Genistein alters growth factor signaling in transgenic prostate model (TRAMP)

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Received 2 October 2003; accepted 12 December 2003

Abstract

Genistein, a component of soy, has been reported to protect against spontaneously developing prostate tumors in the transgenic adenocarcinoma of mouse prostate (TRAMP) model. This is consistent with reports showing that Asians eating a diet high in soy have reduced incidence of clinically manifested prostate cancer. In order to understand the mechanism of action of genistein, we have investigated the expression of androgen and estrogen receptors, four growth factor receptors that signal via tyrosine protein kinases, and specific growth factor proteins in the dorsolateral prostates of TRAMP mice fed 250 mg genistein/kg diet, starting at 5 weeks of age. These analyses were carried out at 12 weeks, prior to the development of solid tumors, allowing us to readily investigate cell proliferation and biomarkers in premalignant tissue. Cell proliferation, AR, ER-alpha, EGFR, ErbB2, EGF, IGF-1R, IGF-1, VEGFR2, ERKs-1 and 2 proteins and TGF-alpha mRNA, but not ER-beta and VEGF, were significantly increased in prostates of TRAMP compared to C57BL/6 mice. Genistein in the diet significantly down-regulated cell proliferation, EGFR, IGF-1R, ERK-1 and ERK-2, but not AR, ER-alpha, ER-beta, ErbB2, EGF, TGF-alpha, IGF-1, VEGF and VEGFR in prostates of TRAMP mice. Serum testosterone and dihydrotestosterone concentrations were not significantly different in C57BL/6 or TRAMP male mice fed control or genistein-containing diets. The up-regulation of sex steroid receptors and multiple growth signaling pathways in TRAMP mice supports the concept of multiple dysregulation contributing to carcinogenesis. Down-regulation of the tyrosine kinase regulated proteins, EGFR and IGF-1R, and of the downstream mitogen-activated protein kinases, ERK-1 and 2, with genistein in the diet provides a possible mechanism for prostate cancer chemoprevention.

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Keywords: Genistein; Prostate; Androgen-signaling; Estrogen-signaling; EGF-signaling; IGF-signaling; VEGF-signaling; ERK-signaling; Mouse

1. Introduction

Asian men, consuming a traditional diet high in soy products, have a low incidence of clinically manifested prostate cancer (Dhom, 1983; Zaridze et al., 1991; Armstrong and Doll, 1995). Yet, Asians who immigrate to the United States and adopt a Western diet lose this protection (Haenszel and Kurihara, 1968; Shimizu et al., 1991; Whittemore et al., 1995; Cook et al., 1999). This suggests that nutrition may influence susceptibility for prostate cancer. While the role of fat intake (Willett et al., 1992; Rose, 1997) and genetics (Lichtenstein et al., 2000) for cancer susceptibility are unclear, other nutritional components may play a significant role. Soy-based diets are high in phytochemicals and quantitative results indicate that isoflavonic phytoestrogens are normal constituents of human urine, blood and prostatic fluid from subjects consuming large amounts of soy products (Adlercreutz et al., 1991; Morton et al., 1997). The predominant phytoestrogen component of soy is genistein. Recently, we demonstrated that dietary exposure to physiological concentrations of genistein alters growth factor signaling in transgenic prostate model (TRAMP).
genistein protects against chemically-induced and spontaneously developing prostate cancer in rodent models (Mentor-Marcel et al., 2001; Wang et al., 2002). The etiology of prostate cancer is still not resolved. Prostate cancer may be a consequence of mutations occurring from genetic instability, viral infections, oxidative-DNA damage, loss of regulation of oncogenes/suppressor genes or control of promotion/progression mechanisms, etc. One of the most promising models for studying prostate cancer chemoprevention is the TRAMP model (Greenberg et al., 1995). TRAMP mice were developed by using the prostate specific probasin promoter to drive expression of the simian virus 40 large tumor antigen-coding region. The SV40 T antigen acts as an oncprotein through interactions with the p53 and retinoblastoma tumor-suppressor gene products. Cancerous lesions have been detected specifically in the prostate by 10–12 weeks of age (Greenberg et al., 1995; Hsu et al., 1998). All TRAMP mice develop premalignant changes resembling human PIN and poorly differentiated tumors, ultimately developing prostatic adenocarcinomas that metastasize to distant sites, primarily the lymph nodes, bone and lungs. While the TRAMP model is appropriate for carrying out chemoprevention, there is the need to further characterize the model so we can understand how chemopreventive agents act.

Since the ontogenicity of prostate cancer in TRAMP mice reasonably mimics that found in men, and the growth of prostate tumor foci can be stimulated by growth factors (Cunha et al., 1987), we hypothesized that sex steroid and specific growth signaling would be dysregulated in these transgenic animals. Sex steroid-induced epithelial cell proliferation and differentiation have been associated with the coordinated induction of several peptide growth factors and their receptors, including some that are tyrosine kinase dependent (Boonstra et al., 1995). Our early studies showed that genistein in the diet could down-regulate the expression of the EGFR and ErbB2 in rats (Dalu et al., 1998). This study investigated AR and ER, four growth factor receptors that signal via protein tyrosine kinases, and specific growth factor proteins associated with cell proliferation and cancer in the prostates of 12-week-old C57BL/6 mice and TRAMP mice fed genistein in the diet. C57BL/6 mice are used as the nontransgenic background in the breeding of these animals.

2. Materials and methods

2.1. Animals

Animal care and treatments were conducted in accordance with established guidelines and protocols approved by the UAB Animal Care Committee. TRAMP mice were obtained from our UAB colleague, Dr. Ada Elgavish, who had previously obtained a transgenic male from Dr. Norman Greenberg (Baylor College of Medicine, TX). In our studies, TRAMP mice were developed on a pure C57BL/6 background, heterozygous for probasin-Tag transgene. Transgenic females were bred with nontransgenic males because transgenic males tend to develop prostate tumors. At age of 3–4 weeks, the isolation of mouse-tail DNA and PCR-based screening assay for transgene incorporation was performed as described previously (Greenberg et al., 1995; Mentor-Marcel et al., 2001). Nontransgenic and TRAMP mice were fed AIN-76A diet until 5 weeks old, at which time one group of 24 TRAMP mice was fed 250 mg genistein/kg diet. An equal number of TRAMP and nontransgenic C57BL/6 mice were fed AIN-76A diet only. The dietary genistein treatment is the same as the one used in our previous chemoprevention study (Mentor-Marcel et al., 2001). At necropsy, conducted at 12 weeks of age, dorsolateral prostates of mice were rapidly removed, frozen in liquid nitrogen and stored at −80°C until processed for Western blot analysis, ELISA or mRNA analysis by RT-PCR. In our studies, we focused our attention on the dorsolateral prostate, because the dorsolateral lobes of the murine prostate are embryologically homologous to the peripheral zone of the human prostate (Price, 1963) where approximately 68% of human prostate cancers originate (McNeal et al., 1998).

2.2. PCNA staining

Prostate glands were removed from the animals and processed for detecting PCNA by commercially available ZYMED PCNA Staining Kit (Zymed Laboratories Inc., South San Francisco, CA) according to the manufacturer’s protocol. Briefly, the paraffin-embedded tissues were deparaffinized in xylene and rehydrated in a series of graded alcohols. The heat induced epitope retrieval (HIER) method was used to enhance the staining. After blocking, the specimens were incubated with biotinylated mouse anti-PCNA, then streptavidin-peroxidase was used as a signal generator and DAB as the chromogen, to stain PCNA-containing nuclei a dark brown. The slides were analyzed using Image-Pro software (Media Cybernetics, Carlsbad, CA). PCNA positive and normal cells from five random areas of each DLP tissue were counted with 1450, 3500 and 4500 total cells/mouse prostate in nontransgenic mice and transgenic mice on AIN-76A diet, and transgenic mice fed genistein in AIN-76A diet, respectively. The PCNA index was defined as number of PCNA positive cells divided by total number of cells × 100.

2.3. Protein expression

Dorsolateral prostates (three per sample, eight samples/treatment group) were homogenized in lysis buffer (1% Triton X-100, 10 mM Tris (pH 7.4), 1 mM EDTA, 1 mM EGTA, 1 mM Hepes (pH 7.6), 2 mM Na vanadate, 0.2 mM PMSF, 2 µg/ml leupeptin, 2 µg/ml aprotinin). Protein concentration of each sample was determined using the Pierce BCA Protein Assay (Pierce, Rockford, Chicago, IL) according to the manufacturer’s protocol. Protein samples were subjected to SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked for 1 hour in TBS-T (0.1% Tween 20 in Tris-buffered saline) with 5% nonfat dried milk, then incubated overnight with primary antibodies against AR and ER (1:500). Membranes were washed with TBS-T and incubated with horseradish peroxidase-conjugated secondary antibodies (1:5000) for 1 hour. Membranes were washed with TBS-T and developed using the ECL Western Blotting Detection System (Amersham Biosciences). Densitometry was performed using Image-J software (National Institutes of Health, Bethesda, MD). The values were normalized against the total cellular protein. Each experiment was performed in triplicate.
The same quantity of protein from each sample was separated by SDS-PAGE and transferred to a nitrocellulose membrane (BioRad, Hercules, CA). The membranes were blocked and immunoblotted with appropriate antibodies including a polyclonal antibody to the amino acid portion of the human AR (N-20) (Santa Cruz, CA); anti-ER-alpha monoclonal IgG (MC-20) (Santa Cruz); anti-EGFR (1161–1186) (Calbiochem, San Diego, CA); anti-IGF-IR (#3022) (Cell Signaling Technology, Beverly, MA) and anti-ErbB-2/Her-2/Neu (#2242) (Cell signaling Technology); anti-VEGFR2 (PIK-1; C-20) (Santa Cruz); and anti-p44/42 MAP Kinase (p44/42 MAP Kinase, 9102) (Cell Signaling Technology). Appropriate blocking peptides from the respective companies were used as positive controls. After incubation with HRP-conjugated secondary antibody (Cell Signaling Technology), bands were detected with chemiluminescence (Pierce) and exposed to X-ray radiography film. Band intensity was quantified using scanning densitometry.

Because the proteins for EGF, IGF-1, ER-beta and VEGF have small molecular weights and/or no reliable antibodies that can be used to evaluate these proteins by Western blot analysis, they were quantitated via enzyme-linked immunosorbent assay (ELISA) (Crowther, 2001). ELISA kinetic analysis was performed for each marker so that the RNA was further checked by 1% agarose gel electrophoresis to confirm the integrity of the RNA for the presence of intact 28S and 18S RNA bands. Complementary DNA was reverse transcribed using the Superscript Preamplification System (Gibco BRL). The primers used in PCR were synthesized by UAB Oligo Core Facility. The primers for amplification of the housekeeping gene, beta-actin, and TGF-alpha cDNAs were designed based on the sequence of mouse beta-actin (Tokunaga et al., 1986) and mouse TGF-alpha (Berkowitz et al., 1996) using GeneJockey Sequence Processor (Biosoft, Cambridge, UK) and Amplify: beta-actin-forward (5′) CTT TGC AGC TCC TTCGTT G (3′) and beta-actin-reverse (5′) TGC CAA TAG TGA TGA CCT G (3′) which generate an 802 bp PCR product; TGF-alpha-forward (5′) AAT CAG GAG CAC TCT TAG CAG C (3′) and TGF-alpha-reverse (5′) GGT TCT GTG TAT GTC CAC CTG G (3′) which generate a 460 bp PCR product. As previously described by us (Brown et al., 1998), kinetic analysis was performed for each marker so that the number of cycles selected corresponded to the exponential range of amplification. PCR products were electrophoresed on agarose gels containing ethidium bromide. Bands were photographed under UV light and scanning densitometry was used to quantitate the intensity. The densitometric value for TGF-alpha was normalized to beta-actin expression.

2.5. Androgen hormone analysis

Sera were prepared and frozen at −80 °C until analysis. Serum testosterone and dihydrotestosterone concentrations were measured with a kit (DSL, Webster, TX) according to the manufacturer’s protocols.

2.6. Statistical analysis

Data analysis were carried out between transgenic mice and nontransgenic mice on AIN-76A diet only, and transgenic mice fed genistein to transgenic mice fed AIN-76A diet only by using one-way ANOVA (Sigma Stat, Jandel Scientific, San Rafael, CA). For androgen concentrations, each serum sample was from a pool of three mice (eight samples/group); data analysis for Western blots, ELISA and PCR were from three dorsolateral prostates/sample (eight samples/group), and for PCNA analysis, one dorsolateral prostate was used as a sample (eight samples/group).

3. Results

Analysis of PCNA staining, showed that the PCNA labeling index was increased nine-fold in prostates of TRAM compared to C57BL/6 mice (Fig. 1). However, genistein in the diet compared to control diet resulted in significantly reduced cell proliferation (47%).
Measurement of serum androgen levels showed that testosterone concentrations (5.02 ± 1.54, 4.89 ± 1.10 and 5.31 ± 1.64 ng/ml) and dihydrotestosterone concentrations (64.00 ± 14.08, 56.96 ± 14.72 and 56.32 ± 17.28 pg/ml) were not significantly different in serum of C57BL/6 mice and TRAMP mice fed control diet, and TRAMP mice fed genistein in the diet, respectively.

Since the early stage of prostate cancer is usually considered to be androgen dependent, we measured AR expression in the dorsolateral prostates of these 12-week-old mice. As evidenced by Fig. 2, Western blot analysis demonstrated that AR level was significantly increased (26-fold) in dorsolateral prostates of TRAMP mice compared to C57BL/6 mice. Also, we measured ER-alpha and found that it was significantly increased by five-fold in prostates of TRAMP versus C57BL/6 mice. Genistein in the diet to TRAMP mice did not alter AR or ER-alpha expressions. Measurement of ER-beta, by ELISA, suggested no change in this receptor in TRAMP versus C57BL/6 mice, or of genistein in TRAMP mice (Fig. 3).
One protein tyrosine kinase signaling pathway associated with cell proliferation is the EGF-signaling pathway. EGFR and ErbB2 were approximately four-fold higher in TRAMP compared to C57BL/6 mice (Fig. 4). Genistein in the diet was able to significantly decreased EGFR, but not ErbB2, protein expression in prostates of TRAMP mice.

Next, we investigated the expression of EGF and TGF-alpha, protein products that serve as ligands to the EGFR. EGF protein and TGF-alpha mRNA levels were significantly increased by 30 and 70%, respectively, in prostates of TRAMP compared to C57BL/6 mice (Fig. 5). Genistein in the diet did not significantly alter EGF and TGF-alpha expressions.

The IGF- and VEGF-signaling pathways have been associated with cell proliferation and prostate cancer. Indeed, IGF-1R and VEGFR2 were found to be significantly increased (19- and 10-fold, respectively) in prostates of TRAMP compared to C57BL/6 mice (Fig. 6). Interestingly, genistein in the diet significantly reduced IGF-1R, but not VEGFR2 protein expression in TRAMPs. While IGF-1 was significantly increased (1.5×), VEGF levels were not altered in TRAMP compared to C57BL/6 mice (Fig. 7). Genistein did not alter IGF-1 and VEGF levels in TRAMP mice.

Protein tyrosine kinases phosphorylate downstream mitogen-activated protein kinases, proteins that play a
significant role in stimulating cell proliferation. ERK-1 and ERK-2 were approximately two to three-fold higher in TRAMP mice compared to C57BL/6 mice (Fig. 8). Genistein significantly down-regulated ERK-1 and ERK-2 by 37 and 46%, respectively, in TRAMP mice.

4. Discussion

We have reported that genistein in the diet suppressed the development of large poorly differentiated tumors (adenocarcinomas) in TRAMP mice (Mentor-Marcel et al., 2001). Greenberg et al. (1995) and Hsu et al. (1998) previously showed that TRAMP mice demonstrated premalignant lesions by 10–12 weeks of age. To us, this appeared to be an appropriate sampling time to investigate cell proliferation and biomarkers as mechanisms of action prior to the time that the tumors become large and compromise oxygen and nutrient to the tissue. Measuring PCNA expression, we observed that the dorsolateral prostates of TRAMP compared to C57BL/6 mice had significantly increased cell proliferation, an event that can lead to hyperplasia and cancer. Genistein in the diet to TRAMP mice reduced cell proliferation by 46%. This is in keeping with slower progression of the disease in genistein fed rats and mice (Mentor-Marcel et al., 2001; Wang et al., 2002), and reported genistein action on cells (Peterson and Barnes, 1993; Naik et al., 1994; Schleicher et al., 1999; Zhou et al., 1999).

Fig. 9 illustrates sex steroid and growth factor signaling pathways found to be altered in dorsolateral prostates of TRAMP mice. These alterations start with the simian virus 40 large tumor antigen acting as an oncoprotein to inactivate p53 and retinoblastoma tumor-suppressor gene products (Lane and Crawford, 1979; DeCaprio et al., 1988; Greenberg et al., 1995). It has become clear that loss of these two suppressor genes contribute to the loss of cell regulation.

While androgen and estrogen receptor levels have not been reported to be altered in human prostate cancer, we found that 12-week-old TRAMP compared to C57BL/6 mice had significantly increased AR and ER levels. These results are consistent with the report of Raghow et al. of increased AR in 15- and 20-week (C57BL/6 TRAMP × FVB) F1 mice (Raghow et al., 2002). Normally, steroid receptor levels are regulated by inducer and feed-back mechanisms whereby increased receptor levels following growth stimulatory factors are controlled by proteolysis and receptor internalization. However, spontaneous somatic AR variants have been reported in TRAMP mice (Han et al., 2001) and in human prostate cancer (Culig et al., 1993; Elo et al., 1995; Culig et al., 1996; Fenton et al., 1997). Variant receptors may not be under such stringent control in TRAMP mice, therefore the bioassays (Western blot analysis, ELISA or RT-PCR) probably do not discriminate between wild-type and variant receptors depending on the recognized epitope or gene sequence. In 28–30-week-old TRAMP mice, poorly differentiated prostate tumors do develop and AR mRNA levels have been reported to be down-regulated at this age (Mentor-Marcel et al., 2001), possibly an indication of androgen-independent prostate cancer (Kaplan et al., 1999).

Availability of androgens for biological action can affect normal and cancer development in the prostate. Inhibition of dihydrotestosterone synthesis from testosterone has been suggested to play a significant role in regulating cell proliferation. Genistein has been reported to inhibit 5-alpha reductase, the enzyme that converts testosterone to...
dihydrotestosterone, in benign prostatic hyperplastic tissue (Evans et al., 1995). Measurements of testosterone and dihydrotestosterone in these mice did not show differences between groups. This suggests that the activity of 5-alpha reductase is not altered in TRAMP compared to C57BL/6 mice, or from genistein treatment.

Although measurement of receptor levels does not provide evidence of binding or activation, it has been hypothesized that the degree of ER receptor expression is proportional to the degree in which the tissue responds to estrogen. ERs are found in both prostatic stroma and epithelium, and estrogen action is important for prostate growth (Prins, 1997). On the other hand, estrogenic stimulation contributes to the genesis of prostatic dysplasia and subsequent prostate cancer (Habenschicht and el Etreybi, 1988). It has been postulated that the relative proportion of ERs-alpha and -beta within a given tissue may be responsible for the differential gene expression in response to ligands (Kuiper et al., 1997). While both receptors bind estrogen, ER-alpha is strongly associated with cell proliferation and ER-beta has been postulated to act as a tumor suppressor (Kege et al., 1998). Consistent with increased cell proliferation, we found that ER-alpha, but not ER-beta, was increased in prostates of TRAMP mice. However, genistein in the diet did not reduce ER-alpha or ER-beta expression in TRAMP mice.

One class of growth factor receptors associated with signal transduction and cell proliferation is characterized as protein tyrosine kinases. Since in vitro studies have reported that genistein inhibits protein tyrosine kinases (Akiyama et al., 1987), we investigated the ability of genistein to regulate two receptor signaling pathways that act via this signal transduction mechanism in rat prostate. The first receptor protein recognized to be a specific protein tyrosine kinase is the EGFR. In TRAMP mice, we found significantly increased levels of EGFR, and of EGF protein and TGF-alpha mRNA. The EGF and TGF-alpha proteins are the primary ligands that bind and activate the EGFR tyrosine kinase. Also, there were significantly increased levels of ErbB2 in prostates of TRAMP compared to C57BL/6 mice. ErbB2 is an oncprotein belonging to the EGFR family, one that can be activated without ligand binding, but capable of forming heterodimers with EGFR and enhancing signal transduction via receptor phosphorylation. Also, it has been observed that EGFR is co-expressed with the up-regulated EGF and TGF-alpha in human prostate tissue (Cohen et al., 1994). Increased expression of EGF and TGF-alpha has been linked to the development of human prostate cancer (Harper et al., 1993; Yang et al., 1993). Our finding of genistein down-regulating EGFR and ErbB2 in the TRAMP prostate is consistent with our earlier report that genistein in the diet down-regulated EGFR and ErbB2 in the rat prostate (Dalu et al., 1998). Modulation of the EGF-signaling pathway is consistent with genistein suppressing chemically-induced prostate cancer in rats (Wang et al., 2002) and spontaneously developing prostate cancer in TRAMP mice (Mentor-Meruel et al., 2001).

Signal transduction also occurs via the IGF-signaling pathway, of which the IGF-1R is a protein tyrosine kinase. There are two IGF ligands (IGF-1 and IGF-2) synthesized primarily in the liver but also in other tissues, including the prostate, which can promote cell proliferation and differentiation (Steiner, 1993; Jones and Cleemons, 1995; Grimberg and Cohen, 2000). These ligands mediate their actions via their receptors, IGF-1R and IGF-2R. IGF-1R is a transmembrane tyrosine kinase receptor that binds IGF-1 with higher affinity than IGF-2. IGF-1R stimulates mitogenesis and protects against apoptosis. IGF-1R overexpression has been shown to be necessary for cell transformation (Grimberg and Cohen, 2000). IGF-2R has no apparent intracellular signaling actions, but it does bind IGF-2 and mediates its uptake and degradation. In our studies, we chose to measure IGF-1R and IGF-1 because of their documented mechanisms for enhancing cell proliferation and cancer. We did find that IGF-1R and IGF-1 were up-regulated in prostates of TRAMP mice. However, Kaplan et al. reported that IGF-1R and IGF-1 mRNAs did not change during primary cancer progression in TRAMP mice (Kaplan et al., 1999). Two possible explanations for the difference in results are (1) Kaplan et al. measured mRNA transcripts and we measured protein expression, and (2) our TRAMP mice were developed on a pure C57BL/6 background while Kaplan et al. used [C57BL/6 TRAMP × FVB] F1 mice. Our results of increased IGF-1 receptor and ligand support the increased potential for cell proliferation and tumorigenesis. Interestingly, genistein in the diet resulted in down-regulated IGF-1R in prostates of TRAMP mice. This probably contributes to the chemopreventive actions of genistein (Mentor-Meruel et al., 2001).

The VEGF-signaling system acts on endothelial cells to stimulate cell proliferation, motility and angiogenesis that can provide blood supply to tumor cells, and VEGF expression has been demonstrated in human prostate tumors (Ferrer et al., 1997). In our studies VEGFR2, but not VEGF, was significantly increased in TRAMP prostate. The reason we investigated VEGFR2 is because it is believed to signal through the MAPK cascade and influence signal transduction (Kroll and Woltenberger, 1997; Huss et al., 2001). Its increase confirms that this pathway can play a significant role in the development of prostate cancer (Huss et al., 2001). Importantly, we found that genistein down-regulated VEGFR2. Modulation of endothelial cell proliferation by VEGF-signaling pathway can influence angiogenesis, the latter an event that genistein has previously been reported to regulate (Fotis et al., 1993).

Huss et al. have reported increased VEGF mRNA in PIN, however in their studies using [C57BL/6 TRAMP × FVB] F1 males, VEGF protein was not detectable until the prostates contained poorly differentiated tumors (Huss et al., 2001). It appears that increased VEGF-signaling is correlated with the process of tumor progression. This is consistent with the report of Ferrer et al. where VEGF expression was not found present in benign prostatic hyperplasia and...
or normal prostate cells in vitro (Ferrer et al., 1997). The fact that genistein did modulate VEGFR2, but not IGF-1, expression may explain why prostate tumors do develop in TRAMP mice, albeit at a slower rate (Mentor-Marcel et al., 2001).

Protein tyrosine kinase receptors can undergo autophosphorylation upon ligand binding and dimerization, and utilize their intrinsic kinase activity to phosphorylate downstream mitogen-activated protein kinases, eventually leading to regulation of transcription (Boonstra et al., 1995). Our data demonstrates that TRAMP compared to F344Bl/6 mice have increased ERK-1 and ERK-2 expression, a finding recently corresponded by Greenberg (Uzagare et al., 2003). Up-regulation of ERKs-1 and 2 in TRAMP mice is indicative of altered signal transduction and has been reported to be increased in prostate tumors with increasing Gleason score (Gioeli et al., 1999). Both proteins are regulated by dual phosphorylation in specific tyrosine and threonine sites, regulating nuclear transformation factors and controlling gene expression. The ERKs are particularly important to the EGF-, IGF- and VEGF-signaling pathways, including nuclear transcription factor regulation, ultimately controlling gene expression. Also, prolonged activation of ERK-2 and mitogenesis by EGF has been shown to require a functional IGF-1R (Swantek and Baserga, 1999). In TRAMP mice, not only is the IGF-1R present, its expression is significantly increased. Very importantly, we found that genistein in the diet down-regulated ERK-1 and ERK-2 expression in prostate tissue. Attenuation of ERK expression by genistein in the prostate may be a critical step in regulation of cell proliferation and prostate cancer chemoprevention.

The interaction of sex steroid and growth factor signaling pathways is thought to be critical in the process of development of hormone-responsive tissues, and for cancer causation in the prostate (Cunha et al., 1987). “Cross-talk” between signaling pathways can link processes occurring in different cellular compartments, increase regulatory diversity, and provide multiple opportunities for cell- and tissue-specific responses. Alterations in sex steroid and multiple growth signaling pathways in the TRAMP model support the concept of multiple disregulation contributing to carcinogenesis.

Soy and genistein have been shown to protect against prostate cancer in rats and mice (Pollard and Luckert, 1997; Zhou et al., 1999; Mentor-Marcel et al., 2001; Wang et al., 2002), reports that are consistent with Asian men consuming a traditional diet high in soy products having a low incidence of clinically manifested prostate cancer (Dhom, 1983; Zandet et al., 2001; Adlercreutz et al., 1994; Adelercreutz et al., 1995; Armstrong and Doll, 1995). Earlier studies have shown that genistein has estrogenic and anti-oxidant properties, inhibits S-alpha reductase, topoisomerase II, tyrosine kinases, angiogenesis, and cell multiplication in a dose-dependent manner, and induces cell differentiation (reviewed in Lamartiniere et al., 2002). Most of these investigations were carried out in vitro and/or used pharmacological doses of genistein. This is the first extensive report of genistein’s mechanism of action during the early phase of prostate cancer development using dietary and physiologic doses, the latter being based on similar blood levels in rodents (139–861 nM) (Dalu et al., 1998; Fritz et al., 1998; Mentor-Marcel et al., 2001; Wang and Lamartiniere, 2002) and in humans (276 nM) eating a traditional diet high in soy (Adlercreutz et al., 1993; Morton et al., 2002). Evidence of genistein reaching the prostate is that comparable concentrations of genistein (263–775 pmol/g tissue) are found in the target tissue and the blood (Dalu et al., 1998; Wang et al., 2002).

Down-regulation of the growth factor receptors, EGFR and IGF-1R, and of the downstream mitogen-activated protein kinases, ERKs-1 and 2, with genistein in the diet demonstrates a potential mechanism for prostate cancer chemoprevention. Future studies with genistein should investigate its potential to regulate the actual protein tyrosine kinases as opposed to the protein levels of these receptors. Selectivity of genistein action is supported by finding that AR, ER-alpha, ER-beta, ErbB2, EGF, TGF-alpha, IGF-1 and serum testosterone and dihydrotestosterone levels were not altered by dietary genistein. Furthermore, genistein’s ability to modulate, but not shut down, several signaling pathways is consistent with components of natural products protecting against the progression of cancer.

Acknowledgements

This research was supported by NIH 1 RO1 ES-11743-01 to CAL. The authors gratefully acknowledge the permission from Dr. Norman Greenberg (Department of Molecular and Cellular Biology, Baylor College of Medicine) to use the TRAMP model, and of our colleague, Dr. Ada Elgavish (Department of Genomics and Pathobiology, UAB) for furnishing us with TRAMP females to initiate our breeding colony.

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