Translocator Protein Blockade Reduces Prostate Tumor Growth

Arlee Fafalios,1 Ardavan Akhavan,3 Anil V. Parwani,2 Robert R. Bies,4 Kevin J. McHugh,1 and Beth R. Pflug1

Abstract Purpose: The transmembrane molecule, translocator protein (TSPO), has been implicated in the progression of epithelial tumors. TSPO gene expression is high in tissues involved in steroid biosynthesis, neurodegenerative disease, and in cancer, and overexpression has been shown to contribute to pathologic conditions including cancer progression in several different models. The goal of our study was to examine the expression and biological relevance of TSPO in prostate cancer and show that the commonly prescribed benzodiazepine lorazepam, a ligand for TSPO, exhibits anticancer properties.

Experimental Design: Immunohistochemical analysis using tissue microarrays was used to determine the expression profile of TSPO in human prostate cancer tissues. To show the effect of TSPO ligands (lorazepam and PK11195) in prostate cancer, we used cell proliferation assays, apoptosis ELISA, prostate cancer xenograft study, and immunohistochemistry.

Results: TSPO expression is increased in prostatic intraepithelial neoplasia, primary prostate cancer, and metastases compared with normal prostate tissue and benign prostatic hyperplasia. Furthermore, TSPO expression correlates with disease progression, as TSPO levels increased with increasing Gleason sum and stage with prostate cancer metastases demonstrating the highest level of expression among all tissues examined. Functionally, we have shown that lorazepam has antiproliferative and proapoptotic properties in vitro and in vivo. Additionally, we have shown that TSPO overexpression in nontumorigenic cells conferred susceptibility to lorazepam-induced growth inhibition.

Conclusion: These data suggest that blocking TSPO function in tumor cells induces cell death and denotes a survival role for TSPO in prostate cancer and provides the first evidence for the use of benzodiazepines in prostate cancer therapeutics. (Clin Cancer Res 2009;15(19):6177–84)

Translocator protein (TSPO), previously known as the peripheral benzodiazepine receptor, is a transmembrane molecule that is best known for transporting cholesterol across the mitochondrial membrane for cell signaling and steroid biosynthesis (1, 2). TSPO has been shown to be overexpressed in numerous malignancies, including those of the breast, prostate, colon, ovary, and endometrium (3–7). Furthermore, a correlation has been shown between TSPO overexpression and the progression of breast, colorectal, and prostate cancers (8). Functionally, TSPO has been shown to take part in the regulation of apoptosis through its interactions with the mitochondrial permeability transition pore (9, 10). TSPO also plays a role in cell proliferation, as a correlation between TSPO expression and cancer cell proliferation has been observed in human astrocytomas (11) and breast cancer (12), whereas TSPO antagonism inhibits cell proliferation (13–16).

As its former name suggests, the peripheral-type benzodiazepine receptor, now called TSPO, has the ability to bind benzodiazepines with relatively high affinity (17). Benzodiazepine receptors are found in both the central and peripheral nervous systems of the body, and their role in the regulation of various biological processes is still being explored. TSPO has been implicated in various diseases, including neurodegenerative disorders and cancer, making it an interesting target for therapeutic intervention. This study provides evidence for the use of benzodiazepines in prostate cancer therapeutics, highlighting the potential of TSPO blockade as a novel therapeutic strategy.
Although benzodiazepines have been used clinically for over 50 years, their application as a form of cancer therapy is largely unexplored. Here, we show that lorazepam, a benzodiazepine commonly prescribed to treat anxiety disorders and which acts on both central and peripheral receptors, inhibits prostate cancer cell growth and survival. Our studies further elucidate the mechanism by which translocator protein antagonists alter cancer cell function. Antagonists for translocator protein are already used in the clinic for other indications and show very minor side effects. Because lorazepam is a commonly prescribed Food and Drug Administration-approved drug, the translation of our preclinical results to the prostate cancer patient population could be readily achieved. Our studies could lead to a significant change in the management of prostate cancer by providing a treatment option with minimal toxicity for use after failure of androgen-deprivation therapy and could ultimately prevent prostate cancer deaths.

**Translational Relevance**

**Materials and Methods**

**Cell lines and culture conditions.** Human prostate cancer cell lines PCC-1, LNCaP, and DU145 were maintained in RPMI 1640 (Invitrogen) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. LNCaP and DU145 cells were purchased from American Type Culture Collection and the PCC-1 cell line is a subline of the PC3 prostate cancer cell line (23). LN05, an androgen-deprived LNCaP cell line, was maintained in RPMI without phenol red with 10% charcoal-stripped FBS and 1% penicillin/streptomycin. The human prostate cancer cell line LAPC4 was maintained in Iscove’s modified Dulbecco’s medium (Invitrogen) supplemented with 10% FBS and 1% penicillin/streptomycin. The androgen-deprived LAPC4 cell line, LA98, was maintained in IMDM without phenol red (Invitrogen) supplemented with 10% charcoal-stripped FBS and 1% penicillin/streptomycin. Human embryonic kidney cells, HEK293, and human cervical cancer cells, HeLa, were maintained in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin.

**Tissue microarrays and TSPO immunocytochemistry.** For these studies, we used prostate tissue arrays (progression array, metastasis array, and prostate-specific antigen failure array) from the in-house Western Pennsylvania Tumor Bank to directly compare TSPO staining intensity in the tissue specimens. Samples from benign kidney, breast, colon, testis, and adrenal were included as positive and negative tissue controls for TSPO and were sampled in duplicate with sections at diagonally opposite ends of the block to eliminate positional staining artifacts.

There were 16 cases of normal donor prostate, 24 cases of nonneoplastic prostate tissues adjacent to malignant glands (NAT), 24 cases of BPH, 22 cases of prostatic intraepithelial neoplasia (PIN), 86 cases of prostatic adenocarcinoma (PCa), and 86 metastatic prostate carcinoma specimens from 35 patients with 25 separate sites of metastases. Samples of benign testis and adrenal were also included on each tissue microarray (TMA) as positive controls for TSPO expression (n = 2 each).

Immunohistochemical stains were done on 5-μm sections of TMA blocks. The sections from all of the groups were deparaffinized and rehydrated through a graded series of ethanol incubations. Heat-induced epitope retrieval was done using a decloaker, followed by rinsing in TBS buffer for 5 min. Slides were then loaded on a Dako Autostainer. The primary anti-TSPO (working dilution, 1:350) was a polyclonal rabbit antibody (Trevisen). The immunolabeling procedures were done using Dako EnVision-Labeled Polymer-HRP antirabbit (Dako) and were carried out according to the instructions of the manufacturer. Slides were then counterstained in hematoxylin, step-dehydrated, and coverslipped.

A prostate optimization MRA block was used as a positive control for each antibody. Both the extent and intensity of immunopositivity were considered when scoring the expression of TSPO. Briefly, the intensity of positivity was scored from 0 to 3 as follows: 0, nonstained; 1, weak; 2, moderate; and 3, strong positive control. The extent of positively stained cells was estimated using the same 0 to 3 scale. Semiquantitative analysis of TSPO expression in the human tissues was carried out in a blinded fashion by a board-certified genitourinary pathologist (A. Parwani) using a four-tier scoring method for intensity (0, 1, 2, 3) added to the percentage of expression in epithelia [intensity + (% × 3)]. The final composite score (0-6) was determined after adding the intensity and extent of positivity in the respective lesions.

**Western blot analysis.** Human prostate cancer cells (PCC-1, DU145, LAPC4, LA98, LNCaP, and LN05), HEK293, and HeLa cells were lysed in lysis buffer (20 mmol/L Tris-HCl, 135 mmol/L NaCl, 10% glycerol, and 1% Triton X). Human hepatocyte lysate was obtained from Dr. Steven Strom, University of Pittsburgh, Pittsburgh, PA, and Jurkat cell lysate was obtained from Upstate (now Millipore). Protein concentration was determined and an equal amount of protein (10 μg) was separated on 10% SDS-PAGE under reducing conditions. Proteins were electrotransferred onto polyvinylidene difluoride membranes (Millipore)
and blocked in 4% milk in PBS supplemented with 0.2% Tween 20 for 1 h at room temperature. Immunodetection of TSPO was carried out using the goat anti-TSPO polyclonal antibody (Novus Biologicals) at a dilution of 1:1,000 at 4°C overnight. The membrane was washed in PBS supplemented with 0.2% Tween 20 and incubated with a donkey anti-goat secondary hors eradish peroxidase–linked antibody (Santa Cruz Biotechnology) at a dilution of 1:4,000 as a control for protein loading.

**Cell viability assay.** PPC-1 human prostate cancer cells were plated at 5 × 10^3 cells/well in 96-well plates (Falcon-BD Biosciences) and adhered overnight at 37°C. The next day, cells were treated with TSPO antagonists PK11195 (Sigma-Aldrich) and lorazepam (Sigma-Aldrich) at varying concentrations (0.1-100 μmol/L), or with vehicle (0.1% ethanol or DMSO) for 48 h at 37°C. The cells were then incubated for 4 h with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasodium bromide (Chemicon) according to the protocols of the manufacturer. Absorbance was measured at a wavelength of 570 nm using the SpectraMax M2e absorption spectrophotometer (Molecular Devices). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrasodium bromide assays were repeated in three independent experiments.

**Cell proliferation assay.** PPC-1 cells were plated at a density of 5 × 10^3 cells/well in six-well plates. Cells adhered overnight and were then treated with TSPO antagonists PK11195 or lorazepam at 10, 50, or 100 μmol/L, or vehicle for 72 h. Cells were trypsinized and cell proliferation was measured by direct cell counting using a Coulter counter (Beckman-Coulter). Cell proliferation assays were repeated in three independent experiments.

**Apoptosis ELISA.** PPC-1 cells were plated at a density of 5 × 10⁴ cells/well in six-well plates. Cells adhered overnight and were then treated with TSPO antagonists PK11195 or lorazepam at 10, 50, or 100 μmol/L, or vehicle for 18 h. A spectrophotometric apoptosis of ELISA was used to quantify histone-associated DNA fragments present in the cell lysates, and was done according to the instructions of the manufacturer (Roche Diagnostics). Briefly, the standard solution and samples were added to the wells of a 96-well plate coated with a monoclonal antibody. After incubation, the plate was washed, and an enzyme-labeled antibody was added. After further incubation, the plate was washed again and treated with the substrate and the absorbance was determined at 405 nm using the SpectraMax M2e absorption spectrophotometer (Molecular Devices). Apoptosis assays were repeated in three independent experiments.

**Annexin V staining and flow cytometry.** Human LNCaP and PPC-1 prostate cancer cells were plated in 100 mm plates and once cells reached ~70% confluence, cells were treated with 50 or 100 μmol/L of PK11195 TSPO antagonist or vehicle for 18 h. For flow cytometry using the Annexin V assay, cells were collected and double-stained

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**Fig. 1.** Increased TSPO expression in prostate cancer. A, relative expression of TSPO by immunohistochemistry in normal donor prostate, NAT, BPH, PIN, primary PCa (PCA), and prostate cancer metastases (MET) by scoring of TMA cores. TSPO expression is significantly (*, *P < 0.05) increased in PIN, PCA, and PCA metastases, compared with normal prostate tissue and BPH. B to G, representative results of immunohistochemical staining of TSPO in normal donor prostate (B), NAT (C), BPH (D), PIN (E), PCA (F), and PCA metastasis (G). H, total cell lysates (10 μg) were used for Western blot analysis to determine the expression levels of TSPO in human prostate cancer cell lines PPC-1, DU145, LAPC4, LA98, LNCaP, and LNCaP compared with human embryonic kidney cells (HEK293), human T lymphocytes (Jurkat), human cervical cancer cells (HeLa), and human liver hepatocytes (Hep).
with FITC-conjugated Annexin V (PharMingen) and propidium iodide. Cells were counted and Annexin V was added at 1 × 10⁶ cells for each condition (in 100 μL of Annexin V binding buffer, according to the recommendations of the manufacturer) in duplicate with propidium iodide at a final concentration of 5 μg/mL. Annexin V–positive cells were considered apoptotic and the percentage of total number of cells was calculated. Cells taking up vital propidium iodide dye were considered dead. Samples of 10,000 cells were analyzed by FACSscan flow cytometer with LYSIS II software package (Becton Dickinson).

Transfection. A vector containing TSPO cDNA (pCMV6-TSPO) was purchased from Origene and the empty vector was used as a negative control for all experiments. HEK293 cells were transfected using LipofectAMINE (Invitrogen), stable clones selected by neomycin resistance, and TSPO expression levels were analyzed by immunoblot analysis as described above. The HEK293 cells overexpressing TSPO will be referred to as HEK293 TSPO.

HEK293 TSPO susceptibility to lorazepam. HEK293 and HEK293 TSPO cells were plated at a density of 3 × 10⁵ cells/well in six-well plates. Cells adhered overnight and were then treated with 50 μmol/L of lorazepam for 48 h. Following treatment, cells were trypsinized and counted using a Coulter counter. These experiments were repeated in three independent experiments.

TSPO antagonism in human prostate cancer xenografts. PPC-1 cells were grown to 80% confluence in growth medium. Cells were dissociated with trypsin, washed twice in HBSS, and 20 male athymic nu/nu mice (Charles River Laboratories) received subcutaneous flank injection of 1 × 10⁶ cells per 100 μL of HBSS. Mice were weighed and tumor volumes were measured with calipers twice a week and tumor volumes calculated (tumor volume = length × width × height ÷ 0.5236). When the average tumor size reached 100 to 200 mm³, the mice were randomized to the following treatment groups: lorazepam or vehicle (DMSO). Treatments were administered i.p. at 40 mg/kg lorazepam for 48 h. Following treatment, cells were trypsinized and counted using a Coulter counter. These experiments were repeated in six independent experiments.

Immunohistochemistry. Five-micrometer sections of paraffin-embedded tissues were quenched in 3% hydrogen peroxide for 15 min and stained following citrate-steam antigen retrieval with TEC-3 Ki-67 (M7249; Dako) or PECAM (Santa Cruz Biotechnology) primary antibodies. A biotinylated secondary antibody was used, followed by streptavidin-conjugated horseradish peroxidase and 3,3′-diaminobenzidine chromogen (K0690; Dako). For labeling apoptotic nuclei, tissues were deparaffinized and treated with 0.3% H₂O₂ for 30 min to eliminate endogenous peroxidases. The DNA nick-labeling reaction was carried out using 50 units/mL of Klenow (Roche Diagnostics), 2 mmol/L of deoxyribonucleotide triphosphate (Promega) with 0.5 mmol/L of biotin-16-dUTP (Roche Diagnostics) in buffer A [0.05 mol/L Tris (pH 7.5), 5 mmol/L MgCl₂, 0.058 mmol/L mercaptoethanol sulfonic acid, and 0.05% bovine serum albumin] for 60 min at 37°C. The sections were then rinsed in PBS and incubated with 50 mg/mL of ferroxidase-Z-Avidin (Zymed Laboratories) in PBS with 0.5% bovine serum albumin for 30 min at 37°C. After rinsing, the labeling was visualized using a dianisobenzidine solution [90 mg 3,3′-diaminobenzidine (Sigma) in 150 mL PBS, 600 μL NiCl₂, +60 μL H₂O₂ 30%]. As a positive control, adjacent tissue sections were treated with DNsae (0.1 mg/mL). All of the tissues were scored in a blinded fashion.

Results

**TSPO expression is increased in prostate cancer.** To determine relative expression levels of TSPO in human tissue, we did immunohistochemical analyses of prostate cancer TMAs. As shown in Fig. 1A-G, we observed significantly increased expression of TSPO in PIN (average scores; 3.0/6), primary prostate cancer (4.1/6), and prostate cancer metastases (4.8/6) compared with normal donor (2.0/6), NAT (2.1/6), and BPH (1.8/6). Furthermore, TSPO expression increases with progression, as prostate cancer metastases have the highest expression levels. Testes and adrenals are steroidogenic tissues documented as having relatively high TSPO expression and were therefore used as positive controls.

**Table 1. TSPO immunostaining in human prostate tissue by Gleason, stage, and PSA failure**

<table>
<thead>
<tr>
<th></th>
<th>PCa</th>
<th>PIN</th>
<th>NAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>3.07 ± 0.86</td>
<td>3.01 ± 1.06</td>
<td>2.85 ± 0.92</td>
</tr>
<tr>
<td>Stage II</td>
<td>4.14 ± 1.04</td>
<td>3.52 ± 0.95</td>
<td>2.58 ± 0.93</td>
</tr>
<tr>
<td>Stage IV</td>
<td>4.46 ± 0.88*</td>
<td>3.63 ± 0.51</td>
<td>2.65 ± 0.79</td>
</tr>
<tr>
<td>PSA failure</td>
<td>4.17 ± 0.97</td>
<td>3.34 ± 0.98</td>
<td>2.66 ± 0.88*</td>
</tr>
<tr>
<td>Disease-free</td>
<td>4.02 ± 1.13</td>
<td>2.91 ± 0.75</td>
<td>2.07 ± 0.82</td>
</tr>
</tbody>
</table>

*Indicates statistical significance, <0.05. Gleason 9 specimens showed higher TSPO levels than Gleason sum 6 and 7 but not Gleason 8. Stage IV PCa had higher TSPO than both stage II and III tumors.
Increased expression of TSPO is also observed in vitro with elevated expression in prostate cancer cell lines PPC-1, DU145, LAPC4, LA98, LNCaP, and LN05 compared with a nontumorigenic, human embryonic kidney cell line (HEK293), T lymphocytes (Jurkat), a tumorigenic cervical cancer cell line (HeLa), and human hepatocytes (Hep; Fig. 1H). It is important to note that the 36-kDa band observed is not unique to our studies, as higher molecular weight bands have previously been reported in Western blots using antibodies against TSPO (24, 25).

Analyses of prostate tumor Gleason sum and stage were carried out to identify whether TSPO is altered with disease progression. TSPO levels were high in all tumor specimens compared with normal adjacent glands, and TSPO expression increased with increasing grade and stage in the TMA specimens (Table 1). TSPO levels in adenocarcinoma were significantly higher than PIN or NAT when matched for stage except in stage II specimens in which PIN regions showed TSPO levels equivalent to regions of NAT. There was also a significant change in TSPO levels in patients with high Gleason sum (Table 1).

There were 25 separate organ sites for prostatic tumor metastasis analyzed from 35 patients. All sites of PCa metastases showed high TSPO levels compared with primary PCa, PIN, or NAT (data not shown). The highest TSPO levels were found in prostate metastatic lesions to the bladder (5.7 ± 0.65) and seminal vesicles (5.5 ± 0.21), whereas slightly lower levels were present in metastases to the soft tissue next to bone (3.7 ± 0.52) as well as the thyroid (4.3 ± 1.3) and scapula (4.4 ± 0.48), but all PCa metastases had higher TSPO levels than NAT (2.0 ± 0.82) or PIN (3.0 ± 0.85), and all metastatic sites but one had higher TSPO (soft tissue next to bone) than organ-confined PCa (4.0 ± 1.11).

Our assessment of TSPO expression in the PSA failure array shows a significant difference in the NAT glands of patients with PSA failure compared with the NAT of patients who remained disease-free (Table 1). However, there was no difference in the PIN or adenocarcinoma expression of TSPO in the primary tumors of patients with PSA failure compared with disease-free patients. There was also no change in TSPO levels with age in prostate tumor specimens (data not shown). The PSA failure array did not contain specimens from patients that have remained disease-free, so the samples on the progression array were used for this comparison, and matching control tissue was used as the comparison between TMAs to assure that immunohistochemical scoring remained the same across separate arrays.

**TSPO antagonism has antiproliferative and proapoptotic effects in vitro.** We began our preliminary functional studies to identify cancer cell sensitivity to TSPO receptor blockade by

![Fig. 2. TSPO antagonism decreases cell proliferation and increases apoptosis in prostate cancer cells in vitro.](Image)

A, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasodium bromide assay following 48 h of treatment of human prostate cancer cells PPC-1 with PK11195, lorazepam (0.1-100 μmol/L), or vehicle (ethanol or DMSO). B, direct cell counting of PPC-1 and LN97 cells treated with varying concentrations of PK11195, lorazepam, or vehicle for 48 h. C, cell death ELISA following 18 h of treatment of PPC-1 and DU145 cells with varying concentrations of PK11195, lorazepam, or vehicle (*, P < 0.05).
screening a series of potential TSPO antagonists, including benzodiazepines temazepam, lorazepam, estazolam, and Ro5-4864, and the isoquinoline carboxamide, PK11195. Among all of the compounds examined, the benzodiazepine lorazepam and PK11195 showed the most significant antagonistic properties based on cell proliferation assays. To further examine the antagonistic effects of these compounds, PPC-1 human prostate cancer cells were treated with varying concentrations of PK11195 or lorazepam (0.01-100 μmol/L) for 48 h and cell viability was analyzed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide assay. Figure 2A shows that treatment with PK11195 or lorazepam significantly decreases cell viability at micromolar concentrations. Additionally, we observed a decrease in cell number following treatment with either PK11195 or lorazepam (Fig. 2B).

In other in vitro cancer models, TSPO antagonists have been shown to reduce cell survival through apoptosis and we assessed whether the decrease in cell proliferation with treatment using PK11195 or lorazepam is through induction of an apoptotic pathway. Prostate cancer cells were treated with PK11195, lorazepam, or vehicle for 18 h. An apoptosis ELISA was used to quantify histone-associated DNA fragments present in the cell lysates. Figure 2C shows a dose-dependent increase in apoptosis following treatment with PK11195 or lorazepam. Using Annexin V staining and flow cytometry, we also observed a significant dose-dependent increase in apoptosis in the PPC-1 and LNCaP cells treated with PK11195 (data not shown).

Overexpression of TSPO receptor modulates the effect of TSPO antagonism. Overexpression of TSPO in HEK293 cells (Fig. 3A) did not significantly alter the growth rate (data not shown) but significantly increased the susceptibility of cells to TSPO antagonism (Fig. 3B). HEK293-TSPO cells, vector only controls, and wild-type HEK293 cells were treated with 50 μmol/L of lorazepam or PK11195, and cell counts were analyzed after 48 hours. Lorazepam (Fig. 3B) and PK11195 (data not shown) significantly reduced cell numbers in HEK293-TSPO cells but not in the controls. However, PK11195 also induced significant cell death in wild-type and vector control cells indicating potential off-target effects.

TSPO antagonism has antiproliferative and proapoptotic effects in vivo. To examine the in vivo efficacy of TSPO inhibition, 20 athymic male mice received subcutaneous flank injections of prostate cancer cells. When tumors reached ~100 to 200 mm³, the mice were stratified into two treatment groups (10 mice per arm) such that each mouse received a daily dose of either 40 mg/kg of lorazepam or vehicle (1% DMSO). The tumor measurements show a divergence in tumor growth between lorazepam-treated and vehicle-treated mice: by week 9, lorazepam-treated mice exhibited a significantly smaller average tumor volume (2,682 ± 539 mm³) when compared with vehicle-treated mice (7,392 ± 346 mm³; Fig. 4A).

A nonlinear mixed effects approach to examine tumor growth longitudinally was implemented to describe the growth characteristics of the PPC-1 cells under vehicle-treated and lorazepam-treated arms. The Gompertz model resulted in the lowest Akaike information criterion and objective function values by ~25 points under the first-order conditional estimation method (FOCE) interaction estimation method ($P < 0.001$ for the objective function with 2 df). Individually, treatment groups were distinguishable with a covariate representing lorazepam treatment for the $\kappa$ (growth rate), $\gamma$ (time of maximum growth), and $\alpha$ (maximum tumor size) terms ($P < 0.001$). However, once multiple factors were added, the effect of treatment on the $\alpha$ term (i.e., the projected maximum size asymptote for the tumor) was greatest, and the effect on the other two terms were no longer significant. Specifically, the objective function changed from 1,791.3 to 1,728.8 with the addition of lorazepam treatment as a covariate on the $\alpha$ term. This represents a statistically significant change with $P < 0.0001$ for 1 df. In addition, the presence of lorazepam resulted in a predicted maximum tumor size approximately 1/2 as large as that predicted in the presence of vehicle (14,900 versus 28,400 mm³).

Once the tumor burden reached 2 cm, the mice were sacrificed 2 hours after the last dose of vehicle or lorazepam, and tumors were removed and processed for analysis. Tissue sections were stained for TSPO to determine if lorazepam treatment altered TSPO density (26, 27). Based on immunohistochemical analysis, we did not observe a significant difference in TSPO expression between the lorazepam-treated and vehicle groups (data not shown). Lorazepam did have an effect on cell proliferation, as there is a significant decrease in expression of the proliferation-associated protein Ki67 in mice treated with lorazepam compared with the vehicle group (Fig. 4B). Furthermore, lorazepam treatment did not affect vascularization, as the number of vessels per field was not significantly different between the two groups (Fig. 4B). Terminal nucleotidyl transferase–mediated nick end labeling analysis reveals that lorazepam has proapoptotic actions in vivo, with the lorazepam-treated group having significantly more apoptotic cells compared with the vehicle group (Fig. 4B).

Discussion

The goal of this study was to characterize TSPO expression in human prostate cancer samples and to determine its role as a potential therapeutic target for advanced disease. We have shown that TSPO expression is increased early in the neoplastic process, as PIN has significantly increased levels of TSPO compared with normal prostate tissue and BPH. Moreover, we have shown that TSPO expression increases with progression, as prostate cancer metastases have significantly more TSPO expression than other tissues examined, including PIN and primary prostate cancer. Expression analysis in vitro suggests that
TSPO is highly expressed in prostate cancer cell lines differing in their invasive abilities and androgen sensitivity. Together, our data support previous studies reporting that TSPO density is elevated in high-grade astrocytomas (11), glioblastomas (28), and highly aggressive breast cancer cell lines (29) compared with low-grade brain lesions and nonaggressive breast cancer cell lines. Similarly, Beinlich et al. reported that the TSPO ligand Ro5-4864 has the highest affinity-binding capacity in highly aggressive, estrogen receptor–negative, progesterone receptor–negative breast cancer cell lines BT-20 and MDA-MB-435-5, but binds with low capacity in estrogen receptor-positive, progesterone receptor–positive nonaggressive MCF-7 and BT-474 breast cancer cell lines (30).

Several studies in the past decade have suggested that TSPO may play a role in carcinogenesis through its action as a modulator of cell proliferation. TSPO is highly expressed in steroidogenic cells, such as those of the testes and adrenals, of which TSPO ligands have been shown to regulate cell proliferation (31). TSPO ligands have also been shown to affect proliferation in various tumors, such as astrocytomas, breast, esophageal, and colorectal cancers (11–14). For our studies, we used one of the most common TSPO ligands, PK11195, as well as the benzodiazepine lorazepam, which has not been previously considered a TSPO antagonist. We show that both PK11195 and lorazepam have antiproliferative properties in prostate cancer cells in vitro. Because lorazepam is a clinically approved drug that could easily be translated from preclinical studies to the prostate cancer patient population, we wanted to determine if lorazepam also exhibits antiproliferative actions in vivo. We observe a decrease in the length of time it took for the prostate cancer xenograft tumors to reach maximum size in the lorazepam-treated mice compared with the vehicle group. Additionally, Ki67 expression, a protein marker for cell proliferation, is decreased in the lorazepam-treated group compared with mice given vehicle only. In our tumor xenograft studies, the mice would become sedated for ~2 hours postinjection with lorazepam but could still ambulate when stimulated and remained responsive during the entire time. There were no deaths associated with dosing once a day with lorazepam at 40 mg/kg for the entire study. The half-life of lorazepam in mice is ~12 hours. The LD50 in mice for lorazepam is 3,178 mg/kg (oral) and we have observed significant effects on induction of apoptosis at 40 mg/kg in mice. The concentration of lorazepam needed to induce apoptosis in tumor cells in patients would likely be achievable. This study further confirms that TSPO ligands modulate cell

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![Figure 4](https://example.com/fig4.png)

**Fig. 4.** TSPO antagonism decreases cell proliferation and increases apoptosis in prostate cancer cells in vivo. A, average tumor volume over time of athymic nude mice bearing PPC-1 xenograft tumors treated daily with lorazepam (40 mg/kg) or DMSO. Tumor volume was measured twice weekly as described in Materials and Methods. Columns, mean; bars, SE. B, immunohistochemical staining of lorazepam-treated or vehicle-treated PPC-1 xenograft tumors for cell proliferation (Ki67), microvascular density (CD31), and apoptosis (TUNEL). Columns, average values of positive signal counted in four random fields (magnification, ×40; *, P < 0.05).
References


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