CHAPTER I

INTRODUCTION

Breast cancer is the common female cancer and being the second leading cause of cancer death in women. In 1998, it was estimated that there were 178,000 new cases of breast cancer and 43,000 deaths in the USA (American Cancer Society, 2001). It is believed that certain lifestyle can reduce the risk of breast cancer. Asian women consuming a traditional diet, high in soy products, have a low incidence of breast cancer (Wu *et al.*, 1998). This evidence induces an interest in soy and soy products.

Soy contains large amounts of phytoestrogens, for example, genistein. Genistein is a phytoestrogen of greatest interest at present. It has several biological activities, including estrogenic, antiestrogenic and anticarcinogenic activities. Possible mechanisms for the anticarcinogenic actions of phytoestrogens, especially genistein, are: a reduction of cancer cell proliferation, an antioxidant, an inhibition of angiogenesis and an induction of cancer cell apoptosis. In spite of a large number of studies supporting the chemoprevention effect of genistein on cancer, some studies have suggested an alternative effect. It must be kept in mind that phytoestrogens are weak estrogens, and under certain experimental conditions it can stimulate cell proliferation and estrogen-dependent gene expressions.

Neonatal and prepubertal animals treated with genistein had reduced the incidence and multiplicity of DMBA-induced mammary adenocarcinomas (Larmartiniere *et al.*, 1995, Murrill *et al.*, 1996, Hilakivi-Clarke *et al.*, 1999). Study in the mammary whole mounts revealed that genistein treatment in neonatal and prepubertal rats decreased numbers of terminal end buds and increased numbers of lobular structures. According to the study of Russo *et al.* (1979) terminal end buds and terminal ducts are undifferentiated and are most susceptible to chemical carcinogens, while lobules are progressively more differentiated and less susceptible to the formation of adenocarcinomas. Thus, genistein treatment in neonatal and prepubertal rats altered

the ontogeny of the mammary gland and rendered the adult animals less susceptible to chemically-induced mammary cancer. These results are consistent with an epidemiological study that young women partaking of a traditional Asian diet high in soy have a low incidence of breast cancer (Shu *et al.*, 2001). Thus, it is possible that the chemoprotective action of the soy isoflavone against breast cancer depends on the timing of exposure. Early exposure to phytoestrogen (during postnatal period) may cause precocious maturation of breast terminal end buds to be more differentiated lobules and subsequently protect the breast cancer. In contrast, increment of the exposure time during post-pubertal period may potentially increase breast cancer risks. In adult animals, however, the effects of soy isoflavones on mammary tumor development are still controversial. Some report indicates that adult rats exposed to soy protein were prevented the development of breast cancer (Hawrylewicz *et al.*, 1991). Other studies reported that the soy isoflavone did not inhibit the development of chemically induced mammary tumor (Cohen *et al.*, 2000, Appelt and Reicks, 1999). Furthermore, the study in adult mice showed that genistein increased mammary tumorigenesis (Day *et al.*, 2001). Dietary feeding of genistein in ovariectomized athymic nude mice, giving the plasma concentration similar to those in human consuming a soy product, enhanced estrogen-dependent tumor growth (Hsieh *et al.*, 1998, Ju *et al.*, 2001). From the above mention, treatment of phytoestrogens may stimulate breast tumor growth and it leads to the concerns for the safety of using soy products in women with breast cancer.

The majority of breast cancers are estrogen dependent, and it is likely that many women with breast cancer are being treated with tamoxifen. Tamoxifen, the most commonly used antiestrogenic drug, has been shown to provide a 26% annual reduction in recurrence and 14% annual reduction in death of breast cancer (American Cancer Society, 2001). It also reduces the incidence of breast cancer among high-risk women who have never been diagnosed with the diseases (Fisher *et al.*, 1998). Some researchers suggested that combination of genistein and tamoxifen treatment may exert beneficial effects. Gotoh *et al.* (1998) found that tamoxifen combined with a diet containing 10% miso (fermented soybean paste) synergistically inhibited the development of N-nitroso-N-methylurea (NMU)-induced rat mammary cancer. Tumor incidences were 91, 77, 68 and 10% and the tumor multiplicities were 4.5, 2.4, 1.4 and 0.2 in the control, miso, tamoxifen and miso plus tamoxifen groups, respectively. In addition, the combination of miso and tamoxifen inhibited tumor growth by approximately 50% for over 6

weeks, whereas tamoxifen itself was ineffective. Constantinou *et al.* (2001) reported that the tumor multiplicity was reduced 29% by tamoxifen treatment, 37% by soy protein isolate and 62% by the two combination and the tumor latency was increased only in the combination group in DMBA-induced mammary carcinogenesis in rats. In contrast, Ju *et al.* (2002) reported in mice that dietary genistein could negate or antagonize the inhibitory effects of tamoxifen on estrogen-dependent tumor growth *in vivo*. These controversial reports raise concerns about the consumption of dietary isoflavone supplements along with tamoxifen therapy in human.

The aims of the present study were

1) To determine whether supplement of genistein, at the human consumption dose, has the effects on NMU-induced tumorigenesis in adult female rats.

2) To investigate the effects of genistein, tamoxifen, and the combination of tamoxifen and genistein on mammary tumor growth in adult female rats.

3) To understand the mechanism of action of genistein and tamoxifen at the level of gene expression.