

Isoflavone intake inhibits the development of 7,12-dimethylbenz(a)anthracene(DMBA)-induced mammary tumors in normal and ovariectomized rats

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To determine the associations between isoflavone (49.72% genistin, 5.32% daidzin, 34.54% glycitin) and breast cancer risk, 150 rats were given 5 mg 7,12-dimethylbenz(a)anthracene and half of them were ovariectomized. Then normal rats and ovariectomized rats were divided into 5 groups: control group, isoflavone high (HI), middle (MI), or low (LI) dose group consuming 100, 500, or 1000 mg isoflavones/kg diet, estrogen group (2.5 mg stilboestrol/kg diet). After 24 weeks, tumor incidences were 73% in control group, 7% in HI, 7% in MI, 27% in LI, and 80% in estrogen group for normal rats; 60% in control group, 13% in HI, 7% in MI, 13% in LI, and 73% in estrogen group for ovariectomized rats. Isoflavone treatment decreased tumor incidence and mean tumor number per rat and increased mean latent period compared with those in control group and estrogen group significantly ($p < 0.05$). The mRNA and protein expression of estrogen receptor β were significantly higher in isoflavone treatment groups than those in control group. Moreover, isoflavone treatment significantly decreased 8-hydroxydeoxyguanosine content and increased superoxide dismutase level in normal rats and decreased malondialdehyde concentrations in ovariectomized rats compared with control group. In conclusions, isoflavone intake significantly inhibited the development of premenopausal and postmenopausal mammary tumors.

Key Words: isoflavones, mammary tumors, ovariectomized rats, estrogen receptor

Breast cancer incidence rates among women in Asian countries have long been noted to be substantially lower than those among women in Western countries, but rapidly increase in Asian women following emigration to the United States.^(1,2) Because changes in cancer risk following emigration are thought to reflect lifestyle changes, particularly in dietary patterns, these observations have led to a lot of searches for protective factors in the Asian diet. Daily isoflavone intake in the United States and Europe is typically < 3 mg.⁽³⁾ In contrast, soy intake among older adults in Japan and Chinese cities such as Shanghai is about 25–50 mg isoflavones per day.⁽³⁾ A large number of epidemiological and experimental studies have indicated that high intake of soy foods contributes to the low breast cancer risk in Asian countries.^(4,5)

However, this idea has been challenged by the apparent estrogenicity of soy isoflavones.⁽⁶⁾ Adipose-derived estrogens, and estrogenic compounds, e.g., in the diet, promote the growth of estrogen receptor (ER) positive breast cancer cells *in vitro* and *in vivo*.⁽⁷⁾ Soy isoflavones can bind to estrogen receptors in our body and have either pro-estrogenic effects or anti-estrogenic effects on

the target tissues, which may depend on tissue-type, status of the receptor, and circulating endogenous estrogen level.^(8,9) Some researchers have suggested that soy isoflavones may act as a dietary estrogen by binding unoccupied estrogen receptors during conditions of low circulating endogenous estrogen to alleviate the symptoms of menopause of postmenopausal women.⁽¹⁰⁾ However, epidemiological studies and experimental data suggest that soy isoflavones can be estrogenic and potentially increase risk of breast cancer.^(11,12) Animal studies on soy isoflavones have generated conflicting data regarding the ability of reducing mammary tumorigenesis in different menopausal animals. Some investigators reported that soy isoflavones reduced growth of mammary tumors that were induced by carcinogen in premenopausal rat models and others reported that, in ovariectomized rat models of postmenopause, dietary isoflavones can promote both carcinogen-induced estrogen-dependent mammary tumorigenesis and growth of ER-positive human breast cancer xenograft.^(13,14)

To determine the effect of soy isoflavones on breast cancer at the different intake stages, we induced the mammary tumors in normal rats as premenopausal rat models and in ovariectomized rats as postmenopausal rat models using 7,12-dimethylbenz(a)anthracene (DMBA) to assess the effects of soy isoflavone intake on the development of mammary tumors. Moreover, we examined the levels of malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OHdG), superoxide dismutase (SOD) in blood serum and analyzed the mRNA and protein expression of two ERs, ER α and ER β to clarify the underlying mechanisms.

Material and Methods

Animals. A total of 150 female Sprague-Dawley rats were obtained at 5 weeks of age from Peking University Laboratory Animal Center (Beijing, China). Animal experimental procedure and care of laboratory animals followed the Guidelines for Animal Experiments of Peking University.

Design. After two weeks of acclimation to commercial powder chow and water, all of the rats were given a single dose of 5 mg DMBA (Sigma-Aldrich, St. Louis, MO) dissolved in 0.5 ml corn oil by intragastric intubation at postnatal day 50. The treatment at postnatal day 50 was based on carcinogenesis studies that indicate that rats at this age have high density of terminal end buds; ductal structures that are more sensitive to DMBA-induced mammary tumors.⁽¹⁵⁾ After 2 weeks post-carcinogen treatment,

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rats were divided into two groups randomly, and were either bilaterally ovariectomized or the ovaries remained intact. The rationale for treating the animals with the carcinogen before ovariectomy is based on the fact that sensitivity of the rat to mammary tumor induction by DMBA is in part dependent on the hormonal state of the animal.⁽¹⁶⁾ Twenty-four hours after ovariectomy, the normal rats and ovariectomized rats were assigned at random to 1 of the 5 groups of 15 animals each and given 1 of the 5 tested dietary foods: modified AIN-93G diet (control group, CG), 100 mg soy isoflavones/kg diet (low isoflavone group, LI), 500 mg soy isoflavones/kg diet (middle isoflavone group, MI), 1000 mg soy isoflavones/kg diet (high isoflavone group, HI), or 2.5 mg stilboestrol/kg diet (estrogen group, EG). During the acclimatization stage, rats were maintained on a modified AIN-93G diet in which soy oil was replaced by corn oil to minimize the amount of extraneous phytoestrogens (The Chinese Academy of Preventive Medicine, Beijing, China). Soy isoflavones containing 49.72% genistin, 5.32% daidzin, and 34.54% glycitin were obtained from North China Pharmaceutical Company (Beijing, China).

Food intake and body weight were recorded weekly. Rats were palpated weekly to monitor tumor development. The 2 largest perpendicular diameters of each tumor were measured with calipers (Mitsutoyo CD-15 CP, Kanagawa, Japan) and the mean of the 2 measures was used to estimate the tumor size. During the 24 weeks after DMBA administration, complete autopsies were performed. All organs were examined for gross abnormalities. Visible mammary tumors were rapidly excised and weighed. To carry out histological examination, tumor tissue sections were cut at 2 μ m and stained with hematoxylin-eosin staining. Two pathologists performed the histological diagnosis according to the classification criteria described by Russo and Russo independently.⁽¹⁷⁾

RT-PCR. Total RNA was isolated from frozen rat mammary tumor tissues using TRIzol (Invitrogen, Carlsbad, CA). Specific PCR primers targeted for ER α , ER β , and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as an internal control were designed as follows: left primer 5'-GGTCCAATTCTGACAATC-GAGC-3' and right primer 5'-TTTCGTATCCCGCCTTTCATC-3' for ER α ; left primer 5'-AACACTTGCGAAGTCGGCAG-3' and right primer 5'-AACCTCAAAGAGTCCTTGGTGTG-3' for ER β ; left primer 5'-CCACCACCATCTTCCAGGAG-3' and right primer 5'-CCTGCCTCACCACCTTCTTG-3' for GAPDH. We performed amplification using HotStarTaq DNA polymerase kit (Qiagen, Tokyo, Japan). Negative controls without cDNA along with appropriate positive controls were included in each PCR reaction. Every gene was detected in 3 tumors in each group. In addition, we measured the densitometric values of ER α and ER β expression band of the 3 mammary tumors using Scion Image and calculated the mean in each groups.

Western blotting. Rat mammary tumor tissues were homogenized using a polytron homogenizer in a radioimmunoprecipitation assay lysis buffer with proteinase inhibitors. Equal amounts of protein (30 μ g) solubilized in sample buffer were separated on 10% SDS polyacrylamide gels and transferred electrophoretically to polyvinylidene difluoride membranes. Membranes were blocked in Tris-buffered saline containing 0.05% Tween 20 plus 5% nonfat dried milk for 1 h at room temperature and then probed with primary antibodies at 4°C overnight. Primary antibodies of rabbit anti-ER α polyclonal antibody, rabbit anti-ER β polyclonal antibody, mouse anti- β -actin monoclonal antibody were used at 1:200 dilutions (Santa Cruz Biotechnology, Santa Cruz, CA). Membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse secondary antibody at 1:10000 dilutions (Rockland, Montgomery, PA) for 1 h at room temperature. Antibody complexes were visualized using Odyssey imaging system (Li-Cor Biosciences Company, Superior Street, Lincoln, Nebraska). Every protein was detected in 3 tumors in each group.

8-OHdG, SOD activity, and MDA measurement. Before autopsy, blood was collected from femoral artery of 15 rats in each group and immediately centrifuged at 3,500 rpm for 15 min. To determine the effects of DMBA on oxidative stress maker and SOD activity, we also collected the blood from femoral artery in 15 DMBA untreated rats at 30 weeks of age. Then the blood serum was stored at -80°C until analysis. 8-OHdG concentrations were measured using a commercial ELISA kit (Da An Gene Company, Guangzhou, China). The ELISA method was described previously.⁽¹⁸⁾ At first, we pre-processed all serum samples using Millipore Microcon filters. Filters were damped with 100 μ l of distilled water and subsequently centrifuged for 5 min. Then filters were turned around and centrifuged for another 5 min to remove remaining water. Damped filters were moved to new tubes and 200 μ l of serum sample was added into each tube and then centrifuged for 30 min. Next, the primary antibody was reconstituted with the primary antibody solution. Then 50 μ l of sample or standard were added to wells, doing duplicate for each. After that, 50 μ l of reconstituted primary antibody was added to each well. Plate was shaken and covered with adhesive strip and then incubated at 4°C for overnight. After incubation, the contents of the wells were poured off and each well was washed with 250 μ l of washing solution three times. Then secondary antibody was reconstituted with the secondary antibody solution; 100 μ l of constituted secondary antibody was added to each well. Next, the plate was shaken, covered with adhesive strip and then incubated for 1 h at room temperature. Washing was repeated at the end of the incubation period. After that, a substrate solution was prepared and 100 μ l of it was added to each well and the plate was shook. The plate was incubated in the dark for 15 min at room temperature. Then 100 μ l of reaction terminating solution was added to each well and the plate was shook. Absorbance was measured at 450 nm in a plate reader and standard curve was used to determine the amount of 8-OHdG in samples.

Serum SOD activity (xanthinoxidase method) and MDA (thiobarbituric acid method) were all measured by an assay kit. MDA was assayed using an assay kit (Nanjing Jiancheng Company, Nanjing, China) according to the method described by Ohkawa *et al.*⁽¹⁹⁾ Briefly, MDA was measured as thiobarbituric-acid-reacting substance production in the following manner. 0.1 ml of sample was added to a 1:1:1 (vol/vol/vol) solution of trichloroacetic acid (15%, weight/vol), thiobarbituric acid (0.375%, wt/vol), and hydrochloric acid (0.25 M). The mixture was heated at 100°C for 30 min. The mixture was immediately cooled and then centrifuged at 3,500 rpm to remove un-dissolved materials. Then the absorbance at 532 nm was determined. SOD activity was measured immediately by xanthinoxidase method using a colorimetric assay kit of SOD (Nanjing Jiancheng Company). Assay was based on monitoring the autooxidation rate of hematoxylin. In the presence of SOD, the rate of autooxidation was inhibited and the percentage of inhibition was linearly proportional to the amount of SOD present within a specific range. Sample SOD activity was determined by measuring the ratios of autooxidation rates in the presence and absence of the sample. Intra-assay and inter-assay coefficient of variation were 8% and 12%, respectively. Data were expressed as U of SOD per 1 ml of blood. Independent experiments were performed 5 times. And the 8-OHdG, SOD, and MDA activity in the serum of all of rats were measured.

Statistic analysis. Fisher's exact probability test was used to compare the percentages of animals with tumors in each group. Mean number of tumors per rat, mean latent period, mean size of the tumors, and the concentrations of MDA, 8-OHdG, and SOD among the different groups were analyzed by one-way ANOVA followed by least significant difference (LSD) multiple comparison tests. Statistical significance was set at $p < 0.05$. Data analyses were performed using SPSS ver. 16.0 for Windows (SPSS Inc., Tokyo, Japan).

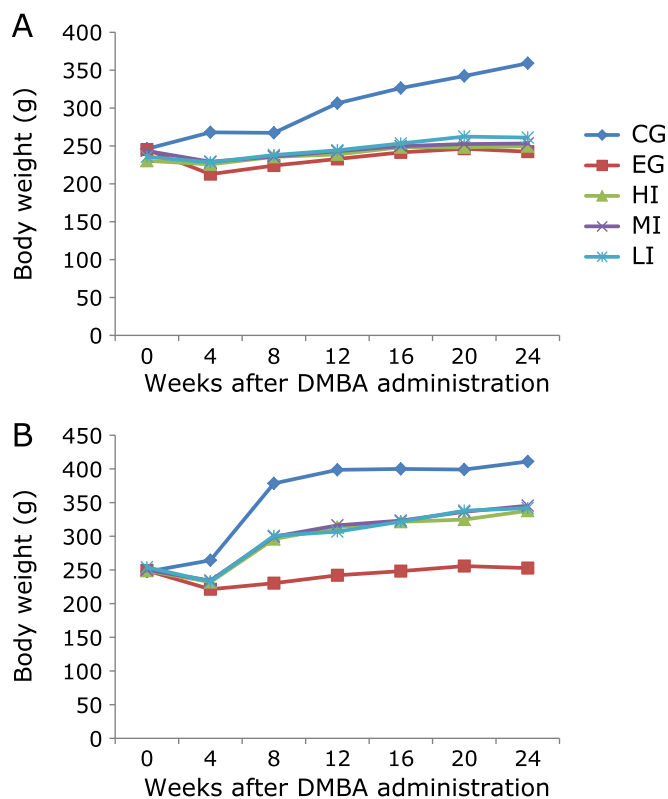


Fig. 1. Body weight curves per week of rats in the 5 groups during the experimental period for normal rats (A) and ovariectomized rats (B). CG; control group, EG; estrogen group, HI; high isoflavone group, MI; middle isoflavone group, LI; low isoflavone group.

Results

Previous studies have reported that ovariectomy and estrogen treatment could decrease the body weight and food intake.⁽²⁰⁾ Similar with the previous studies, the food intake and body weight of the ovariectomized rats decreased during the 2 week acclimatization after ovariectomy in the present study. Administration of isoflavone and estrogen significantly lowered the food intake and the body weight in normal rats and ovariectomized rats during the period of observation. However, no significant differences were observed in the food intake and the body weight among the three isoflavone treatment groups in normal rats and ovariectomized rats (Fig. 1).

During the period of observation, 29 normal rats (39%) and 25 ovariectomized rats (33%) produced mammary tumors. Multiple tumors of different sizes were frequently found in the same rat. Results of mammary tumor development were shown in Table 1. Fig. 2 showed the tumor incidence (A) and the number of tumors (B) in the experimental groups in normal rats, as detected by palpation. In the normal rats, the first tumors were detected at week 10 after DMBA administration in the CG, LI, and EG groups. However, palpable tumors in the HI and MI groups did not appear until week 16 and week 22 after DMBA administration, respectively. The mean latent periods in HI and MI groups were longer than that in CG group and EG group ($p < 0.05$). At week 24 after DMBA administration, tumor incidence were 73% (11/15) in CG group, 7% (1/15) in HI group, 7% (1/15) in MI group, 27% (4/15) in LI group, and 80% (12/15) in EG group. Isoflavone treatment groups showed significantly lower incidences in tumor development compared with CG group and EG group ($p < 0.05$). The mean number of tumors per rat in the CG, HI, MI, LI, and EG groups were 4.9, 0.3, 0.2, 1.4, and 5.5, respectively. Mean tumor number per rat in isoflavone treatment groups was significantly lower than that in the CG and EG groups. There were no significant differences on the tumor incidence among the three isoflavone treatment groups. Tumor incidence, mean number of tumors per rat, and mean latent period of EG group were comparable to those of CG group. We did not find any significant differences in the mean tumor size among the five groups for normal rats. All of tumors induced by DMBA in the normal rats were classified into adenocarcinoma by the two pathologists according to the histological findings. Adenocarcinoma is characterized by the loss of the tubular-alveolar pattern. Solid sheets of neoplastic epithelial cells are interrupted by secondary lumina of round, oval or irregular shape and variable size, representing a cribriform pattern (Fig. 3).

In the ovariectomized rats, 60% rats produced mammary tumors in CG group. The mean tumor number per rat in CG group was 4.2. The tumor incidence and the mean tumor number per rat in CG group in ovariectomized rats were lower than those in CG group in normal rats, but no significance. The first tumors were detected at week 9 after DMBA administration in CG, and EG groups in the ovariectomized rats. However, palpable tumors in the HI, MI, and LI groups did not appear until week 15, 21, and 14 after DMBA administration, respectively (Fig. 2C). The mean latent period in the isoflavone treatment groups was longer than that in CG group and EG group ($p < 0.05$). At week 24 after DMBA administration, tumor incidence was 13% (2/15) in HI group, 7% (1/15) in MI group, 13% (2/15) in LI group, and 73% (11/15) in EG group (Table 1). Isoflavone treatment groups showed significantly lower incidences in tumor development compared with the CG group and the EG group ($p < 0.05$). In addition, mean tumor

Table 1. Prevalence of DMBA-induced mammary tumors in normal rats and ovariectomized rats.

| Groups | No. of rats | Mean latent period (weeks) | Tumor incidence of rats (%)* | Mean tumor No./rat | Mean size of the tumors (cm ³) |
|----------------------------|-------------|----------------------------|------------------------------|------------------------|--|
| <i>Normal rats</i> | | | | | |
| CG | 15 | 11.11 ± 1.54 | 73.3 | 4.9 ± 4.0 | 4.83 ± 2.74 |
| EG | 15 | 10.78 ± 1.30 | 80 | 5.5 ± 4.1 | 4.22 ± 1.86 |
| HI | 15 | 16 ± 0 [#] | 6.7 [#] | 0.3 ± 1.2 [#] | 4.25 ± 6.39 |
| MI | 15 | 22 ± 0 [#] | 6.7 [#] | 0.2 ± 0.6 [#] | 4.48 ± 2.68 |
| LI | 15 | 12.33 ± 3.21 | 26.7 [#] | 1.4 ± 3.3 [#] | 2.9 ± 0.56 |
| <i>Ovariectomized rats</i> | | | | | |
| CG | 15 | 10.12 ± 1.74 | 60 | 4.2 ± 3.0 | 4.54 ± 2.72 |
| EG | 15 | 9.83 ± 2.56 | 73.3 | 4.9 ± 4.1 | 4.32 ± 1.45 |
| HI | 15 | 15.24 ± 2.74 [#] | 13.3 [#] | 0.8 ± 1.2 [#] | 5.23 ± 5.29 |
| MI | 15 | 21 ± 0 [#] | 6.7 [#] | 0.3 ± 0.6 [#] | 4.28 ± 2.38 |
| LI | 15 | 14.83 ± 1.73 [#] | 13.3 [#] | 0.8 ± 1.3 [#] | 2.54 ± 0.76 |

*incidence was measured at 24 weeks after DMBA administration. [#] $p < 0.05$ compared with CG group. CG; control group, EG; estrogen group, HI; high isoflavone group, MI; middle isoflavone group, LI; low isoflavone group.

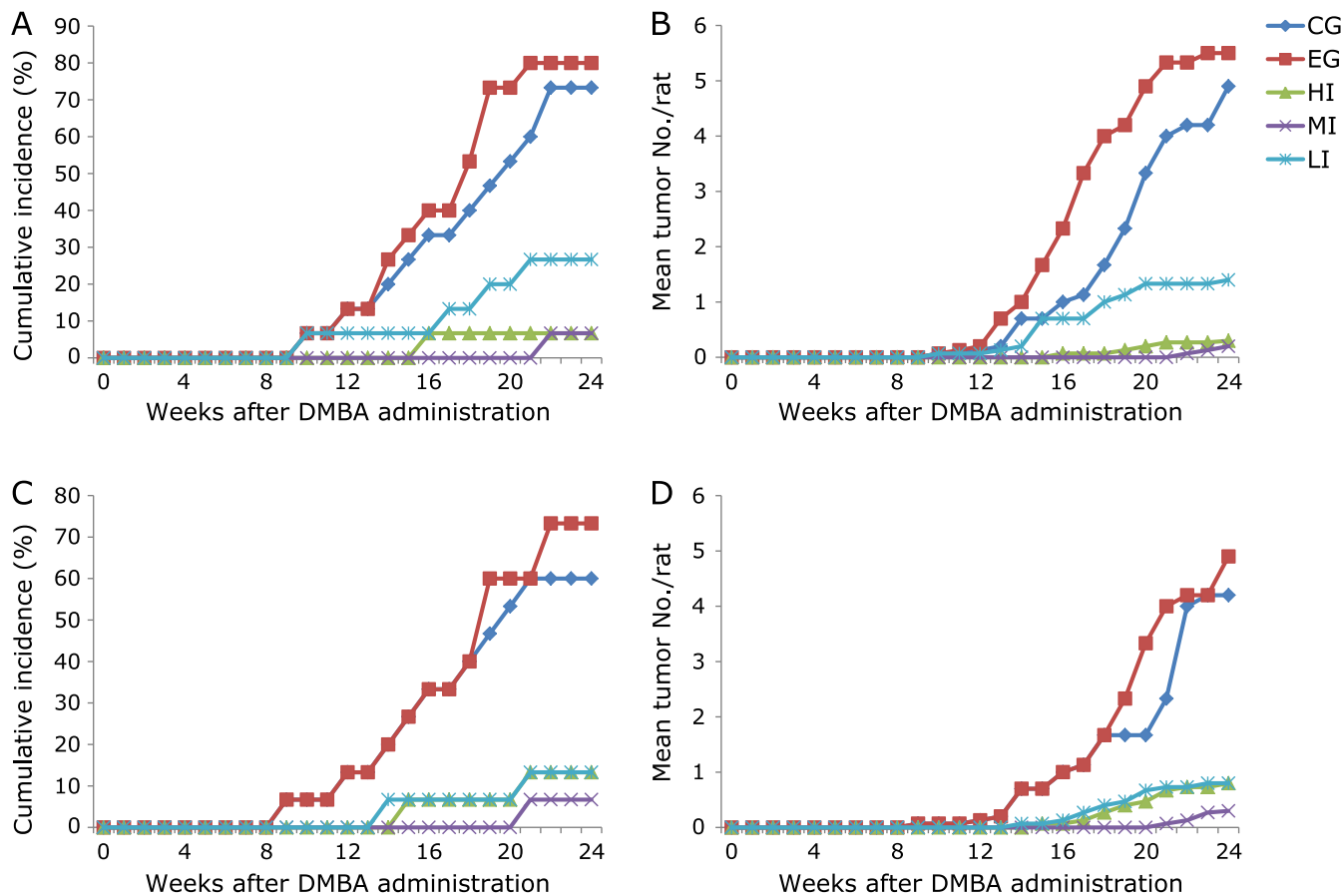


Fig. 2. Time course of palpable DMBA-induced mammary tumor appearance during the experimental period: tumor cumulative incidence for normal rats (A) and ovariectomized rats (C); mean tumor number per rat for normal rats (B) and ovariectomized rats (D). CG; control group, EG; estrogen group, HI; high isoflavone group, MI; middle isoflavone group, LI; low isoflavone group.

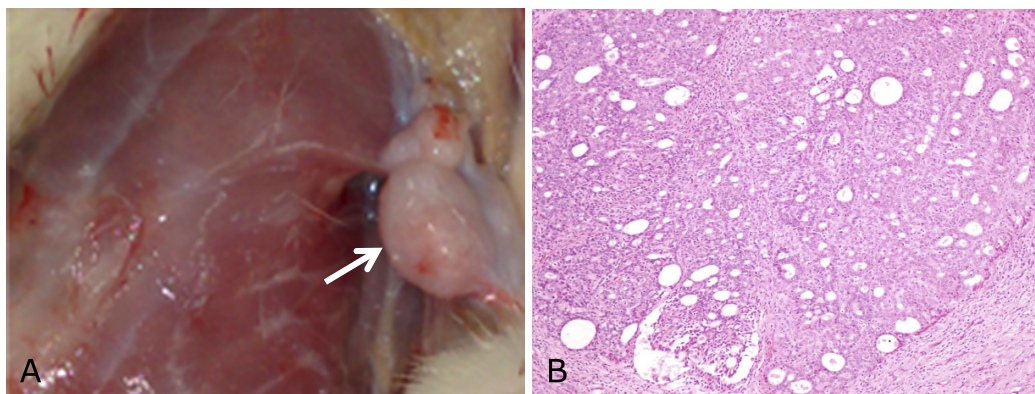


Fig. 3. The typical image of the tumor and the HE staining of rat mammary tumor. Numerous secondary lumina can be seen in the adenocarcinoma. HE ($\times 250$).

number per rat in isoflavone treatment groups was significantly lower than that in the CG and EG groups (Fig. 2D). There were no significant differences in the tumor incidence and the mean tumor number per rat among the three isoflavone treatment groups. Tumor incidence, mean number of tumors per rat, and mean latent period of EG group were comparable to those of CG group. We did not find any significant differences about the mean tumor size among the five groups for ovariectomized rats. All of tumors

induced by DMBA in the ovariectomized rats were classified into adenocarcinoma by the two pathologists according to the histological findings.

In this study, we detected the expression of ER α and ER β in the mammary tumors in different groups. As shown in Fig. 4C, the mRNA expression of ER β was significantly higher in the isoflavone treatment groups than that in CG group in normal rats. Compared with CG group, EG group significantly decreased the

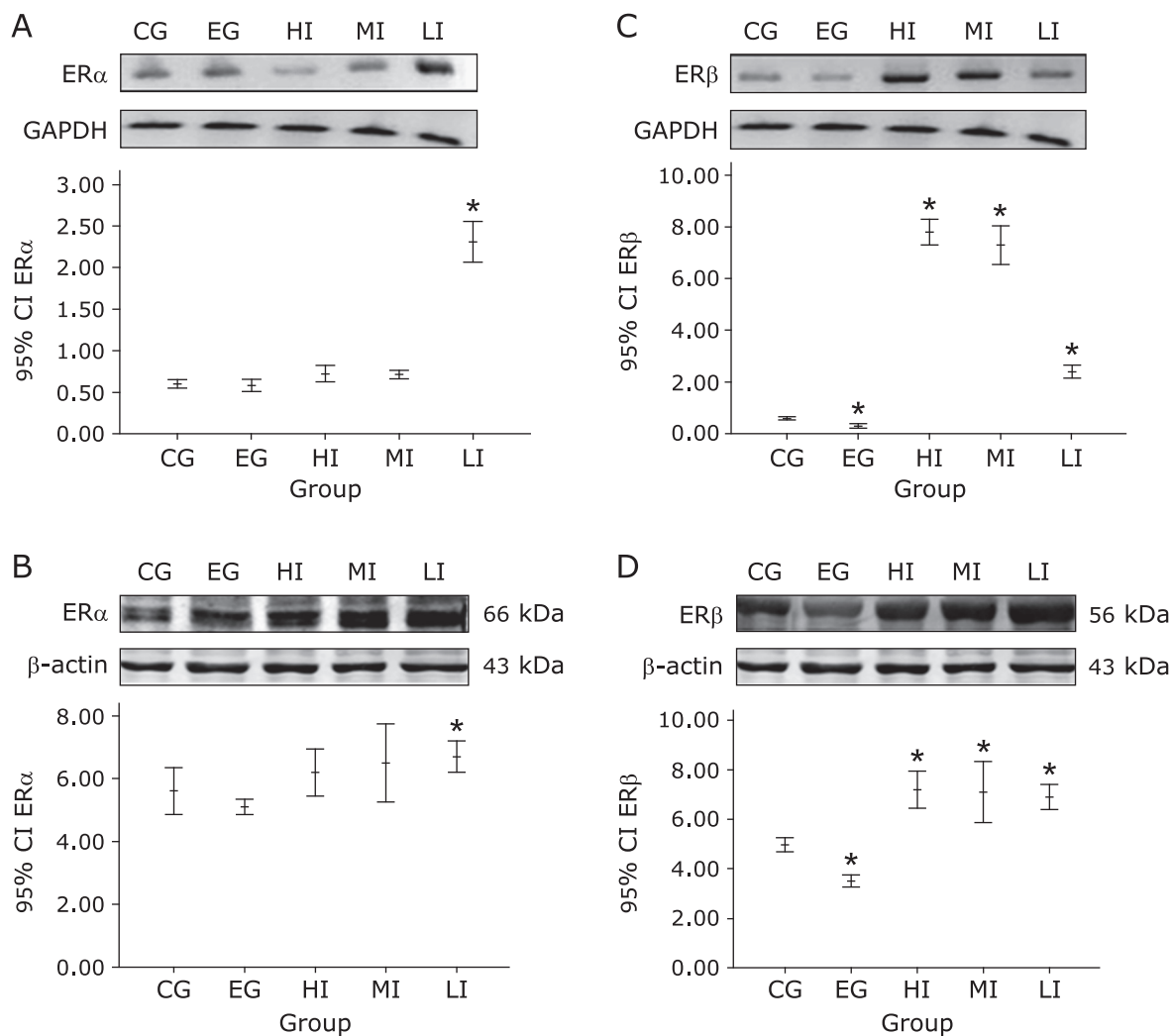


Fig. 4. Expression profiles of ER α and ER β in the mammary tumors in the 5 groups in normal rats. (A and B), ER α . Only LI group presented stronger expression than that in CG group in the mRNA (A) and protein (B) level (* p <0.05). (C and D), ER β . The mRNA expression (C) and protein expression (D) of ER β was significantly higher in the isoflavone treatment groups than those in CG group in normal rats (* p <0.05). Error bar, mean and 95% confidence interval of the densitometric values of ER α and ER β expression in the mammary tumors in the five groups. CG; control group, EG; estrogen group, HI; high isoflavone group, MI; middle isoflavone group, LI; low isoflavone group.

expression of ER β . Western blotting confirmed that ER β protein was up-regulated in the mammary tumor tissues in isoflavone treatment groups compared with that in CG group (Fig. 4D). For ER α , only LI group presented stronger expression than that in CG group in the mRNA and protein level (Fig. 4A and B). In the ovariectomized rats, the mRNA and protein expression of ER β were significantly increased in the isoflavone treatment groups than those in CG group (Fig. 5C and D). ER α expression in HI and MI groups is significantly higher than that in CG group (Fig. 5A and B). Similar with the results in normal rats, EG group significantly decreased the expression of ER β in comparison with that in CG group (Fig. 5C and D). There were no significant differences in the mRNA and protein expression of ER α and ER β in the mammary tumor tissues among the three isoflavone treatment groups in normal rats and ovariectomized rats.

To determine the antioxidant activity of the isoflavone treatment, we evaluated the content of MDA, 8-OHdG, and SOD in the plasma in different groups (Table 2). Compared with that in normal rats without DMBA treatment, DMBA treatment significantly increased the 8-OHdG content and decreased the SOD activity in CG group. However, in the normal rats, isoflavone

treatment significantly decreased the content of 8-OHdG and increased the content of SOD compared with CG group in a dose-dependent manner. Comparing with the CG group, EG group significantly decreased the content of MDA and 8-OHdG and increased the content of SOD. In the ovariectomized rats, the MDA content was significantly decreased in the plasma in the HI and MI groups compared with that of the CG group. Isoflavone treatment decreased the 8-OHdG level compared with that of CG group, but no significance. EG group vs CG group significantly decreased the MDA and 8-OHdG level of the plasma. There were no significant differences in the level of SOD between the isoflavone treatment groups and CG group.

Discussion

Previous studies have reported that ovariectomy of rats prior to the administration of DMBA suppresses the development of rat mammary carcinomas.⁽¹⁶⁾ In the present study, tumor incidence was 73% (9/15) in CG group in normal rats and 60% in CG group in ovariectomized rats, which suggested that treating the animals with the DMBA two weeks before ovariectomy to produce tumor

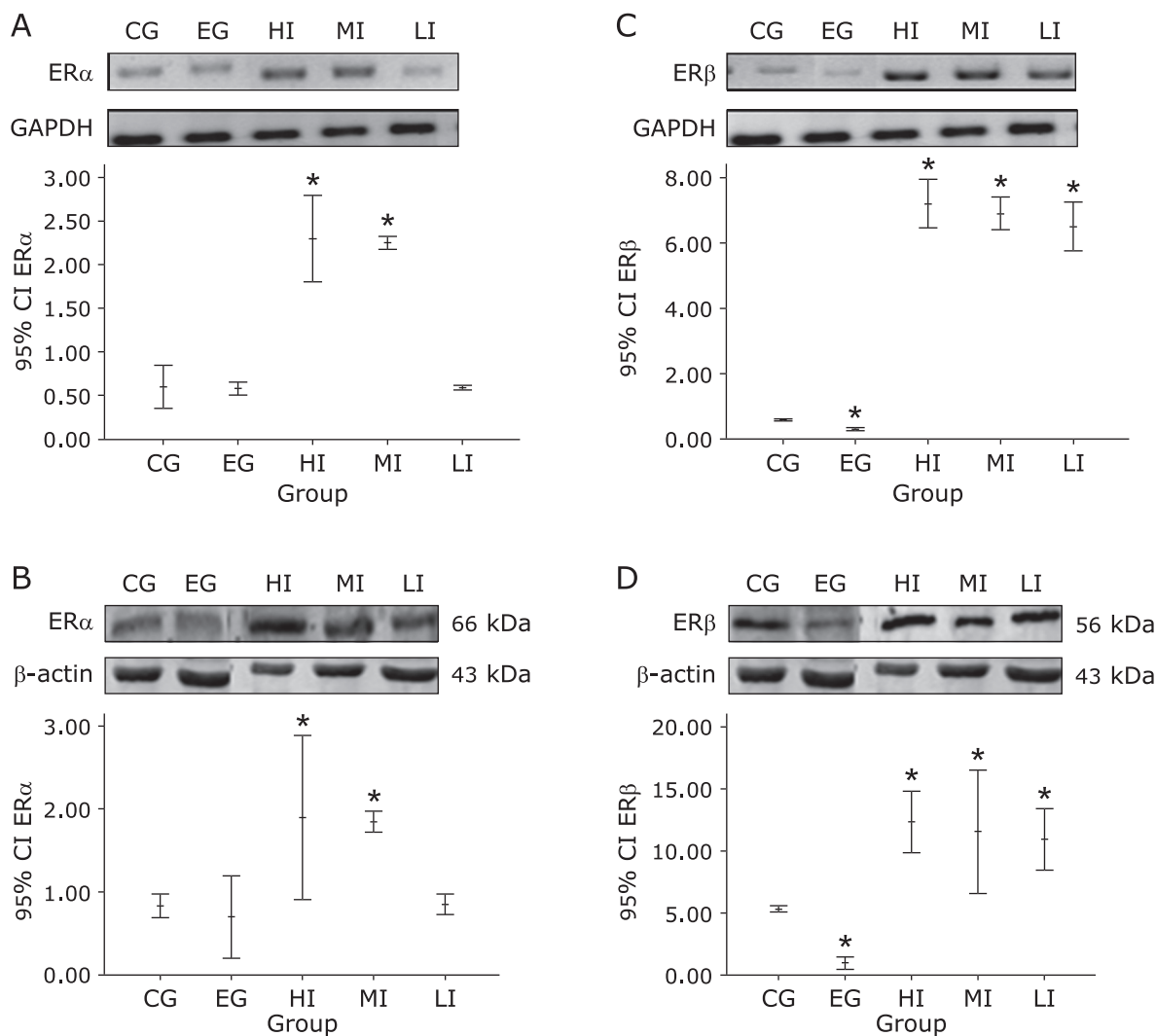


Fig. 5. Expression profiles of ER α and ER β in the mammary tumors in the 5 groups in ovariectomized rats. (A and B), ER α . ER α expression in HI and MI groups is significantly higher than that in CG group in the mRNA (A) and protein (B) level ($*p < 0.05$). (C and D), ER β . The mRNA expression (C) and protein expression (D) of ER β was significantly higher in the isoflavone treatment groups than those in CG group in ovariectomized rats ($*p < 0.05$). Error bar, mean and 95% confidence interval of the densitometric values of ER α and ER β expression in the mammary tumors in the five groups. CG; control group, EG; estrogen group, HI; high isoflavone group, MI; middle isoflavone group, LI; low isoflavone group.

Table 2. Concentrations of malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OHdG), and superoxide dismutase (SOD) in blood serum at autopsy in different groups after DMBA administration.

| Groups | MDA (nmol/ml) | 8-OHdG (ng/L) | SOD (U/ml) |
|----------------------------|-------------------|-------------------|---------------------|
| <i>Normal rats</i> | | | |
| NR | 18.89 \pm 6.96 | 3.62 \pm 0.74* | 146.34 \pm 8.59* |
| CG | 22.92 \pm 5.08 | 4.22 \pm 0.55 | 140.95 \pm 6.10 |
| EG | 17.19 \pm 3.74* | 3.65 \pm 0.40* | 156.37 \pm 4.56** |
| HI | 19.72 \pm 3.58 | 1.95 \pm 0.34** | 161.29 \pm 2.31** |
| MI | 24.86 \pm 6.17 | 2.88 \pm 0.57** | 159.23 \pm 3.00** |
| LI | 20.28 \pm 2.06 | 3.04 \pm 0.45** | 156.59 \pm 2.99** |
| <i>Ovariectomized rats</i> | | | |
| CG | 19.20 \pm 5.81 | 15.65 \pm 1.06 | 156.71 \pm 6.05 |
| EG | 14.34 \pm 1.37* | 13.10 \pm 2.06* | 160.99 \pm 7.44 |
| HI | 14.56 \pm 2.52* | 14.39 \pm 1.79 | 159.39 \pm 5.43 |
| MI | 16.05 \pm 5.71* | 15.43 \pm 1.87 | 150.36 \pm 7.86 |
| LI | 16.64 \pm 1.96 | 15.32 \pm 1.63 | 146.58 \pm 8.36 |

* $p < 0.05$ compared with the CG group; ** $p < 0.01$ compared with the CG group. NR; normal rats without the DMBA treatment. CG; control group, EG; estrogen group, HI; high isoflavone group, MI; middle isoflavone group, LI; low isoflavone group.

model was successful. In the present study, we clearly demonstrated that isoflavone has a significant inhibiting effect on the development of DMBA-induced mammary tumors in normal rats and ovariectomized rats.

Considerable epidemiological studies have shown a negative association between soy intake and breast cancer risk.⁽²¹⁾ Recently, a meta-analysis of prospective studies found that soy isoflavone consumption was not only inversely associated with risk of breast cancer incidence (RR = 0.89, 95% CI: 0.79–0.99), but also was inversely associated with risk of breast cancer recurrence (RR = 0.84, 95% CI: 0.70–0.99).⁽²²⁾ Animal studies have generated conflicting data regarding the ability of isoflavone to reduce mammary tumorigenesis.^(23,24) In general, most previous studies reported that dietary isoflavone exposure may increase tumor latency.^(13,14,23) Our present research proved that isoflavone intake can not only increase the mean latent period, but also decrease the tumor incidence. The same results were reported in other animal studies. Hewitt *et al.*⁽²⁵⁾ reported that female Balb/c mice injected with F3II cells and fed diets supplemented with 0.6% soy extract exhibited a significant 90% reduction in mammary tumor weight compared to controls. Moreover, Kang *et al.*⁽²⁶⁾ found that soy

phytochemical extraction exerts significant antitumor and anti-angiogenic activity in a postmenopausal animal model with breast cancer.

The mechanisms by which isoflavone could alter mammary tumorigenesis are not completely understood. The rat, mouse and human ER exist as two subtypes, ER α and ER β . The difference in function between the two ER remains to be established, but it is generally assumed that ER α mediates the proliferative actions of estrogens and ER β may inhibit cellular proliferation by antagonizing the actions of ER α .⁽²⁷⁾ It is to be noted that ER β is expressed at a significantly higher level than ER α during early development and in normal adult breast, while in the breast tumor ER α expression is higher than ER β expression.⁽²⁸⁾ In addition, isoflavones bind more strongly to ER β than to ER α .⁽²⁸⁾ Some studies reported that genistein can exhibit potential anti-carcinogenesis activities by the induction of mammary epithelial cell differentiation and activation of ER β .⁽²⁹⁾ Recently, Lattrich *et al.*⁽³⁰⁾ tested the effect of ER β agonists on the growth and gene expression of different ER β -positive human breast cancer cell lines and found that the ER β agonists only inhibited the growth of the ER α , ER β -positive breast cancer cell lines, which suggested that the antiproliferative effects of the ER β agonists might be dependent on the presence of both ERs. In the present research, we found that soy isoflavone significantly changed the estrogen receptor expression profiles including the increasing of ER β expression and ER α expression, which suggested that both ER α and ER β may be responsible for the inhibition of mammary tumors.

Oxidative stress and increased production of reactive oxygen species (ROS) are involved in various processes of carcinogenesis.⁽³¹⁾ High level of ROS has been reported to damage many biomolecules and exert diverse cellular changes in gene expression that leads to initiation and promotion of carcinogenesis. Similar with the results in the present research, Deepalakshmi *et al.*⁽³²⁾ have reported that the plasma level of the thiobarbituric acid reactive substances including MDA and 8-OHdG was significantly increased in DMBA-induced breast cancer rats through the overproduction and diffusion of free radicals from the

damaged tumor tissues when compared with DMBA-rats. The antioxidant activity of all the soy isoflavones have been proved by ferric-reducing ability of plasma assay and Trolox equivalent antioxidant capacity assay in a study.⁽³³⁾ Since lipid peroxidation is one of the most important expressions of oxidative stress induced by ROS, its end products MDA and 8-OHdG were determined as the oxidative stress indicator in our study. In the present study, the results showed that isoflavone treatment significantly decreased the 8-OHdG content in normal rats and MDA concentrations in ovariectomized rats compared with CG, implicating that this protective effect is probably based on the antioxidant activity of the soy isoflavones which reduces the oxidative damage by blocking the production of free radicals and inhibits lipid peroxidation. Studies have reported that SOD family played a crucial role in ROS scavenging.⁽³⁴⁾ We found that isoflavone intake significantly increased SOD content in normal rats but not in ovariectomized rats.

In conclusion, our results suggested that isoflavone intake can significantly inhibit the development of mammary tumors in normal rats or in ovariectomized rats through changing the estrogen receptor expression profiles. In addition, antioxidant activity of isoflavone may be also responsible for the carcinogenesis suppression.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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