

Targeted molecular-genetic imaging and ligand-directed therapy in aggressive variant prostate cancer

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Aggressive variant prostate cancers (AVPC) are a clinically defined group of tumors of heterogeneous morphologies, characterized by poor patient survival and for which limited diagnostic and treatment options are currently available. We show that the cell surface 78-kDa glucose-regulated protein (GRP78), a receptor that binds to phage-display-selected ligands, such as the SNTRVAP motif, is a candidate target in AVPC. We report the presence and accessibility of this receptor in clinical specimens from index patients. We also demonstrate that human AVPC cells displaying GRP78 on their surface could be effectively targeted both in vitro and in vivo by SNTRVAP, which also enabled specific delivery of siRNA species to tumor xenografts in mice. Finally, we evaluated ligand-directed strategies based on SNTRVAP-displaying adeno-associated virus/phage (AAVP) particles in mice bearing MDA-PCa-118b, a patient-derived xenograft (PDX) of castration-resistant prostate cancer bone metastasis that we exploited as a model of AVPC. For theranostic (a merging of the terms therapeutic and diagnostic) studies, GRP78-targeting AAVP particles served to deliver the human *Herpes simplex virus thymidine kinase type-1 (HSVtk)* gene, which has a dual function as a molecular-genetic sensor/reporter and a cell suicide-inducing transgene. We observed specific and simultaneous PET imaging and treatment of tumors in this preclinical model of AVPC. Our findings demonstrate the feasibility of GPR78-targeting, ligand-directed theranostics for translational applications in AVPC.

aggressive variant prostate cancer | ligand-directed theranostics | molecular imaging | gene therapy | AAVP

Prostate cancer, the most common carcinoma in men and a major cause of morbidity and mortality (1), is a clinically heterogeneous disease. Although a majority of prostate cancers are highly responsive to androgen receptor (AR)-directed therapies, a subset is relatively resistant to these therapies and carries a dismal prognosis (2–5). At the extreme of this spectrum are the small-cell or neuroendocrine carcinomas, a predominantly AR-negative histological variant of the disease that is associated with atypical clinical features (such as visceral metastases, predominantly lytic bone metastases, low levels of prostate-specific antigen relative to tumor burden, bulky primary tumors, and high levels of lactic dehydrogenase and carcinoembryonic antigen) and responds to platinum-based chemotherapies (6). Although small-cell or neuroendocrine carcinomas of the prostate are rare at initial diagnosis, they are increasingly recognized in the castration-resistant phases of disease progression. Moreover, a larger group of morphologically heterogeneous prostate cancers share the clinical features

of the small-cell neuroendocrine carcinomas and, likely, their underlying biology (7, 8); these have been grouped under the term aggressive variant prostate carcinomas (AVPC). The molecular underpinnings of the AVPC are likely shared with subsets of other

Significance

Aggressive variant prostate cancer (AVPC) is a clinically defined tumor with neuroendocrine or small-cell differentiation, visceral metastases, low prostate-specific antigen, androgen receptor insensitivity, and poor/brief responses to androgen-deprivation or platinum-based chemotherapy. AVPC incidence has markedly increased, yielding an unmet diagnostic/therapeutic need. Here we adapted a patient-derived xenograft model and tumor samples to demonstrate ligand-directed theranostics of AVPC in vivo. We engineered human *Herpes simplex virus thymidine kinase type-1* as a noninvasive imaging reporter/suicide transgene into adeno-associated virus/phage (AAVP) particles displaying motif ligands to cell surface-associated glucose-regulated protein 78kD (GRP78), toward a clinic-ready system. Although individual components of the AAVP system have been extensively investigated, this study is evidence of successful application in relevant preclinical models of untreatable and hard to diagnose aggressive tumor variants.

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Conflict of interest statement: W.A. and R.P. are founders of AAVP BioSystems, which has licensed intellectual property related to the adeno-associated virus/phage (AAVP) technology, and they are inventors on patent applications and entitled to standard royalties if commercialization occurs. The University of Texas M. D. Anderson Cancer Center and the University of New Mexico Health Sciences Center currently manage these arrangements in accordance with their established institutional conflict of interest policy.

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epithelial tumors, such as the triple-negative breast cancers. However, molecular-imaging tools and specific therapeutic approaches tailored to the AVPC are lacking.

To evaluate potential “theranostic” (a merging of the terms therapeutic and diagnostic) strategies for AVPC, we exploited SNTRVAP, a peptide motif isolated by screening of combinatorial phage display libraries in the MDA-PCa-118b patient tumor-derived xenograft (PDX) in vivo (9, 10) (Fig. 1). SNTRVAP is a ligand to a functional supramolecular complex present on the cell surface and containing the 78-kDa glucose-regulated protein (GRP78), an endoplasmic reticulum chaperone involved in prosurvival mechanisms (11). Altered glucose metabolism of cancer cells, as well as glucose starvation in poorly vascularized tumors, are associated with GRP78 overexpression and translocation to the cell surface (12–14). Overexpression of GRP78 has been correlated with poor survival in patients with breast (15–17) and prostate cancer (10, 18–20). Its tumor-specific localization and stress-response attributes make cell surface GRP78 an attractive candidate receptor for ligand-directed strategies in solid tumor management (10, 21). Chimeric adeno-associated virus/phage (AAVP) particles (22–25) combine eukaryotic virus transduction efficiency to phage-targeting attributes. In this hybrid construct, the targeting agent (a ligand peptide) is expressed as a fusion within the phage pIII capsid protein, whereas the effector agent (a transgene) is inserted into the phage genome as a fully functional expression cassette under the control of a constitutive *CMV* promoter. Ligand-directed AAVP particles have been successfully evaluated in several preclinical transgenic and xenograft tumor-bearing mouse models, including carcinomas of the breast and prostate (22), soft-tissue sarcomas (26), and glioblastomas (27). Moreover, AAVP-mediated tumor vascular delivery of TNF has proven efficacy in human melanoma xenografts (28) and pancreatic neuroendocrine transgenic tumors (29), as well as in native tumors in dogs (30). Here we investigated the GRP78-targeting properties of SNTRVAP (10), exploiting its specific binding to cell surface GRP78 for tumor targeting in the MDA-PCa-118b PDX. SNTRVAP-directed, GRP78-targeting AAVP particles were used to deliver the human *Herpes Simplex* virus thymidine kinase type-1

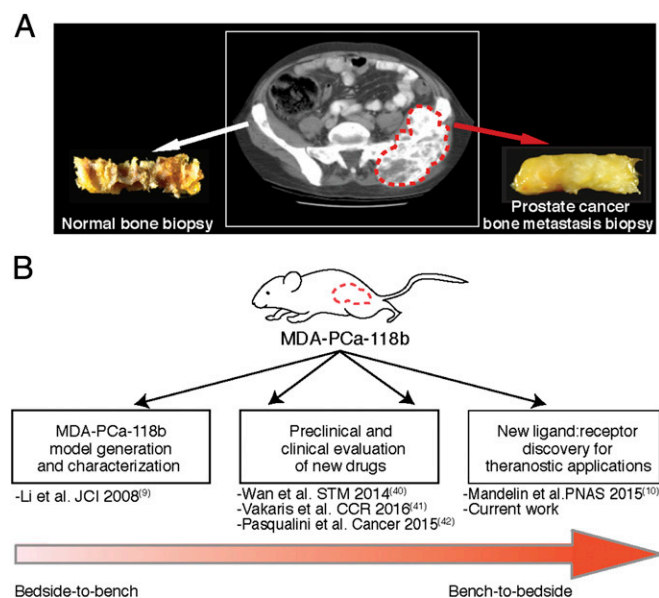


Fig. 1. Schematic representation of the MDA-PCa-118b PDX model generation and applications. (A) Index patient CT scan illustrating the original bone metastasis from which the MDA-PCa-118b PDX was obtained and propagated by subcutaneous injection in immunodeficient mice. (B) Sequential examples of translational research with this PDX model toward the present characterization of a ligand-directed theranostic approach for AVPC.

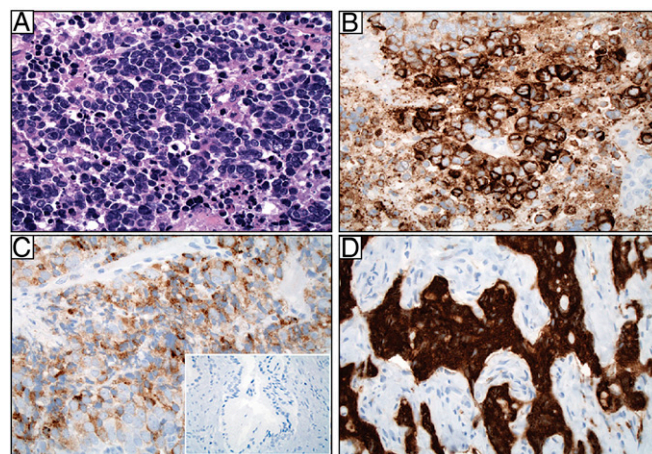


Fig. 2. GRP78 protein is expressed both in the cytoplasm and at the cell surface in AVPC specimens from patients. (A) Pathological evaluation of a representative specimen with high-grade cytology, small cell features, nuclear molding, and necrosis (H&E). (B) The same tumor stained for GRP78, moderate-to-strong (2–3+) intensity in tumor cells. (C) GRP78 staining of a representative case showing weak (1+) but diffuse positivity. (D) GRP78 staining of a representative case showing strong (3+) intensity. (Inset) Staining of normal prostate gland. (Magnification: A–D, 40 \times ; Inset, 5 \times .)

(*HSVtk*) gene to act as both a noninvasive molecular-genetic imaging sensor/reporter, in the presence of a radiolabeled substrate, and a cell suicide-inducing transgene in the presence of ganciclovir (GCV), which is converted to a cytotoxic by *HSVtk*-expressing cells. This strategy proved to be effective as a targeted theranostic in preclinical models. GRP78-targeting AAVP particles are essentially “clinic-ready” for translation into urologic oncology.

Results

The Ligand Peptide SNTRVAP Targets Cell Surface GRP78 in AVPC. We first investigated the amount and localization of GRP78 in a pilot cohort of de-identified patients with primary small cell prostate carcinoma ($n = 4$). This initial analysis revealed a range from moderate-to-strong GRP78 immunostaining in both cytoplasm and cell surface in all patient-derived tumor samples examined (Fig. 2), a promising result in the light of translational applications. We next mapped the SNTRVAP:GRP78 interaction interface with an alanine-scanning site-directed mutagenesis approach (31). Phage particles displaying the parental SNTRVAP motif were compared with mutant particles in which each residue was individually converted to an alanine (or to glycine in the case of alanine itself in the parental motif) for binding to recombinant GRP78 in vitro. These assays showed that none of the residues were dispensable for the interaction: decreased binding to GRP78, ranging from partial (50–75%; site-directed mutagenesis of Ser¹, Thr³, Arg⁴, or Ala⁶) to complete (>75%; site-directed mutagenesis of Asn², Val⁵, or Pro⁷) inhibition was observed for all mutants compared with the parental motif (Fig. 3A). These results are consistent with the concept that a three-residue motif in the primary structure is necessary and sufficient for peptide-protein recognition (32). SNTRVAP specificity was further validated in DU145, a human prostate cancer-derived cell line developed from a brain metastases—which is AR-negative—Tp53 and p16 mutant, and results in lytic bone metastases when implanted in mice, all features that overlap with clinical and molecular features of AVPC (7, 8). Importantly, these cells express high levels of GRP78, and are therefore a suitable model for ligand-directed approaches. To obtain an internal control for specificity, GRP78 was silenced by small-hairpin RNA (shRNA). We observed that two of five GRP78 shRNA vectors (i.e., clones #3 and #5) effectively down-modulated GRP78 (Fig. 3B), and consequently led to a

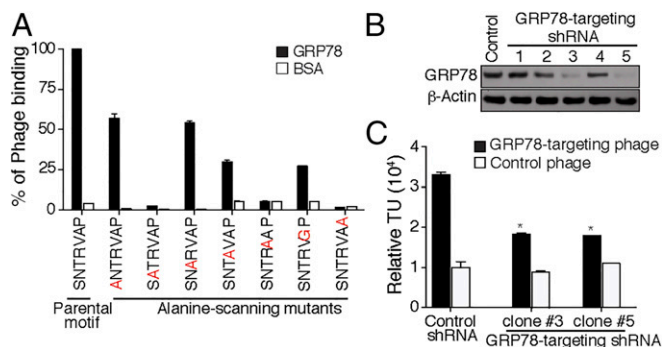


Fig. 3. In vitro validation of SNTRVAP and GRP78 as a ligand-receptor. (A) Ala-scanning site-directed mutagenesis of SNTRVAP. Each residue in the SNTRVAP sequence was mutated and the resulting phage particles were tested for binding to recombinant GRP78. Results are shown after normalization as the mean \pm SEM in triplicate wells. (B) In vitro GRP78 silencing. DU145 cells were infected with lentiviral vectors expressing different GRP78 shRNA constructs, and GRP78 levels were assessed by Western blot. β -Actin served as a loading control. (C) The GRP78-targeting phage specifically binds to DU145 cells expressing cell surface GRP78. Binding is shown as transducing units (TU) relative to the control phage, * $P < 0.05$ by two-tailed Student's t test.

commensurate decrease in cell surface binding of GRP78 to SNTRVAP-displaying phage particles (Fig. 3C) ($P < 0.05$ by two-tailed Student's t test). Taken together, these results confirm a specific interaction between the SNTRVAP and GRP78, and support the possibility of exploiting this ligand-receptor in AVPC.

SNTRVAP Targets GRP78-Expressing Tumor Xenograft Models in Vivo.

We next validated the targeting efficacy of SNTRVAP in immunodeficient mice implanted with either parental or GRP78-silenced DU145 tumor cells. Differential GRP78 expression levels in tumor xenografts were confirmed by immunohistochemistry (IHC) (Fig. 4A) and quantification of specific signals (Fig. 4B). GRP78-targeting or control phage particles were administered intravenously into DU145-derived tumor-bearing mice, and phage particle localization was evaluated after 24 h by staining with an antiphage antibody (33) (Fig. 4C), followed by quantification of DAB signals (Fig. 4D) and titration of phage particles by quantitative PCR (qPCR) (34) (Fig. 4E). The GRP78-targeting phage particles localized in parental DU145-derived tumors but not in size-matched GRP78-silenced DU145-derived tumors. Regardless of cell surface GRP78 levels, control phage particles did not accumulate in tumor xenografts. GRP78-targeting or control phage particles were present in the liver because of the long-recognized virus retention by the reticuloendothelial system (35–37), whereas neither was detectable in control tissues. Taken together, these results establish that the ligand peptide SNTRVAP targets AVPC-like cells that express cell surface GRP78 in vivo.

Targeting Cell Surface GRP78-Expressing Cells for in Vitro and in Vivo Small-Interfering RNA Delivery.

As another step toward potential applications of the GRP78-targeting peptide motif, we explored the possibility of delivering small-interfering RNA (siRNA) and shRNA species to tumors that express cell surface GRP78. Transduction of parental GRP78-expressing DU145 cells was first evaluated in vitro with a custom GAPDH-specific siRNA conjugated to the GRP78-targeting peptide SNTRVAP, in comparison with GAPDH-specific siRNA administered by a standard transfection system (NeoFX). Quantification of GAPDH mRNA showed that the GRP78-targeting system is twice as effective as the standard reference. Equimolar concentrations of either an unconjugated GAPDH siRNA or a GRP78-targeting control siRNA were ineffective, further supporting the ligand-receptor specificity (Fig. 5A). We next coupled the GRP78-targeting

peptide to a GRP78 siRNA sequence (Fig. 5B). The efficacy of this GRP78-targeting GRP78 siRNA was evaluated in vivo in comparison with a control unconjugated siRNA. Forty-eight hours after siRNA administration into DU145 tumor-bearing mice, either GRP78-targeting or control phage particles were administered intravenously and allowed to circulate for 24 h, followed by tumor collection. Quantification of tumor-retrieved phage particles revealed an inhibition (>40%) of GRP78-targeting particles

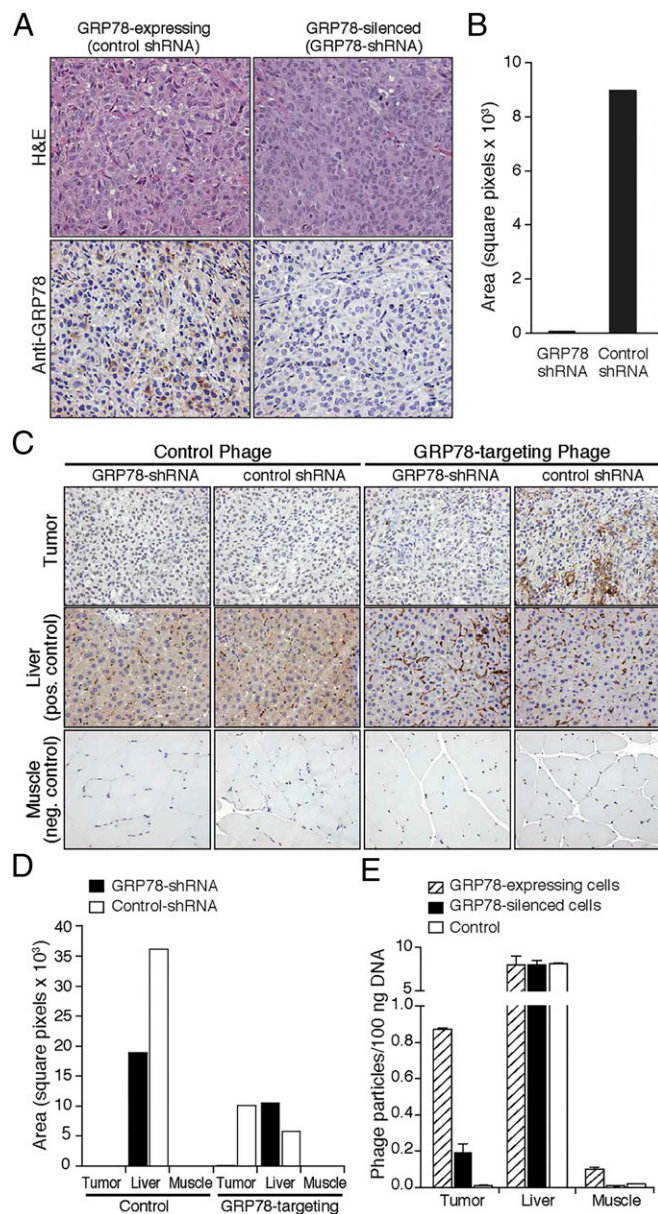


Fig. 4. In vivo validation of SNTRVAP and GRP78 as a ligand-receptor. (A) Animal models of GRP78-expressing and -silenced AVPC. DU145 cells transduced with control or GRP78 shRNA were injected subcutaneous into the flank of athymic BALB/c mice and tumors were grown for 6–8 wk. H&E staining (Upper) and GRP78 IHC (Lower) are shown. (B) Quantification of GRP78 staining was obtained by deconvolution with ImageJ and is expressed as number of pixels per optical field. (C) In vivo accumulation of GRP78-targeting phage. Phage localization was evaluated on excised tumor masses by IHC. Liver and muscle were used as control organs. (D) Quantification of IHC signals was obtained as in B. (E) Phage DNA was extracted and quantified by qPCR. Numbers of phage particles per 100 ng of total DNA are represented as mean \pm SEM. Magnification in A and C is 20 \times .

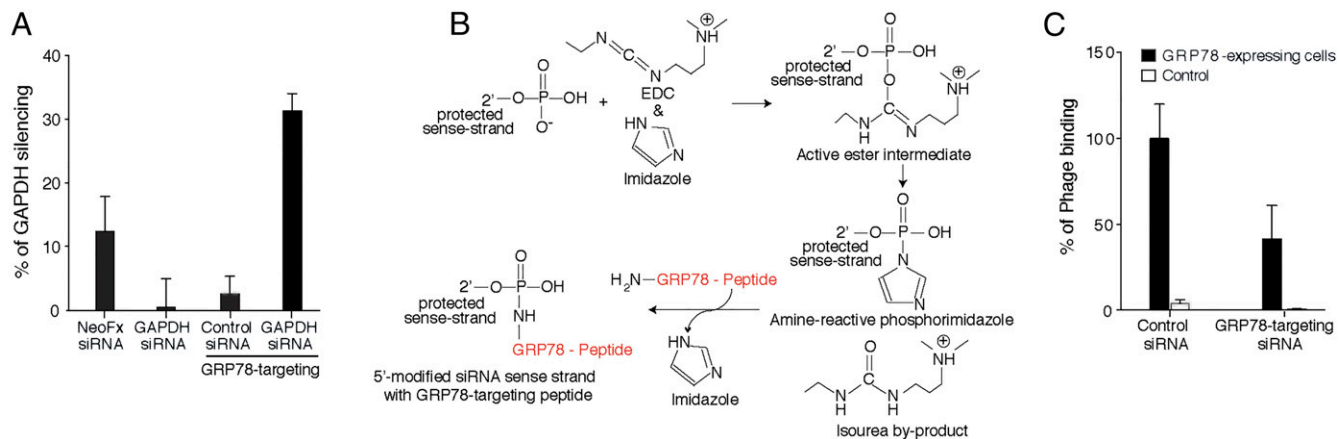


Fig. 5. In vitro and in vivo ligand-directed silencing of GRP78. (A) The GRP78-targeting peptide, chemically conjugated to a GAPDH siRNA, was incubated with GRP78-expressing DU145 cells followed by evaluation of GAPDH mRNA levels. GAPDH siRNA transfection with NeoFX was used as a standard. An unrelated siRNA and an unconjugated GRP78-targeting peptide were used as controls. Values are expressed as percent silencing of GAPDH. (B) Conjugation scheme of GRP78-targeting peptide to the 5'-modified GRP78 sense-strand siRNA. (C) GRP78-targeting siRNA or control siRNA were injected intravenously into DU145 tumor-bearing mice 48 h before intravenous injection of either untargeted or GRP78-targeting phage. After 24 h, tumors were excised and phage was quantified by bacterial infection. Values are expressed as percent accumulation compared with GRP78-targeting phage on nonsilenced cells, and are shown as mean \pm SEM of three experiments.

in tumor-bearing mice pretreated with GRP78-targeting GRP78-siRNA, compared with the control siRNA. Finally, the corresponding control particles were not detected in tumors of treated mice (Fig. 5C). These results demonstrate that the SNTRVAP can target a GRP78-specific siRNA and effectively down-modulate GRP78 on AVPC cells in vivo.

SNTRVAP Mediates GRP78 Targeting for Transgene Delivery to a PDX Model.

Having confirmed the efficacy and specificity of GRP78 targeting via the ligand peptide SNTRVAP in vitro and in vivo, we evaluated the efficacy of GRP78-targeting AAVP particles for in vivo genetic transduction of GRP78-expressing tumors (22). As mentioned above, the *HSVtk* gene product (thymidine kinase) functions as (i) an imaging tool in the presence of a radiolabeled substrate, and (ii) a cell suicide-inducing transgene in the presence of GCV (38). We evaluated both functions toward theranostic anti-AVPC strategies. Mice bearing the MDA-PCa-118b PDX model were randomized into four cohorts receiving: (cohort 1) control AAVP plus saline; (cohort 2) GRP78-targeting AAVP plus saline; (cohort 3) control AAVP plus GCV; (cohort 4) GRP78-targeting AAVP plus GCV. Daily treatments with either saline or GCV were started 5 d after intravenous administration of AAVP particles, as indicated, and continued for 7 d. Twenty-four hours after the last treatment, mice received [18 F]-fluorodeoxyglucose ([18 F]-FDG) to assess tumor viability as a function of glucose metabolism by PET/CT. Expression of the *HSVtk* transgene was evaluated after another 24 h by PET immediately after intravenous administration of 2-[18 F]-fluoro-2-deoxy-1-D-arabino-furanosyl-5-ethyluracil ([18 F]-FEAU) (26, 39). (See [Supplemental Discussion: Comparison Between the \[\$^{18}\$ F\]-FEAU and \[\$^{124}\$ I\]-FIAU Substrates](#) for more on the [18 F]-FEAU substrate). Cohorts 1 and 2 were evaluated in protocols for ligand-directed molecular-genetic imaging. In these PDX-bearing mice, GRP78-targeting AAVP particles specifically localized at sites superimposable with metabolically active tumors, as visualized by [18 F]-FDG PET/CT scans, whereas the control AAVP particles did not show specific accumulation (Fig. 6A). Nonspecific localization of both [18 F]-FDG and [18 F]-FEAU in the kidneys and urinary bladder was observed in all cohorts as a result of renal clearance. Quantification of [18 F]-FEAU (represented here as percentage of the [18 F]-FEAU dose administered) confirmed that *HSVtk* tumor expression in mice receiving GRP78-targeting AAVP particles was significantly higher

than in mice receiving control AAVP particles ($P < 0.001$ by two-tailed Student's *t* test) (Fig. 6B). PDX-bearing mouse cohorts 3 and 4 were evaluated in protocols for ligand-directed therapy. PET/CT scans demonstrated decreased tumor size in GRP78-targeting AAVP-administered mice treated with GCV, compared with the control AAVP plus GCV (Fig. 6C). This inhibition of tumor growth in PDX-bearing mice treated with GRP78-targeting AAVP plus GCV was independently confirmed by measuring gross tumor sizes with calipers, which revealed an average 50% reduction in tumor size ($P < 0.001$ by two-tailed Student's *t* test) (Fig. 6D). Excised tumors were also subjected to microtomography to quantify the percentage of mineralized tissue, a typical feature of osteoblastic metastases from prostate cancer. The growth of densely mineralized bone was significantly suppressed in animals treated with GRP78-targeting AAVP plus GCV, compared with treatments with GRP78-targeting AAVP plus saline or control AAVP ($P < 0.05$ by 2-way ANOVA followed by Bonferroni's test) (Fig. 7A). PDX tumors from mice treated with GRP78-targeting AAVP plus GCV were highly apoptotic as visualized by TUNEL staining, as opposed to tumors in mice treated with either GRP78-targeting AAVP plus GCV or control AAVP (Fig. 7B). These data were confirmed by the quantification of specific IHC signals (Fig. 7C). These results demonstrate the efficacy of the peptide SNTRVAP for ligand-directed strategies that target GRP78 as a cell surface receptor in a clinically-relevant model of AVPC.

Discussion

The combination of improved management and longevity of prostate cancer patients, along with increasing awareness of AVPC (7, 8), has led to a need for more specific tools for detection and treatment of this aggressive phenotype. Given that patients with AVPC are relatively insensitive to AR-directed therapies, and respond only transiently to chemotherapy (2–8), the development of a strategy that would work across molecular and cellular diversity in AVPC remains an unmet need. New technological approaches, such as the emerging field of theranostics, may prove useful to enable biopsy-free spatiotemporal tumor monitoring. As a proof-of-concept for serial ligand-directed theranostics, we herein provide preclinical applications of simultaneous noninvasive molecular-genetic imaging and therapy of AVPC based on targeting the tumor cell surface receptor GRP78.

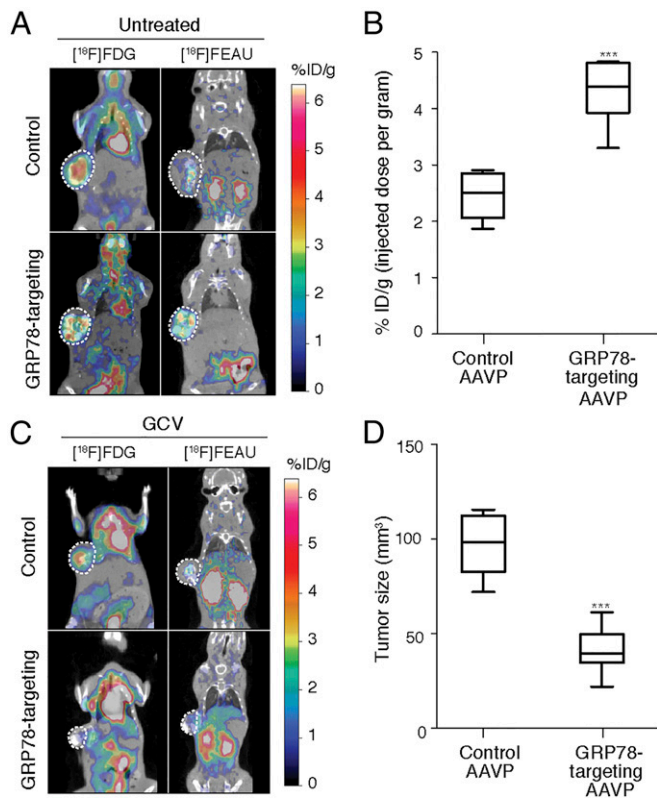


Fig. 6. Ligand-directed theranostics of the MDA-PCa-118b PDX model. (A) PET/CT imaging of MDA-PCa-118b PDX-bearing mice administered intravenously with GRP78-targeting or control AAVP. The pseudocolor scale represents signal intensity of [¹⁸F]-FDG (metabolic activity) or [¹⁸F]-FEAU (*HSVtk* activity). ****P* < 0.001 by two-tailed Student's *t* test. (B) Quantification of *HSVtk* gene expression by micro-PET imaging of [¹⁸F]-FEAU substrate. ****P* < 0.001 by two-tailed Student's *t* test. (C) PET/CT imaging of MDA-PCa-118b PDX-bearing mice administered as in A, and 7 d later treated with GCV for 5 d. (D) Tumor sizes were assessed by caliper measurement. ****P* < 0.01 by two-way ANOVA followed by Bonferroni's test.

The MDA-PCa-118b PDX used in this work was unique in being a fully characterized *in vivo* model of osteogenic prostate cancer (9), and it has subsequently been used to unveil molecular mechanisms in metastatic tumors of the prostate (10, 40–42). The

MDA-PCa-118b PDX is both castration-resistant and capable of developing soft-tissue and osteogenic tumors in mice (9), indicating that it is indeed representative of the highly aggressive pathology spectrum of AVPC; therefore, one may assume that the inherent mechanisms of disease progression, experimental findings, and targeting concepts uncovered by using the MDA-PCa-118b PDX are generalizable to the large “universe” of AVPC patients. By *in vivo* selection of phage-display random peptide libraries in the MDA-PCa-118b PDX, we have recently identified SNTRVAP as a highly specific ligand to GRP78 on the surface of cells within and adjacent to bone-like tumors (10). GRP78 up-regulation and relocation to tumor cell surface are associated with aggressive growth and invasive properties, and have been previously reported in human cancer cell lines and tissues (12). In prostate cancer, GRP78 expression levels correlate with the development of a metastatic disease in which circulating anti-GRP78 auto-antibodies are indicative of poor survival (21, 43). These observations support a potential translation of GRP78-based theranostics for the management of AVPC, including the widespread metastatic burden that remains a major clinical feature of AVPC patients (7, 8, 44). Additional GRP78-binding sequences, such as Trp-rich peptide motifs, have been reported (21, 45). Among these peptides, WIFPWQL targets cell surface GRP78 in tumor-bearing mice and in human specimens of prostate cancer (10, 21, 22). Similar applications of this peptide are reported in our companion article on aggressive variants of breast cancer (46). Here, we chose the peptide SNTRVAP (10) to target AVPC for several reasons: (i) its original selection in the MDA-PCa-118b PDX itself; (ii) its selectivity for a prostate cancer-specific cell surface supramolecular complex that incorporates GRP78, prostate-specific antigen, and $\alpha 2$ microglobulin (10, 47); (iii) its ability to bind AVPC cells either *per se* or loop-grafted into an antibody backbone; and (iv) its superior hydrosolubility relative to the GRP78-binding Trp-rich motifs, which generally harbinger cost-effective production yields in aqueous formulations.

Our studies validate the application of GRP78-targeting AAVP particles as molecular-genetic imaging tools for PET/CT applications, in agreement with the increased expression of cell surface GRP78 during metastatic progression and, in general, in the most aggressive castration-resistant forms of prostate cancer collectively included in the broad definition of AVPC (7, 8). Besides staging and active surveillance of prostate cancer during treatment, such a biomarker-annotated theranostic strategy is expected to provide specific ligand-directed detection of biochemical relapse: that is, to reveal the earliest possible recurrence of GRP78⁺ tumor cells.

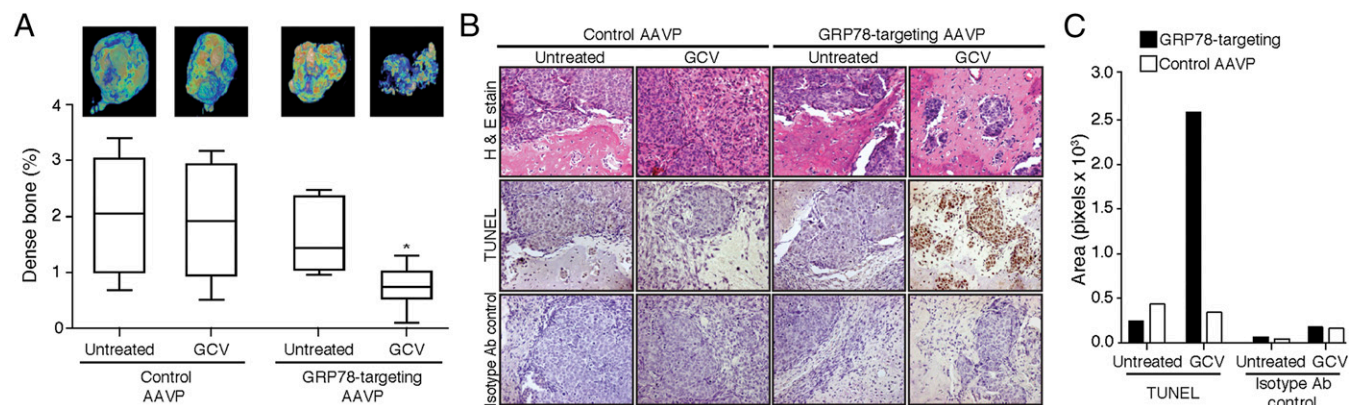


Fig. 7. GRP78-targeting AAVP particles reduce bone density and induce apoptosis in GCV-treated MDA-PCa-118b PDX models. (A) Evaluation of bone density of implanted tumors. (B) H&E staining and TUNEL assays were performed on MDA-PCa-118b tumor sections from mice administered intravenously with control or GRP78-targeting AAVP particles, and treated with saline or GCV. Control, secondary antibody only. (Magnification: 20 \times .) (C) DAB signals were isolated by deconvolution and quantified with ImageJ, and are represented as number of pixels per optical field. **P* < 0.05 by two-way ANOVA followed by Bonferroni's test.

Remarkably, this theranostic tool enables simultaneous therapeutic options that address the composite disease burden in valuable models of human AVPC. In summary, GRP78-targeting AAVP particles provide an efficient approach to address preclinical models of human AVPC for imaging and therapy and, as such, should be considered for translation into clinical applications in patients with this aggressive disease phenotype. Given that AVPC patients share complex genetic and epigenetic lesions (48), AAVP-based transcriptomic reporter analysis of promoter-mediated gene expression may be incorporated into the ligand-directed theranostic approach introduced here.

Materials and Methods

The use of human samples has been reviewed and approved by the Clinical Ethics Service, Institutional Biohazard Committee, Clinical Research Committee, and

Institutional Review Board of the University of New Mexico Comprehensive Cancer Center. The animal experiments were reviewed and approved by the Institutional Animal Care and Utilization Committee of M. D. Anderson Cancer Center. Reagents and all experimental procedures, such as the design of targeted AAVP and all in vivo and in vitro applications, are fully described in *SI Materials and Methods*.

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