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Inhibition of NMU-induced mammary tumorigenesis by dietary soy☆

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Abstract

We previously demonstrated that female Sprague–Dawley rats fed AIN-93G diets containing soy protein isolate (SPI^+) had lower DMBA-induced mammary tumor incidence than those fed diets containing casein (CAS), due partly to altered Phase I metabolism with soy. Here, we evaluated the tumor protective effects of these same diets to the direct-acting carcinogen *N*-methyl-nitrosourea (NMU). Tumor incidence was reduced and tumor latency was enhanced, in NMU-administered female rats lifetime exposed to SPI^+, relative to the CAS group. Tumor multiplicity did not differ with diet, while tumor grade tended to be more advanced with SPI^+. Normal mammary glands of CAS and SPI^+ tumor-bearing rats had comparable proliferative and apoptotic status. However, mammary expression of HER-2/neu and progesterone receptor (PR) genes was higher for SPI^+ rats. Moreover, tumored SPI^+ rats had lower serum progesterone levels than those fed CAS, while serum estrogen did not differ. Serum from tumored SPI^+ rats had higher apoptotic activity towards mammary epithelial MCF-7 cells, than CAS serum. Thus, dietary soy protects against mammary tumorigenesis induced by a direct-acting carcinogen and alters signaling pathways involving PR and HER-2/neu.

Keywords: Mammary carcinoma; NMU; Soy proteins; Apoptosis; Progesterone

1. Introduction

Breast cancer is a heterogenous disease that has been suggested to arise as a result of both genetic and epigenetic modifications [1–3]. The extent to which these pathways contribute to the development of mammary tumors is presently unclear, although an estimated 5–10% of breast cancers are associated with autosomal dominant genetic predisposition [4–5].

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Reproductive factors (parity, age of menarche, age of first full-term pregnancy) and diet have been suggested to confer protection against breast cancer in women [6–8]. Numerous studies to address the underlying protective mechanisms using animal models have so far shown the diversity in these mechanisms, which alter mammary differentiation, proliferation, apoptosis, capacity for DNA repair, and gene expression, among others [9–12].

Our laboratory is interested in understanding the interaction of diet during early development and adult risk of chronic diseases including breast cancer. In this regard, we have used rat models of chemically-induced mammary carcinogenesis to evaluate soy proteins in soy-based infant formulas for protective effects against adult onset mammary cancer. In a previous study we documented the protection by soy protein isolate (SPI^+), relative to casein (CAS) of DMBA-induced mammary tumor development [13]. A significant reduction in levels of Phase I enzymes that metabolize the procarcinogen DMBA was observed with SPI^+ [14], suggesting lowered levels of activated mutagen as one possible mechanism contributing to cancer protection. The present study extends this investigation to rat mammary tumorigenesis induced by NMU, a direct-acting carcinogen requiring no activation and identifies potential molecular markers that may be relevant to diet-associated mammary tumor protection.

2. Materials and methods

2.1. Animals, diets, and tissue collection

All animal procedures were approved by the University of Arkansas for Medical Sciences Animal Care and Use Committee. Time-mated Sprague–Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) were kept individually in polycarbonate cages in rooms under controlled temperature (24 °C), humidity (40%), and light (12 h light/dark cycle). Rats at gestation day (GD) 4 were randomly assigned to semi-purified AIN-93G isocaloric diets [13], made with corn oil and contained as sole protein source, either Casein (CAS, New Zealand Milk Products, Santa Rosa, CA) or Soy protein isolate (SPI^+) (Solae Company, St Louis, MO). Amino acid content between the diets was equalized, as previously described [13]. Animals were provided food and water ad libitum.

At delivery, pups from dams of the same diet group were pooled and 10 pups (five for each sex) were randomly assigned to each dam for suckling. Female pups ($n = 50$ per diet group) were weaned at postnatal day (PND) 21 to the same diets as their dams. At PND50, rats were administered *N*-methyl-*N*-nitrosourea (NMU Lot # ASI-701; Ash Stevens Inc., Detroit, MI) at a dose of 50 mg/kg body weight, via tail vein injection [15]. Rats were weighed weekly, and beginning at 3 wk after NMU-treatment, were palpated twice weekly for tumors. The initial detection date of tumor for each rat, and the subsequent detection of new tumors and their locations were recorded. Rats from all diet groups were killed at 115 d post-NMU. Tumors were weighed, fixed and analyzed for pathology by a board-certified pathologist (S.K.). In rats having tumors in only one mammary gland at the fourth position (MG#4), the corresponding non-tumorigenic tissue was collected, fixed in 10% neutral-buffered formalin for paraffin-embedding or immediately homogenized in TRIzol reagent for RNA extraction.

2.2. Immunohistochemistry and TUNEL

Proliferative cell nuclear antigen (PCNA) was localized in mammary tissues using a commercially available antibody [16]. TUNEL assay to detect apoptotic cells was performed following the manufacturer's instructions (Oncogene, La Jolla, CA). Cells showing dark brown color were counted from three randomly selected fields (200× magnification) per slide, with two slides evaluated for each mammary section. Tissue sections from 3 to 4 animals of each diet group were analyzed.

2.3. RNA isolation and quantitative RT-PCR

Total RNA was extracted in TRIzol Reagent (Life Technologies Inc., USA) and its integrity confirmed by using the RNA6000 Nano LabChip kit with the Agilent 2100 Bioanalyzer System (Agilent Biotechnologies, Palo Alto, CA). cDNA synthesis and quantitative real-time PCR were conducted under previously described conditions [16]. The nucleotide