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Original Article

Mammalian cell display for rapid screening scFv antibody therapy

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Human antibodies are beginning to draw attention for use in immune gene therapy. The efficient generation of effective therapeutic monoclonal antibodies suitable for the treatment of cancers and infectious diseases would be enormously valuable. Antibody display methods are increasingly used to screen human monoclonal antibodies. Here we report the construction of a mammalian cell display method derived from a naive antibody repertoire, for which human single-chain variable fragments (scFv) have been transiently displayed on 293T cell surfaces based on a pDisplay vector. The sizes of the current pDisplay–scFv antibody repertoires have been estimated to be 0.74×10^7. An immunoblot assay confirmed the expression of the scFv antibody library. The subcellular distribution of ErbB3–scFv expression plasmid facilitated the display of ErbB3 scFv on the cell membrane surface and the efficiency of the display was evaluated by fluorescence-activated cell sorting. This method of mammalian cell display was verified by successfully screening ErbB3 scFv candidates. A published scFv control was used to confirm the feasibility of the ErbB3 scFv screening process. Three ErbB3 scFv candidates were produced and they were found to have affinity similar to the published scFv candidate. Thus, the present screening system provided an optimal alternative for rapid acquisition of a novel candidate scFv sequence to target genes with high affinity *in vitro*.

Keywords human scfv antibody; mammalian cell display; erbb3; FACS; human embryonic kidney cell

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Introduction

Human antibodies have considerable potential for use in immune gene therapy. The production of effective therapeutic monoclonal antibodies against targets in cancers and infectious diseases is an important and challenging task. Methods involving antibody display are widely used in the screening of human monoclonal antibodies. The recombinant antibody display technologies available today can be divided into *in vitro* display techniques [1], including phage display [2,3] and ribosome display [4,5], and *in vivo* display platforms, such as bacterial, yeast, and mammalian cell-surface displays [6,7]. Antibodies screened from the mammalian cell system have been used to perform glycosylation modifications and have shown higher affinity than those screened from other systems. In this way, mammalian antibody library technology is an alternative method of screening human antibodies.

Mammalian cells have been recently established as methods for displaying antibody repertoires [7–9]. In mammalian screening system, non-viral and viral expression vectors systems were used to generate antibody display libraries derived from peripheral blood mononuclear cells (PBMCs) of human donors [10] or spleen cDNA of chickens immunized with human and mouse interleukin 12 [11].

There have been few reports describing the applications of mammalian cell surface antibody display libraries. However, many human antibodies have gone through clinical studies and have been approved by the Food and Drug Administration of USA. Therefore, investigation of screening human antibody using mammalian system should be encouraged. The main limitation of mammalian cell display system is its small repertoire sizes. Furthermore, the more complex the antibody library, the less stable the display system, because of the involvement of inconsistent expression of heavy chain and light chains, or incorrect antibody assembly. In addition, rapid acquisition of antibody sequences is difficult. These obstacles must be overcome before antibody therapeutics can be developed. In contrast, a basic single-chain variable fragment (scFv) display system appears to be stable, simple,

and robust, which may rapidly yield antibody sequences. Furthermore, scFv antibody can be manipulated to produce diverse products such as full-length antibody constructions, scFv–antibody drug conjugates, and others. Therefore, the scFv sequence is important for the gene therapy-based antibody therapeutics.

Here, the scFv-displaying antibody repertoires were constructed to efficiently supply *de novo* isolated antigen-specific antibodies. Then, ErbB3 scFv antibody was successfully screened within 2–3 weeks, which confirmed the potential application of the scFv–pDisplay system for screening other optimal targets for antibody-based gene immunotherapy.

Materials and Methods

Construction of scFv-expressing plasmid library

One hundred and twenty peripheral blood samples were harvested from 82 healthy donors, 8 colon cancer patients, 10 liver cancer patients, 12 breast cancer patients, and 8 ovarian cancer patients to construct a non-immune antibody library. Clinical samples were obtained from the General Hospital of the Chinese Armed Police, with the informed consent of patients and with the approval of the Ethical Committee of the General Hospital of Chinese Armed Police and Beijing Institute of Radiation Medicine. The study was carried out according to the principles in the Declaration of Helsinki.

The scFv library was constructed as shown in **Fig. 1**. A total of 120 RNA samples were prepared from PBMCs collected from 120 patients using a lymphocyte separation medium (Solarbio, Beijing, China). A total of 120 cDNA reverse transcription reactions were carried out as described previously [12]. The cDNAs were divided into 12 pools, and each pool contained ~8–12 cDNA samples according to the classification of diseases. The 12 cDNA pools were used as templates to amplify the heavy chain/light chain variable region (VH/VL) genes using primers listed in **Table 1**. These primers were synthesized by Sangon (Beijing, China). The primers sets were synthesized to assure that they could amplify all possible variable region genes in V BASE [13]. The $(Gly4Ser)_3$ linker (GGTGGTGGTGGATCAGGTGGAGGAGGTTCTGGAGGTGGTGGATCC) was used to construct scFv. To amplify VH and VL fragments, a polymerase chain reaction (PCR) mixture (25 μl) containing 16.75 μl ddH_2O, 5 μl buffer, 2 μl dNTP, 0.5 μl forward primer (10 μM), 0.5 μl reverse primer (10 μM), and 0.25 μl primestar (TaKaRa, Tokyo, Japan) was prepared. Then 1 μl cDNA was added and the reaction was carried out under the following conditions: 98°C for 8 min (98°C for 30 s, 55°C for 30 s, and 72°C for 30 s), 28 cycles, 72°C for 7 min.

The scFv genes were obtained by overlapping PCR, which was performed using recovered VH and VL as templates using PTfw and PTrv primers (**Table 1**). A 25-μl PCR mixture was mixed with 1 μl VH and 1 μl VL, respectively, and the reaction was carried out under the following conditions: 98°C for 8 min (98°C for 30 s, 55°C for 30 s, and 72°C for 1 min), 28 cycles, 72°C for 7 min.

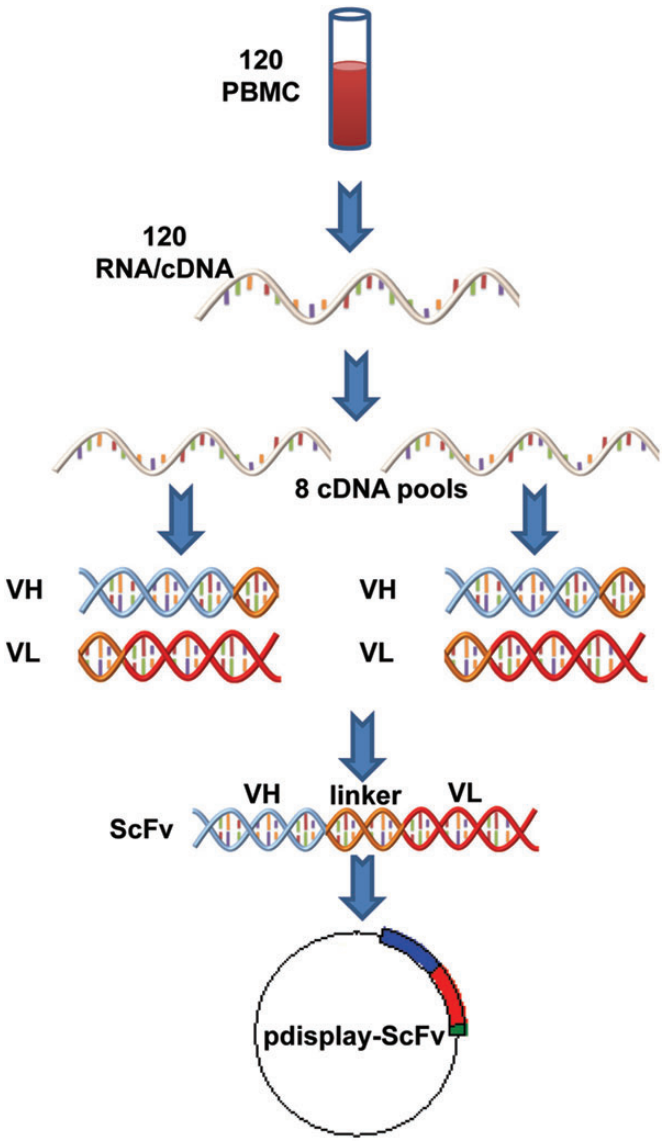

Figure 1. scFv-expressing plasmid library construction A total of 120 PBMCs were extracted. RNAs and cDNAs were isolated separately. The cDNAs were divided into 12 pools and used as templates for the amplification of VH/VL gene pools. Overlapping PCR was performed to construct scFv, which was cloned into pDisplay vector.

The scFv mammalian library was constructed by inserting the recovered scFvs into the pDisplay vector (Invitrogen, Carlsbad, USA) using *Sfi*I and *Sal*I digestion enzymes (Invitrogen). The scFv candidates targeting ErbB3 were screened to confirm the function of the scFv library. In addition, a published ErbB3 scFv was used as a positive control to monitor the quality of the mammalian library. The ErbB3 scFv-positive sequence, which was specific for the extracellular domain of ErbB3 [14] (GenBank accession No: AF048774.1), was selected from a phage human scFv

Table 1. Primes sequences for the amplification of scFv antibody library

Primer	Sequence (5′→3′)
VH5-1	CCTTTCTATGCGTCGCGGCCCAGCCGGCCATGCAGGTGCAGCTGCAGGAGTCSG
VH5-2	CCTTTCTATGCGTCGCGGCCCAGCCGGCCATGCAGGTACAGCTGCAGCAGTCA
VH5-3	CCTTTCTATGCGTCGCGGCCCAGCCGGCCATGCAGGTGCAGCTACAGCAGTGGG
VH5-4	CCTTTCTATGCGTCGCGGCCCAGCCGGCCATGGAGGTGCAGCTGKTGGAGWCY
VH5-5	CCTTTCTATGCGTCGCGGCCCAGCCGGCCATGCAGGTCCAGCTKGTRCAGTCTGG
VH5-6	CCTTTCTATGCGTCGCGGCCCAGCCGGCCATGCAGRTCACCTTGAAGGAGTCTG
VH5-7	CCTTTCTATGCGTCGCGGCCCAGCCGGCCATGCAGGTGCAGCTGGTGSARTCTGG
VH3-1	ACCTCCAGAACCTCCTCCACCTGATCCACCACCACCTGAGGAGACRGTGACCAGGGTG
VH3-2	ACCTCCAGAACCTCCTCCACCTGATCCACCACCACCTGAGGAGACGGTGACCAGGGTT
VH3-3	ACCTCCAGAACCTCCTCCACCTGATCCACCACCACCTGAAGAGACGGTGACCATTGT
VH3-4	ACCTCCAGAACCTCCTCCACCTGATCCACCACCACCTGAGGAGACGGTGACCGTGGTCC
VH3-5	ACCTCCAGAACCTCCTCCACCTGATCCACCACCACC GGT TGG GGC GGA TGC ACT CC
VH3-6	ACCTCCAGAACCTCCTCCACCTGATCCACCACCACC SGA TGG GCC CTT GGT GGA RGC
Vκ5-1	GGATCAGGTGGAGGAGGTTCTGGAGGTGGTGGATCCGACATCCRGDTGACCCAGTCTCC
Vκ5-2	GGATCAGGTGGAGGAGGTTCTGGAGGTGGTGGATCCGAAATTGTRWTGACRCAGTCTCC
Vκ5-3	GGATCAGGTGGAGGAGGTTCTGGAGGTGGTGGATCCGATATTGTGMTGACBCAGWCTCC
Vκ5-4	GGATCAGGTGGAGGAGGTTCTGGAGGTGGTGGATCCGAAACGACACTCACGCAGTCTC
Vκ3-1	CAGTCATTCCGGACGCGTCGACTTTGATTTCCACCTTGGTCC
Vκ3-2	CAGTCATTCCGGACGCGTCGACTTTGATCTCCASCTTGGTCC
Vκ3-3	CAGTCATTCCGGACGCGTCGACTTTGATATCCACTTTGGTCC
Vκ3-4	CAGTCATTCCGGACGCGTCGACTTTAATCTCCAGTCGTGTCC
Vλ5-1	GGATCAGGTGGAGGAGGTTCTGGAGGTGGTGGATCC CAG TCT GTS BTG ACG GAG CCG CC
Vλ5-2	GGATCAGGTGGAGGAGGTTCTGGAGGTGGTGGATCC TCC TAT GWG CTG ACW CAG CCA C
Vλ5-3	GGATCAGGTGGAGGAGGTTCTGGAGGTGGTGGATCC TCC TAT GAG CTG AYR CAG CYA CC
Vλ5-4	GGATCAGGTGGAGGAGGTTCTGGAGGTGGTGGATCC CAG CCT GTG CTG ACT CAR YC
Vλ5-5	GGATCAGGTGGAGGAGGTTCTGGAGGTGGTGGATCC CAG CCW GKG CTG ACT CAG CCM CC
Vλ5-6	GGATCAGGTGGAGGAGGTTCTGGAGGTGGTGGATCC TCC TCT GAG CTG AST CAG GAS CC
Vλ5-7	GGATCAGGTGGAGGAGGTTCTGGAGGTGGTGGATCC CAG TCT GYY CTG AYT CAG CCT
Vλ5-8	GGATCAGGTGGAGGAGGTTCTGGAGGTGGTGGATCC AAT TTT ATG CTG ACT CAG CCC C
Vλ5-9	GGATCAGGTGGAGGAGGTTCTGGAGGTGGTGGATCC CAG DCT GTG GTG ACY CAG GAG CC
Vλ3-1	CAGTCATTCCGGacgcGTCGAC TAG GAC GGT SAS CTT GGT CC
Vλ3-2	CAGTCATTCCGGacgcGTCGAC GAG GAC GGT CAG CTG GGT GC
PTfw	CCTTTCTATGCGTCGCGGCCCAGC
PTrv	CAGTCATTCCGGACGCGTCGACTT

R = A/G, Y = C/T, M = A/C, K = G/T, S = C/G, W = A/T, H = A/C/T, B = C/G/T, V = A/C/G, D = A/G /T, N = A/C/G/T.

antibody library. It was synthesized and cloned into the designated pDisplay vector as described above.

Determination of the sizes of pDisplay–scFv antibody repertoires

To preliminarily evaluate the sizes of the pDisplay–scFv repertories, bacterial solutions were plated on Luria–Bertani (LB)-ampicillin plates and cultured at 37°C overnight. Colonies were counted to ensure a rough antibody repertoires size. Sequencing was performed (Sangon) and the sequence data were submitted to IgBlast software (http://www.ncbi.nlm.nih.gov/projects/igblast/) to confirm the production of scFv antibody.

Scfv antibody library expression and display characteristics

Transfection was performed in 35-mm tissue culture dishes using HEK293T cells. The day before transfection, 2×10^5 cells were seeded in each tissue culture dish. pDisplay–scFv plasmid repertoires (~2–3 μg) were transfected using

polyethylenimine (PEI) according to the manufacturer's instructions (Invitrogen). Six hours later, medium was exchanged with fresh medium containing 5% fetal bovine serum (FBS; CWBIO, Beijing, China). Approximately 60 h post-transfection, antibody expression was analyzed by immunoblot assay with antibodies against c-myc (1 : 5000 dilution, Proteintech, Chicago, USA), and GAPDH (1 : 5,000 dilution; Santa Cruz, USA).

The display of scFv antibody was investigated using immunofluorescence assay. In brief, cells grown on glass coverslips were fixed, permeabilized, and blocked in normal goat serum. Cells were also labeled with FITC-anti-c-myc antibody (Sigma-Aldrich, St Louis, USA). Nuclei were counterstained with DAPI (Bio-Rad, Hercules, USA). Confocal images were collected using a Radiance 2100 a confocal microscope (Bio-Rad).

The efficiencies of transfection and display were determined using fluorescence-activated cell sorting (FACS). HEK293T cells were transfected as described above. Approximately 60 h later, cells were collected and incubated simultaneously with 1 μg FITC-anti-c-myc antibody (Sigma-Aldrich) per 1×10^6 cells at room temperature for 1 h. Then cells were washed three times with phosphate buffer solution (PBS) before detection.

Display of ErbB3 scFv antibody on the surfaces of mammalian cells

The mammalian cells surface display and FACS protocols are illustrated schematically in **Fig. 2**. Transfection was performed in six 100-mm tissue culture dishes using HEK293T cells as described previously [15]. Plasmid pools were prepared by mixing and 2×10^6 cells were seeded in each tissue culture dish. After overnight incubation, 30 μg pDisplay-scFv DNA pools spiked with ErbB3 scFv positive control (0.3% in 30 μg plasmid) were transfected into each dish with 30 μl PEI according to the manufacturer's instructions.

Cells were cultured in 5% FBS for an additional 60 h. Sorting was performed by FACS (MoFlo XDP; Beckman Coulter, Pasadena, USA) using FITC-His-ErbB3-ECD, an extracellular domain of the ErbB3 (His-tagged) purified in our own laboratory and labeled with FITC in Uscn (Wuhan, China).

Approximately 60 h later, 1×10^6 cells were suspended in 1 ml PBS with 1 μg FITC-His-ErbB3-ECD. After incubation at room temperature for 1 h, the cells were washed three times with PBS and resuspended in 100 μl of PBS. A total of 6×10^7 cells were sorted and 0.1%–0.4% of the population was collected per round. At the end of each sorting event, plasmid DNA was extracted from the collected cells

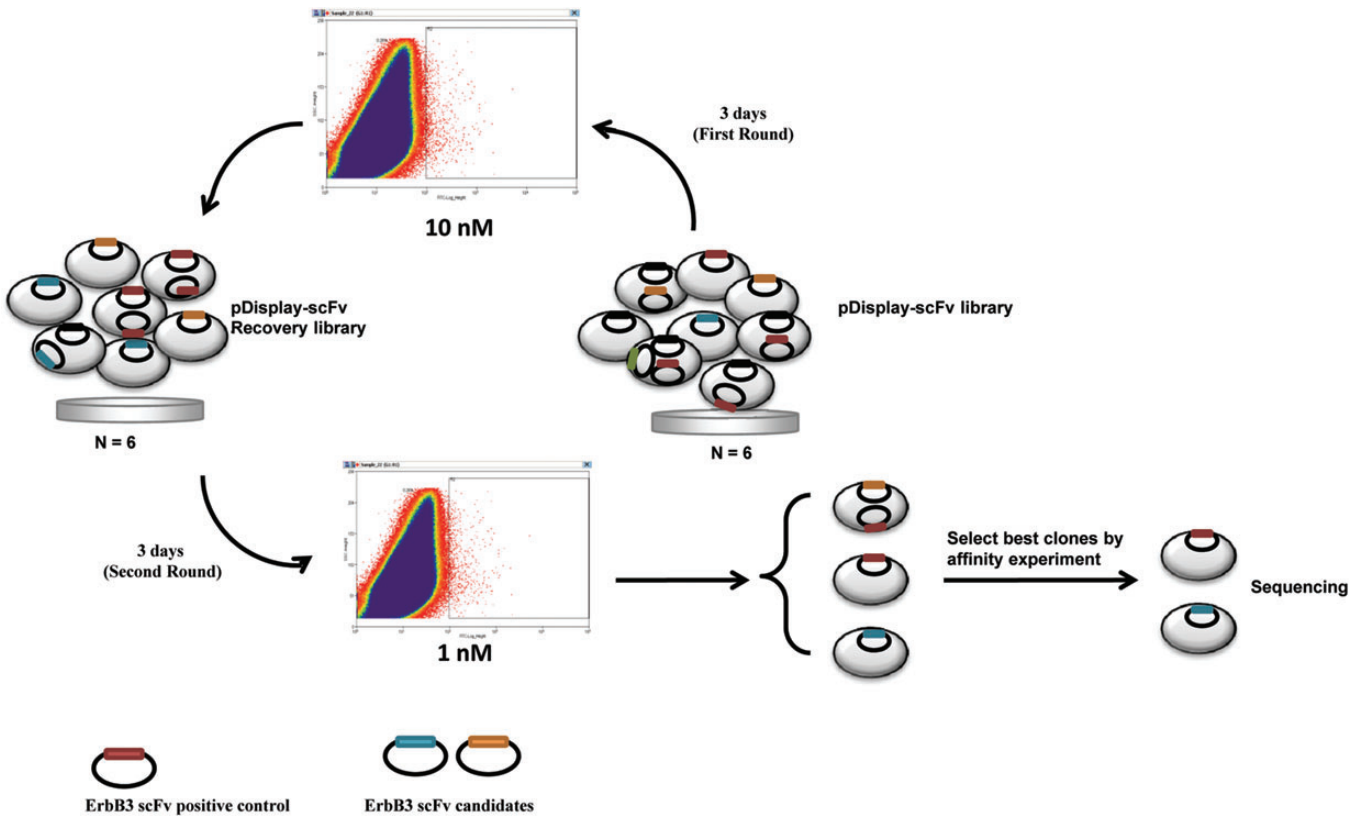

Figure 2. Schematic representation of the pDisplay–scFv display system used to screening ErbB3 scFv antibodies HEK293T cells were co-transfected with pDisplay–scFv plasmid library. These cells display human platelet-derived growth factor receptor on their surfaces. The scFv displaying mammalian cells were incubated with fluorescent-conjugated antigen (10 and 1 nM, generally) and sorted by flow cytometry. The whole screening process took only about 2 weeks.

and transformed into DH5α for amplification. Then, the extracted plasmid DNA were amplified and used as the pool for the next round sorting.

ErbB3 antigen concentration was decreased during the FACS sorting process. For the first sorting, 1×10^6 cells were incubated with 1 μg/ml of FITC-His-ErbB3-ECD (final concentration 10 nM). For the second sorting, 1×10^6 cells were incubated with 0.1 μg/ml of FITC-His-ErbB3-ECD (final concentration 1 nM). At the end of the screening, clones were sequenced and repeated clone sequences were considered as ErbB3 candidate scFvs for further affinity determination.

Apparent affinity constant (apparent K_D) determination by flow cytometry

Apparent K_D was measured by determining the antigen concentration when half of the scFv on the HEK293T cell surface bound to the antigen according to previously published methods [15]. The day before transfection, 2×10^5 cells were seeded in 35-mm tissue culture dish. Multiple ErbB3 candidate scFv plasmids were transfected into the tissue culture dishes. pDisplay–VEGF scFv (non-specific sequence) served as the negative control. Then, approximately 60 h post transfection, 1×10^5 cells were incubated with various concentrations (0, 0.4, 1.6, 6.4, 25.6, 50, and 100 nM) of FITC-His-ErbB3-ECD as described above. MFI in HEK293T cells was measured on a Guava flow cytometer (Millipore, Boston, USA). Equilibrium constants were determined using the Marquardt-Levenberg algorithm for non-linear regression with GraphPad Prism (Origin 7.5) according to the developer's instructions.

Results

Size of pDisplay–scFv antibody repertoires

Construction of the plasmid vector pDisplay–scFv is described in **Fig. 3A**. The creation of large, diverse antibody libraries from natural sources relies on primers that can amplify as many variable region genes as possible. Primers selected in this study are used to amplify as many as possible the functional variable regions [13]. They were designed using the following criteria: at least 16 bp homology of the

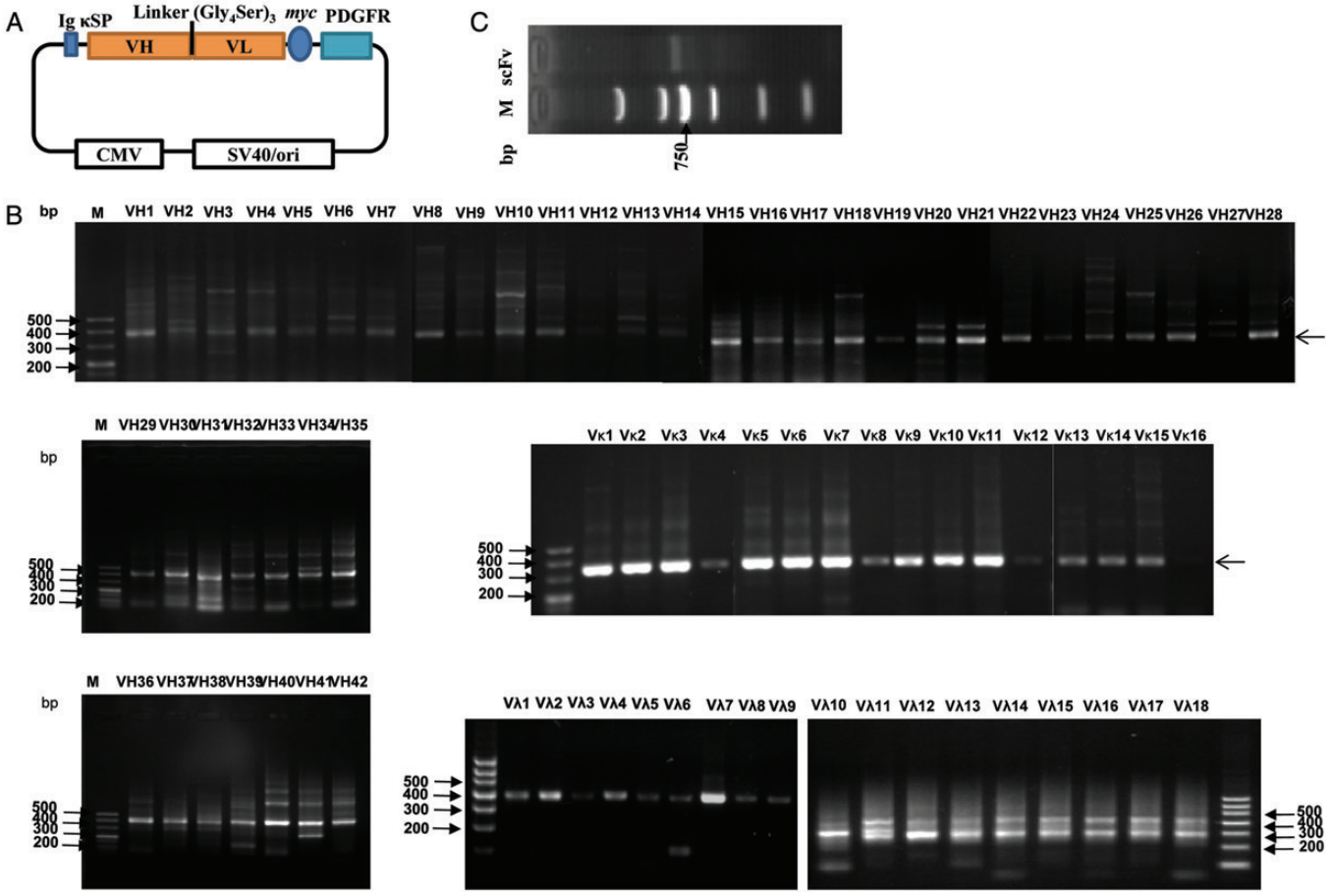

Figure 3. Construction of scFv antibody library (A) Expression plasmid for display of scFv on mammalian cells. The vector was based on pDisplay vector. P_{CMV} cytomegalovirus promoter; IgκSP, murine Igκ chain signal peptide; myc, an epitope tag to measure the scFv expression level; $P_{SV40/ori}$, SV40 promoter and origin facilitating episomal replication in mammalian cells expressing SV40 large T antigen. (B) VH and VL fragments were recovered in agarose electrophoresis. (C) Overlapping polymerase chain reaction (PCR) was performed to produce the scFv fragment. (D) Sequences were compared using DNAMAN to verify the diversity of the scFvs.

3′ end of the primer to the variable region genes, no more than 8-fold total degeneracy and minimum primer dimer formation. VH and VL (Vλ and Vκ) fragments were amplified and recovered as shown in **Fig. 3B**. Overlapping PCR was performed to obtain the scFv fragment (**Fig. 3C**).

The antibody diversity and repertoire size were calculated as follows. A total of 10^4 μl scFv fragments were obtained after multiple operation runs. The 4 μl scFv fragments and digested display vector were ligated by T4 DNA ligase and the ligated products were transformed into DH5α, half of which were plated onto LB plate with ampicillin. A total of 530 clones were grown. Of these, 20 clones were selected for PCR determination and sequencing. The immunoactivity analysis of VH and VL sequences was performed using IgBLAST-imgt and shown in **Supplementary Fig. S1**. Of these 20 clones, 14 were found to be productive, and DNAMAN analysis indicated that they differed in sequence, which indicated diversity (**Supplementary Fig. S2**). For this reason, these 14 clones were treated as positive clones. The current size of the repertoire of productive antibody library was calculated as follows: $(14/20) \times 530 \times 2 \times 10^4 = 0.74 \times 10^7$.

Expression and display characteristics of the scFv antibody library

Immunoblot assay confirmed the correct expression of the scFv antibody library (**Fig. 4A**). To ascertain the display function of scFv antibody on the cell surface for the following FACS sorting processes, cellular location of antibody pools was determined. Confocal images at 60 h after transfection revealed that the scFv was mainly located on the cell membrane (**Fig. 4B**). The efficiency of display was evaluated using FACS (**Fig. 4C**). The results showed that the mammalian antibody was expressed and displayed on the cell surface at an efficiency level of 20%. This established a foundation for the following scFv antibody screening processes targeting certain antigen.

FACS sorting

To confirm the feasibility of the pDisplay–scFv antibody library for screening a designated target gene, scFv targeting ErbB3 was screened. Some of the positive scFv plasmid was spiked during the screening process (0.3% total DNA plasmid transfected). This could theoretically be sorted and selected from the primary antibody library. After two rounds of selection, 35 clones were selected to be sequenced and submitted to IgBlast. The candidate scFv 22# was repeated nine times and scFv 9# five times. The positive control was repeated three times. IgBlast results showed that VH and VL genes were all productive and contained no stop codons. The VLs were κ-type. The ErbB3 scFv sequences (such as candidate scFv 9# and scFv 22#) differed in sequence from the positive control as shown in **Fig. 5A**. The data confirm the efficacy of the pDisplay–scFv antibody library.

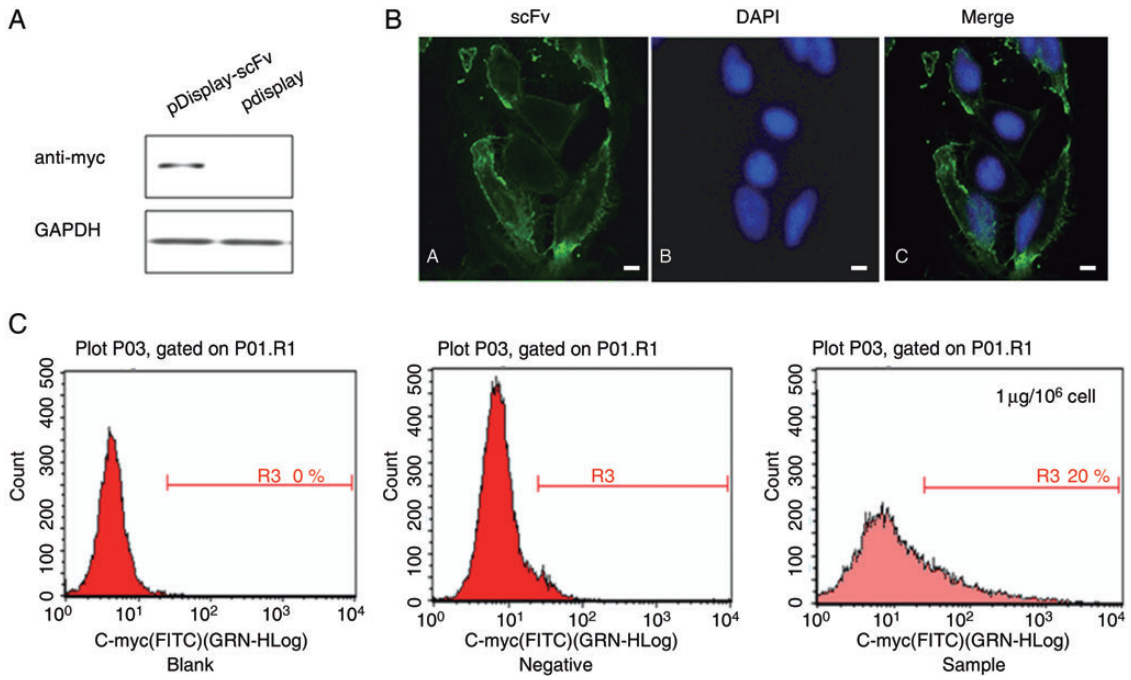

Figure 4. Expression and display characteristics of the scFv antibody library (A) Immunoblot assay confirmed the correct expression of the scFv antibody library using anti-myc and GAPDH antibodies. (B) Confocal microscopic images of HEK293T cells displaying scFv. 293T cells transfected with scFv-expressing plasmid were grown on coverslips. (A) The scFv were detected with FITC (green). (B) DAPI nuclear staining (blue). (C) Merged staining patterns are shown (scale bar = 10 μm). (C) scFv-expressing plasmid pools and labeled with PBS. Negative: cells were transfected with pDispay empty vectors and labeled with FITC-anti-c-myc antibody. Sample: cells were transfected with scFv-expressing plasmid pools and labeled with FITC-anti-c-myc antibody.

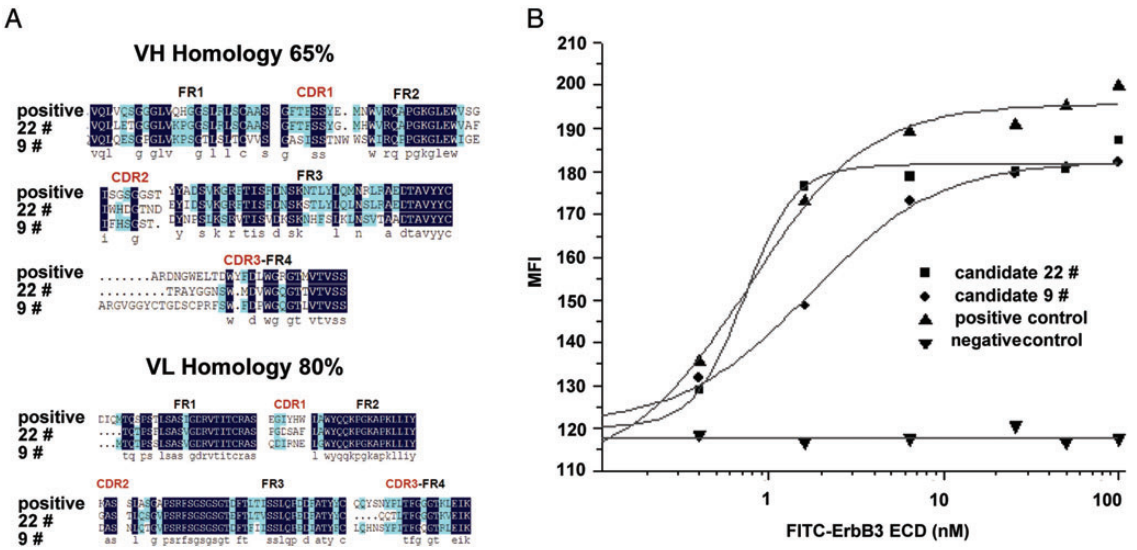

Figure 5. Apparent affinity of ErbB3 scFv candidates Apparent affinity was determined using median fluorescence intensity (MFI) of FITC. Results are plotted versus various concentrations of FITC-labeled ErbB3-ECD used to label surface-displayed scFv candidate 22 (K_D app = 0.72 nM), candidate 9 (K_D app = 1.72 nM), and positive control (K_D app = 0.76 nM). Data were fit with nonlinear fitting. pDisplay–VEGF scFv (non-specific sequence) served as the negative control.

The apparent affinity of ErbB3–scFv antibody

Apparent binding affinity of antibody was monitored by FACS after several pDisplay–scFv candidates were transfected into HEK293T cells. As shown in **Fig. 5B**, the apparent binding affinity (K_D app) was 0.72 nM for the candidate 22 and 1.72 nM for candidate 9, which was similar to that of the positive control (K_D app = 0.76 nM).

Discussion

We constructed a stable, simple scFv antibody library for rapid screening of any designated target. Our system has several advantages. First, we need only 2 weeks to obtain the ErbB3 scFv candidates containing the positive scFv control, suggesting the feasibility and efficacy of our display antibody library. In addition, the succeeding antigen binding affinity can be easily performed using the candidate scFv-expressing plasmid without the soluble scFv antibody. In this study, scFv candidates targeting ErbB3 were successfully obtained. In the subsequent experiments, scFv candidates targeting PD1 and B lymphocyte stimulator were also successfully screened (data not shown). The present work demonstrated the robustness of the antibody library. If conditions are permitted, it may be possible to perform multiple scFv sorting selection, simultaneously targeting multiple antigens as in the case of differently labeled antigens using multiple pathway FACS. Our screening system provided an optimal alternative for rapid acquisition of candidate scFv targeting potential genes with high affinity *in vitro*.

There are some characteristics of FACS as follows. Generally, for FACS selection mode, the sort window was drawn to include the top 0.1% of total cells in terms of ratio of PE/FITC fluorescence [9,15]. Regarding FACS, there are several setting parameters that may affect the results of the sorting process including the specific antibody, cell treatment, and FACS compensation adjustment. Results showed that the single- and double-labeled sorting results were the same. It was therefore concluded that the single-labeled sorting was sufficient for sorting selection as long as the sorting window was appropriately set. Of note, because the strong fluorescence, the PE-stained single sorting mode usually leads to more false-positive clones, so a relatively weak fluorescence is recommended for the single-labeled sorting. Thus, we recommend that when the pDisplay and FACS system were used together, different antigens should be labeled with different fluorescent molecules, and simultaneous selection should be done with a multiple selecting FACS system. Different sorting windows should be selected according to the intensity of the labeled fluorescent molecule. In addition, when using the display system, fresh sorting and plasmid recovery are necessary. Though the fixed process did not affect sorting and selection in FACS, we failed to recover plasmids due to fixation.

Antibody screening systems can be divided into stable and transient transfection systems, both of which have their pros and cons. Though stable transfection systems usually involve only one sequence per cell, obtaining candidate antibody-secreting cell clones is usually time consuming and labor intensive. In comparison, for transient transfection system, some cells have different copy numbers of target genes. It is also likely that the scFv with the most pronounced affinity may be expressed at relatively low level that leads to false-negative results. Similarly, certain scFvs with medium affinity may be highly expressed; this may lead to false-negative

results. However, this problem could be overcome by reducing the antigen concentration in FACS screening and increasing the number of selection runs. scFv sequences may be obtained in this way. For transient transfection systems, as described in this study, candidate scFv sequences targeting specific antigens were obtained rapidly and efficiently, rather than cells that specifically expressed a single scFv candidate. In this study, after the second run with the 1 nM FITC-His-ErbB3-ECD concentration, scFv candidate sequences with considerable affinity to the positive control sequence were obtained. Certainly, scFv sequences with very high binding potency could be obtained by reducing the antigen concentration and adding more selection runs.

Although phage display is the most widely accepted up to now, and eukaryotic expression screening system has its advantages such as protein maturation, folding and modification, we are still not sure which repertoire type and which display platform will yield the best antibodies for any particular target antigen. Thus, many formats and platforms need to co-exist to assure obtaining the optimal antibodies. Our high throughput methods of selection and screening allowed for time- and cost-effective discovery of scFvs specifically binding to designated antigens in the scFv display library.

Supplementary Data

Supplementary Data are available at *ABBS* online.

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