The Insulin-like Growth Factor Axis and Prostate Cancer: Lessons from the Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) Model

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ABSTRACT

We have characterized the temporal expression of the insulin-like growth factor (IGF) axis in the transgenic adenocarcinoma of mouse prostate (TRAMP) model as prostate cancer progression in this model closely mimics that observed in the human disease, and the model provides samples representing the earliest stages of prostate cancer that are clinically the most difficult to obtain. We report that prostate-specific IGF-I mRNA expression increased during prostate cancer progression in TRAMP mice and was elevated in the accompanying metastatic lesions, whereas prostatic IGF-I mRNA remained at nontransgenic levels in androgen-independent disease. Expression of IGF-II mRNA, however, was reduced in primary prostate cancer, metastatic lesions, and androgen-independent disease. Expression of type-I IGF receptor (IGF1R) mRNA, encoding the cognate receptor for both IGF-I and IGF-II, as well as type-2 IGF receptor (IGF2R) mRNA was not found to be altered during primary prostate cancer progression in intact TRAMP mice but was dramatically reduced in metastatic lesions and in androgen-independent disease. Similar to reports from clinical disease, serum IGF-I levels were observed to increase precociously in TRAMP mice early in disease progression but remained at nontransgenic levels after castration. Elevated serum levels of IGF-binding protein 2 were observed to correlate with advanced prostate cancer in the TRAMP model. Together these observations implicate IGF-I as an important factor during the initiation and progression of primary prostate cancer and provide evidence that there is a strong selection against expression of IGF1R and IGF2R in metastatic and androgen-independent disease.

INTRODUCTION

The IGF axis is an important modulator of growth and development, and changes in this axis may have important implications in malignant growth (1, 2). There are two IGF ligands (IGF-I and IGF-II) synthesized primarily by the liver that can promote cell proliferation and differentiation and inhibit apoptosis in distant tissues acting in an endocrine manner (2). IGFs are also produced locally by most tissues, in which they may act in an autocrine or paracrine manner (3). The biological functions of these ligands are mediated primarily by the type-I IGF receptor (IGF1R), a tyrosine kinase transmembrane receptor that binds IGF-I with higher affinity than IGF-II. The IGF2R, also known as the mannose-6-phosphate receptor, binds IGF-II but with no apparent intracellular signaling actions, although it seems to function as a scavenger receptor that mediates the uptake and degradation of extracellular IGF-II (2). The IGF2R also binds to mannose-6-phosphate containing glycoproteins and can target them to lysosomes (2). The IGF ligands circulate in plasma complexed to IGFBPs 1–6, which function to transport IGFs and modulate IGF activity (2–4).

Deregulation of the IGF axis has been specifically implicated in clinical prostate cancer. Recent epidemiological studies have demonstrated that elevated serum IGF-I, in particular, is associated with prostate cancer risk (5–7). In a prospective case-control study of men participating in the Physician’s Health Study, it was found that men with serum IGF-I concentrations in the upper quartile were at increased risk of developing clinically evident prostate cancer within the next 5–10 years. The study also indicated that plasma IGF-I concentration may be a better predictor of prostate cancer than serum PSA (5). The levels of IGFBP-2 and IGFBP-3 are also found to be altered in the serum and prostate tissue of prostate cancer patients. In these patients, levels of IGFBP-2 are often increased, whereas levels of IGFBP-3 are often decreased (8–12). Because IGFBP-3 is a substrate for PSA, a member of the kallikrein family of serine proteases, it is postulated that rising PSA levels during the natural history of prostate cancer facilitate disease progression by proteolytically cleaving IGFBP-3, thereby increasing the level of bioavailable IGF at the cellular level (13).

Despite the evidence establishing a close relationship between the IGF axis and prostate cancer, it has been difficult to comprehensively examine changes in the IGF axis at the molecular level throughout the natural history of clinical disease, in part due to the paucity of clinical samples representing the earliest forms of this disease as well as the heterogeneity of both the disease and of the patient population. We have, therefore, used the autochthonous Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) model to facilitate molecular characterization of the changes in the IGF axis during the initiation, progression, and metastasis of prostate cancer as well as in androgen-independent disease (14–16).

The TRAMP model was previously generated using the minimal PB –426/+28 regulatory sequence to specifically target SV40 early gene (Tag) expression to prostatic epithelium (14) commencing at sexual maturity. By 10–12 weeks of age, TRAMP mice generally develop PIN and/or well-differentiated prostate cancer. All TRAMP mice ultimately develop prostatic adenocarcinoma that metastasizes to distant sites, primarily the lymph nodes and lungs. This generally occurs by 24–30 weeks of age in [C57BL/6 × FVB]F1 TRAMP mice (14, 15). Following androgen ablation, 20–35% of TRAMP mice remain tumor free, whereas 65–80% develop androgen-independent disease. Tumors that develop in castrated mice uniformly progress to poorly differentiated adenocarcinoma. Castrated mice that develop tumors also exhibit twice the incidence of metastasis (16). Here we report evidence that local expression of IGF-I in the prostate correlates with, and may in fact facilitate, early disease progression. Furthermore, we demonstrate that independence from IGF1R-mediated signaling correlates with—and may, therefore, be a prerequisite for—metastasis and androgen independence.
A question related to the image is not provided. If you have a specific question or need assistance with something else, please let me know!
TRAMP mice, expression of IGF-I mRNA remained at nontransgenic or precastration levels in the prostate tumors of the castrated TRAMP mice as well as in the accompanying metastatic lesions (Fig. 2). These data indicate that the development of prostate cancer in intact TRAMP mice is distinct from androgen-independent disease with respect to the regulation of IGF-I expression in the prostate.

As demonstrated in Fig. 3, the expression of IGF1R mRNA was not observed to change significantly during primary cancer progression in intact TRAMP tumors. It is interesting to note, however, that although expression of IGF1R mRNA remained at nontransgenic levels in the tumors from intact TRAMP mice, expression of IGF1R mRNA was significantly reduced ($P < 0.05$) in tumors from castrated TRAMP mice (85%) as well as in metastatic lesions (97%) when compared with prostate levels in nontransgenic mice. These observations implicate a selection against expression of IGF1R in advanced and disseminated disease.

Although expression of IGF-II mRNA in the prostate did not change significantly with age in nontransgenic mice, IGF-II mRNA levels were significantly reduced (75–95%; $P < 0.05$) in the prostates of TRAMP mice when compared with nontransgenic mice (Fig. 4). This decrease (80%) in expression of IGF-II mRNA was observed as early as 12 weeks of age in TRAMP mice and persisted as the cancer progressed. Expression of IGF-II mRNA was also significantly reduced ($P < 0.05$) in tumors from castrated TRAMP mice (80%) as well as in metastatic lesions (75–95%) when compared with IGF-II mRNA levels in prostates of nontransgenic mice.

As shown in Fig. 5, changes in expression of IGF2R mRNA did not occur during prostate cancer progression in the TRAMP model. Similar to the pattern of expression of IGF1R mRNA, expression of IGF2R mRNA remained at nontransgenic levels during cancer progression in tumors from intact TRAMP mice. Expression of IGF2R mRNA was significantly reduced ($P < 0.05$) in tumors from castrated TRAMP mice (62%) and in metastatic lesions (47–66%) when compared with prostate levels in nontransgenic mice, which suggests that—like IGF1R and IGF-II—reduced expression of IGF2R correlates with advanced disease.

Analysis of Serum IGF-I Levels during Cancer Progression in TRAMP Mice. Radioimmunoassays were used to determine whether changes in the concentration of serum IGF-I correlated with prostate cancer progression in an independent cohort of TRAMP and nontransgenic mice. As shown in Fig. 6, the serum IGF-I concentration in

![Fig. 2. IGF-I expression during cancer progression in TRAMP mice. Relative IGF-I mRNA levels were quantitated by QRT-PCR, and mean ($\pm$SE) levels are expressed relative to the prostate from 30-week-old nontransgenic mice (N30), which was assigned a value of 100%. N12, prostate from 12-week-old nontransgenic mice; N30, prostate from 30-week-old nontransgenic mice; T12, prostate from 12-week-old TRAMP mice; T18, prostate from 18-week-old TRAMP mice; T30, prostate from 30-week-old TRAMP mice; T4; prostate from 24-week-old castrated TRAMP mice; TM, metastatic lesion from TRAMP mice. Bar, the mean ($\pm$SE) IGF-I mRNA level normalized to L19 ($n = 4$; except $n = 6$). T30, *, significant difference ($P < 0.05$) from T12; $\Delta$, significant difference ($P < 0.05$) from TM by Fisher’s protected least significant difference ANOVA.

![Fig. 3. IGF1R expression during cancer progression in TRAMP mice. Relative IGF1R mRNA levels were quantitated by QRT-PCR and mean ($\pm$SE) levels are expressed relative to the prostate from 30 week old TRAMP mice (N30), which was assigned a value of 100%. Cohort abbreviations are as defined in Fig. 2. Bar, the mean ($\pm$SE) IGF1R mRNA level normalized to L19 ($n = 4$; except $n = 3$, T12, and $n = 5$, T30). *, significant difference ($P < 0.05$) from T12, T18, and T30; $\Delta$, significant difference ($P < 0.05$) from N30 by Fisher’s PLSD ANOVA.](Image321x112 to 547x269)
nontransgenic mice increased between 6 weeks of age (178 ± 8 ng/ml) and 18 weeks of age (232 ± 36 ng/ml) reaching a maximal level by 18 weeks of age. In contrast, the serum IGF-I concentration in TRAMP mice reached a level corresponding to the maximal nontransgenic mice by 12 weeks of age, a time when the serum IGF-I concentration in TRAMP mice reached a level corresponding to the maximal nontransgenic mice (178 ± 8 and 172 ± 11 ng/ml, respectively). Although we cannot rule out that the reduced serum IGF-I level is not a direct consequence of castration, the fact that tumors still developed suggests that normal levels of IGF-I are not required for tumor progression in androgen-independent disease.

**Analysis of Serum IGFBPs Levels during Prostate Cancer Progression in TRAMP Mice.** To assess whether changes in serum IGFBPs were induced by the prostatic tumors, we subjected serum samples of control (n = 9), TRAMP (n = 9), and castrated TRAMP (n = 4) mice to WLB with radiolabeled IGFs and to WIB with an IGFBP-2 specific antibody. Using this method, we observed no differences in any of the serum IGFBPs between control and TRAMP mice prior to 24 weeks of age, at which time serum IGFBP-2 levels were approximately 2-fold higher than in control mice (P < 0.05; Fig. 7). This is similar to reports in human prostate cancer patients (10, 11). It was interesting to note that a 50% reduction in serum IGFBP-3 level was observed as a consequence of castration of TRAMP mice, a finding remarkably similar to that reported previously in castrated baboons (24).

**DISCUSSION**

Recent studies have indicated that an elevation in the level of serum IGF-I is associated with an increased risk for prostate cancer (5, 7, 25). On meta-analysis, there was an approximate 8% increase in the serum IGF-I level in prostate cancer patients (6), and elevated serum IGF-I could be observed in men at least 5 years prior to a clinical diagnosis of prostate cancer (5). In part on the basis of this data, serum IGF-I has been proposed as a candidate marker for early detection of prostate cancer, although the role and origin of the elevated serum IGF-I remains to be elucidated. Unfortunately, it has been difficult to comprehensively characterize changes in the IGF axis in prostate tissue at the molecular level during clinical disease progression. Hence, the goal of this study was to use the TRAMP model to assess whether specific alterations in expression of the IGF axis reproducibly occur in the prostate gland as well as in serum during the progression of autochthonous prostate cancer and androgen-independent disease.
We observed that the expression of IGF-I mRNA in the prostate increased with prostate cancer progression in intact TRAMP mice. This is consistent with previously reported observations that the SV40 early genes, specifically large Tag, are known to influence the transcription of IGF-I (26). This is accomplished, in part, by preventing the binding of an inhibitory E2F complex to the IGF-I promoter (27). Although IGF-I is important in the development of prostate cancer, it has previously been demonstrated that when the insulin gene regulatory region was used to target Tag to the pancreas expression of IGF-II but not expression of IGF-I was found to be increased (28), which indicated that IGF-II is important in the development of pancreatic cancer and suggests that up-regulation of IGF-I is not a general property of the transgene but rather a consequence of the transformation of prostate epithelial cells.

Concurrent with the observed increase in expression of prostatic IGF-I, serum IGF-I was found to be precociously elevated in TRAMP mice early in cancer progression. Although the level of prostatic IGF-I mRNA per cell was found to be reduced, it should be noted that the prostates of TRAMP mice are, on average, 20% larger by wet weight than those of nontransgenic littermates at 12 weeks of age (16). It is possible that those cells expressing IGF-I may have a growth advantage, and that the observed increase in serum IGF-I may reflect the increased number of IGF-I expressing prostatic epithelial cells. It is interesting to note that the increase in serum IGF-I levels coincided with the time at which the majority of TRAMP mice displayed either PIN and/or well-differentiated prostate cancer. Hence, the integrity of the prostate ductal structures may have been compromised as proposed for the mechanism whereby PSA enters the circulation (29), and the small foci of invasive cells could have allowed IGF-I to “leak” into the circulation. Because no significant changes in serum IGFBP levels were observed in intact TRAMP mice until 24 weeks of age, this essentially rules out the possibility that elevations in serum IGF-I were an artifact caused by binding protein interference in the assay. This further indicates that the observed increase in serum IGF-I was probably due to prostatic IGF-I and not due to a systemic response.

It is curious that, as prostatic IGF-I levels continued to rise in the TRAMP mice, the serum IGF-I levels did not rise above the level observed in nontransgenic adults. The level of serum IGF-I in TRAMP mice is most likely maintained via a feedback loop because IGF-I is known to negatively regulate secretion of growth hormone by the pituitary, and hepatic expression of IGF-I—the major source of serum IGF-I—is primarily responsive to growth hormone (2, 3). Therefore, serum IGF-I, synthesized by the prostate, possibly down-regulates the secretion of pituitary growth hormone and thereby decreases IGF-I secretion by the liver to maintain serum IGF-I at an optimal level.

Although our data suggest that prostatic IGF-I contributes to serum IGF-I, this finding does not preclude the possibility that the prostate is also a direct target of circulating IGF-I and that elevated basal levels of serum IGF-I may directly contribute to early prostate cancer progression. It was demonstrated (30) that systemic administration of IGF-I to rats for 1 week resulted in an increase in the mean wet weight of the prostate gland. Likewise, acromegaly patients with elevated growth hormone and IGF-I serum levels also have increased prostate volumes, and when these patients are treated with octreotide to suppress GH/IGF-I levels, prostate volumes were observed to decrease (31). On the basis of these observations, it should be interesting to determine whether genetic differences in serum IGF-I bioavailability contribute to a predisposition to prostate cancer. In fact, it has been demonstrated that African-American men have a higher incidence of clinically significant prostate cancer than Caucasian-American men (32). Although both populations exhibit similar basal levels of serum IGF-I, African-American men generally have lower levels of IGFBP-3, the major binding protein in the serum (33). Hence, African-American men may be predisposed to prostate cancer because they actually have a higher level of bioavailable IGF-I in their serum and/or tissues. By crossing TRAMP mice with mice that have systemic alterations in IGF-I levels, for example, li/lit mice that have reduced serum IGF-I levels (34) and MT-GHRH transgenic mice that have elevated serum IGF-I levels (35), it should be possible to further test this hypothesis.

It is interesting to note that expression of IGF1R, IGF-II, and IGF2R mRNA was significantly reduced (85–96%, 75–95%, 47–66%, respectively; P < 0.05) in both metastatic and androgen-independent disease. The dramatic reduction (85–96%, P < 0.05) in IGF1R expression in these poorly differentiated tumors is particularly interesting because it supports the hypothesis that the loss of IGF1R expression may be associated with the loss of differentiation and increased tumorigenicity (36). Thus, the data from the TRAMP model suggest that organ-confined disease seems to be IGF-I-dependent, whereas metastatic and androgen-independent disease are IGF1R-independent. Although this study focused on mRNA expression,
future studies will examine signaling molecules downstream of the IGF1R to determine the functional consequence of the loss of IGF1R mRNA expression in metastatic and androgen-independent disease.

The IGF2R gene is lost in a variety of cancers including liver and breast tumors and is thought to be a tumor suppressor gene (37, 38), and IGF2R expression is significantly reduced (47–66%, \( P < 0.05 \)) in metastatic and androgen-independent disease in the TRAMP model. There are a number of possible events that could arise as a consequence of the loss of IGF2R expression. Because IGF2R regulates intracellular trafficking of lysosomal enzymes including cathepsins that are IGFBP proteases (39), the loss of IGF2R expression could result in increased cathepsin activity that may proteolytically cleave the IGFBPs and thereby increase the bioavailability of the IGFs. In addition, increased secretion of cathepsins may, in part, facilitate metastasis by degradation of basement membranes (39). Because IGF2R is also involved in the activation of latent TGF\( \beta \) (37, 38), the loss of IGF2R expression would reduce TGF\( \beta \) activity. Curiously, it has been demonstrated previously (40) that levels of TGF\( \beta \) can be elevated in prostate cancer, which indicates that TGF\( \beta \) must be activated by an alternate mechanism in prostate cancer cells lacking IGF2R.

It is tempting to speculate on a mechanism whereby IGF-I contributes to the initiation and progression of prostate cancer. One possibility is that elevated levels of IGF-I may promote angiogenesis because it is known to induce VEGF (41). In fact, in our model, the increase in serum IGF-I seems to correlate with the increase in mean vessel density associated with the development of high-grade PIN lesions.\(^4\) Given this relationship (Fig. 8), we propose that IGF-I is involved in the “angiogenic switch” (42) leading to prostatic neovascularization. Although it is unclear whether the elevated levels of serum IGF-I in prostate cancer patients is of prostatic origin, it is possible that both systemic and prostatic IGF-I may contribute to the initiation and/or progression of the disease, which could explain why men with elevated baseline levels of serum IGF-I may be predisposed to prostate cancer.

Cohen (6) has recently put forth three hypotheses regarding the reported increase in serum IGF-I levels observed during prostate cancer progression: (\( a \)) that elevated serum IGF-I could promote symptomatic benign prostatic hyperplasia (BPH) and thereby create an ascertainment bias for the diagnosis of subclinical prostate cancer; (\( b \)) that serum IGF-I could be a marker of prostatic tissue IGF-I that contributes to prostate cancer development; and (\( c \)) that serum IGF-I could directly contribute to the risk of developing prostate cancer. Although we have not addressed the first hypothesis, our new findings using the TRAMP model tend to support the second and third hypotheses.

In summary, our results using TRAMP mice support the epidemiological data that elevated serum IGF-I correlates with prostate cancer progression and that the prostate can be a source of IGF-I. Furthermore, our data demonstrate that specific changes in the IGF axis correlate with the initiation and/or early progression of the disease. To

\(^4\) Unpublished observations.
directly test this hypothesis, we have now generated transgenic mice targeting the DES-IGF-I ligand [which has a reduced affinity for the IGFBPs (43)] to the prostate under the control of the prostate epithelial cell-specific rat PB promoter. Preliminary observations with the PB-DES mice demonstrate that they develop PIN-like lesions, which supports the hypothesis that deregulated IGF-I expression in the prostate is causally related to neoplastic transformation,5 and we anticipate that these mice will allow us to better characterize the consequence of IGF-I deregulation. The TRAMP model also predicts that the loss of IGFR1 is a hallmark of advanced androgen-independent and metastatic disease and provides the rationale that therapies designed to reduce IGF-I levels, as with a somatostatin analogue, may be efficacious prior to androgen ablation therapy or the occurrence of metastasis but will mostly likely not be effective in patients with metastatic disease or those who have undergone androgen ablation therapy. Lastly, because the data from the TRAMP model predict that the loss of IGFR1 and IGFR2 is a hallmark of advanced androgen-independent and metastatic disease, these molecules should be examined as possible prognostic tools to differentiate between patients whose cancer will remain dormant and those whose cancer will progress to advanced disease.

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