Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols

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Development of effective chemopreventive agents against prostate cancer (CaP) for humans requires conclusive evidence of their efficacy in animal models that closely emulates human disease. The autochthonous *tr*ansgenic *a*denocarcinoma of the *m*ouse *p*rostate (TRAMP) model, which spontaneously develops metastatic CaP, is one such model that mimics progressive forms of human disease. Employing male TRAMP mice, we show that oral infusion of a polyphenolic fraction isolated from green tea (GTP) at a human achievable dose (equivalent to six cups of green tea per day) significantly inhibits CaP development and increases survival in these mice. In two separate experiments, the cumulative incidence of palpable tumors at 32 weeks of age in 20 untreated mice was 100% (20 of 20). In these mice, 95% (19 of 20), 65% (13 of 20), 40% (8 of 20), and 25% (5 of 20) of the animals exhibited distant site metastases to lymph nodes, lungs, liver, and bone, respectively. However, 0.1% GTP (wt/vol) provided as the sole source of drinking fluid to TRAMP mice from 8 to 32 weeks of age resulted in (*i*) significant delay in primary tumor incidence and tumor burden as assessed sequentially by MRI, (*ii*) significant decrease in prostate (64%) and genitourinary (GU) (72%) weight, (*iii*) significant inhibition in serum insulin-like growth factor-I and restoration of insulin-like growth factor binding protein-3 levels, and (*iv*) marked reduction in the protein expression of proliferating cell nuclear antigen (PCNA) in the prostate compared with water-fed TRAMP mice. The striking observation of this study was that GTP infusion resulted in almost complete inhibition of distant site metastases. Furthermore, GTP consumption caused significant apoptosis of CaP cells, which possibly resulted in reduced dissemination of cancer cells, thereby causing inhibition of prostate cancer development, progression, and metastasis of CaP to distant organ sites.

prostate cancer | chemoprevention | apoptosis

Prostate cancer (CaP) is an important public health problem, accounting for more than 184,000 estimated new cases and ≈40,000 deaths in the year 2000 alone in the United States (1). In the absence of satisfactory treatment options for CaP, chemoprevention could be an effective approach to reduce the incidence of the disease (2, 3). For a variety of reasons, there is greater emphasis on identifying naturally occurring dietary substances as cancer chemopreventive agents (3–6). Indeed, CaP is an excellent candidate disease for chemoprevention because it is typically diagnosed in elderly men; therefore, even a modest delay in the neoplastic development achieved through pharmacological or nutritional intervention could result in a substantial reduction in the incidence of the clinically detectable disease.

Green tea, a popular beverage consumed worldwide, has been shown to possess cancer chemopreventive effects in a wide range of target organs in rodent carcinogenesis models (4–8). The chemopreventive effects of green tea against tumorigenesis and tumor growth have been attributed to the biochemical and pharmacological activities of its polyphenolic constituents, most notably (−)-epigallocatechin-3-gallate, present therein (7–10). Epidemiological studies, although inconclusive, suggest a protective effect of tea consumption on some cancer types in humans (11, 12). Limited epidemiological studies indicate that people who consume tea regularly may have a lower risk of CaP (13, 14). Further, the Japanese and Chinese populations who regularly consume tea, especially green tea, have one of the lowest incidences of CaP in the world (15, 16). In addition, the incidence of CaP is also low in other Asian men, who consume a traditional low-fat diet and tea (15).

For relevance to humans, CaP chemoprevention studies should be conducted in animal models that closely emulate human disease and possess surrogate endpoint biomarkers for rapid evaluation of chemopreventive and/or therapeutic agents. Recent developments of genetically manipulated animals provide new scope for chemoprevention studies and for developing strategies to offset specific genetic susceptibilities to cancer (17, 18). The major advantage of these models is that in these animals, cancer arises in their natural tissue microenvironment and progresses through multiple stages, as does human cancer.

The *tr*ansgenic *a*denocarcinoma of the *m*ouse *p*rostate (TRAMP) is one such model for CaP that closely mimics progressive forms of human disease. In this model, expression of the SV40 early genes (T and t antigen, Tag) are driven by the prostate-specific promoter probasin that leads to cell transformation within the prostate (19). One-hundred percent of male TRAMP mice develop CaP without any chemical or hormonal treatment (19, 20). Further, CaP in this model progresses from prostatic intraepithelial neoplasia to histologic cancer to carcinoma metastatic to lymph nodes, lungs, liver, and bone sequentially over 12–28 weeks with median survival of 42 weeks (20). Recent studies from our laboratory (21) and elsewhere (22, 23) have established the utility of these mice for CaP chemoprevention studies. In the present study, we determined the consequence of oral infusion of a polyphenolic fraction isolated from green tea (hereafter referred to as GTP) on CaP development and progression in this model at a human-achievable dose. Our results demonstrate that oral infusion of GTP causes a significant inhibition in the development, progression, and metastasis of CaP to distant organ sites.

Materials and Methods

TRAMP Mice. The male and female TRAMP mice developed on a pure C57BL/6 background, heterozygous for the probasin-Tag transgene, were bred and maintained in the Animal Care Facility (School of Medicine, Case Western Reserve University). Transgenic males and the nontransgenic littermates were routinely obtained as [TRAMP C57BL/6 × FVB Breeder] F_1. The isolation of mouse-tail DNA and PCR-based screening assay were performed as described (19). All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Abbreviations: CaP, prostate cancer; GTP, green tea polyphenols; TRAMP, transgenic adenocarcinoma mouse prostate; GU, genitourinary; PCNA, proliferating cell nuclear antigen; IFG-I, insulin-like growth factor-I; IGFBP-3, insulin-like growth factor binding protein-3.

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Study Design for GTP Chemoprevention. GTP (>95% enriched preparation) was obtained from Natural Resources & Products (Charlottesville, VA). Chromatographic analysis of this mixture showed that it contains four major polyphenolic constituents: epigallocatechin-3-gallate (62%), epicatechin-3-gallate (24%), epigallocatechin (5%), epicatechin (6%), and caffeine (≈1%). The effect of GTP consumption on prostate carcinogenesis in TRAMP mice was studied in two separate experiments. Throughout each experiment, the animals had access to laboratory chow ad libitum. For each experiment, 20 male TRAMP mice of 8 weeks of age were equally divided into two groups. A freshly prepared solution of 0.1% GTP in tap water was supplied every Monday, Wednesday, and Friday to experimental animals as the sole source of drinking fluid for 24 weeks (GTP-infused group), whereas the control group of animals was supplied with the same tap water throughout the experiment (water-fed group). This feeding regimen has been used in mice in many prior chemoprevention studies from this and other laboratories (24, 25). The feeding protocol mimics an approximate consumption of six cups of green tea per day by an average adult human (25). Additional untreated and treated nontransgenic controls were also included in the study. After completion of the experiment, the animals from both experimental and control groups were killed by cervical dislocation, and the prostate gland was carefully removed under the microscope for further studies.

To investigate the effect of GTP consumption on tumor-free survival, in a third experiment, 36 male mice of 8 weeks of age were equally divided into two groups. The control group of animals was supplied with tap water, whereas animals in the experimental group were infused with 0.1% GTP (wt/vol) in drinking water exactly as in the first two experiments. Animals of both groups were monitored biweekly for tumor development by abdominal pelvic palpation and survival. For these studies, the animals were killed by CO_2 asphyxiation when obviously moribund.

Magnetic Resonance Imaging. Five animals each from both experimental and control groups were randomly selected and monitored for tumor growth and volume by MRI at 20 and 30 weeks of age. Imaging in these animals was performed by using a whole body 1.5 Telsa imager with 25 mT/m gradient strength, 150-ms rise time, and a custom-built 1-cm small animal receiver coil. T1-weighted (TR/TE = 400 ms/14 ms), double-echo T2-weighted (TR/TE = 1900 ms/20, 84 ms), and CISS T2-weighted (TR/TE/Flip angle = 12.3 ms/5.9 ms per 70°) gradient echo volumetric scans with a field of view between 2 and 5 cm and in plane resolution of 78–200 μm were obtained with a slice thickness of 500-2000 μm. Images were filmed for subjective analysis and/or transferred to a free-standing imaging workstation for volumetric analysis of prostate tumor.

Insulin-Like Growth Factor-I (IGF-I) and Insulin-Like Growth Factor Binding Protein-3 (IGFBP-3) Assay. Serum was separated from the whole blood obtained from the retro-orbital venous plexus with heparinized capillary tubes, and IGF-1 and IGFBP-3 levels were determined by commercially available ELISA kits (Diagnostic Systems Laboratories, Webster, TX) according to the manufacturer's protocol. The sensitivity of the assay was 0.04 ng/ml, and virtually no crossreactivity was visible with other members of the group.

Preparation and Analysis of Tissue. At the time of sacrifice, the lower GU tract, including the bladder, testes, seminal vesicles, and prostate, was removed *en bloc*. The GU wet weight was recorded to the nearest 0.01 g. Tissues collected at necropsy were fixed in 10% (vol/vol) phosphate-buffered formalin for 12 h and then transferred to 70% ethanol before standard tissue processing. Sections of the prostate (4 μm) were cut from paraffin-embedded tissues and mounted on ProbeOn-Plus slides (Fisher Scientific). Sections were stained with hematoxylin and eosin and were reviewed by light microscopy for the presence of CaP. Distant site metastases were examined as described (20, 21).

Immunoblotting and Immunohistochemistry. Prostate glands were removed from the animals and processed for immunoblotting and immunohistochemistry as described (21) by using appropriate antibodies for PCNA, SV-40 T-antigen, and β-actin obtained from Santa Cruz Biotechnology.

Image Analysis. Sections were visualized on a Zeiss-Axiophot DM HT microscope. Images were captured with an attached camera linked to a computer. Images and figures were composed by using ADOBE PHOTOSHOP 5.5 (Adobe Systems, Mountain View, CA).

Immunofluorescence Analysis and Apoptosis Detection. Four-micrometer-thick sections were cut from paraffin-embedded tissues. Immunofluorescence was performed by using M30 CytoDEATH antibody (Boehringer Mannheim) with a fluorescence microscope (Axiophot, Zeiss). Scoring of apoptotic cells in these sections was done by using the OPTIMAS 6 software program (Optimas, Bothell, WA). Apoptotic index (%) was calculated by dividing the number of apoptotic cells (fluorescence positive) by the total number of cells counted per cross-section of a sample of the prostate.

Apoptosis by ELISA. Apoptosis was also assessed by Cell Death Detection ELISA^{PLUS} kit (Roche Molecular Biochemicals) according to the manufacturer's protocol.

Statistical Analysis. All statistical analyses were carried out with STATISTICAL ANALYSIS SYSTEM software (SAS Institute, Cary, NC) and P values less than 0.05 were considered significant. The Kaplan–Meier method was used to estimate survival, and differences were analyzed by the log-rank test.

Results

MRI Analysis of TRAMP Mice Infused with GTP. To assess the effect of GTP infusion in TRAMP mice on prostate carcinogenesis, we first measured the prostate growth by using MRI. MRI is considered a powerful tool for imaging internal organs and for diagnosis of certain cancer types in humans (26). We used this technique for monitoring the effect of GTP infusion on CaP development and progression in TRAMP mice (Fig. 1*A*). As shown by MRI scans, water-fed TRAMP mice demonstrated the presence of prostate tumor at 20 weeks of age, when the tumor was also detectable by abdominal pelvic palpation (Fig. 1*Ac*). As evident by MRI, at 30 weeks the water-fed TRAMP mice were found to have fully developed tumor (Fig. 1*Ad*). In sharp contrast, 0.1% GTP infusion to TRAMP mice was found to result in significant prevention or delay in prostate cancer development (Fig. 1*A e* and *f*). Compared with water-fed TRAMP mice, GTP-infused animals exhibited a marked reduction in the growth of prostate tumor at 20 weeks of age (44% inhibition) and at 30 weeks of age (42% inhibition), respectively, as observed by the volumetric analysis of the prostate (Fig. 1*B*). This was also evident from abdominal pelvic palpation.

Effect of GTP Infusion on Serum IGF-I and IGFBP-3 Levels. In clinical practice, to monitor CaP progression in humans, levels of prostate-specific antigen, IGF-I, and IGFBP-3 in serum are determined. Because in TRAMP mice, like other mice, the murine equivalent of prostate-specific antigen has not yet been identified and/or isolated, we monitored the effect of GTP infusion on growth and development of CaP by determining the levels of IGF-I and IGFBP-3 in serum. Recent studies have demonstrated that elevated levels of IGF-I with concomitant lowering of IGFBP-3 levels in serum is associated with CaP risk and could be excellent predictors of CaP progression in humans (27). We monitored serum IGF-I and IGFBP-3 after 8, 16, and 24 weeks of GTP infusion. For comparison, these levels were also measured in nontransgenic littermates that did not develop CaP. As shown in Fig. 2*A*, compared with nontransgenic animals, increasing levels of IGF-I were

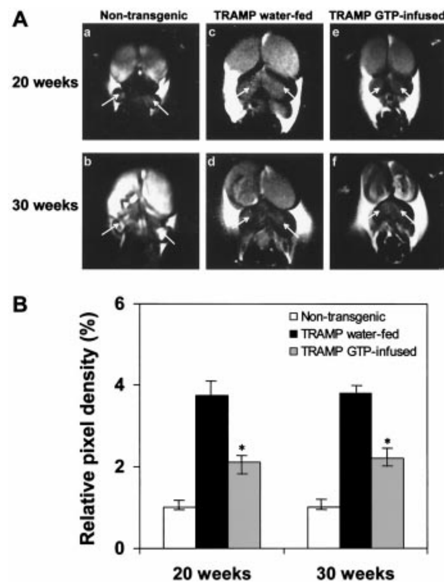

Fig. 1. Effect of GTP infusion on prostate cancer development in TRAMP mice evaluated by longitudinal MRI analysis. (*A*) MRI was used to assess the growth of primary tumor in TRAMP mice followed longitudinally in individual animal. Details are described in *Materials and Methods*. A marked reduction in prostate development was observed in these mice after 0.1% (wt/vol) GTP infusion between 8 to 32 weeks. Representative images of nontransgenic (panels a and b) and water-fed (*c* and *d*) TRAMP mice and GTP-infused TRAMP mice (*e* and *f*) are shown here at 20 (*a*, *c*, and *e*) and 30 (*b*, *d*, and *f*) weeks of age. Arrows indicate prostate. (*B*) Volumetric analysis of the TRAMP mice prostate after GTP infusion. The data are represented as percent change in relative pixel density observed at 20 and 30 weeks of age where nontransgenic mice prostate is taken as control. Values represent mean ± SE of five animals. *, $P < 0.001$ compared with TRAMP water-fed mice.

observed in water-fed TRAMP mice that were significantly lowered in GTP-infused mice. In contrast, serum IGFBP-3 levels, the major binding protein for IGF-I, were lower in water-fed TRAMP mice and were significantly restored in GTP-infused mice (Fig. 2*B*).

Effect of GTP Infusion on Prostate Tumorigenesis. GTP infusion for 24 weeks to TRAMP mice did not exhibit any symptoms of toxicity or apparent signs of ill health. No significant affect was observed in the body weight profile in nontransgenic littermates infused with 0.1% GTP when compared with the water-fed nontransgenic controls. However, TRAMP mice receiving GTP infusion registered a slight decrease in body weight (≈5%) compared with their corresponding control group (data not shown). This difference may be the result of more tumor growth and hyperproliferation of the accessory sex organs in the abdominal region that occurs in control TRAMP mice.

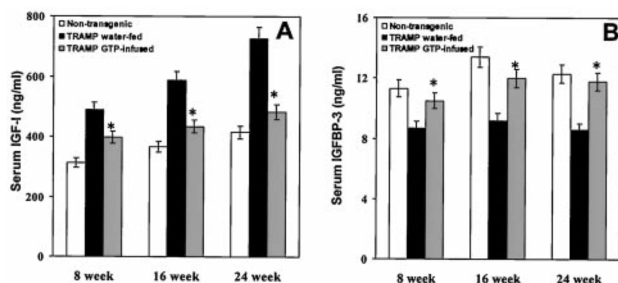

Fig. 2. Effect of GTP infusion on serum levels of IGF-I (*A*) and IGFBP-3 (*B*) in TRAMP mice. Eight-week-old TRAMP mice were infused with 0.1% GTP (wt/vol) as sole source of drinking fluid for 24 weeks. Blood was withdrawn at 8, 16, and 24 weeks after GTP infusion, and serum IGF-I and IGFBP-3 levels were analyzed by enzyme-linked immunoabsorbant assay. Details are described in *Materials and Methods*. A marked inhibition in serum IGF-I and restoration in serum IGFBP-3 levels were observed after GTP infusion. Values represent mean ± SE of 10 animals. *, $P < 0.001$ compared with TRAMP water-fed mice.

To investigate the effect of GTP infusion on CaP growth and progression in TRAMP mice, in two separate experiments, 0.1% GTP was supplied to the animals as the sole source of drinking fluid for 24 weeks starting at the age of 8 weeks. As summarized in Table 1, in the first experiment, as expected all of the 10 mice in the water-fed group developed severe CaP with marked local invasiveness in the abdominal region, which was assessed by abdominal pelvic palpation and MRI. In contrast, only 3 of the 10 (30%) GTP-infused TRAMP mice developed palpable tumors. Similarly, in the repeat experiment, all 10 mice in the control group developed fully malignant and palpable tumors, whereas in the GTP-infused group only 4 of the 10 (40%) animals exhibited palpable tumors. Importantly, in these GTP-infused mice, the invasiveness of CaP was much less as compared with water-fed mice. Further, we studied the effect of GTP infusion on the metastases to different site organs. The cumulative data at the termination of the experiment (32 weeks of age) from 20 animals in the water-fed group showed 100% invasive tumors, which metastasize to lymph (95% animals), lungs (65% animals), liver (40% animals), and bone (25% animals), respectively. In sharp contrast, in the GTP-infused group, none of the 20 mice exhibited metastases to any of the distant organs studied. Further, to determine the effect of GTP infusion on CaP in TRAMP mice, gross biological indicies (wet weights) were used to assess the tumorigenicity (Table 1). As observed visibly, GTP infusion resulted in complete absence of hyperplasia in the GU apparatus, especially in the seminal vesicles. An important observation in this experiment was that GTP infusion resulted in a significant decrease in prostate weight (≈64%) and GU weight (≈72%) compared with the water-fed TRAMP group (Table 1; Fig. 3 *A* and *B*).

Effect of GTP Infusion on Prostate Histology. Histological examination of a typical TRAMP mouse prostate tissue at 32 weeks of age revealed prostatic neoplasia characterized by a pronounced proliferation of papillary structures lined by pseudostratified neoplastic cells with marked hyperchromasia and scattered apoptosis. In contrast, the experimental group of GTP-infused mouse exhibited glands lined by uniform columnar cells with dispersed chromatin and minimal luminal infoldings. GTP infusion also resulted in a significant increase in the number of apoptotic cells in the prostate (Fig. 3 *C* and *D*).

Effect of GTP Infusion on Proliferation Marker. We next determined the effect of GTP infusion on cellular proliferation in prostate as assessed by following the ubiquitous and molecular proliferation marker PCNA. PCNA serves as a requisite auxiliary protein for DNA polymerase δ-driven DNA synthesis and is cell cycle regulated (28, 29). GTP infusion for 24 weeks resulted in a marked reduction in PCNA protein expression in the prostate of TRAMP mice compared with the water-fed group (Fig. 4*A*). These results were further confirmed by immunohistochemical analysis of the tissue (Fig. 4*B*). Further, the effect of GTP infusion on Tag expression (T-antigen) was determined in prostates of TRAMP mice. GTP infusion did not result in any significant alteration in the levels of Tag protein expression, and they are detectable in both GTP-infused and water-fed groups (data not shown).

Effect of GTP Infusion on the Extent of Apoptosis. Because green tea is known to induce selective apoptosis in cancer cells (30), we hypothesized that the observed inhibition of prostate tumorigenesis by GTP infusion is mediated by increased apoptosis of cancerous cells. To test our hypothesis, we used multiple approaches of apoptosis determination. In our first approach, ELISA was per-

Table 1. Effect of oral infusion of GTP on the morphology of prostate and genito-urinary (GU) weight in TRAMP mice and their nontransgenic littermates

Group*	Number of animals	Palpable tumor†	Animals with metastasis‡ Lymph	Lungs	Liver	Bone	Prostate weight mg	GU weight g
Experiment 1								
Non-TG control	10	0/10	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	17.4 ± 1.4	0.45 ± 0.05
TRAMP water-fed	10	10/10	9/10 (90%)	7/10 (70%)	4/10 (40%)	3/10 (30%)	68.2 ± 8.4	3.76 ± 0.48
TRAMP GTP-infused	10	3/10	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	32.6 ± 2.8§	1.08 ± 0.12§
Experiment 2								
Non-TG control	10	0/10	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	15.6 ± 1.2	0.52 ± 0.04
TRAMP water-fed	10	10/10	10/10 (100%)	6/10 (60%)	4/10 (40%)	2/10 (20%)	83.7 ± 11.6	4.36 ± 0.58
TRAMP GTP-infused	10	4/10	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	36.2 ± 3.8§	1.20 ± 0.14§
Cumulative								
Non-TG control	20	0/20	0/10 (0%)	0/20 (0%)	0/10 (0%)	0/10 (0%)	16.5 ± 1.8	0.49 ± 0.05
TRAMP water-fed	20	20/20	19/20 (95%)	13/20 (65%)	8/20 (40%)	5/20 (25%)	76.0 ± 10.8	4.06 ± 0.64
TRAMP GTP-infused	20	7/20	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	27.5 ± 3.2§	1.14 ± 0.16§

The data represented in each experiment are the mean ± SE of 10 mice.

*Mice (8 weeks of age) received plain drinking water (control group) or GTP (0.1% wt/vol) infusion in drinking water for 24 weeks. At the age of 32 weeks, the animals were killed and studied for prostate tumorigenesis and metastases.

†Prostate tumor was assessed by abdominal pelvic palpation.

‡Metastases in the lymph, liver, and bone were examined under the microscope, whereas metastasis in lungs was examined by the India ink method. Details are described in *Materials and Methods.*

§, $P < 0.001$, water-fed, control TRAMP compared with GTP-infused TRAMP, Student's t test.

formed for detection of apoptosis. As shown in Fig. 5*A*, GTP infusion for 24 weeks resulted in a significant increase in apoptosis in the prostate of TRAMP mice. In the second approach, these results were further confirmed by immunofluorescence detection in the prostate tissue by M30 CytoDEATH antibody that binds to a caspase-cleaved formalin-resistant epitope of the cytokeratin 18 cytoskeletal protein, a marker for apoptosis (Fig. 5*B*). A significant increase in apoptotic index (2.12 ± 0.1 vs. 27.7 ± 3.2% control vs. GTP-infused) was observed in GTP-infused mice prostate compared with water-fed TRAMP mice.

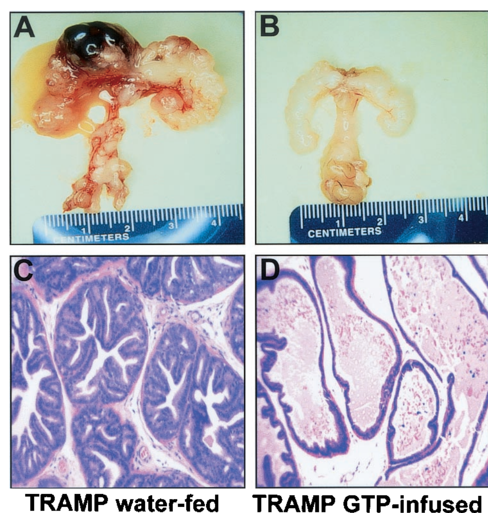

Fig. 3. Effect of GTP infusion on GU apparatus and prostate histology in TRAMP mice. (*A*) Photograph of typical GU apparatus of TRAMP mice exhibiting hyperproliferation. (*B*) GU apparatus of TRAMP mice with 0.1% GTP infusion (wt/vol) for 24 weeks. A marked decrease in GU weight and volume was observed in TRAMP mice after GTP infusion. (*C*) Histologic examination of a typical TRAMP mouse prostate at 32 weeks of age revealed moderately differentiated neoplastic cells with extensive cribriform structures, marked thickening, remodeling, and hypercellularity of the fibromuscular stroma. (Magnification, ×40.) (*D*) GTP infusion (0.1%, wt/vol) to TRAMP mice resulted in a marked reduction in epithelial stratification and cribriform structures, and the glands remained simple without epithelial thickening or surface complexity. (Magnification, ×40.) Representative figures are shown.

Effect of GTP Infusion on Tumor-Free Survival and Survival Probability in TRAMP Mice. Extended tumor-free survival and survival probability is the most desirable effect of any chemoprevention regimen. Therefore, in the next series of experiments, we evaluated whether or not GTP infusion leads to tumor-free survival and prolongs life expectancy of TRAMP mice. Our data indicated (Fig. 6) that continuous GTP infusion to TRAMP mice actually resulted in the prolongation of the lifespan of these mice. The continuous GTP infusion to TRAMP mice significantly increased the tumor-free survival ($P < 0.001$, log-rank test) inasmuch as 50% of the animals remain tumor-free up to 40 weeks of age (Fig. 6*A*). In addition, GTP-infused TRAMP mice exhibited a significant increase (70% higher) in life expectancy ($P < 0.001$, log-rank test) with a median survival of 68 weeks compared with the 42 weeks in water-fed TRAMP mice (Fig. 6*B*).

Discussion

Limited available options for the treatment of CaP and its increasing incidence have spurred the search for novel preventive ap-

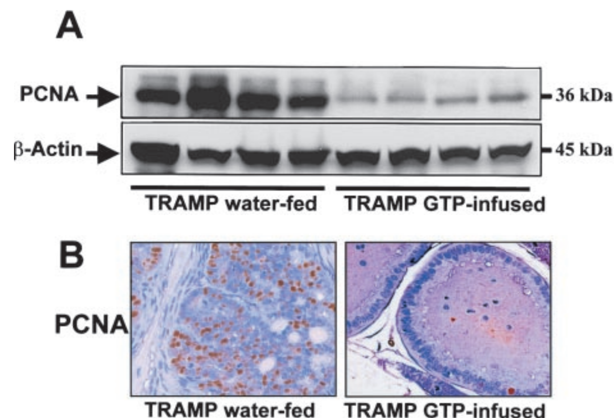

Fig. 4. Effect of GTP infusion on the protein expressions of PCNA in the TRAMP mice prostate. (*A*) Protein expression of PCNA by Western blot. (*B*) Immunohistochemical analysis is shown. In water-fed TRAMP mice, an extensive PCNA staining was observed in the nuclei. GTP infusion (0.1%, wt/vol) resulted in the marked reduction in the protein expression of PCNA in these mice. Equal loading of the protein in the lanes was confirmed by stripping the membrane and reprobing it with β-actin. Details are described in *Materials and Methods*.

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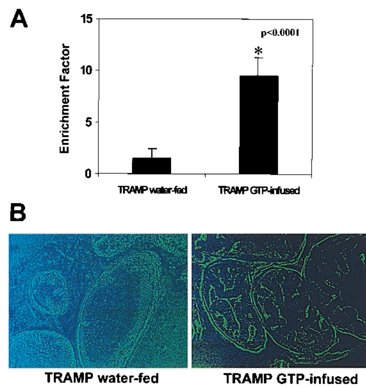

Fig. 5. Effect of GTP infusion on the extent of apoptosis in the TRAMP mice prostate. (*A*) Apoptosis was determined by cell-death ELISA[PLUS] as per vendor's protocol. Data are expressed as Enrichment factor. Values represent mean ± SE of 10 animals. *, $P < 0.0001$ compared with water-fed TRAMP mice. (*B*) Immunofluorescence detection of prostate tissue in water-fed and GTP-infused TRAMP mice by M30 CytoDEATH antibody that binds to caspase-cleaved epitope of the cytokeratin 18 cytoskeletal protein, a marker of apoptosis. A marked increase in M30 fluorescence was observed after 0.1% GTP infusion (wt/vol), compared with water-fed TRAMP mice. A representative figure from each group at ×80 magnification is shown. Details are described in *Materials and Methods*.

proaches for the management of this disease. Chemoprevention by the use of dietary agents or synthetic compounds could be one such strategy that may block the neoplastic inception or delay disease progression (4–6). Because CaP is typically diagnosed in men aged 50 years and older, even a slight delay in the onset and subsequent progression of the disease through the use of chemopreventive agent(s) could have important health benefits. Ideally, the efficacy of such chemopreventive agents should be verified in animal models that emulate human disease before recommending their use for humans. The most notable implication of our work is that oral infusion of a human-achievable dose of green tea results in significant inhibition in development and progression of CaP along with increased survival in an animal model that emulates human disease. These data, therefore, suggest that green tea consumption may have inhibitory effects on prostate carcinogenesis in humans.

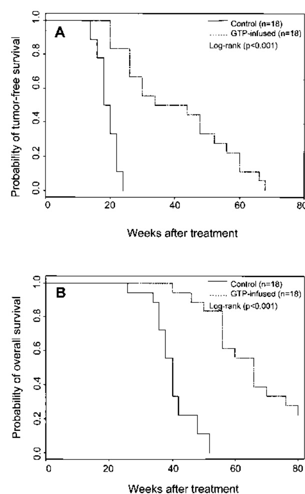

Fig. 6. Effect of GTP infusion on tumor-free survival (*A*) and survival probability (*B*) in TRAMP mice. A significant increase in tumor-free survival ($P < 0.001$, log-rank test) and survival probability ($P < 0.001$, log-rank test) in GTP-infused TRAMP mice was observed.

Epidemiological studies, although not conclusive, have suggested the protective role of green tea against CaP development (10–14). Recent laboratory studies have indicated that green tea and its polyphenolic constituents impart inhibitory effects on the activities of many enzymatic, metabolic, and signaling pathways that have relevance to cancer development and progression (31–36). A number of studies have shown the growth-inhibitory effects of green tea against many animal tumor bioassay systems including lung, skin, and forestomach (37–40). Studies from our laboratory have shown that green tea polyphenols show promising testosterone-mediated cell growth inhibitory effects and anchorage-independent growth of human prostate carcinoma cells LNCaP *in vitro* as well as GTP-infused Cpb:WU rats and C57BL/6 mice *in vivo* (41). Cell culture studies from this laboratory (42) and elsewhere (43–45) have shown that GTP and epigallocatechin-3-gallate, the major polyphenolic constituent of green tea, inhibit growth of several types of human CaP cells. Our studies in cell culture have shown that (−)-epigallocatechin-3-gallate was effective in imparting growth inhibition cell-cycle deregulation and apoptosis of both androgen-sensitive as well as androgen-insensitive human CaP cells (46). Notably, (−)-epigallocatechin-3-gallate has been shown to cause growth inhibition and regression of human prostate tumors in athymic nude mice (47). Earlier *in vivo* studies have not assessed the effect of green tea infusion on CaP chemoprevention in a prostate carcinogenesis model, partly because of the lack of appropriate animal models that could mimic the progressive forms of human prostatic disease. The TRAMP model possesses similarity to human disease in the development and progression to metastatic CaP. Recent studies from our laboratory and elsewhere have established the utility of TRAMP mice for CaP chemoprevention studies (21–23). In the present study, we assessed the chemopreventive potential of GTP against prostate carcinogenesis in the TRAMP model. Our results suggest that GTP infusion was significantly effective in inhibiting CaP development and completely abolished distant site metastases. Prior published studies have shown that polyphenols present in green tea and caffeine possess cancer chemopreventive effects (48). Although the role played by caffeine in observed CaP chemopreventive effects could not be ruled out, we believe that the observed effects in this study may be because of the polyphenolic constituents rather than caffeine because of its presence in low concentration (≈1%) in the GTP mixture.

In the present study, we have used multiple noninvasive techniques to monitor the chemopreventive potential of GTP for the prevention of CaP. Our first effort was to use the noninvasive technique of MRI for the monitoring of CaP development in these mice. Our studies demonstrate for the first time that MRI may be used as an efficient tool for assessing the effectiveness of a chemopreventive agent against CaP in animals. Next, we found that GTP infusion to TRAMP mice caused significant inhibition of serum IGF-I and restoration of serum IGFBP-3 levels. This is consistent with recent epidemiological studies implicating deregulation of the IGF axis in CaP progression (49) and showing that serum IGF-I could be a better predictor of CaP risk than serum prostate-specific antigen (50). Our results are also consistent with the previous observation where prostate-specific IGF-I was found to be increased during prostate cancer progression in TRAMP mice (51). This is an important observation because recent studies have shown that high circulating levels of IGF-I are associated with increased risk of several common cancers, including those of the breast, prostate, lung, and colorectum (49). The level of IGF-binding protein (IGFBP-3), a major IGF-I binding protein in serum that, in most situations, suppresses the mitogenic action of IGF-I, has been shown to be inversely associated with the risk of these cancers. Taken together, the IGF axis, particularly IGF-I and IGFBP-3, could be developed as endpoint biomarkers for monitoring CaP chemoprevention.

Increased proliferation of CaP cells ultimately results in tumor invasion and metastasis leading to significant mortality in humans (52). Unfortunately, over 60% of the newly diagnosed cases of CaP develop metastatic forms of the disease (53). In the present study, GTP was found to be effective in completely abolishing distant site metastases and cellular proliferation as shown by the proliferation markers *viz*. PCNA. An important observation of the study is that GTP infusion to TRAMP mice did not alter the expression of t/T-antigen, as they are readily detectable in both GTP-infused and water-fed groups. These results confirm that mechanism of CaP inhibition by GTP infusion was not through down-regulation of the transgene.

Another important observation of our study was a marked induction of apoptosis in the prostate by GTP infusion. In recent years, apoptosis has gained much attention as a preferential way of eliminating the unwanted cancerous cells (42–46, 53). At present, only a few agents are known to possess the potential for selective elimination of cancer cells (ref. 54 and references therein). Recent studies from our laboratory have shown that GTP selectively induces apoptosis of various human carcinoma cells without affecting the normal cells (42). This observation has been verified from many laboratories worldwide (43–46). Our results show that GTP infusion to TRAMP mice results in massive apoptosis of neoplastic prostatic cells and further suggest that GTP could be an effective agent for a preferential elimination of cancerous and precancerous cells via a programmed cell death. Based on our data, we believe that the observed inhibition of CaP tumorigenesis and subsequent metastasis by GTP infusion is caused by selective apoptotic death of cancerous cells; however, further studies are needed to substantiate this suggestion.

Studies indicate that 23% of CaP patients undergoing surgical intervention still show the evidence of disease progression (52). Once the disease becomes hormone refractory, most treatment is palliative and the median lifespan of these patients is less than 12 months. Therefore, agents that may prolong the survival and quality of life of such patients could have immediate clinical importance. In the present study, GTP infusion to TRAMP mice resulted in a significant increase in tumor-free survival and survival probability. Based on these results, we suggest that regular consumption of green tea may prolong life expectancy and quality of life in CaP patients.

In summary, our results suggest that GTP, a mixture of various polyphenols inhibit the growth and progression of CaP in TRAMP mice. Our data support the epidemiologic reports that green tea may reduce CaP risk in humans. It is important to emphasize that during the course of CaP development and progression the effectiveness of green tea is not yet certain in humans. However, based on the present study, it is tempting to suggest that green tea in general and polyphenols present therein may prove to be a useful supplement in the prevention or slower progress of CaP in humans.

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