Inhibitory Effects of Genistein on 7,12-Dimethylbenz(a)anthracene (DMBA)-Induced Mammary Tumor Development in Human c-Ha-ras Proto-Oncogene Transgenic Rats

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I. Introduction

Genistein, a major component of isoflavone contained in high concentration in soybean, plays a role in inhibitory effects of breast cancer (1,2). It reduced incidence and multiplicity of mammary cancer and incidences of carcinoma in the prostate and stomach (3). In vitro studies, it inhibited growth of mammary carcinoma and other carcinoma cell (4-6). Several possible mechanism for chemopreventive effects of genistein have proposed. It has been identified as a tyrosine kinase inhibitor (7). which was to play a key role in regulation of cell growth. It recently has been reported to induce apoptosis by reduction of the mitochondrial potential and release of cytochrome c (8). More importantly, it has been shown to suppress angiogenesis by decreasing the levels of vascular endothelial growth factor and transforming growth factor-β_1 (4).

The *ras* family genes involved in the regulation of cell proliferation and differentiation have been implicated in development of various tumors in human as well as experimental animal (9-11). Transgenic (Tg) rats carrying *c-Ha-ras* proto-oncogene have been highly susceptible to mammary gland and urinary bladder carcinogenesis (12,13). In addition, the Tg rats than Tg mice have advantage of tumor development within short experimental period (14).

In the present studies, we investigated chemopreventive effects of genistein on mammary gland and skin carcinogenesis induced by DMBA in the c-Ha-ras proto-oncogene Tg and non-Tg (non-transgenic).

II. Materials and methods

The *c-Ha-ras* proto-oncogene Tg rats were generated by injecting the human *c-Ha-ras* proto-

oncogene into pro-nuclei of fertilized rat oocytes from Spraugue-Dawley rats described by *Ota et al* (13). A total of 123 male and female c-Ha-ras proto-oncogene Tg and non-Tg rats divided into 3 groups. Rats in group 1 received one dose of DMBA (25mg/kg body weight) by gavage at 50 days of age. They then placed on powered basal diet containing genistein for 12 weeks for female and 20 weeks for males. Rats in group 2 were given DMBA and 25 ppm genistein. Rats in group 3 were given DMBA alone. They were sacrificed at weeks 12 and 20. Periodical palpation of mammary tumor incidence and multiplicity, and body and organ weights were recorded. Cell proliferation of genistein measured by the modified tetrazolium (MTT) method in the human *c-Ha-ras* proto-oncogene derived mammary tumor cell.

III. Results and Discussion

In the Tg rats, the incidence and multiplicity of mammary gland adenocarcinoma were significantly decreased by 250 ppm genistein (46% and 0.73±0.27) as compared to the DMBA alone group (92% and 3.91±0.76) ($P<0.05$ and $P<0.01$, respectively). In non-Tg rats, the incience of mammary tumor in the group fed 250 ppm genistein was also significantly decreased as compared to DMBA alone group ($P<0.05$). No toxic effects were observed in the genistein fed groups. These results of the present study demonstrated that dietary feeding of genistein significantly reduce the development of mammary gland tumor induced by DMBA in the human *c-Ha-ras* proto-oncogene Tg and non-Tg rats. Similarly, it significantly decreased the incidence and multiplicity of mammary adenocarcinoma in the Tg rats, whereas decreased only the incidence of mammary tumor in the non-Tg rats. Our data shows more inhibitory effects of genistein on mammary adenocarcinoma than adenoma in Tg and non-Tg rats, suggesting that genistein have the inhibitory effects on the tumor progression. It was reported to reduce the adenocarcinoma incidence and multiplicity of DMBA-induced mammary gland in rats (16). According to these results, genistein exerts anti-invasive effects as well as anti-proliferative effects. The anti-invasive effect might be related to the transcriptional down-regulation methyl metalloproteinase-9 (MMP-9) mediated by AP-1 activity (4). In our studies, genistein suppressed cell proliferation of *ras* related mammary tumor cells, suggesting that genistein reduced mammary tumor induction by making influence of *ras* gene, modulating cell proliferation and differentiation.

Genistein has anti-oxidant and anti-promotional effects in 12-O-tetradecanoyl phorbol-13-acetate (TPA) induced mouse skin carcinogenesis model (15). It enhanced activities of anti-oxidant enzymes in the skin and small intestine of mice (16). However, It was reported to not have any modifying effects on skin carcinogenesis induced by DMBA and TPA in mice (17), consistent with our results.

The *Ha-ras* gene mutation was reported to show high tumor induction in chemically induced rat mammary tumor. In our earlier reports, the Tg rats can be used as a more effective medium-term mammary carcinogenesis model. In this study, the incidence of palpable tumors in the female Tg rats

firstly occurred at 4 week, rapidly increasing the number and size from week 6, and in all rats at week 12. It remains unknown why Tg rats are high susceptible to the mammary carcinogenesis.

In conclusion, genistein effectively reduced the tumor incidence and multiplicity of mammary gland in both the human c-Ha-ras proto-oncogene Tg rats and non-Tg rats. Particularly, it showed more inhibitory effect on the development of malignant tumor in the Tg rats. Thus, it has the potential for good chemopreventive agent of mammary carcinogenesis. Regarding the mechanism, it inhibited cell proliferation associated with *ras* gene. To correctly elucidate the mechanism on inhibitory effect of mammary tumor development, we are trying to further studies.

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