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

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

Liu Xiongxiong and Li Qiang

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).

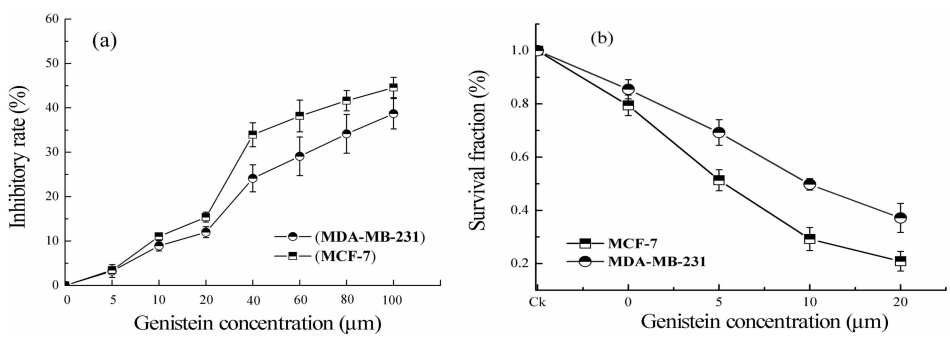

Fig. 1 The effect of genistein on the cell growth of MCF-7 and MDA-MB-231 cells. Cells in logarithm growing period were treated with genistein of various concentrations. (a) After incubation for 48 h, the proliferation of the cells was detected by MTT assay, (b) After pretreated with genistein for 24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

cells at G_2/M phase was increased from (18.5±0.87)% to (32.7±1.2)%, and the MDA-MB-231cells at G_2/M phase was increased from (19.9±0.44)% to (30.9±1.89)%. Thus, Our experimental data clearly indicate that genistein arrests the cell cycle at G_2/M phase and exerts an inhibitory effect on the proliferation of both breast cancer cell lines, they may play an important role in cancer prevention.

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3 - 79 Radiation-induced Delayed Microsatellite Instability in Human Normal Liver HL-7702 Cells

Zhao Ting, Ye Fei, Liu Xiongxiong and Li Qiang

The paradigm in which the deleterious effects of ionizing radiations are attributed to chemical alterations of DNA through energy depositions is well established. However, accumulating evidence suggests that other phenomena like heritable phenotype of genomic instability (radiation-induced genomic instability, RIGI) may also occur when cells are irradiated. The manifestation of this instability may be delayed for many cell divisions; it will then show itself in a variety of ways depending on the cell system used. Delayed lethal mutations (delayed reproductive cell death), delayed apoptosis, non-clonal cytogenetically detectable chromosome aberrations, often involving chromatid lesions, microsatellite instability, micronucleus formation and, potentially, transformation, are all well documented end-points[1]. The delayed nature of onset may be of particular relevance to neoplasia, but the relationship is still unclear[2]. In our experiment, human normal liver HL-7702 cells were irradiated with high linear energy transfer (LET) carbon ions (30 keV/(m) and low-LET X rays, respectively. Delayed effect in terms of microsatellite instability (MSI) in progenies of the directly irradiated cells and bystander cells, obtained in the way of medium transfer, at the 8th passage postirradiation was examined. Shown in Figs. 1～3 are the results acquired in our experiment.

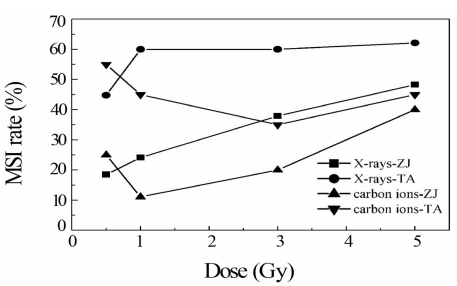

Fig. 1 MSI rate varies with exposure dose and ray type.

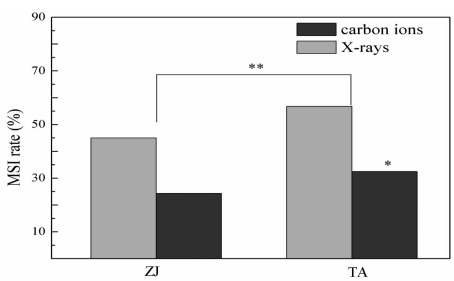

Fig. 2 MSI rate of different ray type and treatment.

These experimental data exhibit that the delayed effect induced by the high-LET carbon ions was different from that induced by the low-LET X rays, and a higher incidence of MSI was observed in the progenies of the cells after exposure to the X-rays than to the carbon ions. We also found that the delayed effect in the progenies of the bystander cells was much more severe than those of the directly irradiated cells. Furthermore, the events of MSI and loss of heterozygosity (LOH) induced by the ionizing radiations were not randomly distributed throughout the genome and specific loci existed indeed. These results imply that the radiation risk to normal tissues is lower in heavy ion therapy than in conventional X-ray radiotherapy. Probably the analysis of microsatellite loci with MSI high-frequency occurrence can be applied to access long-term survival conditions and second cancer risks for the patients after radiotherapy.