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

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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 (Tbx21) 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.

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 = 0.00083; 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). 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−/− animal (SP-KO14; A, ×40; B, ×400). C, high-grade (grade 3) tumors show little to no gland formation and sheets of highly atypical cells with abundant mitoses and apoptosis, as seen in a TRAMP transgenic T-bet−/− animal (SP-KO8, ×600). D, metastatic tumor deposits in the kidney of a TRAMP transgenic T-bet−/− animal (SP-KO19, ×200). Renal tubules and glomeruli are noted in the background. E, metastatic tumor deposits in the lung of a TRAMP transgenic T-bet+/+ animal (SP-WT4, ×100). The deposits are adjacent to bronchioles and may represent lymphatic spread. The background alveolar lung is unremarkable. F, metastatic tumor deposits in the lung of a TRAMP transgenic T-bet−/− animal (SP-KO20, ×100). Some deposits are adjacent to bronchioles and may represent lymphatic spread. The background alveolar lung is unremarkable. G, metastatic lesions in the liver of a TRAMP transgenic T-bet−/− animal (SP-KO20, ×200). Nodules of crowded tumor cells replace the normal liver without a surrounding inflammatory reaction. H, metastatic lesions in the salivary gland of a TRAMP transgenic T-bet−/− animal (SP-KO22, ×100). Nodules of crowded tumor cells replace the normal salivary gland without a surrounding inflammatory reaction. These images are representative of the respective gradings and lesions described in Table 1 and Fig. 2.

*Unpublished data.*
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.

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References


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