3 - 77 A Causal Link between Radiation-induced Oxidative Stress and Late Effect of DNA Methyltransferase Expression in Human Normal Liver HL-7702 Cells

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Carcinogensis is a multistage and complex process characterized by molecular alterations. These molecular events include alterations in gene expression that are regulated by both genetic and epigenetic mechanisms. ROS-induced oxidative stress has been shown to regulate both genetic and epigenetic cascades underlying altered gene expression in human disease including cancer[1]. Although a causal link between chronic oxidative stress and radiation-induced late normal tissue injury remains to be established, a growing body of evidence appears to support the hypothesis that chronic oxidative stress might serve to drive the progression of radiation-induced late effects. The aim of this study was to investigate the molecular mechanisms between ionizing radiation induced chronic oxidative and late effects of DNA methyltransferase expression alterations in human normal liver HL-7702 cells.

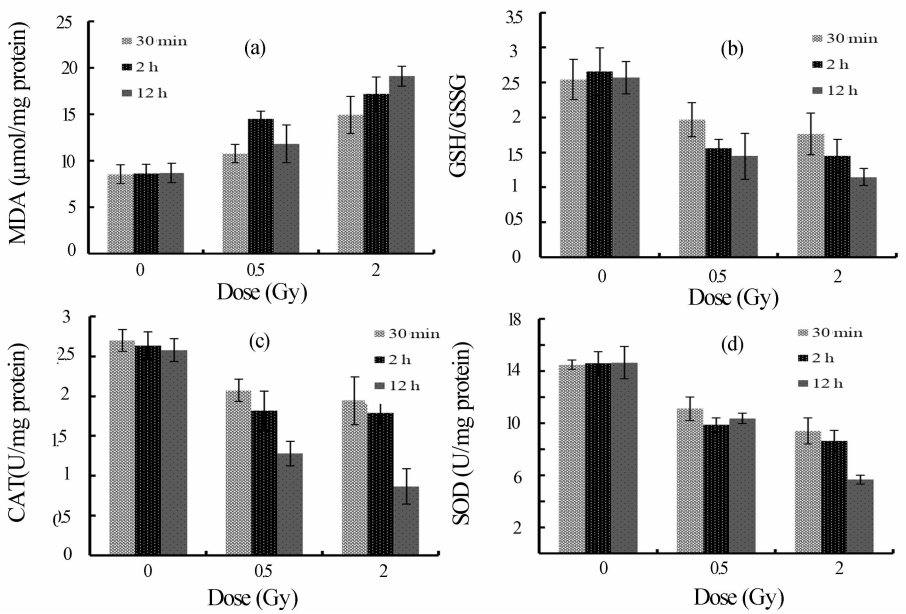

Fig. 1 Radiation-induced changes in oxidative stress: (a) malondialdehyde; (b) GSH/GSSG; (c) CAT; (d) SOD contents in HL-7702 cells irradiated with X-rays of different doses. Each value is presented as mean ± SEM.

In our experiment, HL-7702 cells were irradiated with X-rays of different doses. The results showed that oxidative stress presented in the irradiated cells depended on the dose of the X-rays and the time after irradiation (Fig. 1). The MDA content increased significantly at 12 h post irradiation in comparison with the control group, thus indicating that the irradiation obviously triggered the production of ROS and subsequent oxidative stress. The X-ray irradiation resulted in a prominent decrease in SOD and CAT activities as well as GSH/GSSG levels compared with the control group, suggesting that antioxidant levels in the irradiated cells were insufficient to counter the accumulated ROS.

Fig. 2 The expression of DNA methyltransferases DNMT1 and DNMT3a in HL-7702 cells passaged successively for 8 times after irradiation.

In mammalian cells, the enzymes critical for DNA methylation reactions are DNA methyltransferases 1 (DNMT1), 3a (DNMT3a), and 3b (DNMT3b). These enzymes catalyze the transfer of the methyl donor group from S-adenosylmethionine (SAM) to the 5'-carbon of cytosines within CpG dinucleotide islands (regions of 0.5-4.0 kb in length) in genomic DNA. More specifically, these enzymes are involved in DNA methylation by means of either maintenance (DNMT 1) and/or de novo methylation (DNMT3a and DNMT 3b). Thus, they could potentially contribute not only to increased promoter methylation status (through de novo methylation) but also ensure inheritance of gene silencing (through maintenance methylation) both of which could account for the acquisition of a malignant transformation phenotype[2]. In our experiment, the directly irradiated cells were passaged successively for 8 times. Our western blot data revealed that the expression of DNA methyltransferases DNMT1 and DNMT3a changed depending on the dosage of the X-ray irradiation (Fig. 2). Meanwhile, this alteration was correlated with the oxidative stress mentioned above. These results suggest that there is a causal link between radiation-induced oxidative stress and late effect of DNA methyltransferase expression change in HL-7702 cells.

References

[1] T. Ueno, M. Yamada, Y. Sugita, et al., J. Dent. Res., 90(2011)353.
[2] M. J. Hitchler, F. E. Domann, Free Radic. Biol. Med., 43(2007)1023.

3 - 78 Effects of Genistein on Cell Survival and Cell Cycle in Two Human Breast Cancer Cell Lines

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Genistein (5,7,4'-trihydroxyisoflavone) is a major isoflavonoid in dietary soybean, which may play a prominent role in cancer prevention[1]. Previous studies have shown that genistein can inhibit the growth of leukemia, esophageal and prostate cancers in vivo and in vitro[2-3]. The aim of this study was to investigate the potential anticancer function of genistein in two human breast cancer cell lines (MCF-7 and MDA-MB-231).

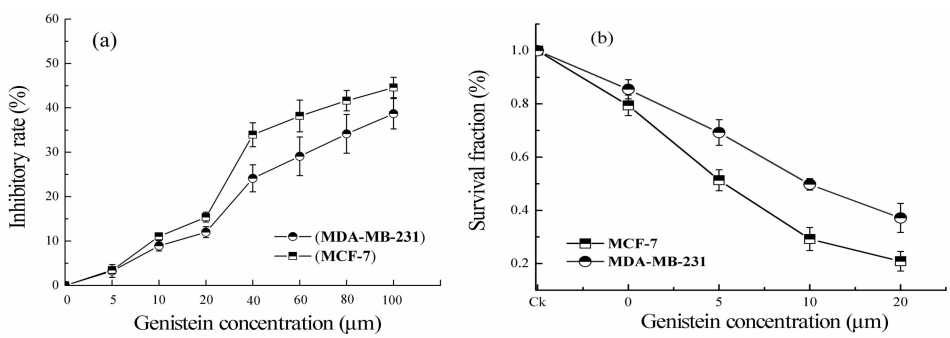

Fig. 1 The effect of genistein on the cell growth of MCF-7 and MDA-MB-231 cells. Cells in logarithm growing period were treated with genistein of various concentrations. (a) After incubation for 48 h, the proliferation of the cells was detected by MTT assay, (b) After pretreated with genistein for 24 h, the cells were irradiated with X-rays of 4 Gy.

MTT assays showed that genistein inhibited the growth of MCF-7 and MDA-MB-231 breast cancer cells in concentration-dependent manners (Fig. 1a). After the concentration of genistein was more than 40 μM, the inhibitory rate increased up to 20%. Therefore the genistein concentration within 20 μM was used to do the following experiments. Clonogenic survival data exhibited that genistein combined with X-ray irradiation decreased the cell survival significantly for all the cell lines compared with the irradiation of 4 Gy X-rays alone (Fig. 1b). Flow cytometry analyses revealed that both cells were arrested in the G_2/M phase after treated with various concentrations of genistein for 24 h, compared to the control group, the MCF-7