Novel single-chain variant of antibody against mesothelin established by phage library

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Abstract
Mesothelin (MSLN) shows increased expression in various cancer cells. For clinical application of antibodies as a positron emission tomography (PET) imaging reagent, a human shortened antibody is essential both for avoiding redundant immune responses and for providing rapid imaging. Therefore, we cloned a single-chain fragment of variable regions (scFv) from a human-derived gene sequence. This was achieved through the construction of a naïve phage library derived from human tonsil lymphocytes. Using a column with human recombinant MSLN, we carried out biopanning of phage-variants by colony formation. We first obtained 120 clones that were subjected to selection in an ELISA using human recombinant MSLN as a solid phase antigen, and 15 phage clones of scFv with a different sequence were selected and investigated by flow cytometry (FCM). Then, six variants were selected and the individual scFv gene was synthesized in the V_L and V_H domains and expressed in Chinese hamster ovary cells. Mammalian cell-derived human-origin scFv clones were analyzed by FCM again, and one MSLN highly specific scFv clone was established. PET imaging by 89Zr-labeled scFv was done in mice bearing xenografts with MSLN-expressing cancer cells, and tumor legions were successfully visualized. The scFv variant established in the present study may be potentially useful for cancer diagnosis by PET imaging.

Keywords
89Zr (zirconium-89), mesothelin, phage library, positron emission tomography (PET) imaging, single-chain fragment of variable region
INTRODUCTION

Mesothelin (MSLN) is a cell differentiation-related and cell surface glycoprotein (40 kDa) that includes a glycosyl-phosphatidylinositol (GPI) anchor. In normal tissues, MSLN is expressed only in mesothelial cells in the pleura, peritoneum, and pericardium. However, in malignant tumors, MSLN is overexpressed in diverse types of cancers, such as ovarian cancer, gastric cancer, non-small cell lung cancer, breast cancer, pancreatic cancer, and malignant mesothelioma. MSLN and its variant, soluble MSLN-related peptide (SMRP), are possible biomarkers for malignant tumors. Commercially available ELISA to diagnose malignant mesothelioma based on recognition of SMRP are available. A clinical trial of anti-MSLN mAb, amatuximab (MORAb-009), for the treatment of mesothelioma and pancreatic cancer is now underway.

Monoclonal antibodies have been applied as powerful clinical tools for diagnosis (for cancer imaging) and treatment. However, full-length IgG has a long circulation time in the blood and undesirable characteristics, such as accumulation in the liver. In contrast, small-sized antibody variants, such as single-chain fragment of variable region (scFv), diabody, minibody, and F(ab’)2 can penetrate tumors and other tissues faster and more uniformly than full-length IgG. A deletion of Fc domain allows evasion of immune detection. As a result, in vivo clearance of specific antibody to MSLN. The 96-well microtiter plates were immobilized to the purified scFv preparations were estimated by SDS-PAGE. We also measured the molecular weight of the scFv with MALDI-time of flight mass spectrometry (MALDI-TOF-MS) (AXIMA Performance; Shimadzu).

MATERIALS AND METHODS

2.1 | Reagents

Deferoxamine-p-SCN (DFO) was purchased from Macrocyclics. Amicon Ultra 0.5 centrifugal filter units were purchased from Merck Millipore. Other chemicals were reagent grade.

2.2 | Construction of phage antibody library

Phage antibody library was constructed by using human tonsils removed from 15 patients with tonsil hypertrophy and inflammation as shown on schematic diagrams in Figure 1. Single-chain Fv form of an antibody was fused to truncated cp3 (scFv-cp3) expressed on the phage surface. Two types of antibody libraries were constructed and used in this study. One was scFv-cp3 consisting of VH and VL sequences recovered from lymphocytes in the tonsils which was designated as H1. The other was scFv-C-cp3 consisting of VH and VL sequences from the same sample and designated as H2 (Figure 1C). This study was approved by the Okayama University Ethics Committee and Medical & Biological Laboratories Co., Ltd Ethics Committee and was implemented according to the Ethical Guidelines for Human Genome/Gene Research enacted by the Japanese Government and the Helsinki Declaration.

2.3 | Screening of antihuman MSLN-scFv-cp3

Phages displaying scFv-cp3 which showed MSLN-binding activity were selected by a panning method. Recombinant soluble form (amino acids 297-580) of human MSLN (r-MSLN) was prepared according to Iwahori et al. The phages recovered by the fourth or fifth round of panning with columns loaded with r-MSLN beads were infected into Escherichia coli and spread on a plate. Colonies were selected and grown in a liquid culture. Supernatants containing scFv-cp3 were used for screening by ELISA to evaluate specificity to MSLN. The 96-well microtiter plates were immobilized to the same r-MSLN. Epidermal growth factor receptor (EGFR) was used as a negative control. To select antihuman MSLN scFv-cp3s, rabbit anti-cp3 polyclonal antibody (Medical Biological Laboratories [MBL]) was used as the first antibody, and HRP-conjugated goat anti-rabbit IgG (H + L chain) was used as the second antibody. Finally, tetramethylbenzidine (TMB) was added and optical density (OD) was measured at 450 nm. Then the selected anti-MSLN scFv-cp3 were further selected by flow cytometry based on reactivity with cancer cell lines expressing MSLN on the cell surface (NCI-H226, NCI-N87 and BxPC-3). PANC-1 was used as an MSLN-negative cell line.

2.4 | Preparation of antihuman MSLN-scFv-His-Tag in mammalian cells

From the DNA sequence of a highly reactive scFv-cp3, we synthesized a scFv gene with a His-Tag sequence and expressed scFv using mammalian cells. Overall workflow for the preparation of humanized antihuman MSLN-scFv is shown in Figure 1C.

We synthesized an artificial gene of the VH and VL sequences of an anti-MSLN scFv-cp3 selected by ELISA and flow cytometry (FCM), and a linker and His-Tag were added to it. This gene was inserted into the mammalian cell expression vector pCex17.4 (Lonza) and genetically transfected into CHO-K1-GSKO. Molecular sizes of the purified scFv preparations were estimated by SDS-PAGE. We also measured the molecular weight of the scFv with MALDI-time of flight mass spectrometry (MALDI-TOF-MS) (AXIMA Performance; Shimadzu).
2.5 | Cell culture

Cancer cell lines established from various tissue types were purchased from ATCC and Japanese Collection of Research Bioresources (JCRB). Names of cell lines, tissue origins, and disease cases are shown in Table S1. All cells were cultured at 37°C in an incubator humidified with 5% CO₂.

2.6 | Flow cytometry analysis of antibodies

Human cancer cells (Table S1) were processed with enzyme-free cell dissociation buffer (Gibco/Life Technologies). Antihuman MSLN cp3-scFv, His-Tag scFv or full-length antihuman MSLN IgG were used for primary antibody. For antihuman MSLN cp3-scFv, we used antibody against Protobothrops flavoviridis venom hemorrhagic factor HR1-007 as a negative control, and rabbit anti-cp3 polyclonal antibody (MBL) as a secondary antibody. For the tertiary antibody, Alexa Fluor 488-labeled goat antirabbit polyclonal antibody (Invitrogen) was used. As a control, we processed the cells without His-Tag scFv. Full-length antihuman MSLN or a negative control anti-KLH (IgG2b, isotype-matched) as the first antibody, and a goat polyclonal antihuman IgG labeled with Alexa Fluor 488 (Invitrogen) as a secondary antibody were used. Finally, the cells were suspended in PBS containing 7-AAD and EDTA for the exclusion of dead cells and examined with a BD FACSAria III flow cytometer (BD Biosciences). All antibodies were allowed to react for 1 hour on ice and, between the steps of the reaction, cells were washed twice. In addition, mean fluorescence intensity and exclusion of dead cells

FIGURE 1 | Schematic diagrams of the preparation and structure of the anti-mesothelin (MSLN) single-chain fragment of variable regions (scFv). A, Schematic diagram of the preparation of a naïve human scFv phage library using human tonsil lymphocytes. B, The scFv has a variable region of heavy and light chains and a structural linker with a cp3 sequence and a light chain constant region with PeiB and cp3 sequences. PT7 has a T7 promoter. The variable region is shown in black, and the constant region is shown in gray. C, Schematic diagram of the preparation of an scFv with a His-Tag sequence and the concentration and selection of an scFv with a cp3 sequence (cp3-scFv). The cp3-scFv was concentrated by four to five rounds of bio-panning in a His-Tag column or mouse anti-His-Tag monoclonal antibody and Protein G column that uses beads to which human recombinant MSLN was bound. Individual cp3-scFv were cloned, and the binding ability to MSLN was evaluated by ELISA. scFv that were highly reactive according to ELISA were selected for their ability to bind to cancer cells, which was confirmed by flow cytometry (FCM). Based on the sequence information for selected scFv, we synthesized DNA of the scFv with a His-Tag sequence and expressed with Chinese hamster ovary (CHO) cells, and confirmed the ability to bind to cancer cells using FCM. scFv with a His-Tag sequence that was highly reactive based on FCM were evaluated by positron emission tomography (PET) imaging. V₇ is the heavy chain variable region, V₅ is the light chain variable region, and C₅ is the light chain constant region. The variable region is shown in black, and the constant region is shown in gray.
were determined using BD FACSDiva software and calculated using Microsoft Excel.

2.7 | Deferoxamine-p-SCN conjugation and radiolabeling of His-Tag scFv

For DFO conjugation of His-Tag scFv, the ratio of dissolved chelating agent DFO (p-SCN-Bn-DFO) : His-Tag scFv was adjusted to a molar ratio of 3:1 and incubated for 1 hour in bicarbonate buffer (pH 9.0) at 37°C. 89Zr was produced by a cyclotron (HM-125; Sumitomo Heavy Industries, Ltd), purified from excess target material 89Y, and obtained in 1 mol/L oxalic acid. 89Zr-oxide solution was neutralized by the addition of 2 mol/L Na2CO3, followed by 0.5 mol/L HEPES buffer (pH 7.0). The resultant 89Zr solution was mixed with a DFO-modified His-Tag scFv clone in 0.9% sodium chloride/5 mg/mL gentisic acid (pH 5.0) and incubated at 37°C for 30 minutes to obtain 89Zr-DFO-scFv. Unbound 89Zr was removed through ultrafiltration using an Amicon Ultra 10K centrifugal filter and saline containing gentisic acid. Radiochemical purity was determined with TLC autoradiography (TLC-ARG) and HPLC (LC-20; Shimadzu) using a Superdex 200 10/300 column (GE Healthcare). Radioactivity was detected with a GABI Star detector (Raytest) connected to HPLC. In vitro stability of radiolabeled scFv after a 6-hour incubation in 50% human plasma/PBS at 37°C was also analyzed with HPLC. Furthermore, to evaluate changes in binding ability after DFO conjugation and 89Zr marking, equilibrium dissociation constants (Kd) were measured with a BLItz intermolecular interaction analyzer (ForteBio, Inc.) with second-generation amine-reactive (ARG2) biosensor probes.

2.8 | Xenografts

All animal experiments were conducted in accordance with the guidelines of Okayama University and approved by the Institutional Animal Care and Use Committee (IACUC) at Okayama University (OKU-2013098). Five-week-old male BALB/c nu/nu mice were purchased from Charles River and were maintained under specific pathogen-free conditions at Department of Animal Resources, Advanced Science Research Center, Okayama University before use. For PET imaging, BALB/c nu/nu mice were grafted with NCI-N87 (3 × 106) cells s.c. in the right shoulder as well as PANC-1 (1 × 107) cells in the left shoulder. Imaging experiments were carried out when the tumor size reached approximately 8 mm in diameter.

2.9 | Positron emission tomography, computed tomography imaging and biodistribution of mice

Each mouse inoculated with NCI-N87 and PANC-1 cells was anesthetized by inhalation of isoflurane. 89Zr-DFO-scFv was given into the tail vein of mice (n = 3), and a dynamic emission scan was acquired for 3 hours using a PET system (Clairivivo PET; Shimadzu). Mean amount of scFv given was 6.0 MBq/8.3 μg for H1a050 and 4.1 MBq/11.5 μg for H2a064. After the PET scan, computed tomography (CT) data were obtained using a PET/CT system (Eminence Stargate; Shimadzu). Acquired PET data were reconstructed using 3-D-DRAMA. PET and CT images were converted to DICOM format and fused using PMOD software version 3.3 (PMOD Technologies Ltd). Three-dimensional volumes of interest (VOI) were manually drawn on PET/CT images to determine the mean percentage of the injected dose per gram of tissue (%ID/g) in heart as blood pool and tumors. After CT scanning, mice were dissected for biodistribution studies. Tumors and major organs were collected and weighed, and the radioactivity was measured with a gamma-counter (AccuFLEX γ7001; Hitachi Aloka Medical). Biodistribution data are expressed as %ID/g. Specificity of 89Zr-DFO scFv H1a050 accumulation in NCI-N87 xenografts was tested by competition with excess amount of unlabeled scFv H1a050. NCI-N87 tumor-bearing mice (n = 6 mice/group) were injected with 1 μg (38 kBq) of 89Zr-DFO scFv H1a050 with or without 49 μg unlabeled scFv H1a050. The mice were dissected at 3 hours after injection and the radioactivity of NCI-N87 xenograft was measured.

2.10 | Statistical analysis

Data are presented as the mean ± SD. Statistical analysis was carried out using a nonpaired Student’s t test for comparison of two groups. Statistical significance was established at P < .05.

3 | RESULTS

3.1 | Construction of phage antibody library and screening of anti-human MSLN-scFv-cp3

From palatine tonsillar lymphocyte cDNA of patients with tonsil hypertrophy and inflammation, an antibody phage library was constructed. Through four to five rounds of bio-panning, 120 anti-MSLN cp3-scFv clones were obtained. Reactivity of the anti-MSLN cp3-scFv clones was examined in ELISA with solid-phase r-MSLN (Figure 2), allowing selection of 15 scFv clones with high reactivity to r-MSLN. The Vh sequence of each clone was confirmed (data not shown), and some clones had almost the same sequence. Among the homologous clones, the clones with the best specificity and reactivity to rMSLN were selected. For example, as H1a003, H1a005, H1a006, H1a012, H1a024, and H1a054 had the same sequence as H1a050, H1a050 was selected as a representative clone.

Reactivity of these anti-MSLN cp3-scFv clones was confirmed by FCM analysis. As target cells expressing MSLN, NCI-H226 (lung cancer cell line), NCI-N87 (gastric cancer cell line), and BxPC-3 (pancreatic cancer cell line) were used. As a negative control, pancreatic cancer cell line, PANC-1, which showed very weak expression of MSLN, was used. Through FCM analysis, we selected six scFv clones with high reactivity to MSLN-positive cancer cells and extremely weak or negative reactivity to PANC-1 (Figure 3). Some clones, such as H2a002, showed high reactivity in FCM but were excluded from selection as they showed nonspecific binding in ELISA. We found that there was a correlation between the ELISA and FCM data of cp3-scFv clones, which suggested that the anti-MSLN scFv clones prepared in
this study recognize the MSLN molecule on live cell membranes as well as the immobilized MSLN molecule on plastic plates (Figure S1).

3.2 | Preparation of antihuman MSLN-scFv-His-Tag in mammalian cells

In order to obtain structural authenticity and correct glycosylation pattern, scFv were generated by mammalian cells. Antihuman MSLN-scFv-His-Tag variants that can be produced in mammalian cells were prepared from the DNA sequences of the selected anti-MSLN cp3-scFv clones, inserted into a vector, and transfected into Chinese hamster ovary (CHO) cells (Figure 4A,B). We prepared six antihuman MSLN-scFv-His-Tag clones and carried out FCM analysis using each cancer cell line. Among them, clones H1a050 and H2a064 presented specific activity to MSLN-expressing cell lines. In particular, clone H1a050 showed high reactivity to MSLN expressed in cancers derived from various tissues (Figure 5). In addition, scFv H1a050 showed reactivity similar to that of full-length IgG anti-MSLN antibody 11-25 (Figure 5). FCM results of all six scFv clones with various cancer cell lines are shown in Figure S2. Structure of the antihuman MSLN His-Tag-scFv and amino acid sequence of the six scFv clones are shown in Figure 4A,B, respectively.

Antihuman MSLN-scFv-His-Tag molecular weight estimated from DNA sequences was calculated to be 27 063.24 Da for H1a050 and 26 684.87 Da for H2a064. The approximate molecular weights of the six antihuman MSLN-scFv-His-Tag clones was confirmed by reduced SDS-PAGE (Figure 4C). The molecular weights determined by MALDI-TOF-MS were 26 926.58 Da for His-Tag scFv H1a050 and 26 547.88 Da for His-Tag scFv H2a064. Although there is a difference in molecular weight of approximately one amino acid residue, a peptide of nearly the estimated molecular weight was obtained.

3.3 | Deferoxamine-p-SCN conjugation and radiolabeling of His-Tag scFv

His-Tag scFv were modified with DFO and labeled with $^{89}$Zr. Specific activity after $^{89}$Zr labeling was 0.496 MBq/μg and 0.365 MBq/μg for H1a050 and H2a064, respectively. In vitro stability of $^{89}$Zr-labeled scFv after 6-hour incubation in 50% human plasma at 37°C was...
FIGURE 3 Evaluation of antihuman mesothelin (MSLN) single-chain fragment of variable regions (scFv) with a cp3 sequence (cp3-scFv) clones with flow cytometry (FCM). Using lung cancer cells (NCI-H226), gastric cancer cells (NCI-N87), pancreatic cancer cells (BxPC-3), and PANC-1, we analyzed the reactivity of antihuman MSLN cp3-scFv clones by FCM. Black indicates antihuman MSLN cp3-scFv clones, and gray shows the HR1-007 anti-Protobothrops flavoviridis venom scFv. Clones framed in black were selected for their high reactivity using FCM. Numbers indicate the ratio of the mean fluorescence intensity of each antihuman MSLN cp3-scFv clone to that of the HR1-007 anti-P. flavoviridis venom scFv used as a control.
98.3% and 100%, for H1a050 and H2a064, respectively. In addition, the $K_D$ of each scFv before DFO conjugation, after DFO conjugation, and after $^{89}$Zr labeling were as follows: H1a050: $4.68 \times 10^{-9}$ mol/L, $3.38 \times 10^{-9}$ mol/L, and $4.62 \times 10^{-8}$ mol/L; H2a064: $5.96 \times 10^{-8}$ mol/L, $1.14 \times 10^{-7}$ mol/L, and $7.82 \times 10^{-8}$ mol/L.

### 3.4 | Positron emission tomography, CT imaging and biodistribution of mice

Figure 6A shows the representative PET and CT images at 3 hours after giving $^{89}$Zr-labeled DFO-His-Tag scFv clones H1a050 and H2a064. Antihuman MSLN-scFv-His-Tag clone H1a050 showed higher accumulation in transplanted tumors from NCI-N87 compared with H2a064. Time-activity curve of $^{89}$Zr-DFO-scFv clones in the blood (heart) showed rapid clearance with a half-life of approximately 15 minutes for H1a050 and 12 minutes for H2a064 (Figure 6B). $^{89}$Z-DFO-scFv H1a050 had the highest NCI-N87 tumor uptake ($1.24\%$ID/g) approximately 4 minutes after injection, and the uptake decreased slowly and was retained at approximately $0.9\%$ID/g at 3 hours after administration (Figure 6C). Uptake of $^{89}$Z-DFO-scFv H1a050 of PANC-1 tumor was constantly lower than that of NCI-N87 tumor. $^{89}$Z-DFO-scFv H2a002 showed a similar uptake pattern for both NCI-N87 tumor and PANC-1 tumor, although their decline was faster than that of H1a050 (Figure 6D).

Figure 7A shows the biodistribution of $^{89}$Zr-DFO-scFv H1a050 and H2a064 after 3 hours PET and CT imaging. $^{89}$Zr-DFO-scFv H1a050 showed significantly higher accumulation in NCI-N87 tumors ($1.54\%$ID/g) compared with that in PANC-1 tumors ($0.78\%$ID/g) ($P < .05$). High accumulation of radioactivity was observed in the kidneys and liver, as they are major organs for scFv clearance.

Figure 7B shows the in vivo blocking experiment of $^{89}$Zr-DFO-scFv H1a050 and H2a064 after 3 hours PET and CT imaging. $^{89}$Zr-DFO-scFv H1a050 showed significantly higher accumulation in NCI-N87 tumors ($1.54\%$ID/g) compared with that in PANC-1 tumors ($0.78\%$ID/g) ($P < .05$). High accumulation of radioactivity was observed in the kidneys and liver, as they are major organs for scFv clearance.

Figure 7B shows the in vivo blocking experiment of $^{89}$Zr-DFO-scFv H1a050. Tumor uptake of 1 μg of $^{89}$Zr-DFO-scFv H1a050 was significantly blocked by excess amount of unlabeled scFv H1a050 molecules ($P < .005$), indicating the specificity of scFv H1a050 for MSLN-expressing cancer cells.

Mice bearing various sizes of NCI-N87 tumor underwent PET imaging with $^{89}$Zr-labeled H1a050. NCI-N87 tumor was visualized by PET with $^{89}$Zr-labeled H1a050 regardless of the size of the tumor (Figure 8A). Tumor uptake of scFv per weight was nearly consistent...
FIGURE 5 Evaluation of the antihuman His-Tag-single-chain fragment of variable regions (His-Tag scFv) clone in each cancer cell line. We analyzed the reactivity of the antihuman His-Tag scFv clone to three pancreatic cancer cell types, two lung cancer cell types, one type of prostate cancer cells, one uterine cancer cell type, two cervical cancer cell types, two ovarian cancer cell types, and three gastric cancer cell types by flow cytometry (FCM). Black indicates anti-human MSLN His-Tag scFv clone, gray indicates negative control. The negative control uses only the anti-His-Tag antibody as the secondary antibody without the anti-human MSLN His-Tag scFv clone as the primary antibody. Bottom row shows FCM results for the full-length mouse antihuman mesothelin (MSLN) antibody 11-25. Black indicates the full-length mouse antihuman MSLN antibody, and gray indicates the negative control, the IgG isotype control anti-KLH antibody. Values are the ratios of the mean fluorescence intensity (MFI) of each antihuman MSLN cp3-scFv and full-length mouse antihuman MSLN antibody 11-25 to the MFI of the control.

(Figure 8B,D), so the uptake of scFv per tumor correlated with the size of the tumor (Figure 8C,E).

4 | DISCUSSION

In the present study, we prepared scFv against MSLN, a target molecule in cancers. We showed that MSLN-positive tumors in tumor-bearing mice could be visualized by PET imaging with $^{89}$Zr-labeled anti-MSLN scFv (Figure 6). The scFv domains showed high amino acid sequence identities, as well as the hypervariable region. Complementarity determining region (CDR) was rich in L1, L2, L3, and H3 changes. Meanwhile, H2 showed only slight differences in two amino acid residues, demonstrating extremely high homology. In addition, the "RPS" amino acid sequence of the CDRL2 domain and the "Q**DSS" amino acid sequence of the CDRL3 domain were conserved; thus, these amino acid residues strongly contributed to the antibody's affinity to human MSLN (Figure 4B).

Anti-MSLN cp3-scFv clones were selected by their reactivity in ELISA with r-MSLN and in FCM with cancer cell lines. Then the
selected scFv clones were produced in mammalian CHO cells. The reactivity of these clones was confirmed again using FCM, and two highly reactive scFv clones H1a050 and H2a064 were selected. These scFv clones showed specific reactivity to MSLN-expressing cancer cells derived from a wide range of tissues (Figure 5) with a similar reactivity pattern to that of mouse antihuman MSLN full-length IgG antibody (Figure S1).

The selected scFv clones produced in CHO cells were modified with DFO and labeled with $^{89}$Zr through DFO. According to the dissociation constant obtained by BLItz, radiolabeled scFv had sufficient
antigen-binding activity. The radiolabeled scFv clones were also stable in 50% plasma at 37°C for 6 hours (98.3% and 100%). In PET imaging, \(^{89}\text{Zr}\)-labeled scFv clearly showed the tumor derived from NCI-N87 cancer cells strongly expressing MSLN, but did not show the tumor by PANC-1 cells weakly expressing MSLN (Figure 6A). As seen from the distribution in the blood and tumors, \(^{89}\text{Zr}\)-labeled scFv was quickly discharged from the blood and retained in tumors even at 3 hours post-injection. The accumulation of NCI-N87 was almost twofold that of PANC-1 cells weakly expressing MSLN (Figure 6A).

Moreover, effects such as opsonization of the Fc domain inhibit specific examination of antibody molecules as probes. Therefore, in the present study, we imaged with scFv, which is a small molecule and a fragment without the Fc domain. scFv showed fast blood clearance, but tumor clearance is also reported to be fast.\(^{26-29}\) In vivo visualization with
scFv has been demonstrated with anti-tissue factor with a NIRF label. However, with PET imaging, scFv is usually not used, and most imaging uses low molecular fragment antibody molecules such as scFv-Fc or diabody. The scFv prepared in the present study allowed visualization of tumors that express MSLN with PET at 3 hours after injection. As such, our antibodies would be useful for tumor imaging. There was a large amount of $^{89}$Zr accumulation in the kidneys and liver as they are major organs for scFv clearance (Figure 7). Still, as there are reports of a method in which SMRP in blood is neutralized by giving unlabeled anti-MSLN antibody at the same time, and a method that regulates accumulation in the liver through chemical conjugation using Fab and $^{99m}$Tc, we may need to consider these methods to decrease $^{89}$Zr accumulation in the liver.

A metallic positron emitter, $^{89}$Zr, does not emit beta-minus particles. Therefore, radiation to the body can be controlled for greater safety. Moreover, because of its longer half-life (78.41 hours) compared to other PET nuclides, it can be used for various and more complex labeling and purification.

In conclusion, our novel anti-MSLN scFv specifically reacted with MSLN-positive cancer cells in vitro and could clearly visualize MSLN-positive tumors by in vivo PET imaging, which demonstrates its potential as an agent for imaging MSLN-positive cancer.

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DISCLOSURE

Authors declare no conflicts of interest for this article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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