PJ-34 Confers Radiosensitizing Effect on MDA-MB-231 Cells

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Objective

Radiotherapy is one of the common approaches for cancer therapy, however, the radioresistance remains a major obstacle for the effectiveness of treatment. Previous studies have reported that various poly (ADP-ribose) polymerase (PARP) inhibitors demonstrate anti-cancer activity as a single agents and in combination with chemotherapeutic agents or radiotherapy. These findings suggest that PJ-34, one of the PARP inhibitors, could be the potential agent for radiosensitizing breast cancer cells. Thus, the aim of the study was to analyze the radiosensitizing effect of PJ-34 on human triple negative breast cancer cells MDA-MB-231. To achieve this objective, the activation of the Ataxia Telangiectasia Mutation (pATM) and the one of the variants of histone H2A.X (pH2A.X), as the early markers of a cell's response to DNA damage, and cell survival were studied.

Methods

MDA-MB-231 cells were cultured under sterile conditions at 37˚C in a humid environment containing CO_2 (5%) and the culture medium comprised Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL penicillin with 100 µg/mL streptomycin and 2 mM L-glutamine.

Cells were treated with PJ-34 (SigmaAldrich) one hour before irradiation to the single dose of 2 and 4 Gy (Clinac 2100C/D linear accelerator).

To conduct the quantitative analysis of the pATM and pH2A.X positive cells, Muse Multi-Color DNA Damage kit (Merck Millipore) was used one hour following irradiation.

The survival of cells was evaluated by clonogenic assay.

Statistical analyses were performed using Student's t test. P <0.05 was considered to indicate a statistically significant difference.

Results

The percentage of pATM and pH2A.X positive cells significantly increased following 10 µM of PJ-34 and 2 Gy irradiation compared to irradiation alone. Interestingly, the greatest amount of pH2A.X positive cells was following 4 Gy irradiation (0 µM of PJ-34) and then decreased to a lower level with PJ-34. The percentage of pATM positive cells decreased in the similar way following 4 Gy irradiation (Fig. 1).

Moreover, we observed that survival fraction significantly decreased in dose-dependent manner. The significant differences were obtained between 0 µM and 10 µM of PJ-34 in 2 Gy group (Fig. 2).

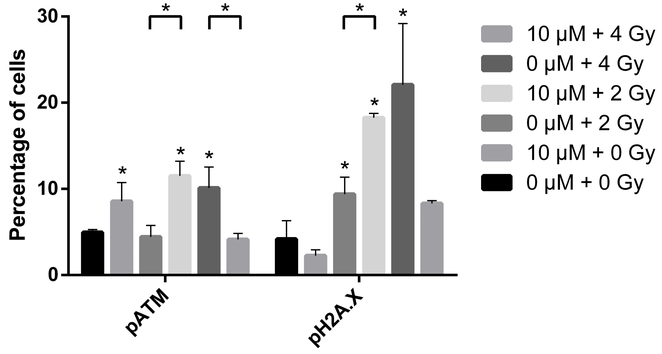

*Figure 1. DNA damage response in MDA-MB-231 cells one hour following irradiation. pATM and pH2A.X positive cells were measured using flow cytometry and Muse™ Multi-Color DNA Damage Kit. All error bars represent the SD. (*) means difference compared with untreated group, *p < 0.05.*

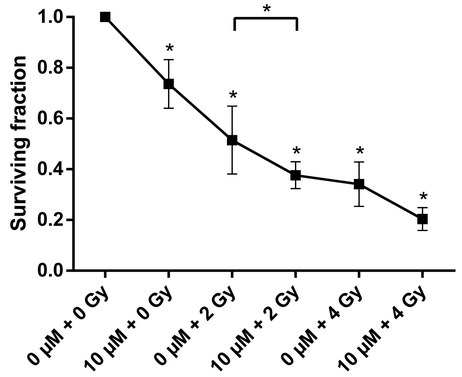

*Figure 2. Determination of MDA-MB-231 cell survival via clonogenic assay. All error bars represent the SD. (*) means difference compared with untreated group, *p < 0.05.*

Conclusions

The level of pATM and pH2A.X, and surviving fraction are the important factors in assessing the radiosensitivity of cells. Our results suggest that PJ-34 at concentration of 10 µM may be appropriate agent for enhancing the effect of 2 Gy radiotherapy.

Key words

radiosensitization, breast cancer, PARP inhibitor

InspireProject

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