PSCA and MUC1 in Non-small-cell Lung Cancer as Targets of Chimeric Antigen Receptor T cells Postprint

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Abstract

In recent years, immunotherapies, such as those involving chimeric antigen receptor (CAR) T cells, have become increasingly promising approaches to non-small-cell lung cancer (NSCLC) treatment. In this study, we explored the antitumor potential of prostate stem cell antigen (PSCA)-redirected CAR T and mucin 1 (MUC1)-redirected CAR T cells in tumor models of NSCLC. First, we generated patient-derived xenograft (PDX) mouse models of human NSCLC that maintained the antigenic profiles of primary tumors. Next, we demonstrated the expression of PSCA and MUC1 in NSCLC, followed by the generation and confirmation of the specificity and efficacy of PSCA-and MUC1-targeting CAR T cells against NSCLC cell lines in vitro. Finally, we demonstrated that PSCA-targeting CAR T cellscould efficiently suppress NSCLC tumor growth in PDX mice and synergistically eliminate PSCA(+)MUC1(+) tumors when combined with MUC1-targeting CAR Tcells. Taken together, our studies demonstrate that PSCA and MUC1 are both promising CAR T cell targets in NSCLC and that the combinatorial targeting ofthese antigens could further enhance the antitumor efficacy of CAR T cells.

Full Text

Preamble

PSCA and MUC1 as Targets of Chimeric Antigen Receptor T Cells in Non-Small-Cell Lung Cancer

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Abstract

In recent years, immunotherapies such as chimeric antigen receptor (CAR) T cell therapy have emerged as increasingly promising approaches for treating non-small-cell lung cancer (NSCLC). In this study, we explored the anti-tumor potential of PSCA-redirected and MUC1-redirected CAR T cells in NSCLC tumor models. First, we generated patient-derived xenograft (PDX) mouse models of human NSCLC that maintained the antigenic profiles of primary tumors. Next, we demonstrated the expression of prostate stem cell antigen (PSCA) and mucin 1 (MUC1) in NSCLC, followed by the generation and validation of PSCA- and MUC1-targeting CAR T cells and confirmation of their specificity

and efficacy against NSCLC cell lines in vitro. Finally, we demonstrated that PSCA-targeting CAR T cells could efficiently suppress NSCLC tumor growth in PDX mice and synergistically eliminate PSCA MUC1 tumors when combined with MUC1-targeting CAR T cells. Taken together, our studies demonstrate that both PSCA and MUC1 are promising CAR T cell targets in NSCLC and that combinatorial targeting of these antigens could further enhance the anti-tumor efficacy of CAR T cells.

Keywords: Non-small-cell lung cancer, Patient-derived xenograft, CAR T, PSCA, MUC1

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide[1],[2], and non-small-cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases[3], . Current therapeutic strategies, including surgery, radiation, and chemotherapy, have not yielded significant survival benefits. Tyrosine kinase inhibitors (TKIs) targeting EGFR and ALK have been widely used to treat NSCLC, but frequent resistance develops due to acquired mutations in EGFR and ALK . Furthermore, recently introduced immune checkpoint inhibitors targeting CTLA4, PD-1, and PD-L1 have shown either no or only moderate effects in NSCLC[1] [13]. Therefore, novel treatment regimens are urgently needed.

Chimeric antigen receptor (CAR) T cells targeting CD19 have generated exciting results in leukemia and lymphoma[1] [1] . However, their broad applicability to solid cancers is limited by the scarcity of truly tumor-specific target antigens. Additionally, the heterogeneity of tumor-associated antigens (TAAs) in solid cancers complicates CAR T cell therapy, as targets may differ not only among various cancer types but also among patients with the same cancer. Consequently, it is essential to define the TAA profile of a solid tumor before implementing TAA-oriented personalized CAR T cell immunotherapies.

Few antigens have been targeted by CAR T cells for NSCLC treatment. Glypican-3 was recently reported as a promising target for lung squamous cell carcinoma[1] . In a phase I clinical trial of anti-EGFR CAR T cells for lung cancer, only 2 of 11 patients achieved partial responses[1] . MUC1, a transmembrane glycoprotein, is aberrantly upregulated in many cancer types, including NSCLC[1] . A trial of MUC1-targeting CAR T cells is currently recruiting patients with four types of solid cancers, including NSCLC (ClinicalTrials.gov Identifier: NCT02587689). Thus, MUC1 represents a promising CAR T cell target in NSCLC.

Prostate stem cell antigen (PSCA) is a glycosylphosphatidylinositol-anchored cell surface antigen[2] that is primarily overexpressed in prostate cancer[21], although its expression has also been reported in other tumors such as gallbladder adenocarcinoma[22] and gastric cancer[23]. Surprisingly, PSCA has been found to be frequently overexpressed in NSCLC[2] , though this observation requires confirmation. Antibody-based PSCA-targeted therapies and peptide vaccines have

been explored for prostate cancer[2] [2] . Furthermore, PSCA-targeting CAR T cells have been used to treat pancreatic cancer in humanized mice[2] , and clinical trials of anti-PSCA CAR T cells for prostate, bladder, and pancreatic cancers are ongoing (ClinicalTrials.gov Identifiers: NCT02092948 and NCT02744287). However, it remains unknown whether anti-PSCA CAR T cells could be effective against NSCLC.

Patient-derived xenograft (PDX) models have been widely used in translational cancer research[3] , as they faithfully recapitulate the original tumors from which they were derived, and this similarity is maintained across passages[31]. In this study, we generated an NSCLC PDX model in which we detected strong histological expression of PSCA and weak expression of MUC1. We subsequently demonstrated the capacity of PSCA- and MUC1-targeted CAR T cells to recognize and kill NSCLC cells expressing the respective target antigens. Finally, we observed enhanced efficacy of a combination of both PSCA- and MUC1-targeted CAR T cells against double-positive NSCLC samples. Our results suggest that PSCA and MUC1 are both viable NSCLC-specific targets for CAR T cells and indicate that combinatorial antigen targeting could enhance the anti-tumor efficacy of these cells.

1. PDX Models Retained the Molecular Phenotype of NSCLC Cells

Using TALEN-mediated gene targeting, we previously generated a NOD-SCID-IL2R / (NSI) mouse strain capable of engrafting and modeling human hematopoietic cells[32]. Here, we generated human NSCLC PDX mice by subcutaneously or intravenously implanting dissected primary tumor masses or cell suspensions that could be serially transplanted and engrafted in NSI mice.

Immunohistochemistry results showed that HLA NSCLC cells from patient P2, which had been engrafted in the lungs of NSI mice, expressed E-cadherin but not vimentin during both the first and second passages after transplantation (Fig. 1A [Figure 1: see original paper]). Moreover, NSCLC cells from the third passage of another patient (patient P1) metastasized from the initial subcutaneous implants to the livers and spleens of NSI mice, and tumor cells from all locations uniformly expressed vimentin but not E-cadherin (Fig. 1B). Therefore, PDX models of NSCLC retained the molecular phenotypes of NSCLC cells across different passages and in different metastatic organs.

2. Frequent Expression of PSCA in NSCLC

NSCLC samples from eight patients were successfully engrafted in NSI mice to generate PDX models (Table 1). We subsequently harvested the tumors and evaluated the expression of PSCA and MUC1. Although MUC1 is frequently overexpressed in NSCLC[1] , only 2 of the 8 patients in our study expressed this antigen. In contrast, 7 patients, including the 2 who expressed MUC1, expressed PSCA (Fig. 2 [Figure 2: see original paper]).

Collectively, our results demonstrate frequent expression of PSCA in human NSCLC cells, consistent with a previous report[2] . The co-expression of PSCA and MUC1 in patients with NSCLC prompted us to evaluate the efficacy of combination CAR T cells for dual antigen targeting in our PDX models.

3. Generation of CAR T Cells Targeting PSCA and MUC1

To redirect T lymphocytes to PSCA and MUC1, we used second-generation PSCA-specific and MUC1-specific CARs, which consisted of single-chain variable fragments (scFvs) derived from the humanized 1G8 anti-PSCA antibody[33] and the anti-MUC1 HMFG2 monoclonal antibody[3] , respectively, and signaling domains from the costimulatory molecule CD28 and CD3 (Fig. 3A [Figure 3: see original paper]). Lentiviral vectors encoding green fluorescent protein (GFP; negative control), CAR-PSCA, and CAR-MUC1 were transfected into pre-activated human T cells to generate GFP T, CAR-PSCA T, and CAR-MUC1 T cells, respectively. Transduction efficiencies were measured as the percentages of GFP cells (Fig. 3B). We used reverse transcription polymerase chain reaction (RT-PCR) analysis of the scFv sequences to further confirm expression of anti-PSCA CAR and anti-MUC1 CAR in T cells (Fig. 3C).

4. CAR-PSCA T Cells and CAR-MUC1 T Cells Specifically Targeted PSCA and MUC1 Lung Cancer Cells, Respectively, In Vitro

We evaluated the specificity and efficacy of CAR-PSCA T cells against lung cancer cell lines in vitro. First, in a PSCA expression analysis of three lung cancer cell lines (A549, H23, and H460), only A549 cells strongly expressed PSCA (Fig. 4A [Figure 4: see original paper]). Immunohistochemistry analyses also consistently detected PSCA in A549 cells (Fig. 2). A luciferase-based in vitro killing assay demonstrated that CAR-PSCA T cells specifically killed A549GL and H23-PSCA-GL cells (Fig. 4B). Enzyme-linked immunosorbent assay (ELISA) results showed PSCA-specific induction of IL-2 and IFN- production in supernatants from the killing assay (Fig. 4C). Taken together, these findings indicate that CAR-PSCA T cells can recognize and kill PSCA cells in vitro.

We also confirmed the specificity and efficacy of CAR-MUC1 T cells. Like PSCA, MUC1 was detected only on A549 cells, but not on H23 or H460 cells (Fig. 4D). CAR-MUC1 T cells killed A549GL and H23-MUC1-GL cells, but not H460GL or H23GL cells, in vitro (Fig. 4E). We additionally observed MUC1-specific induction of IL-2 and IFN- production in culture supernatants (Fig. 4F). Next, A549, H460, H23, and H23-PSCA cells were transduced with a lentiviral vector expressing GFP and luciferase (Fig. 4G), and H23-PSCA-GL and H23-MUC1-GL cells were generated by transducing lentiviral vectors encoding PSCA and MUC1 into H23GL cells (Fig. 4H).

5. CAR-PSCA T Cells Were Efficacious Against PSCA NSCLC in PDX Mice

We used a PDX model generated from the PSCA , MUC1 tumor of patient P2 to further confirm the efficacy of CAR-PSCA T cells against NSCLC (Fig. 5A [Figure 5: see original paper]). Briefly, dissected tumor masses (~2 mm × 2 mm) were subcutaneously transplanted into NSI mice to generate PDX mice, which subsequently received two infusions of T cells (Fig. 5B); tumors were measured until day 40. NSCLC tumor mass growth was significantly suppressed by CAR-PSCA T cells but not by CAR-MUC1 T cells (Fig. 5C). On day 40, the smallest tumors were found in mice treated with CAR-PSCA T cells (Fig. 5D), and tumors treated with CAR-PSCA T cells had much lower weights than those left untreated or treated with GFP T cells (Fig. 5E). No significant difference was observed with CAR-MUC1 T cells, further suggesting that our CAR T cells recognized and killed NSCLC PDX tumors in an antigen-dependent manner. These results demonstrate the efficacy of CAR-PSCA T cells against PSCA NSCLC in PDX mice.

6. CAR-PSCA T and CAR-MUC1 T Cells Synergistically Inhibited NSCLC Growth in PDX Mice

We next evaluated the efficacy of a combination of CAR-PSCA T and CAR-MUC1 T cells in an NSCLC PDX model generated from patient P8, whose tumor expressed both PSCA and MUC1 (Fig. 5A). PDX mice were left untreated (blank) or treated with identical numbers of GFP T, CAR-PSCA T, CAR-MUC1 T, or a 1:1 mix of CAR-PSCA T and CAR-MUC1 T cells (Fig. 5F). Tumor growth was dramatically inhibited by CAR-PSCA T cells, CAR-MUC1 T cells, and the combined T cell treatment (Fig. 5G-H). Furthermore, tumor weights in mice treated with combined CAR T cells were significantly lower than those in mice treated with a single CAR T cell type (Fig. 5J-K). Collectively, the combination of CAR-PSCA and CAR-MUC1 T cells exhibited superior efficacy against NSCLC compared with either cell type alone.

Discussion

Treating most patients with solid cancers will require the development of genetically redirected T cells that target private somatic mutations, or neoantigens[3] . However, this remains an arduous task given the heterogeneity of the mutational landscape within a tumor mass and between metastases. Despite the paucity of tumor-specific antigens shared across various solid cancers and among patients with the same cancer type, the TAAs of a single specific cancer are much less heterogeneous than neoantigens. Therefore, CAR T cell immunotherapy remains important during the development of neoantigen-targeting techniques.

Although numerous TAAs have been detected in NSCLC[3] , few have been targeted by CAR T cells[3] ,[3] . The use of these limited non-cancer-specific antigens,

which include FAP[3] , EGFR , mesothelin [1], and glypican-3[1] , has led to poor or undefined therapeutic outcomes in patients. It is therefore critical to expand the repertoire of NSCLC-specific targets for CAR T cells. PSCA-targeting CAR T cells have been developed[2] , [2], [3] and are being evaluated in clinical trials for safety in patients with prostate, bladder, and pancreatic cancers (ClinicalTrials.gov Identifiers: NCT02092948 and NCT02744287). Additionally, a trial of MUC1-targeting CAR T cells is currently recruiting patients with NSCLC (ClinicalTrials.gov Identifier: NCT02587689). In this study, we frequently detected PSCA and MUC1 expression in NSCLC cells and thereby demonstrated the utility of anti-PSCA and anti-MUC1 CAR T cells.

Dual targeting of ErbB2 and MUC1 by T cells expressing both CARs has been reported to deliver complementary signals, enhance CAR T cell proliferation, but reduce IL-2 production . Combinatorial antigen recognition of PSCA and PSMA by a CAR providing suboptimal activation and a chimeric costimulatory receptor (CCR), respectively, has been reported to improve specificity and reduce off-target effects [2]. This strategy requires two types of chimeric receptors to be expressed simultaneously on T cells.

In this study, we tested dual targeting of PSCA and MUC1 using mixed CAR T cells targeting either PSCA or MUC1 in NSCLC PDX models. Cancer cells within a tumor mass may not uniformly express a single specific antigen, and even the PSCA MUC1 samples in our study might contain single-positive cancerous cells. Therefore, a CAR T cell-based combinatorial targeting strategy may broaden the population of targeted cancerous cells.

Although the CAR-PSCA and CAR-MUC1 T cells used in our study caused considerable reductions in tumor size, they could not completely eradicate NSCLC in PDX mice. This result could be attributed to the poor survival of CAR T cells in the immunosuppressive PDX tumor microenvironment. Strategies to improve the survival and infiltrating capacity of CAR T cells, such as optimized costimulation and cytokine co-expression [1], warrant exploration for all CAR T cells targeting solid tumors.

Overall, we have demonstrated that both PSCA and MUC1 are rational targets in NSCLC, and that PSCA- and MUC1-targeting CAR T cells may comprise novel therapeutic agents for patients with NSCLC. Our results also suggest that a mixture of CAR T cells with different specificities could simultaneously target tumor antigens and lead to better therapeutic outcomes.

Materials and Methods

Vector Design

To generate CAR-PSCA and CAR-MUC1 lentiviral vectors, scFvs derived from the humanized 1G8 anti-PSCA antibody[33] and the anti-MUC1 HMFG2 monoclonal antibody[3] were codon-optimized and synthesized by GenScript (Piscataway, NJ, USA). These scFvs were cloned into a CAR-encoding vector back-

bone comprising the CD8 leader sequence, human IgD hinge, portions of the CD28 transmembrane domain, and the CD28 and CD3 endodomains within the second-generation lentiviral vector pWPXLd. The amino acid (aa) sequences of the CARs were: CD8 leader (aa 1-21), scFv, IgD hinge (aa 187-289), CD28 (aa 153-220), CD3 (aa 52-163).

Lentivirus Production and Transduction of Primary Human T Cells

Lentivirus particles were produced in HEK-293T cells via polyethyleneimine (Sigma-Aldrich, St Louis, MO, USA) transfection. The pWPXLd-based lentiviral plasmid and two packaging plasmids, psPAX2 and pMD.2G, were co-transfected into HEK-293T cells. Lentivirus-containing supernatants were harvested at 48 and 72 hours post-transfection and filtered through a 0.45- m filter. Peripheral blood mononuclear cells (PBMCs) were isolated from the buffy coats of healthy donors using Lymphoprep (Fresenius Kabi Norge, AS, Berg i Østfold, Norway). T cells were negatively selected from PBMCs using a MACS Pan T Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) and activated using microbeads coated with anti-human CD3, anti-human CD2, and anti-human CD28 antibodies (Miltenyi Biotec) at a 3:1 bead:cell ratio for 3 days in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 10 mM HEPES, 2 mM glutamine, and 1% penicillin/streptomycin. On day 3 post-activation, T cells were transduced with CAR lentiviral supernatants in the presence of 8 g/ml polybrene (Sigma). Twelve hours post-transduction, T cells were cultured in fresh media containing IL-2 (300 U/mL); subsequently, fresh media was added every 2–3 days to maintain cell density within the range of 0.5–1 × 10 /mL.

Healthy PBMC donors and all patients who provided primary specimens gave informed consent for the use of their samples for research purposes, and all procedures were approved by the Research Ethics Board of the Guangzhou Institutes of Biomedicine and Health (GIBH).

Cells and Culture Conditions

HEK-293T cells were maintained in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY, USA). A549 (human lung adenocarcinoma), H23 (human lung adenocarcinoma), and H460 (human large cell lung cancer) cell lines were obtained from ATCC (Manassas, VA, USA) and maintained in RPMI-1640. Luciferase-GFP expressing cell lines (A549GL, H23GL, and H460GL) were generated through transfection of the parental cell lines with a lentiviral supernatant containing luciferase-2A-GFP and sorted for GFP expression on a FACS Aria™ cell sorter (BD Biosciences, San Jose, CA, USA). H23-PSCA-GL or H23-MUC1-GL cells were generated by transfecting H23 cells with lentiviral supernatant. DMEM and RPMI-1640 media were supplemented with 10% heat-inactivated FBS (Gibco/Life Technologies), 10 mM HEPES, 2 mM glutamine (Gibco/Life Technologies), and 1% penicillin/streptomycin. All cells were cultured at 37°C in an atmosphere of 5% carbon dioxide.

Flow Cytometry

All samples were analyzed using an LSR Fortessa or C6 flow cytometer (BD Biosciences), and data were analyzed using FlowJo software (FlowJo, LLC, Ashland, OR, USA). The following antibodies were used: PSCA (clone 7F5) from Santa Cruz Biotechnology (Dallas, TX, USA) and anti-human CD227 (MUC-1, clone 16A52) from Biolegend (San Diego, CA, USA). Clone 16A binds to the glycopeptide RPAPGS(GalNAc)TAPPAHG of MUC1 (MUC1-Tn) with high affinity [2]. The scFv of HMFG2 (used to engineer the CAR) can recognize a range of tumor-associated MUC1 glycoforms, such as Tn, STn, T, and ST (binding to MUC1-Tn and MUC1-STn with higher affinity). HMFG2 has the broadest capacity for strong binding to tumor-associated MUC1 glycoforms[3] . Therefore, the CAR-MUC1 T cells we generated can recognize 16A-positively stained cells.

In Vitro Tumor Killing Assays and Cytokine Release Assays

A549GL, H23GL, H23-PSCA-GL, or H23-MUC1-GL target cells were incubated with GFP T, CAR-PSCA T, or CAR-MUC1 T cells at the indicated ratios in triplicate wells of U-bottomed 96-well plates. Supernatants were collected from wells with E:T ratios of 1:1 and analyzed for IL-2 and IFN- concentrations using ELISA kits (eBioscience, San Diego, CA, USA). Cells were treated with 100 l/well of the luciferase substrate D-luciferin (potassium salt, 150 g/ml; Cayman Chemical, Ann Arbor, MI, USA), and target cell viability was monitored using a microplate reader at a 450-nm excitation wavelength. Background luminescence was negligible (<1% of the signal from wells containing only target cells). Therefore, the viability percentage (%) was calculated as (experimental signal/maximal signal) × 100, and the killing percentage was equal to 100 - viability percentage.

PDX Models for CAR T Cell Treatment

We used 6–8-week-old NSI mice according to protocols approved by the Institutional Animal Care and Use Committee of GIBH. All mice were maintained in specific pathogen-free (SPF) cages and provided with autoclaved food and water. Surgical tumor samples were obtained from Sun Yat-Sen University Cancer Center (Guangzhou, China) with informed consent from the patients; tumors were cut into 2 mm × 2 mm pieces and directly transplanted subcutaneously without Matrigel or other additives into 3–6 immunodeficient NSI mice within a 30-min period. Tumors that reached an approximate size of <1000 mm^3 were removed and passaged to other NSI mice. On days 7 and 10, according to doses used in other reports [3], , 5 × 10 total T cells were injected through the tail vein into each NSCLC-burdened NSI mouse. Tumors were measured every 4 days with calipers to determine the subcutaneous growth rate. Tumor volume was calculated using the following equation: (length × $width^2$)/2.

Reverse Transcription (RT-PCR)

mRNA was extracted from cells using the RNeasy mini kit (Qiagen, Stockach, Germany) and reverse transcribed into cDNA using the Prime-Script™ RT reagent Kit (Takara, Shiga, Japan). The following primers were used: -ACTIN forward: 5 -AGAGCTACGAGCTGCCTGAC-3 ; -ACTIN reverse: 5 -AGCACTGTGTTGGCGTACAG-3 ; scFv of CAR-PSCA forward: 5 -CTCTGTGGGGGATAGGGTCA-3 ; scFv of CAR-PSCA reverse: 5 -TCACAAGATTTGGGCTCGCT-3 ; scFv of CAR-MUC1 forward: 5 -TCGGTGGAGGAACCAAACTG-3 ; scFv of CAR-MUC1 reverse: 5 -CCTCCCTTTCACAGACTCCG-3 .

Immunohistochemistry

Tumor tissue sections were fixed with 10% paraformaldehyde, embedded in paraffin, sectioned at a thickness of 4 m, and stained using standard hematoxylin and eosin techniques. Paraffin sections were also immunostained with antibodies specific for E-cadherin (ZA0565), vimentin (ZA-0511), PSCA (ZA-0158), MUC1 (ZM-0391), and HLA (Abcam, Cambridge, UK) overnight at 4°C, followed by secondary staining with goat anti-mouse or goat anti-rabbit IgG (PV-9000) (ZSGB-BIO, Beijing, China). Images of all sections were obtained with a microscope (DMI6000B; Leica Microsystems, Wetzlar, Germany).

Statistics

Data are presented as means ± standard errors of the means. Student' s t-test was used to determine the statistical significance of differences between samples, and a P value <0.05 was considered statistically significant. All statistical analyses were performed using Prism software, version 5.0 (GraphPad, Inc., San Diego, CA, USA).

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Conflict of Interest Statement

The authors declare no competing financial interests.

Authors and Contributors

XW, YL, and JL contributed to conception and design, collection and/or assembly of data, data analysis and interpretation, and manuscript writing. LQ, YX, RZ, and QL contributed to provision of study material or patients and collection and/or assembly of data. BL, SL, SW, and QW provided animal care and administrative support. YY and DP contributed to conception and design and provided financial support. YY, MP, FY, YL, XZ, YW, and PL contributed to conception and design. XW and PL contributed to conception and design, data analysis and interpretation, manuscript writing, final approval of manuscript, and provided financial support. All authors read and approved the final manuscript.

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Figure Legends

Figure 1. Generation and molecular characterization of patient-derived xenograft (PDX) models of non-small-cell lung cancer. (A) Hematoxylin and eosin (H&E) staining and immunohistochemical detection of human leukocyte antigen (HLA), E-cadherin, and vimentin in tumor sections from both the first and second passages of PDX mice for patient P2 (see Figure 2). Although HLA cells from PDX mice also expressed E-cadherin, cells from both passages were negative for vimentin. (B) H&E staining and immunohistochemical detection of HLA, E-cadherin, and vimentin in sections from liver, spleen, and subcutaneous (s.c.) tissue. All sections were from a single mouse from the third passage of PDX mice for patient P1 (see Figure 2). Scale bar = 20 m.

Figure 2. Sections of tumors from eight patient-derived xenograft (PDX) models. Representative images correspond to tumors derived from eight patients; all sections were stained with hematoxylin and eosin (H&E) and antibodies against human leukocyte antigen (HLA), PSCA, and MUC1. The passage numbers of each PDX for the patients are indicated. The negative controls (NC) are liver tissues from the same mouse of the third passage of PDX for patient P3. Scale bar = 20 m. PSCA and MUC1 detection results are also shown in Table 1.

Figure 3. Construction of anti-prostate stem cell antigen (PSCA) and anti-mucin 1 (MUC1) chimeric antigen receptor (CAR) T cells. (A) Structures of the genes used for lentiviral transduction. GFP, control without CAR; CAR-PSCA, anti-PSCA CAR; CAR-MUC1, anti-MUC1 CAR. (B) Representative flow cytometric analysis of transduced T cells. (C) Reverse transcription-PCR detection of the following: -ACTIN in wild type (WT), CAR-PSCA, and CAR-MUC1 T cells (left); anti-PSCA scFv in WT and CAR-PSCA T cells and CAR-PSCA vector as a positive control (middle); and anti-MUC1 scFv in WT and CAR-MUC1 T cells and CAR-MUC1 vector as a positive control (right).

Figure 4. T cells expressing prostate stem cell antigen (PSCA) or mucin 1 (MUC1) chimeric antigen receptor (CAR) specifically killed PSCA or MUC1 lung cancer cell lines, respectively, in vitro. (A) Flow cytometric analysis of PSCA expression on A549, H23, and H460 cell lines. (B) Percentages of lung cancer line cells killed by GFP T cells and CAR-PSCA T cells at the indicated effector (E):target (T) ratios. The ratios represent the absolute number of CAR T cells versus target cells (corrected for transduction efficiency). T cells were cocultured with A549GL, H460GL, H23GL, or H23-PSCA-GL cells for 18 h, and luciferase activities were measured using a D-luciferin substrate. % killing = % (total activities without T cells - activities with T cells)/total activities without T cells. Data are representative of killing assays using T cells from three different donors. (C) Results of enzyme-linked immunosorbent assays (ELISAs) to detect IL-2 and IFN- in the supernatants

of cocultures at an E:T ratio of 1:1. (D) Flow cytometric analysis of MUC1 expression on A549, H23, and H460 cell lines. (E) Percentages of lung cancer line cells killed by GFP T cells and CAR-MUC1 T cells at the indicated E:T ratios. (F) Results of ELISAs to detect IL-2 and IFN- in the supernatants of cocultures at an E:T ratio of 1:1. Data are representative of killing assays using T cells from three different donors. (G) Post-transduction GFP-Luciferase (GL) expression was detected in A549GL, H23GL, and H460GL cell lines by flow cytometry. GFP served as a marker of luciferase expression. (H) Flow cytometric detection of PSCA (left) and MUC1 (right) in H23GL cells after lentiviral transduction. Error bars denote standard errors of the means, and groups were compared using the unpaired t-test. $P < 0.05$, $\boldsymbol{P < 0.01}$, $P < 0.001$.

Figure 5. Prostate stem cell antigen chimeric antigen receptor (CAR-PSCA) expressing T cells inhibit the growth of non-small-cell lung cancer (NSCLC) and exhibit synergistic efficacy with mucin 1 CAR (CAR-MUC1) expressing T cells against NSCLC in patient-derived xenograft (PDX) models. (A) Diagram of the experiment with primary NSCLC tumors from patient P2 or P8 in NSI mice. Mice were inoculated subcutaneously with dissected tumor masses from patient P2 or P8 (2 mm × 2 mm), infused with 5 × 10 total T cells on days 7 and 10, and culled on day 40 for tumor analysis. B-E show results from the PDX model of patient P2. (B) T cells were analyzed for transduction efficiency before infusion into PDX mice of patient P2. (C) Tumor growth curves in groups treated with no T cells (n=3), GFP T cells (n=3), CAR-PSCA T cells (n=4), or CAR-MUC1 T cells (n=4). (D) Tumors from mice treated with no T cells, GFP T cells, CAR-PSCA T cells, or CAR-MUC1 T cells on day 40 are shown. One mouse from both the no T and GFP T groups died when tumors were small, which is not shown. (E) Comparison of the weights of tumors described in D. F-J show results from the PDX model of patient P8. (F) T cells were analyzed for transduction efficiency before infusion into PDX mice of patient P8. (G) Tumor growth curves in groups treated with no T cells (n=3), GFP T cells (n=3), CAR-PSCA T cells (n=4), CAR-MUC1 T cells (n=4), and combinatorial CAR T cells (n=4). (H) Tumors from different groups in G on day 40 are shown. Both the no T and GFP T groups had one mouse die when tumors were small. (I) Comparison of the weights of tumors in H. (J) Tumors from CAR-PSCA T, CAR-MUC1 T, and combinatorial groups were singled out for comparison. Error bars denote standard errors of the means, and groups were compared using the unpaired t-test. $P < 0.05$, $\boldsymbol{P < 0.01}$, $P < 0.001$.

Table 1. Clinical information of the patients and characteristics of the corresponding PDX models

Patient	Gender	Age	Pathology	PSCA (primary/PDX)	MUC1 (primary/PDX)	E-cadherin in PDX	Vimentin in PDX	Metastasis	Route of administration

M, male; F, female; AC, adenocarcinoma; LCC, large cell carcinoma; SCC, squamous cell carcinoma; +, expression; -, no expression; ND, not detected; SC, subcutaneous; IV, intravenous.

Note: Figure translations are in progress. See original paper for figures.