Supporting Information

Toward *In vivo* Imaging of Heart Disease Using a Radiolabeled Single-Chain Fv Fragment Targeting Tenascin-C

Norihiro Kobayashi,[a,*] Kenichi Odaka,[b] Tomoya Uehara,[c] Kyoko Imanaka-Yoshida,[d,e] Yoshinori Kato,[a] Hiroyuki Oyama,[a] Hiroyuki Tadokoro,[f] Hiromichi Akizawa,[c] Shuji Tanada,[g] Michiaki Hiroe,[h] Toshimitsu Fukumura,[b] Issei Komuro,[i] Yasushi Arano,[c] Toshimichi Yoshida,[d,e] Toshiaki Irie[b]

[a] Kobe Pharmaceutical University, 4-19-1, Motoyama-Kitamachi, Higashinada-ku, Kobe 658-8558, Japan, [b] Molecular Imaging Center, National Institute of Radiological Sciences, 4-9-1, Anagawa, Inage-ku, Chiba 263-8555, Japan, [c] Graduate School of Pharmaceutical Sciences, Chiba University, 1-8-1, Inohana, Chuo-ku, Chiba 260-8675 Japan, [d] Mie University Matrix Biology Research Center, 2-174, Edobashi, Tsu 514-8507, Japan, [e] Department of Pathology and Matrix Biology, Graduate School of Medicine, Mie University, 2-174, Edobashi, Tsu 514-8507, Japan, [f] Department of Bio-Medical Engineering, Tokai University, 317, Nishino, Numazu 410-0395, Japan, [g] Department of Radiation Oncology and Nuclear Medicine, International University of Health and Welfare, Mita Hospital, 1-4-3, Mita, Minato-ku, Tokyo 108-8329, Japan, [h] Department of Cardiology, National Center for Global Health and Medicine, 1-21-1, Toyama, Shinjuku-ku, Tokyo 162-8655, Japan, [i] Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, 2-2, Yamadaoka, Suita, Osaka 565-0871, Japan.

Supplementary Experimental Procedures

Buffers. PBS: 50 mM sodium phosphate buffer (pH 7.3) containing 9.0 g/L NaCl; M-PBS: PBS containing 20 g/L skim milk; G-PBS: PBS containing 1.0 g/L gelatin; T-PBS: PBS containing 0.050 (v/v) % Tween 20.

ELISA for Detection and Characterization of ScFv-4F10 (Figure 3A in the text). Microwells in 96-well microplates (no. 3590) (Costar) were coated with a solution of a Tnc (prepared and purified as reported previously)[1] (10 μg/mL) in 0.10 M carbonate buffer (pH 8.6) (100 μL/well) overnight at 4°C. After washing 3 times with PBS, the wells were blocked with M-PBS at 37°C for 1 h. The wells were washed again, and then crude periplasmic extract or purified scFv-4F10 protein, diluted with G-PBS (100 μL/well), was added along with free Tnc dissolved in G-PBS (100 μL/well). After the solutions were mixed and incubated at 37°C for 1 h, they were aspirated off and the wells were washed 3 times with T-PBS. Next, anti-FLAG M2 antibody (Sigma), diluted with G-PBS (5.0 μg/mL) (100 μL/well), was added and incubated at 37°C for 1 h. The wells were washed and horseradish peroxidase (POD)–conjugated rabbit anti-mouse IgG antibody (Fc-specific) (Jackson ImmunoResearch), diluted with G-PBS (100 μL/well), was added and incubated at 37°C for 1 h. Following washing, the bound POD activity in each well was determined colorimetrically using *o*-phenylenediamine as the hydrogen donor.[2,3]

Determination of Binding Affinity and Kinetics of ScFv-4F10 to Tnc. Surface plasmon resonance (SPR) analysis was performed using a Biacore 2000 (Biacore) to determine the association and dissociation rate constants (k_a and k_d) of scFv-4F10. Five different concentrations of scFv (54, 107, 214,

428, 857 nM), diluted in 50 mM phosphate-buffered saline (pH 7.3) containing 0.15 M NaCl, and 0.020% gelatin, were applied to a flow cell equipped with a sensor tip that immobilized the Tnc, followed by washing with the same buffer. The flow rate of the solutions was set at 20 μL/min.

Synthesis of 1-[4-(5-maleimidopentyl)amidobenzyl]diethylenetriaminepentaacetic acid (EMCS-Bz-DTPA).[4] To a chilled (−15˚C) solution of *N*-(carboxypentyl)maleimide[5] (282 mg, 1.34 mmol) in tetrahydrofuran (THF, 1 mL) was added *N*-methylmorpholine (135 mg, 1.34 mmol) and isobutyl chloroformate (183 mg, 1.34 mmol) at the same temperature. After 2 min, a THF solution (1 mL) of *N,N,N',N'',N''*-pentakis[(*tert*-butylcarbonyl)methyl]-1-[(4-aminophenyl)methyl]diethylenetriamine [NH_2-Bz-DTPA($tBu)_5$][6] (950 mg, 1.22 mmol) was added. The reaction mixture was stirred for 30 min at −15˚C and for 2 h at room temperature. After removing the solvent *in vacuo*, the residue was purified by column chromatography on silica gel using a mixture of ethyl acetate and hexane (1:1) as an eluent, to produce EMCS-Bz-DTPA($tBu)_5$ (355 mg, 30 %).

EMCS-Bz-DTPA($tBu)_5$ (117 mg, 0.12 mmol) was treated with a mixture of trifluoroacetic acid (1.9 mL) and anisole (0.1 mL) for 3 h. After removing the solvent, diethyl ether was added to precipitate EMCS-Bz-DTPA as a pale yellow solid (77 mg, 93%). ^{1}H-NMR (DMSO): δ 1.21-1.25 (2H, q, J = 6.8 Hz, CH_2), 1.48-1.58 (4H, m, CH_2), 2.23-2.26 (2H, t, J = 7.2 Hz, NHCO-CH_2), 2.43-2.95 (8H, m, N-CH_2, Bz-CH_2), 3.12-3.58 (13H, m, CH_2-COO, C*H*, CH_2-*N*-maleimide), 6.99 (2H, s, *maleimide*), 7.10-7.13 (2H, d, J = 8.4 Hz, *aromatic*), 7.46-7.48 (2H, d, J = 8.4 Hz, *aromatic*), 9.77 (1H, s, N*H*). FAB-MS: *m/z* 692 $(M+H)^+$.

Determination of reactive SH groups in scFv-Cys molecules before and after DTPA conjugation (Figure 4A in the text). ScFv-Cys was biotinylated with a reagent that contains a maleimide group (Biotin Labeling Kit–SH; Dojindo Molecular Technologies), and the product (~50 ng) in G-PBS (100 μL) was incubated at 37°C for 1 h in the microwells coated with Tnc (1.0 μg/well). After washing the wells 3 times with T-PBS, the POD-conjugated streptavidin (0.010 μg) in T-PBS (100 μL) was incubated in the wells at 37°C for 1 h. The wells were washed similarly and the bound POD activity was measured as described above over the course of a 10-min reaction.

Comparison of the binding activity to Tnc between the scFv-Cys and scFv-DTPA (Figure 4B in the text). ScFv-Cys and scFv-DTPA (each 200 ng) in G-PBS (100 μL) were incubated at 37°C for 1 h in the microwells coated with Tnc (1.0 μg/well). After washing the wells 3 times with T-PBS, POD-conjugated anti-FLAG M2 antibody (0.50 μg) in T-PBS (100 μL) was incubated in the wells at 37°C for 1 h. The wells were washed and the bound POD activity was determined as described above.

Table S1. Nucleotide sequences of PCR primers used in this study.

Primer	Sequence(5′→3′; Restriction site[a])
V_L-IIa	GATGTTKTGATGACCCAAACT
V_L-IIa-ext	ACTAGTCGACGATRTTKTGATRACCCA (*Sal* I)
V_L-For(CDR3)	GGATCCCGGGCGGAAAATGTGTAGTTTGCAC (*Xma* I)
V_H-Rev1	ATTGTTATTACTCGCGGCCCAACCGGCCATGGCCGAGGTCCTGCTGCAACAGTC (*Sfi* I)
V_H-For1	CCGCCGGATCCACCTCCGCCTGAACCGCCTCCACCTGCAGAGACAGAGACCAGAG
V_L-Rev1	CAGGCGGAGGTGGATCCGGCGGTGGCGGATCGGATGTTTTGATGACCCAAACTC
V_L-For1	GCTCAACTTTCTTGTCGACTTTATCATCATCATCTTTATAATCTTTGATTTCCAGCTTGGTGC (*Sal* I)
V_L-For2	GCTCAAACTTTCTTGTCGACTTATCAGCATGCTCCGCCCGCACGTTTGATTTCCAGTTTGGTT TTATCATCATCATCTTTATA (*Sal* I)

[a] Underlined in the nucleotide sequences

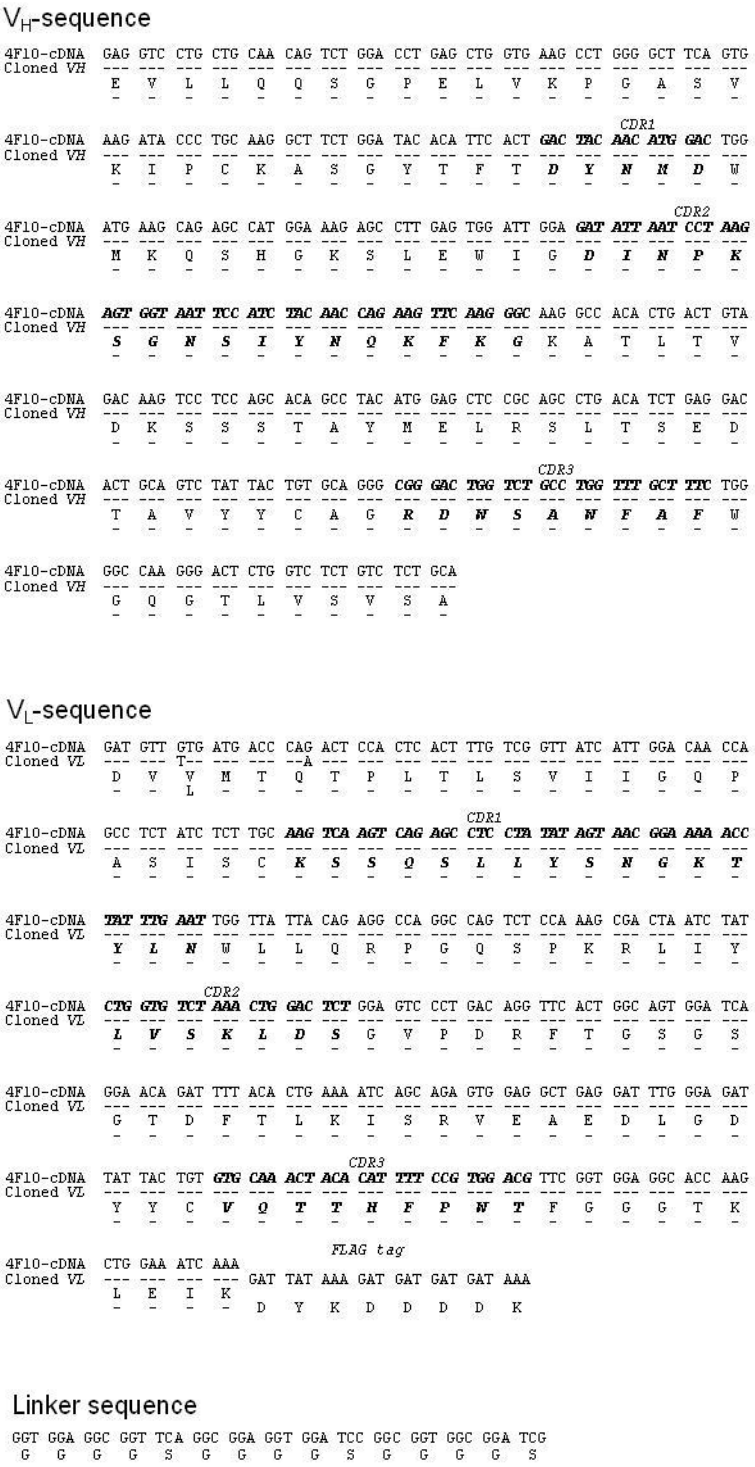

Figure S1. Nucleotide and amino acid sequences of the V_H and V_L domains of the anti-Tnc antibody 4F10.

The upper and lower lines show the sequences of the cDNA and the scFv-4F10 construct, respectively. One silent mutation and one missense (Val3Leu) mutation were introduced in V_L of scFv-4F10, due to the use of degenerate primer in the PCR cloning steps. The complementarity-determining regions (CDRs)[7] are shown in bold italic letters. The sequence of the linker that connects V_H and V_L is also shown.

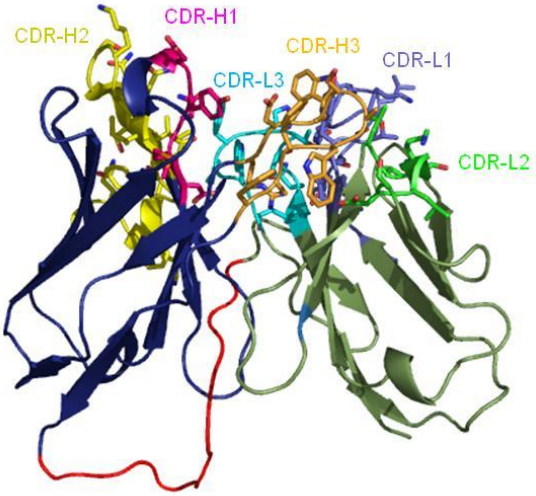

Figure S2. Protein modeling of scFv-4F10.

The protein ribbon structures of scFv-4F10 was generated using the SWISS-MODEL software.[8] The β-sheet structures consisting of the amino acids of the framework regions (FRs) are drawn by bold arrows (dark blue and dark green arrows for V_H and V_L, respectively). Amino acid residues forming the CDRs[7] (CDR-H1, -H2, -H3 in the V_H domain and CDR-L1, -L2, -L3 in the V_L domain) are indicated. The linker peptide is shown in red. This model structure has the characteristic V-domain architecture, *i.e.*, the CDR-composing amino acids form flexible loops that are raised above the surface of the rigid β-sheet scaffold.

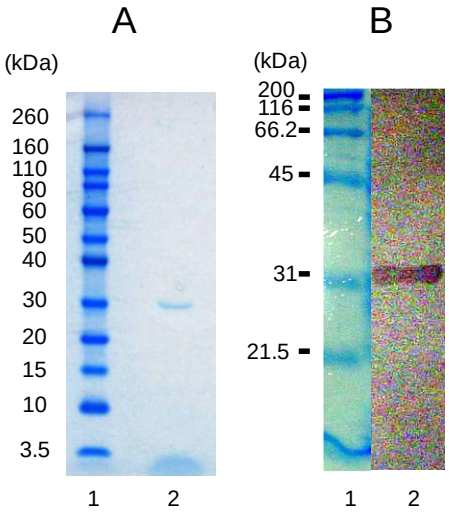

Figure S3. PAGE of scFv-4F10.

(A) SDS-PAGE of affinity-purified scFv-4F10 (Coomassie brilliant blue staining): lane **1**, M_r marker; **2**, scFv. (B) Immunoblotting of the affinity-purified scFv-4F10: lane **1**, M_r marker; **2**, scFv. The scFv was detected by sequential incubation with anti-FLAG M2 antibody followed by POD-conjugated rabbit anti-mouse IgG Fc-specific antibody. Bound POD activity was visualized using hydrogen peroxide and 4-chloro-1-naphthol as substrates.

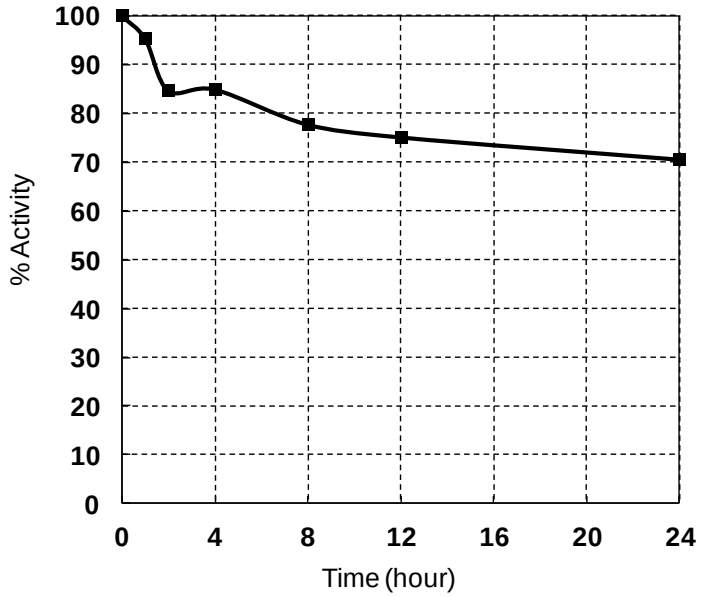

Figure S4. Retention of the binding activity of scFv-Cys against Tnc that has been incubated in plasma.

Normal rat plasma (Jackson ImmunoResearch) (200 μL) containing scFv-Cys (1.0 μg) was prepared in sextuplicate, and each of them was incubated at 37°C for 1, 2, 4, 8, 12, or 24 h. Then, an aliquot (40 μg) was taken from the mixture, diluted 25-fold with PBS, and 100 μL aliquot of the resulting solution (containing 200 ng/mL scFv, if this remained intact) was incubated in duplicate at 37°C for 1 h in microwells coated with a 10 μg/mL Tnc solution as described above. The wells were washed 3 times with T-PBS, and the POD-conjugated anti-FLAG M2 antibody (0.50 μg) in T-PBS (100 μL) was incubated at 37°C for 1 h. After washing the wells, bound POD activity was determined as described in the text.

References (for Supporting Information)

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