Androgen-independent Prostate Cancer Progression in the TRAMP Model

Jeffrey R. Gingrich,2 Roberto J. Barrios, Michael W. Kattan, Hyun S. Nahm, Milton J. Finegold, and Norman M. Greenberg3

Scott Department of Urology [J. R. G., M. W. K., N. M. G.], Department of Pathology [R. J. B.], Information Technology Program [M. W. K.], and Department of Pathology [M. J. F.], Texas Children's Hospital, and Department of Cell Biology, Baylor College of Medicine [H. S. N., N. M. G.], Houston, Texas 77030

Abstract

We previously established the autochthonous transgenic adenocarcinoma mouse prostate (TRAMP) model to facilitate characterization of molecular mechanisms involved in the initiation and progression of prostate cancer. TRAMP mice display high grade prostatic intraepithelial neoplasia or well-differentiated prostate cancer by 10–12 weeks of age. To test the hypothesis that molecular events leading to androgen independence and metastasis can occur early in the natural history of prostate cancer yet remain silent until selective pressures such as androgen deprivation are applied, we have examined the consequences of castration on the initiation and progression to metastatic prostate cancer in TRAMP mice. Cohorts were castrated at 12 weeks of age and sacrificed at 18 (T12/18) or 24 (T12/24) weeks of age, and the development of primary cancer and metastatic disease was compared to noncastrated (T18 and T24), respectively. In addition, T12/24 GU weight was significantly greater than T18 and T24, respectively. All tumors that developed in castrated mice were poorly differentiated in contrast to 27% in noncastrated controls. Although castration significantly decreased GU tumor burden, overall progression to poorly differentiated and metastatic disease was not ultimately delayed. These results demonstrate that prostate cancer in the TRAMP model is heterogeneous with respect to androgen dependence as early as 12 weeks of age; therefore, early androgen ablation may have a variable impact on progression in an individual mouse. Further analysis of this prostate cancer model to identify specific molecular mechanisms that determine androgen sensitivity may facilitate future initiation of appropriate individualized hormonal therapy for the management of human prostate cancer.

Introduction

Androgen action is intimately associated with the normal growth, development, and function of the prostate gland, and in addition, most prostate cancer is believed to be initially androgen dependent. Because of the work of Huggins and Hodges (1) in the early 1940s, androgen ablation continues to be the most effective therapy available today for the treatment of recurrent, locally advanced, and metastatic prostate cancer. Although the majority of patients (80–90%) will have a beneficial response to androgen ablation, the specific indications (biochemical or clinical progression), the timing (early, late, or intermittent), and the impact of androgen ablation on overall cancer-specific survival remain controversial (2–5). In fact, androgen ablation may actually provide selective pressure for the growth and metastasis of androgen-independent forms of prostate cancer. Unfortunately, it has been difficult to systematically investigate the impact of androgen ablation on clinical outcome in a comprehensive manner for several reasons, including the long natural history of the disease, the heterogeneity of both the disease and various patient populations, and differing patient and physician perspectives regarding the indications for and means of androgen ablation in the treatment of prostate cancer. However, with recent advances in transgenic technology, novel animal models that reproducibly develop spontaneous prostate cancer can be used as a paradigm to directly study the impact of androgen ablation on the progression of prostate cancer to the state of androgen independence.

We and our collaborators have developed a system based on the rat probasin promoter to target heterologous gene expression specifically to the mouse prostate in a developmentally and hormonally regulated fashion (6). Subsequently, a recombinant PB-SV40 Tag4 construct was assembled to induce spontaneous neoplastic epithelial transformation by abrogating functional expression of the p53 and Rb tumor suppressor genes. The PB-Tag transgene was introduced into the C57BL/6 inbred strain of mice, and line 8247 was used to establish the TRAMP model (7, 8). In TRAMP mice, expression of the PB-Tag transgene is spatially restricted to the dorsolateral lobes of the prostate, and the temporal pattern of transgene expression correlates with sexual maturity. TRAMP mice reproducibly display evidence of high-grade PIN and/or well-differentiated prostate cancer by 10–12 weeks of age. Ultimately, TRAMP mice spontaneously develop invasive primary tumors that routinely metastasize to the lymph nodes and lungs and less frequently metastasize to the spinal column, kidneys, and adrenal glands (8). By the time TRAMP males are 30–36 weeks of age, 100% will display primary tumors and metastatic disease. In the present study, we investigate the consequences of castration on the initiation, progression, and metastasis of prostate cancer in the TRAMP model. These studies were also designed to test the hypothesis that molecular events leading to androgen independence and metastasis can occur early in the natural history of prostate cancer yet may remain silent until selective pressures such as androgen ablation by chemical and/or physical means are applied. We now report that although androgen ablation at 12 weeks of age significantly decreased median primary prostate tumor burden, overall progression to poorly differentiated and metastatic prostate cancer was not ultimately delayed.

Materials and Methods

Transgenic Animals. Previously described male and female TRAMP mice, heterozygous for the PB-Tag transgene, were maintained in a pure C57BL/6 background (Harlan Sprague Dawley, Inc., Indianapolis, IN). Transgenic males for these studies were routinely obtained as [TRAMP C57BL/6 × FVB Breeder]F1, offspring (8). Isolation of mouse-tail DNA and PCR-based screening assays were performed as described previously (7). Randomly

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2 Present address: Department of Urology, University of Tennessee at Memphis, 956 Court Avenue, H220, Memphis, TN 38163.
3 To whom requests for reprints should be addressed, at Department of Cell Biology, Baylor College of Medicine, One Baylor Plaza M636, Houston, TX 77030.
4 The abbreviations used are: Tag, T antigen; TRAMP, transgenic adenocarcinoma mouse prostate; PIN, prostatic intraepithelial neoplasia; GU, genitourinary.
assigned cohorts of TRAMP mice were sacrificed at 12, 18, and 24 weeks [T12 (12–13 weeks), T18 (16–18 weeks), and T24 (22–27 weeks), respectively] or age. Nontransgenic littermates were used as controls (C12, C18, and C24, respectively). Additional cohorts of TRAMP and control mice were anesthetized and castrated through a scrotal approach at 12 weeks of age and monitored until sacrifice at 18 (T12/18 and C12/18) or 24 (T12/24 and C12/24) weeks of age. All experiments were conducted using the highest standards for humane care in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

**Preparation and Analysis of Tissues.** At the time of sacrifice, the lower GU tract, including the bladder, seminal vesicles, and prostate, were removed *en bloc*. The GU wet weight was recorded to the nearest 0.1 g. Tissues collected at necropsy were routinely fixed in 10% (v/v) phosphate-buffered formalin for 6 h and then transferred to 70% ethanol prior to standard tissue processing. Sections (3–5 μm) were cut from paraffin-embedded tissues and mounted on ProbeOn-Plus slides (Fisher Scientific, Houston, TX). Routine sections were stained with H&E. Histological sections of the prostate were reviewed by light microscopy for the presence of prostate cancer and classified as PIN (epithelial stratification with occasional mitotic figures or cribriform pattern), well-differentiated (multiple epithelial mitotic figures and apoptotic bodies, invasive glands with stromal hypercellularity), moderately differentiated (many acini completed filled with tumor yet still forming some glandular structures), or poorly differentiated (sheets of malignant cells with little or no glandular formation) prostate cancer, or atrophic glands only (no identifiable tumor deposits). Peri-aortic lymph nodes were routinely obtained for microscopic examination at the time of necropsy from mice with grossly visible primary tumor burden and any otherwise enlarged lymph nodes. In addition, random samples of lungs and any other grossly abnormal tissues were also submitted for histological examination. Immunohistochemical analysis for the detection of the Tag oncoprotein was performed on representative sections of tumors and metastases, as described previously, with minor modifications (9). Antigen retrieval was performed by steamer heating of histological sections in Antigen Unmasking Solution for 25 min according to the manufacturer’s protocol (Vector Laboratories, Inc., Burlingame, CA). Histological sections were counterstained with hematoxylin.

**Data Analysis.** Nonparametric ANOVA was performed on the response-variable GU weight, considering time until sacrifice and presence of the transgene as independent variables. Because of a significant interaction, pairwise multiple comparisons were performed using the least squares means method on rank transformed data. Nonparametric ANOVA was also used to test whether the combination of castration and time of sacrifice affected the GU weight of TRAMP mice. Multiple comparisons were used to test whether pathology was affected by castration after controlling for time. Fisher’s exact test was used to determine whether the incidence of lymph node metastasis in TRAMP mice was affected by castration. All statistical analyses were performed using SAS statistical software (SAS version 6.12; SAS Institute, Cary, NC).

**Results and Discussion**

**Impact of Transgene Expression on GU Wet Weight.** A total of 107 mice were stratified among 10 different groups according to presence of the transgene, weeks of castration, and time of sacrifice as shown in Fig. 1A. At the time of sacrifice, GU wet weights were determined in 103 of 107 mice as a measure of primary tumor burden. As shown in Fig. 1B, the median GU weight at 12 weeks of age was 0.4 g for the C12 mice and 0.5 g for the T12 mice. By 18 weeks of age, the median GU wet weight was 0.6 g for the C18 mice and 0.9 g for the T18 mice. At 24 weeks of age, the median GU wet weight remained stable at 0.5 g for the C24 mice, whereas TRAMP GU wet weight increased further to 1.7 g (T24). Nonparametric ANOVA showed that time and presence of the transgene each affected GU weight (*P* = 0.0001 and *P* = 0.0001, respectively) and that the interaction between them was significant (*P* = 0.0006). Multiple comparisons with least squares means indicated that TRAMP mice had significantly larger GU weights than nontransgenic mice at each time point (*P* = 0.0271 at 12 months, *P* = 0.0001 at 18 months, and *P* = 0.0001 at 24 months).

**Impact of Castration on GU Wet Weight.** Cohorts of TRAMP mice castrated at 12 weeks of age were followed until 18 or 24 weeks of age. At 18 weeks of age, the median GU weight of 0.1 g for T12/18 TRAMP mice was significantly less than that of noncastrated T18 TRAMP mice (*P* < 0.05). Similarly, at 24 weeks of age, although several mice displayed substantial primary tumor burden, the median GU weight of 0.5 g for T12/24 TRAMP mice was significantly less than that of noncastrated T24 TRAMP mice (*P* < 0.05). In addition, the median GU wet weight of the T12/24 group was signif-
significantly greater than that of the T12/18 group (P < 0.05). Therefore, although the majority of primary tumors appear to be initially responsive to androgen deprivation, the outgrowth of androgen-independent tumors in several mice was observed to be quite rapid. These results support the hypothesis that prostate cancer in the TRAMP model is a heterogeneous disease with respect to androgen dependence as early as 12 weeks of age and that androgen ablation demonstrates a variable impact on prostate cancer progression. These observations in an autochthonous transgenic model are, therefore, in general agreement with the findings of Isaacs and Coffey (11), who used the transplantable Dunning R-3327-H rat model to demonstrate that androgen-independent prostate cancer progression reflects the basic heterogeneity of androgen-dependent and -independent cells within the primary tumor. Taken together, these findings oppose the concept that androgen-independent cells arise by induced adaptation to an androgen-depleted environment and support the hypothesis that androgen ablation provides strong selective pressure for the growth of the androgen-independent population of cells. It should be possible in future studies to apply this experimental paradigm to investigate the heterogeneous sensitivity of prostate cancer to chemotherapy and radiation therapy.

Effect of Castration on Incidence of Prostate Neoplasia. Representative histological sections of the dorsolateral prostate glands were reviewed for the presence of PIN or invasive neoplasia in 95 of 107 mice (12 entire prostate specimens were reserved for molecular analysis). No spontaneous tumors were observed in any of the control animals. Prostatic adenocarcinoma was graded as well, moderately, or poorly differentiated as described above. Consistent with our previous reports, all TRAMP mice displayed either PIN or well-differentiated adenocarcinoma by 12 weeks of age (Table 1). Over the age range of 18–24 weeks, a relatively even distribution of well (35%), moderate (38%), and poorly (27%) differentiated primary tumors was observed. This relative distribution was maintained in the subset of 22–27-week-old TRAMP mice (data not shown). Time was positively associated with advanced pathology (P = 0.03).

Histological prostate sections from castrated mice, whether nontransgenic (Fig. 2A) or TRAMP (Fig. 2B), typically demonstrated the presence of atrophic prostate glands. In contrast to the castrate nontransgenic mice, many castrate TRAMP mice displayed a much more hypercellular fibromuscular stroma but lacked definitive evidence of prostate cancer. Prostate tumors were observed arising adjacent to typical, albeit atrophic, glands in 3 of 7 (43%) T12/18 TRAMP mice and 8 of 10 (80%) T12/24 TRAMP mice for a total of 11 of 17 (65%) of castrated mice. When all castrated mice (i.e., including those that developed tumors and those that only had evidence of atrophic glands) were analyzed controlling for time, castration did not have a statistically significant impact on primary tumor pathology. However, it is interesting to note that 100% of tumors that developed in castrated TRAMP mice were poorly differentiated in contrast to the 27% of noncastrated TRAMP mice that developed poorly differentiated tumors (Table 1). Similar pathological changes of decreased glandular density and increased Gleason score, associated with preoperative androgen deprivation prior to radical prostatectomy, have been reported (12). As shown in Fig. 2C, many of these poorly differentiated primary tumors were particularly aggressive, invading into the urethral musculature.

Effect of Castration on Incidence of Prostate Cancer Metastases. Peri-aortic lymph nodes from all TRAMP mice were examined grossly and, in the instance of gross primary prostate tumors, histologically for evidence of microscopic metastases. In the absence of gross primary prostate tumor, enlarged lymph nodes were also examined histologically. In addition to poorly differentiated and highly invasive primary tumors, lymphatic metastases were detected in both the castrated and noncastrated TRAMP mice. Fig. 2D displays a representative peri-aortic lymph node from a T18/24 mouse that has been partially replaced by poorly differentiated metastatic prostate tumor cells. Consistent with our previous reports, all of the metastatic lymph node deposits were poorly differentiated. As shown in Table 2, the overall incidence of lymphatic metastases in noncastrated and all castrated TRAMP mice at 18–24 weeks of age was not statistically significant (19 and 29%, respectively). However, it is interesting to note that compared to the noncastrated TRAMP mice, the incidence of lymphatic metastases in the subset of castrated mice that developed histological primary prostate cancer more than doubled from 5 of 26 (19%) to 5 of 11 (45%). A similar trend in detectable pulmonary metastases was also observed (data not shown). These studies suggest that those mice that developed prostate cancer despite early androgen deprivation were predisposed to develop more poorly differentiated, invasive primary prostate tumors with an increased incidence of progression to metastatic prostate cancer.

Expression of Tag Oncoprotein after Castration. Because progbasin promoter-directed expression of a heterologous gene has been demonstrated to be androgen dependent in transgenic mice (6), immunohistochemical analysis was performed to detect expression of the Tag oncoprotein in representative samples derived from androgen-independent TRAMP tumors and metastases. As displayed in Fig. 2, E and F, despite androgen ablation, nuclear Tag protein was readily detectable in both the primary and metastatic tumor deposits, respectively (n = 3 mice). This staining is consistent with our previous observation of uniform Tag expression within primary and metastatic tumor sites. Because TRAMP-C cell lines derived from a primary TRAMP prostate tumor do not express Tag either in culture or following s.c. grafting to C57BL/6 mice, it is apparent that continuous transgene expression is not required to maintain the transformed phenotype (13). Although previous studies had demonstrated down-regulation of a simple probasin-driven reporter construct following castration in transgenic mice (6), these studies suggest that, as a consequence of neoplastic transformation, androgen-independent mechanisms facilitate prostate-specific gene expression in the TRAMP model. It is tempting to suggest that similar events may contribute to progression of human prostate cancer following androgen ablation. Studies to elucidate these molecular mechanisms are presently under way.

Perspective. We chose to castrate TRAMP mice at 12 weeks of age, a time at which high grade PIN or well-differentiated prostate cancer is reproducibly observed, to test the hypothesis that molecular events leading to androgen-independent and metastatic prostate cancer could occur at this early stage. As a consequence of castration at
12 weeks of age, we observed an initial regression of GU prostatic tumor burden. With respect to androgen ablation as curative therapy, however, only 20% of mice castrated at 12 weeks showed a durable prostate cancer regression, whereas the majority of mice demonstrated a rapid outgrowth of androgen-independent disease. Furthermore, androgen deprivation resulted in an increased proportion of poorly differentiated tumors (100%) and more than twice (19% versus 45%) the number of lymphatic metastases in those mice that developed...

Fig. 2. Histological analysis of the impact of castration on dorsolateral prostate growth and tumor development. In A, histological section of a castrated nontransgenic control (C12/18) mouse shows atrophic peri-urethral prostatic acini (H&E; X40). In B, histological section of a TRAMP 12/18 mouse shows atrophic peri-urethral prostatic acini with stromal hypercellularity (H&E; X40). In C, histological section of a TRAMP 12/18 GU tract demonstrates a poorly differentiated prostatic tumor invading into the urethral muscle (H&E; X40). In D, histological section of a peri-aortic lymph node from the mouse shown in C revealed metastatic prostate tumor (T) replacing the normal lymph follicles (L; H&E; X10). In E, immunohistochemistry of a serial section of C for T antigen oncoprotein demonstrates relatively uniform intense nuclear staining within a representative region of poorly differentiated tumor (X40). In F, immunohistochemistry of a serial section of D for Tag oncoprotein demonstrates punctate nuclear staining within a metastatic lymph node deposit (T) adjacent to the normal lymph (L) tissue (X40).
primary prostate tumors compared to noncastrated TRAMP controls. It is interesting to note that approximately 10–15% of human patients can maintain a long-term response to androgen deprivation alone, a number not substantially different from the 20% of TRAMP mice that appeared to achieve prolonged remission in this study. In general, these studies provide support for the hypothesis that androgen independence can occur early in the natural history of prostate cancer and that androgen ablation selects for the growth of androgen-independent disease that may already coexist with the androgen-dependent population. It is tempting to speculate that the androgen-sensitive cells may potentially exert a constraining influence on proliferation of the androgen-independent cells within a heterogeneous tumor.

In the subset of TRAMP mice that developed rapid prostate cancer progression in spite of castration, the primary tumor burden as measured by GU wet weight was similar to or greater than many noncastrated mice. Furthermore, the majority of primary tumors in noncastrated animals were well to moderately differentiated and nonmetastatic, whereas all of the castrated animal tumors were poorly differentiated and more frequently metastatic. It would seem clear from these studies that for many individual mice, early androgen ablation offers little benefit with respect to primary tumor burden and may actually be detrimental with respect to the differentiation of the primary tumor and incidence of metastases. Similarly, there is a subset of patients with metastatic prostate cancer who do not maintain a durable response to androgen ablation therapy. Unfortunately, it is not readily possible at this time to determine the androgen sensitivity of a given tumor. This underscores the need to develop new molecular diagnostics to facilitate analysis of hormonal sensitivity to augment the conventional pathological grading and clinical staging parameters that are available presently.

Lastly, it is unclear at this time whether androgen-independent prostate cancer is the consequence of molecular events mediated by, or independent of, androgen receptor signaling. Although the androgen receptor is required for normal growth and development of the prostate gland and is generally believed to act as a tumor suppressor in that it is required to maintain terminally differentiated function of the prostatic epithelium, it is clear that the androgen receptor is a target for somatic mutation and that deregulated androgen signaling is a potent consequence of such mutations (reviewed in Ref. 14). Hence, a mutated androgen receptor might, much like a mutated p53 gene, function as an oncogene. In fact, Tilley et al. (15) have shown recently that up to 44% of primary human prostate tumors contain detectable mutations in the androgen receptor prior to any endocrine treatment and that they are associated with rapid failure of subsequent hormonal therapies. However, comprehensive analysis of the incidence and nature of spontaneous mutations of the androgen receptor in prostate cancer has been difficult in part because of the size and allelic variation of the androgen receptor itself and because prostate cancer is a heterogeneous disease of a genetically heterogeneous human population. We are presently examining the potential for the androgen receptor to act as either a tumor suppressor or oncogene by characterizing the incidence, nature, and consequence of androgen receptor mutations in the autochthonous TRAMP model.

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References

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