Monoclonal Antibodies to Six-Transmembrane Epithelial Antigen of the Prostate-1 Inhibit Intercellular Communication In vitro and Growth of Human Tumor Xenografts In vivo

Pia M. Challita-Eid, Kendall Morrison, Soudabeh Etessami, Zili An, Karen J. Morrison, Juan J. Perez-Villar, Arthur B. Raitano, Xiao-Chi Jia, Jean M. Gudas, Steven B. Kanner, and Aya Jakobovits

Agensys, Inc., Santa Monica, California

Abstract

Six-transmembrane epithelial antigen of the prostate-1 (STEAP-1) is a novel cell surface protein highly expressed in primary prostate cancer, with restricted expression in normal tissues. In this report, we show STEAP-1 expression in prostate metastases to lymph node and bone and in the majority of human lung and bladder carcinomas. We identify STEAP-1 function in mediating the transfer of small molecules between adjacent cells in culture, indicating its potential role in tumor cell intercellular communication. The successful generation of two monoclonal antibodies (mAb) that bind to cell surface STEAP-1 epitopes provided the tools to study STEAP-1 susceptibility to naked antibody therapy. Both mAbs inhibited STEAP-1–induced intercellular communication in a dose-dependent manner. Furthermore, both mAbs significantly inhibited tumor growth in mouse models using patient-derived LAPC-9 prostate cancer xenografts and established UM-UC-3 bladder tumors. These studies validate STEAP-1 as an attractive target for antibody therapy in multiple solid tumors and provide a putative mechanism for mAb-induced tumor growth inhibition. [Cancer Res 2007;67(12):5798–805]

Introduction

The clinical use of monoclonal antibodies (mAb) as cancer therapeutics has been realized in the last few years with the approval and the successful commercialization of antibody products, such as trastuzumab, rituximab, cetuximab, and bevacizumab (1). These advances stimulate the need for identification of additional cell surface targets suitable for antibody-based therapy. Among the desirable features of such targets are abundant and homogenous target expression in the majority of relevant patient populations, minimal expression in normal vital tissues, and target involvement in the disease pathogenesis.

Previously, we reported the identification of six-transmembrane epithelial antigen of the prostate-1 (STEAP-1), a novel 39–amino acid cell surface antigen (2). Our studies showed significant and homogeneous STEAP-1 expression in all prostate cancer metastases, including hormone-refractory samples, with predominant expression in prostate among normal tissues (2). High levels of STEAP-1 were also detected in multiple epithelial tumor cell lines, suggesting its potential applicability in other cancer indications. Immunohistochemical analysis indicated STEAP-1 localization at cell-cell junctions of the secretory epithelium of prostate and prostate cancer cells. This observation, together with its six-transmembrane topology, suggested that STEAP-1 may function as a channel/transporter protein in cell-cell junctions.

The favorable expression profiling of STEAP-1 in normal and cancer tissues suggested its potential use as a target for immunotherapy. Recent studies have shown STEAP-1 as a suitable antigen for T-cell–based immunotherapy (3–5); however, its utilization for antibody-mediated targeting has not yet been explored.

In this report, we have extended STEAP-1 expression analysis to show its significant expression in all prostate cancer metastases and in the majority of lung and bladder carcinoma specimens tested. We identified the involvement of STEAP-1 in intracellular communication in vitro. Two mAbs, specific to STEAP-1 extracellular loops, were shown to inhibit STEAP-1–mediated intercellular transport in vitro. Furthermore, both antibodies exhibited significant efficacy in inhibiting the in vivo growth of prostate and bladder tumor xenografts.

Materials and Methods

Cell lines and xenografts. All human cancer cell lines used in this study were obtained from the American Type Culture Collection. Cell lines were maintained in DMEM supplemented with 10% fetal bovine serum. The androgen-dependent patient-derived prostate cancer xenograft LAPC-9AD (6) was obtained from Dr. C. Sawyers (Memorial Sloan-Kettering, New York, NY). The tumors were propagated by passage in severe combined immunodeficient (SCID) mice.

Cell line generation. NIH-3T3 and PC3 cells, obtained from American Type Culture Collection, were transduced with a retroviral vector encoding STEAP-1 (2, 7). Cells were selected using G418, expanded, and used in the binding assays. 293T cells were transiently transfected with pcDNA3.1/MycHis vector (Invitrogen) encoding STEAP-1 using a calcium phosphate precipitation method. Cells collected after 48 h were confirmed for STEAP-1 expression by flow cytometry.

Immunohistochemical staining. Sheep polyclonal antibody directed to the NH2 terminus of STEAP-1 was used for immunohistochemical analysis (2). Briefly, formalin-fixed, paraffin-embedded tissue sections were incubated with 1.5 μg/mL antibody followed by sequential incubation with biotinylated rabbit anti-sheep IgG (Vector Laboratories) and streptavidin-conjugated horseradish peroxidase (HRP; BioGenex). The peroxidase reaction was subsequently visualized with 3,3′-diaminobenzidine substrate (BioGenex).

Intercellular communication assay. Intercellular communication was measured using a fluorescent dye transfer assay adapted from previously published studies (8, 9). Briefly, acceptor cells were preloaded with the dye dextran-Texas red (10,000 molecular weight; Invitrogen/Molecular Probes) overnight at 37°C. Donor cells were preloaded with 5 μg/mL of the green dye calcein AM (Invitrogen/Molecular Probes) for 1 h at 37°C. Donor cells...
were layered over acceptor cells at a ratio of 1:3. Cells were incubated at 37°C for 6 to 7 h. Percentage transfer was calculated as the fraction of cells that accepted the green dye (yellow/orange cells) in the total population of acceptor cells (yellow/orange and red cells). Transfer was measured by counting the number of cells in five different fields using fluorescence microscopy (×40).

Small interfering RNA studies. Small interfering RNA (siRNA) oligonucleotide 5′-GAAGACGATTATTTGCATA-3′, obtained from Dharmacon, was selected based on silencing potency and lack of off-target effect on STEAP-1–nonexpressing cells (ST3 and RAT1). PCS-STEAP-1 and PC3-neo cells were transfected with the selected siRNA oligonucleotide at a concentration of 10 nmol/L using LipofectAMINE-2000 (Invitrogen). Forty-eight hours later, the cells were harvested and either stained for STEAP-1 expression by flow cytometry or used in the intercellular communication assay.

mAb generation and purification. BALB/c mice (Taconic) were immunized with 293T cells transiently transfected with a vector encoding STEAP-1. Spleen cells from immunized mice were fused to SP2/0-Ag14 myeloma cells using polyethylene glycol. Supernatants from derived hybridomas were screened for binding STEAP-1–expressing cells by flow cytometry. mAbs were purified using standard Protein G (GE Healthcare/Life Sciences) affinity chromatography.

Western blotting. Cells were lysed using radioimmunoprecipitation assay buffer [150 mmol/L NaCl/50 mmol/L Tris-HCl (pH 7.2)/1% deoxycholate/1% Triton X-100/0.1% SDS/0.5 mmol/L EDTA]. Anti-STEAP-1 mAbs were preincubated with protein-G beads at 4°C for 2 h, washed and incubated with the cell lysates at a concentration of 10 μg/mL overnight at 4°C. The immunoprecipitates were washed thrice with PBS, eluted in sample loading buffer, and then subjected to SDS-PAGE. Proteins were transferred onto nitrocellulose and the blots were stained with 4CBA and analyzed by flow cytometry.

Affinity measurements. Adherent cell monolayers were detached by treatment with 0.5 mmol/L EDTA in PBS, washed, and incubated with anti-STEAP-1 antibodies for 1 h at 4°C. The cells were then washed and stained with phycoerythrin-conjugated goat anti-mouse IgG (Jackson Immunoresearch Laboratories, Inc.) and analyzed by flow cytometry.

Flow cytometry analysis. Adherent cell monolayers were detached by treatment with 0.5 mmol/L EDTA in PBS and incubated with increasing concentrations of STEAP-1 mAbs overnight at 4°C. The cells were then washed and stained with phycoerythrin-conjugated goat anti-mouse IgG and analyzed by flow cytometry. The mean fluorescence intensity (MFI) was plotted against the mAb concentration, and the affinity was determined by nonlinear regression using GraphPad Prism software as the concentration of ligand required to reach half-maximal binding (GraphPad Software).

In vivo studies. Male ICR-SCID mice, 5 to 6 weeks old, were obtained from Taconic. For tumor formation inhibition studies, mice were injected s.c. with 2 × 106 LAPC-9AD cells mixed with Matrigel (BD Biosciences) at a ratio of 1:1. Treatment was initiated on the same day with 500 μg/dose of either control or STEAP-1 mAb by i.p. injection. Mice were treated twice weekly for 6 weeks. For treatment of established tumors, mice were injected s.c. with 2 × 106 UM-UC-3 cells mixed with Matrigel. Treatment was initiated on day 8 when tumor volume reached ~60 mm³. Mice were treated with 1 mg mAb injected i.p. twice weekly for three doses. Tumor length (L) and width (W) were measured with a caliper and tumor volume was estimated using the formula \(V = \frac{L \times W^2}{2}\). The percentage inhibition rate was calculated using the formula:

\[
1 - \left(\frac{\text{mean volume of treated tumors}}{\text{mean volume of control tumors}}\right) \times 100
\]

Serum prostate-specific antigen (PSA) levels were determined using the Human PSA ELISA kit from Anogen according to the manufacturer’s protocol.

Figure 1. STEAP-1 protein expression in prostate, bladder, and lung cancer specimens. STEAP-1 staining of formalin-fixed paraffin-embedded tissue sections from (A) prostate carcinoma, grade 3 (left) and grade 4/5 (right), (B) prostate cancer metastasis to lymph node (LN; left) and bone (right), (C) bladder transitional carcinomas, and (D) lung cancer adenocarcinoma (left) and squamous cell carcinoma (right).

Statistical analysis. For LAPC-9AD xenograft studies, mean tumor volumes among groups were compared using the nonparametric Kruskal-Wallis test. Pairwise comparisons were calculated using the Dunn’s multiple comparison post-test, two sided with \(\alpha = 0.05\). For UM-UC-3 xenograft studies, mean tumor volumes among groups were compared using the parametric ANOVA test, and results of pairwise comparisons of treated groups versus control were obtained using Dunnett multiple comparison post-test. Tests were two sided with \(\alpha = 0.05\).

Results

Expression of STEAP-1 in human lung, bladder, and prostate metastasis tumors. Previously, we showed strong and uniform expression of STEAP-1 protein in all primary prostate cancer specimens tested and in numerous cell lines derived from multiple solid tumors (Fig. 1A; ref. 2). In this study, we have extended the immunohistochemical analysis of STEAP-1 protein expression to metastatic prostate cancer and to bladder and lung...
cancers. All prostate lymph node and bone metastases analyzed (five specimens each) exhibited strong and homogeneous STEAP-1 staining (Fig. 1B). All 14 primary bladder transitional cancer specimens were positive for STEAP-1 with 60% showing strong staining in the majority of the tumor cells (Fig. 1C). STEAP-1 protein expression was also detected in 14 of 16 lung carcinoma tested including lung adenocarcinoma and squamous cell carcinoma (Fig. 1D). Of the positive tumors, 70% expressed STEAP-1 in the majority of the tumor sections. These results validate STEAP-1 as a potential target not only for all stages of prostate cancer disease, but also other epithelial tumors.

**STEAP-1 mediates intercellular communication activity.** We have observed previously that STEAP-1 protein localizes to areas of cell-cell junctions. This observation, together with the predicted secondary structure as a channel protein, suggested that STEAP-1 may be involved in intercellular communication (2). STEAP-1 function as a transporter of small molecules (<1 kDa) between cells was studied using a fluorescent dye transfer assay (see Materials and Methods). The prostate cancer cell line PC3, which does not express STEAP-1, was chosen for the assay due to its minimal intrinsic cell communication properties resulting from low levels of connexin expression (10). Retroviral transduction of STEAP-1 or empty vector was used to generate paired cell lines, PC3-STEAP-1 and control PC3-neo, to study the effect of STEAP-1 on intercellular communication (Fig. 2A). Donor cells loaded with calcein AM and acceptor cells loaded with dextran-Texas red were mixed. Dye transfer, as manifested by intracellular yellow/orange fluorescence, was not detected between donor and acceptor cells that did not express STEAP-1 nor between cells in which only one partner expressed STEAP-1. In contrast, extensive dye transfer was observed in almost all cells in cultures where both donor and acceptor cells expressed STEAP-1. Confirmation of STEAP-1 function in mediating

Figure 2. STEAP-1 mediates intercellular communication in PC3 cells. A, donor and acceptor cells (donor → acceptor) were loaded with calcein AM (donor) or dextran-Texas red (acceptor) dyes and mixed 1:3 to allow intercellular communication to occur as manifested by the appearance of the yellow/orange color (see Materials and Methods). Representative pictures were taken from PC3-neo → PC3-neo, PC3-neo → PC3-STEAP-1, PC3-STEAP-1 → PC3-neo, and PC3-STEAP-1 → PC3-STEAP-1 populations. B, PC3-STEAP-1 cells were transfected with a control siRNA (gray histogram) or a STEAP-1 siRNA (black histogram). Cells were harvested 72 h later, stained with either isotype control mAb (solid histogram) or mAb 120.545 (open histograms), and then analyzed by FACS.
intercellular communication was done by silencing its expression in PC3-STEAP-1 acceptor cells using a STEAP-1 siRNA oligonucleotide. Cells transfected with a STEAP-1 siRNA oligonucleotide showed significant specific decrease in STEAP-1 cell surface expression (Fig. 2B) and exhibited an 87% reduction in dye transfer compared with cells transfected with control siRNA (Table 1).

**Generation of mAbs specific to STEAP-1 cell surface epitopes.** To evaluate STEAP-1 as a target for antibody therapy, we generated mAbs that recognize cell surface STEAP-1 epitopes. Two mouse IgG2a mAbs, 92.30 and 120.545, were derived from cell-based immunizations. Both mAbs bind specifically to STEAP-1 expressed recombinantly by 3T3 cells and endogenously by the prostate cancer cell line LNCaP (Fig. 3), the bladder cell line UM-UC-3, and the prostate cancer xenograft LAPC-9AD (data not shown). In all cell lines, mAb 120.545 exhibited stronger binding to cell surface STEAP-1 as depicted by the higher MFI detected by fluorescence-activated cell sorting (FACS) compared with 92.30 mAb. Both mAbs immunoprecipitated the 32-kDa STEAP-1 protein from 3T3-STEAP-1 cells (Fig. 3B). The mAb affinity to cell surface STEAP-1 was measured by FACS titration on 3T3-STEAP-1 and LNCaP cells. mAb 120.545 exhibited an affinity of 1 nmol/L to both 3T3-STEAP-1 and LNCaP cells. mAb 92.30 exhibited lower affinity, 12 and 45 nmol/L for 3T3-STEAP-1 and LNCaP, respectively. We have found that the two mAbs bind to different STEAP-1 epitopes, based on their inability to compete with each other in competitive binding analysis and the robust binding of mAb 92.30 but not 120.545 to the STEAP-1 mouse orthologue (data not shown).

**STEAP-1 mAbs block intercellular communication.** We investigated the effect of the two mAbs on STEAP-1-mediated intercellular communication activity. Incubation of STEAP-1–expressing donor and acceptor cell populations with each of the STEAP-1 mAbs blocked intercellular communication in a dose-dependent manner (Table 1). Dye transfer was inhibited by 69% and 99% in the presence of 50 μg/mL. 92.30 or 120.545 mAb, respectively, whereas the control mAb did not show a significant effect. These data show that the STEAP-1 mAbs are capable of modulating the function of STEAP-1 as an intercellular transporter protein.

**STEAP-1 mAbs inhibit tumor growth in vivo.** The ability of STEAP-1 mAbs to inhibit tumor growth in vivo was evaluated using the patient-derived androgen-dependent prostate cancer xenograft LAPC-9AD and the bladder cancer cell line UM-UC-3. Both xenograft models express significant levels of STEAP-1 as confirmed by immunohistochemical staining of tumor tissues (Figs. 4A and 5A). SCID mice were injected s.c. with $2 \times 10^6$ LAPC-9AD tumor cells and treated i.p. with either PBS, control mouse mAb, or STEAP-1 mAbs at 500 μg/dose. Treatment was given twice weekly for 6 weeks starting on the day of tumor cell injection. As shown in Fig. 4B, significant inhibition of tumor growth (85% for mAb 120.545 and 92% for mAb 92.30) was detected on day 40. For both mAbs, the inhibition rate was statistically significant compared with control animals ($P < 0.05$). Mice receiving the STEAP-1 mAbs were monitored for an additional 7 days after sacrificing the control-treated mice due to high tumor burden. No significant increase in tumor volume was observed 7 days after the last treatment. Previous studies have shown that in LAPC-9AD tumor-bearing mice, PSA blood levels were directly correlated to tumor burden (6, 11). To further confirm STEAP-1 mAb efficacy, sera obtained from mice at time of sacrifice were tested for PSA levels. As shown in Fig. 4C, significant reduction (>95%) in circulating PSA levels was detected in mice treated with either 120.545 or 92.30 mAb compared with control mice.

The antitumor effect of STEAP-1 mAbs was also evaluated in established bladder cancer UM-UC-3 xenografts (Fig. 5B). UM-UC-3 cells were injected s.c. into SCID mice to establish tumors. STEAP-1 mAb treatment was initiated on day 8 when tumors reached a volume of 50 to 60 mm³. Randomized mouse groups were treated

<table>
<thead>
<tr>
<th>Donor cells</th>
<th>Acceptor cells</th>
<th>Treatment (μg/mL)</th>
<th>% Dye transfer</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC3-STEAP-1</td>
<td>PC3-neo</td>
<td>—</td>
<td>8</td>
<td>NA</td>
</tr>
<tr>
<td>PC3-neo</td>
<td>PC3-STEAP-1</td>
<td>—</td>
<td>5</td>
<td>NA</td>
</tr>
<tr>
<td>PC3-STEAP-1</td>
<td>PC3-STEAP-1</td>
<td>—</td>
<td>97</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Table 1.** STEAP-1–mediated intercellular communication is inhibited by STEAP-1 siRNA and mAbs

**siRNA treatment**

<table>
<thead>
<tr>
<th>Donor cells</th>
<th>Acceptor cells</th>
<th>Treatment</th>
<th>% Dye transfer</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC3-STEAP-1</td>
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<td>Control siRNA</td>
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</tr>
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<td>PC3-STEAP-1</td>
<td>STEAP-1 siRNA</td>
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<td>87</td>
</tr>
</tbody>
</table>

**mAb treatment**

<table>
<thead>
<tr>
<th>Donor cells</th>
<th>Acceptor cells</th>
<th>Treatment</th>
<th>% Dye transfer</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC3-STEAP-1</td>
<td>PC3-STEAP-1</td>
<td>Control IgG2a (20)</td>
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<td>0</td>
</tr>
<tr>
<td>PC3-STEAP-1</td>
<td>PC3-STEAP-1</td>
<td>Control IgG2a (50)</td>
<td>77</td>
<td>0</td>
</tr>
<tr>
<td>PC3-STEAP-1</td>
<td>PC3-STEAP-1</td>
<td>mAb 92.30 (20)</td>
<td>40</td>
<td>51</td>
</tr>
<tr>
<td>PC3-STEAP-1</td>
<td>PC3-STEAP-1</td>
<td>mAb 92.30 (50)</td>
<td>24</td>
<td>69</td>
</tr>
<tr>
<td>PC3-STEAP-1</td>
<td>PC3-STEAP-1</td>
<td>mAb 120.545 (20)</td>
<td>20</td>
<td>75</td>
</tr>
<tr>
<td>PC3-STEAP-1</td>
<td>PC3-STEAP-1</td>
<td>mAb 120.545 (50)</td>
<td>1</td>
<td>99</td>
</tr>
</tbody>
</table>

**NOTE:** Donor and acceptor cells were preloaded with dye as described in Materials and Methods and incubated in the absence or presence of siRNA or mAb at the indicated concentrations. Percentage dye transfer was calculated as the fraction of yellow/orange cells in the total population of acceptor cells. Percentage Inhibition was calculated as the percentage reduction in dye transfer in STEAP-1 siRNA-treated cells or anti-STEAP-1 mAb-treated cells compared with control treatments.

Abbreviation: NA, not applicable.
with either PBS or 1 mg per dose of 92.30 or 120.545 mAb for three doses. The study was terminated on day 20 due to high tumor burden in the control group. Statistically significant inhibition of tumor growth was detected following treatment with mAb 92.30 (inhibition of 51%; \( P = 0.0015 \)) and mAb 120.545 (inhibition of 47%; \( P = 0.0055 \)) compared with the control group. These \textit{in vivo} studies show potent STEAP-1 mAb efficacy in inhibiting growth of both prostate and bladder tumor xenografts. Treatment of mice with mAb 92.30, which binds to the murine STEAP-1 orthologue, did not show any overt toxicity. The mice were well groomed and sustained normal body weight and their movement was normal during the experimental period.

**Discussion**

This report provides validation of STEAP-1 as an attractive target for antibody cancer therapy based on its significant expression in multiple cancer indications and its apparent involvement in tumor growth that can be modulated by naked mAbs. Our overall immunohistochemical analysis of patient samples shows significant STEAP-1 expression in various epithelial tumors. The strong and homogeneous STEAP-1 expression, shown previously in primary prostate cancer, has been extended in this study to prostate cancer metastases to lymph node and bone. STEAP-1 expression was also detected in the majority of tested specimens of lung adenocarcinomas and squamous cell carcinomas as well as bladder transitional carcinomas, thus expanding the repertoire of cancer indications applicable to STEAP-1–targeted therapy.

We describe the successful generation of two unique mAbs specific to the extracellular epitopes of STEAP-1 and their utilization to show antitumor efficacy. Generation of mAbs to STEAP-1 cell surface epitopes was a challenging task due to the predicted exposure of only small peptide loops on the extracellular leaflet. The two mAbs exhibit different affinities and bind to
different epitopes; however, both inhibited STEAP-1–mediated intercellular communication and the growth of two different xenograft tumor models. The antibodies inhibited the formation of patient-derived LAPC-9 prostate cancer xenografts, as manifested by both tumor volume and PSA levels. Furthermore, they inhibited the growth of established UM-UC-3 bladder cancer xenografts. These results support the involvement of STEAP-1 in growth of these two xenograft models and indicate the ability of STEAP-1 antibodies to modulate this function. STEAP-1 mAbs 92.30 and 120.545 were evaluated in proliferation assays in vitro in both LNCaP and UMUC-3 cell lines. The mAbs did not affect the proliferation of the cell lines (data not shown). Because a significant effect of both mAbs on tumor growth in vivo was observed in two different models, we propose that STEAP-1 may play a critical role in tumor growth in vivo but not in vitro due to interaction with neighboring cells within the solid tumor microenvironment.

Of interest is the identification of STEAP-1 function as an intercellular communication protein, which concurs with our previous hypothesis based on its localization to cell-cell boundaries and its projected topology (2). Using a dye transfer assay, we show that STEAP-1 expression in connexin-deficient PC3 cells induces significant dye transport between cells, which is inhibited by specific STEAP-1 siRNA.

The predicted secondary structure of STEAP-1 as a channel protein supports the possibility that STEAP-1 acts as a transporter protein to directly transfer the dye between the cells. Alternatively, STEAP-1 may affect intercellular communication indirectly, by altering intracellular ion concentrations, which in turn regulate gap or adherence junction activity. Such a function was attributed to L-type calcium channels, which were shown to regulate adhesion gap junction activity in human osteoblastic cells via regulation of calcium influx (12). Recently, the family member STEAP-3 (TSAP6) has been shown to promote exosome secretion, which in turn allowed communication between adjacent cells (13). This may be an additional potential mechanism mediating the observed STEAP-1 intercellular communication activity.

The role of cell-cell communication in cancer pathogenesis has been gaining attention (14). Cellular communication facilitates the intercellular exchange of small molecular weight solutes, such as nutrients, metabolites, electrolytes, and second messengers from distant blood vessels, thus supporting tumor growth. Substantial evidence from various models implicates cell-cell communication in the ability of tumor cells to invade and metastasize. Intercellular communication between glioma tumor cells and astrocytes or endothelial cells was shown to increase tumor invasion and to increase vascular endothelial growth factor secretion and vascular tube formation, respectively (9, 15, 16). In prostate cancer, a direct correlation between increased connexin 26 expression levels and cancer progression was observed (17). The ability of STEAP-1 mAbs to inhibit growth of STEAP-1–expressing tumors and its intercellular transport function suggest a potential link between STEAP-1 transport function and its involvement in tumor cell growth. The inhibition of STEAP-1–mediated intercellular communication can further facilitate the effect of the mAbs on tumor growth in an in vivo microenvironment where limited nutrients and interaction with surrounding matrix components require various survival mechanisms.

Structural domains in STEAP-1 may suggest its involvement in tumor cell growth. STEAP-1 contains a heme-binding domain called the apoptosis, cancer and redox associated transmembrane domain, which is present in a structurally related family identified.
recently to include members of the STEAP family, as well as the bacterial NADPH oxidase (Nox) family and the YedZ family of oxidoreductases (18, 19). The heme-binding domain function present in this family may facilitate electron transfer and thereby alter cell growth and metabolism as has been shown for the bacterial proteins (20). Unlike the other members of the STEAP family and the bacterial Nox and YedZ families, STEAP-1 has only a heme-binding domain but not an oxidoreductase domain. The role of this structural feature of STEAP-1 in its intercellular transport function remains to be explored.

The characteristics of STEAP-1 described here validate it as a novel target with the desirable characteristics for antibody therapy. STEAP-1 is one of a few prostate cancer antigens that meet the appropriate criteria and represent potential antibody therapy targets. Other novel antigens include PSCA, a cell surface antigen highly expressed in all stages of prostate cancer (21). PSCA has been successfully targeted with naked mAbs in various xenograft models leading to significant inhibition of tumor growth and spread (11, 22) and is currently being evaluated in clinical trials. In addition, toxin-conjugated anti-PSCA antibody completely eradicated established tumors (23). For other novel prostate cancer targets, such as PSMA and tomoregulin, naked mAb efficacy has not been shown, resulting in the need to use mAbs as a vehicle to deliver payloads, such as radioisotopes and toxins, to affect tumor growth (24–26). The strong and homogeneous expression of STEAP-1 in cancer specimens, together with its restricted expression in normal tissues, also makes it a suitable target for antibody drug conjugate therapy. Taken together, STEAP-1 is an appealing target for antibody therapy that warrants future clinical investigation for multiple solid tumor indications.

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References


Figure 5. STEAP-1 mAbs inhibit the growth of established UM-UC-3 bladder cancer xenografts in vivo. A, UM-UC-3 xenograft tumors were stained by immunohistochemistry using the polyclonal sheep STEAP-1 antibody confirming expression of STEAP-1 in the xenograft. B, UM-UC-3 cells were injected s.c. into SCID mice. When tumors reached 50 to 60 mm³ in volume, mice (n = 10 per group) were treated with PBS, mAb 120.545, or mAb 92.30. Treatment was given by i.p. injection with 1 mg/dose twice weekly for a total of three doses starting on day 8 (arrow). Tumor volume was measured at the indicated time points. Points, mean tumor volume (mm³); bars, SE. Statistical analysis using the Dunnett test shows *P < 0.01 for both mAb 120.545 and mAb 92.30 treatments compared with control treatment.
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