3 - 51 Genistein Combined with X-ray Radiation Induces Oxidative Stress and Oxidative Damage in A549 Cells but Not in MRC-5 Cells

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Selectively killing cancer without harming normal tissue is a fundamental challenge in cancer therapy. Elevated oxidative stress and aberrant redox homeostasis are frequently observed in cancer cells compared with their normal cell counterparts[1]. A small shift toward an oxidizing condition in cells may lead to elevated proliferation and induction of adaptive response. However, a high oxidizing condition often results in cell injury and cell death. Persistent high level of reactive oxygen species (ROS) in cancer cells usually elicits increased cell proliferation and adaptive responses that may contribute to tumorigenesis, metastasis, and treatment resistance. However, normal cells may still maintain redox homeostasis through adaptive responses. Therefore, regulating intracellular redox state may represent an ideal strategy to selectively sensitize cancer cells to oxidative stress–inducing therapy, such as radiotherapy.

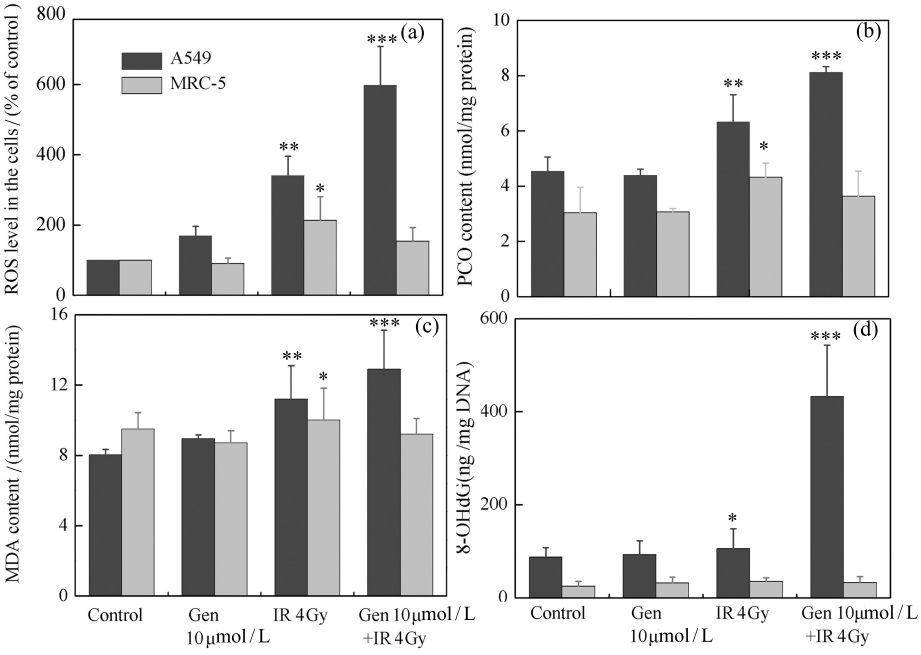

Fig. 1 (color online) Genistein induces oxidative stress and oxidative damage in A549 cells but not in MRC-5 cells. (a) ROS level in the cells, (b) PCO content, (c) MDA content, (d) 8-OHdG content. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control group.

The influence of genistein on intracellular redox states in cancer and normal cells was investigated. We showed that genistein selectively induced oxidative stress and oxidative damage in non-small cell lung cancer (NSCLC) A549 cells but not in normal lung fibroblast MRC-5 cells. First, we compared the effect of genistein on cellular ROS level in both cell lines by DCF assay. As shown in Fig. 1(a), X-rays significantly increased the ROS levels both in A549 cells ($P < 0.01$) and in MRC-5 cells ($P < 0.05$). However, genistein alone induced the increase of ROS level in lung cancer A549 cells but not in normal fibroblast MRC-5 cells. When combined with the radiation, genistein further increased the cellular ROS level in A549 cells. Significantly, in MRC-5 cells, genistein decreased the radiation-induced ROS level (significant at 4 Gy), suggesting an antioxidant response. Oxidative damages to proteins and lipids were measured in terms of PCO and MDA, respectively. The results are shown in Figs. 1(b) and (c). Consistent with the ROS production, 4 Gy X-rays alone significantly increased the MDA contents both in A549 cells ($P < 0.01$) and in MRC-5 cells ($P < 0.05$). However, in the combined-treatment group, the MDA content was increased significantly ($P < 0.001$) in A549 cells but not in MRC-5 cells. Simultaneously, DNA oxidative damage was studied through quantifying the levels of modified base 8-OHdG in both cell lines and the results are shown in Fig. 1(d). The modified base level increased significantly in A549 cells co-treated with genistein and radiation in comparison with the control group ($P < 0.001$) while no obvious change of the modified base level was observed in MRC-5 cells after the co-treatment. Our data suggest that genistein might selectively regulate intracellular redox

state in cancerous cells and sensitize them to radiations.

Reference

[1] Y. Sun, St Clair DK, Y. Xu, et al., Cancer Res, 70(2010)2880.

3 - 52 Unfolded Protein Response Induced by X-rays in Breast Cancer Cells

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Expand ER stress is triggered due to the loss of homeostasis in the ER which causes the accumulation of misfolded proteins within the ER lumen. Severe or prolonged ER stress may induce the unfolded protein response (UPR), which is an adaptive mechanism aimed at reducing levels of unfolded proteins and keeping balance in the ER. CHOP, Bip, JNK, EIF2α are major elements in these pathways.

In this study, we investigated the activation of CHOP, Bip, total JNK and phosphorylated JNK (P-JNK), total EIF2α and phosphorylated EIF2α (P- EIF2α) in response to X-rays in breast cancer MCF-7 and MDA-MB-231 cells using western blot analysis. As shown in Fig. 1, doses of 2 and 8 Gy were given and the detection time points post-irradiation were 0.5, 1, 2, 4, 6, 8, 12 and 24 h, respectively, whilst untreated cells were used as control. Our results show that ER stress was stimulated after X-ray irradiation. At 6, 8, 12 and 24 h post-irradiation, accumulation of CHOP was detected upon 2 Gy, whilst pronounced accumulation of Bip can be detected on both 2 and 8 Gy. Moreover, JNK (at 0.5, 1 and 2 h post-irradiation) and EIF2α were also phosphorylated and activated by the ER stress in a time-dependent manner. Therefore, we propose that ER stress was stimulated in breast cancer MCF-7 and MDA-MB-231 cell lines after X-ray irradiation.

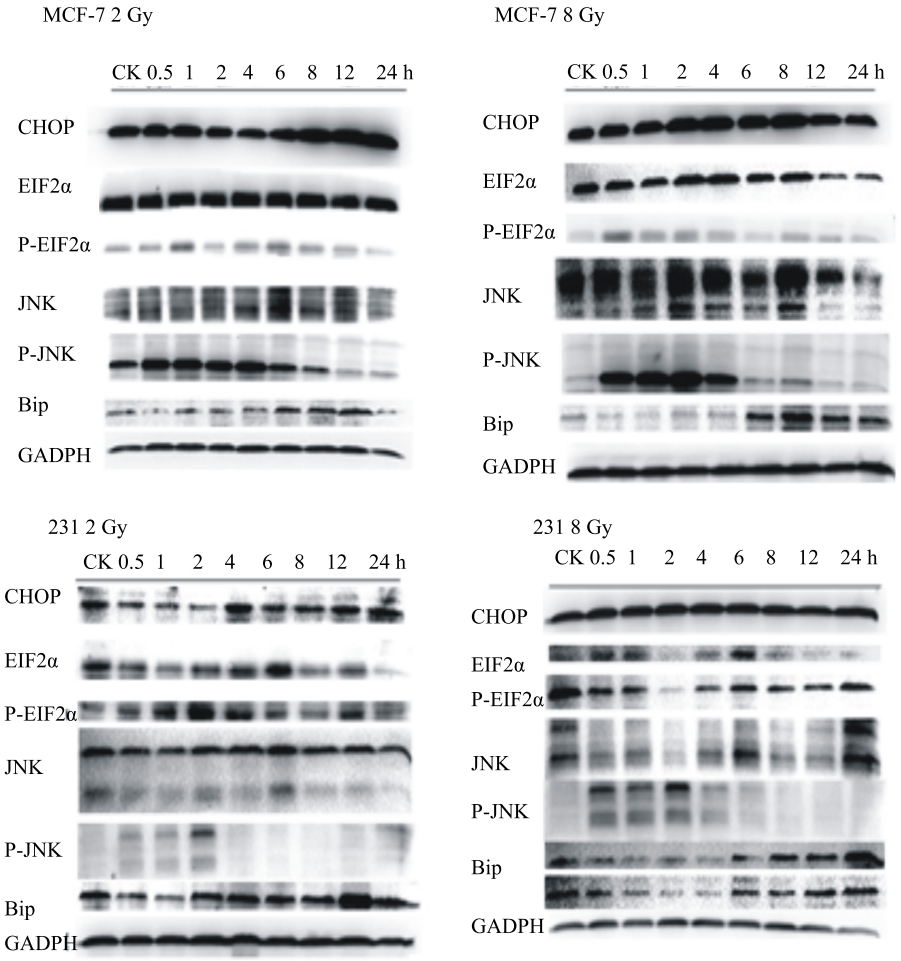

Fig. 1 UPR related key protein expressions of breast cells exposed to X-rays.