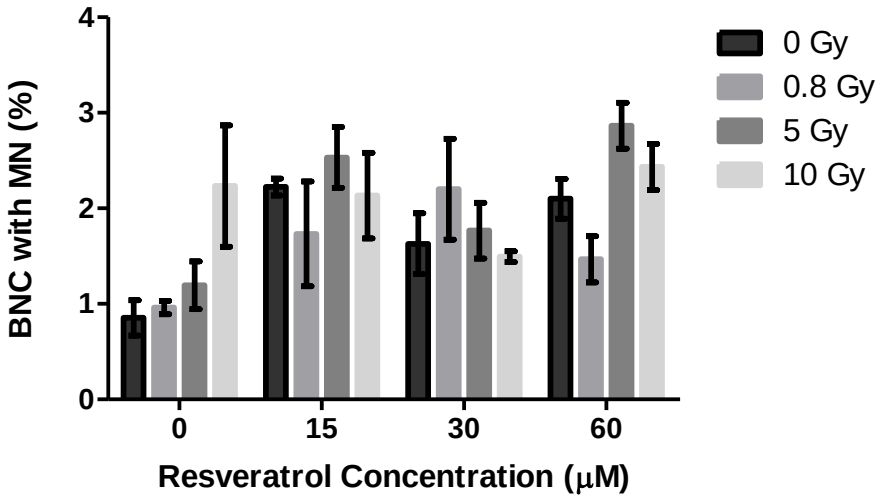

Figure 2: Micronucleus (MN) frequencies in NCI-H292 cell culture exposed to resveratrol and ionizing radiation. Error bars represent standard error means (SEM).

Based on statistical analysis (ANOVA), resveratrol effect in different concentrations was considered significant ($p<0.05$). However, radiation effect and the interaction between resveratrol and radiation effects were considered not quite significant ($p>0.05$). In another statistical analysis (Dunnett post-hoc test), resveratrol effect was considered very significant ($p<0.005$), but radiation effect and the interaction between resveratrol and radiation effects were considered not quite significant ($p>0.05$).

Then, resveratrol (15 and 60 μM) induced significant increase frequency ($p<0.05$) of micronucleus formation in non-irradiated NCI-H292 cell culture and exposed at 5 Gy dose. Resveratrol (30 μM) induced micronucleus formation at 0.8 Gy radiation dose.

Fig. 3 show cytokinesis block proliferation index (CBPI) of culture cell exposed to different resveratrol concentrations and ionizing radiation doses.

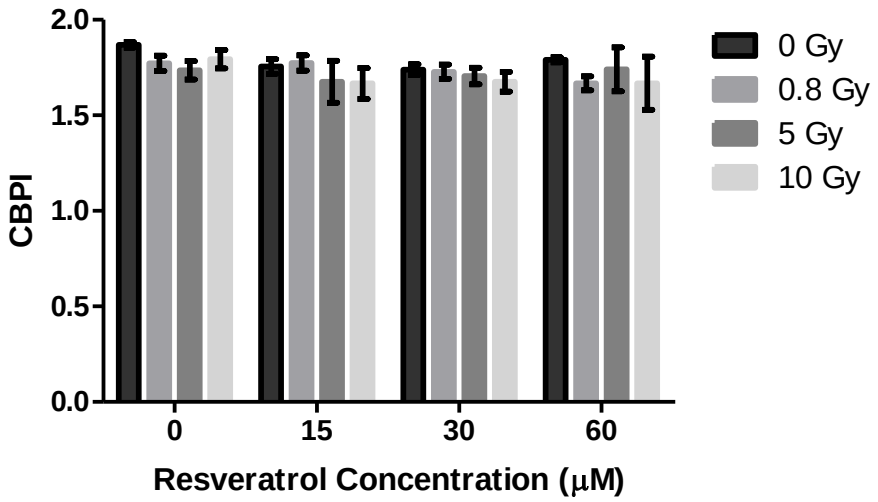

Figure 3: CBPI of NCI-H292 cell culture exposed to resveratrol and ionizing radiation. Error bars represent standard error means (SEM).

Based on statistical analysis (ANOVA and Dunnett post-hoc test) of resveratrol effect, radiation effect, and the interaction between resveratrol and radiation effects were considered not significant ($p>0.05$) to CBPI. Then, it indicates that the number of cell cycles per cell during the period of exposure to cytochalain-B was similar for all groups analyzed.

3. CONCLUSIONS

Cytokinesis-block micronucleus technique is accurate and sensitive for detecting chromosome damage caused by low dose exposures to ionizing radiation.

The results showed that resveratrol at 15 and 60 μM induced significant increase frequency ($p<0.05$) of micronucleus formation in non-irradiated NCI-H292 cell culture and exposed at 5 Gy dose. Moreover, resveratrol at 30 μM induced significant increase frequency of micronucleus formation in just one dose (0.8 Gy).

Therefore, resveratrol demonstrated a great potential to cause DNA damage in mucoepidermoid lung carcinoma cells (NCI-H929) irradiated at a single fraction from a TrueBeam^{TM} linear accelerator in a low doses of ionizing radiation.

More studies about resveratrol radiosensitive potential in tumor cells are necessary for improve the ionizing radiation treatments.

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