T-bet Regulates Metastasis Rate in a Murine Model of Primary Prostate Cancer

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Materials and Methods

Abstract

The local progression of primary tumors is extrinsically controlled by type 1 immune responses, particularly via the cytokine IFN-γ, whose secretion is highly dependent on helper T cells. The T-box transcription factor T-bet (Thbx21) plays a critical role in the development of type 1 helper T cells and is essential for the production of IFN-γ. Here, the T-bet pathway in the autochthonous transgenic adenocarcinoma mouse prostate model is demonstrated to have only a modest effect on the characteristics of primary prostate cancers but rather exerts a significant suppressor function in the development of metastatic disease.

Introduction

The regulation of tumor progression by antitumor immunity requires helper T cell-dependent type 1 (IFN-γ)-related responses to inhibit cell cycle progression and promote tumor immunogenicity (1–4). Recently, the T-box transcription factor T-bet was identified to play a critical role in Th1 helper T cells, which direct type 1 immune responses and IFN-γ production in vivo (5). To test the role of T-bet in primary tumor development and progression, we generated T-bet-deficient animals in the transgenic adenocarcinoma mouse prostate (TRAMP) model, in which a probasin regulatory element directs the expression of SV40 early genes to the prostatic epithelium (6). Surprisingly, T-bet deficiency had little, if any, effect on the development of the primary tumor; instead, T-bet was required to suppress metastatic disease.

Materials and Methods

Mice. TRAMP transgenic mice of the C57BL/6 background (6) were a gift of Norman Greenberg (Fred Hutchinson Cancer Research Center, Seattle, WA). T-bet-deficient mice of the C57BL/6 background were derived in our laboratory (5). TRAMP and T-bet-deficient mice were intercrossed to generate TRAMP transgenic T-bet+/− animals, which were bred against wild-type C57BL/6 animals to generate TRAMP transgenic or nontransgenic T-bet+/− animals or against C57BL/6 T-bet−/− animals to generate TRAMP transgenic or nontransgenic T-bet−/− animals. All animals were housed under specific pathogen-free conditions at the Harvard School of Public Health.

Pathological Assessment. At the ages indicated, appropriate organs were harvested and examined by routine H&E staining. Grading was performed by one of us (J. L. H.) in blinded fashion using previously described criteria for grade of murine prostatic intraepithelial neoplasia and adenocarcinoma (7). The presence of metastasis in nonprostatic organs was determined by visual inspection and confirmed by histopathological analysis. We (S. L. P. and M. J. T.) examined two cohorts of animals, which were combined for analysis. Statistical significance was determined by two-tailed Student’s t test.

Results and Discussion

Primary Prostate Cancer Progression in the Absence of T-bet.

To evaluate the role of T-bet in an autochthonous model of prostate cancer, TRAMP transgenic T-bet+/+ and T-bet−/− animals were examined. When analyzed between 21 and 30 weeks of age, both T-bet-deficient and -sufficient TRAMP transgenic animals developed primary prostate cancers with comparable frequencies (Table 1; Figs. 1 and 2): 100% of TRAMP animals of both T-bet genotypes developed at least high-grade prostatic intraepithelial neoplasia, with 96% (25 of 26) of T-bet-deficient versus 91% (19 of 21) of T-bet-sufficient animals developing overt adenocarcinoma (P was not significant). Interestingly, T-bet-deficient TRAMP animals developed somewhat higher grade adenocarcinomas than their T-bet-sufficient counterparts, although the magnitude of the difference was modest [92% (23 of 25) versus 72% (15 of 21) developing grades 2 or 3, respectively; P = 0.023], whereas one of eight nontransgenic T-bet+/+ animals developed high-grade prostatic intraepithelial neoplasia, two of nine nontransgenic T-bet−/− animals developed overt, low-grade adenocarcinoma (P = 0.088, comparing adenocarcinoma rates). However, overall tumor size, as judged by the size of the prostate, did not differ significantly between T-bet+/+ and T-bet−/− counterparts (1.08 ± 0.53 versus 1.19 ± 0.67 cm, respectively; P was not significant). We therefore concluded that T-bet deficiency had little, if any, effect on primary tumor incidence and may have resulted in a modest increase in the rate of tumor progression.

Increased Metastatic Disease in the Absence of T-bet.

Distant metastases, particularly to the liver, salivary gland, and lung, occur spontaneously in TRAMP transgenic animals, presumably related to hematological dissemination (6, 7). When examined for such lesions, T-bet-deficient animals consistently developed increased frequencies of metastatic disease; higher frequencies of animals developed metastases (3 of 21 T-bet+/+ versus 13 of 25 T-bet−/− TRAMP animals; P = 0.0033), although the numbers of organs in individual animals with metastatic disease was not significantly affected (1.667 ± 0.58 versus 1.62 ± 0.87 organs in metastatic T-bet+/+ versus T-bet−/− TRAMP animals, respectively; P was not significant). Nonetheless, when examined on a per-organ basis, tissues of T-bet-deficient animals clearly developed higher rates of metastasis when compared with their T-bet-sufficient counterparts (24% of 88 versus 6% of 79 organs examined, respectively; P = 0.0083; Fig. 2C). Interestingly, metastasis was not observed in any of the non-TRAMP T-bet+/+ animals, but was seen in one of the non-TRAMP T-bet−/− animals with low-grade prostatic adenocarcinoma (n = 8 and 9, respectively; P was not significant). We therefore concluded that T-bet indeed regulates cancer progression, but primarily by suppressing the rate at which the tumor progresses to metastatic capability.

Mechanisms of Immunoregulation in the TRAMP Transgenic Model.

Previous studies have demonstrated that effective antitumor responses in prostate cancer have been linked to type 1 helper T cells...
and/or their primary effector cytokine, IFN-γ (8–10). In the TRAMP model, tumor progression can be significantly attenuated by immunotherapy via tumor vaccines and/or T cell-directed therapy augmented with CD152 blockade, but the specific immunological mechanisms of this protection remain to be elucidated (11–14). Nonetheless, given the accumulating evidence for the importance of type 1 and IFN-γ-related immune responses in the suppression of cancer progression (1–4), the transcriptional regulators of such responses, such as T-bet (5), would be expected to play critical roles in cancer progression due to their roles in immunity.

Interestingly, however, the present studies indicate that T-bet is not primarily required to regulate the incidence or magnitude of primary cancers, at least in the TRAMP model. Instead, T-bet is critical for the ability of the host to regulate the metastatic capability of the primary
tumor, a finding not likely related simply to a difference in primary tumor grade because the differences between TRAMP transgenic T-bet−/− and T-bet+/+ animals were only modest at best, and there is no correlation between tumor grade and the presence of metastasis in this model (Ref. 6; e.g., note the development of metastasis in the setting of a grade 1 prostate adenocarcinoma in SP-WT4). The precise mechanism by which T-bet participates in tumor suppression remains unproven but is unlikely to involve an intrinsic tumor suppressor effect in the cancer itself because T-bet is undetectable by immuno-histochemistry in normal prostate and prostate cancer tissues from both humans and TRAMP transgenic and nontransgenic mice. Instead, these results likely reflect the importance in helper T cells of production of IFN-γ (5), which plays critical roles in tumor regulation (8–10); however, because T-bet-deficient T cells can produce IFN-γ under certain conditions (15, 16), it is difficult to attribute the phenotype of TRAMP transgenic T-bet-deficient mice simply to IFN-γ deficiency and raises the intriguing possibility that the remaining low amounts of IFN-γ are sufficient to control primary tumor formation but not metastases. At the same time, T-bet also regulates type 1 responses in both dendritic cells (17) and B cells (15) and has been detected in natural killer cells (18), so any or all of these populations may theoretically cooperate with or act independently of T cells in tumor immunoregulation. Indeed, dendritic cells have been proposed as adjuvant therapeutic agents in prostate cancer therapy, although their effect may be mediated via T cells themselves (19). As such, continued investigation into the diverse roles that T-bet plays in various immune cell populations will likely lead to novel approaches to prostate cancer immunotherapy.

On a broader note, our findings suggest that wild-type C57BL/6 mice may have a propensity for neoplasia, which is accentuated in the...
absence of T-bet; however, our study size lacked the sufficient power to establish this finding conclusively (two of nine non-TRAMP T-bet−/− animals versus zero of eight T-bet+/− animals developed prostatic adenocarcinoma; \( P \) was not significant). It will be of interest to examine large cohorts of aging T-bet-deficient mice for the incidence of breast, prostate, lung, and other tumors, as done for mice that lack signal transducer and activator of transcription 1 (2), to establish whether T-bet plays a similar role in tumor surveillance.

**Fig. 2.** Prostate cancer progression in T-bet-deficient transgenic adenocarcinoma mouse prostate (TRAMP) mice. A. Primary cancer grade was evaluated in the prostates of TRAMP transgenic and nontransgenic T-bet+/+ and −/− animals, as unaffected, high-grade prostatic intraepithelial neoplasia (PIN), or adenocarcinoma grades 1, 2, or 3. Left panel shows the absolute number of specimens of each histopathological type in each group; right panel shows the same data represented as percentages of the total number of specimens. B, primary cancer size, as indicated by prostate size, was evaluated on prostates at the greatest diameter. Each square indicates an individual animal, but the squares sometimes overlap; ns are the same as in A. Horizontal bars indicate averages. C, metastatic disease progression was determined by evaluating the percentage of cumulative organs affected at the indicated ages for T-bet+/+ TRAMP nontransgenic, T-bet+/− TRAMP transgenic, T-bet−/− TRAMP transgenic, and T-bet−/− TRAMP-transgenic animals.

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**References**


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